Immunology 1985 **54** 105

Lymphocyte emigration from lymph nodes by blood in the pig and efferent lymph in the sheep

R. M. BINNS, R. PABST* & S. T. LICENCE *AFRC Institute of Animal Physiology, Babraham, Cambridge, and *Centre of Anatomy, Hannover Medical School, West Germany*

Accepted for publication 30 August 1984

Summary. Two types of experiment using local labelling of lymph nodes with FITC showed that lymphocytes emigrate from lymph nodes, predominantly in blood in the pig and in efferent lymph in the sheep. In the first type of experiment with the pig, few cells emigrated via the lymph, while the number of labelled cells in the blood increased progressively and the indices in mesenteric blood were always higher than in jugular blood in simultaneously-drawn samples. However, in the sheep, when efferent lymph flowed freely, very low numbers emerged in blood and continuing large numbers of lymphocytes emerged in efferent lymph. In the second type of experiment carried out wholely under anaesthetic on mesenteric lymph nodes in pigs and sheep, and on superficial inguinal lymph nodes in pigs, the lymph node was isolated, the lymph and venous drainage collected and only the arterial supply maintained. Large numbers of $FITC^+$ lymphocytes emigrated via the vein in pigs with either node cannulation (i.e. up to 7% blood lymphocytes were labelled with an emigration rate of $\sim 10^8$ cells/hr) but in sheep, while lymph contained $\sim$30–80% labelled cells and the emigration rate was also $\sim 10^8$ cells/hr, the mesenteric blood contained very few labelled cells ($\sim$0·2%, giving a mean venous emigration rate of $2{\cdot}7 \times 10^6$/hr). Study of the type of lymphocytes emerging from labelled pig lymph nodes and spleen during the phase of major emigration showed that sIg^+ B and E rosette-forming T cells, but almost no Null cells, are involved.

Correspondence: Dr R. M. Binns, Dept. Immunology, AFRC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT.

INTRODUCTION

The efficient recirculation of lymphocytes through lymph nodes, spleen and other lymphoreticular tissues is of great importance, providing the means of interaction of antigen-reactive lymphocyte subpopulations with antigen on specialized presenting cells, and the wide dissemination of the resulting effector and memory cells. The route of entry of lymphocytes into lymph nodes is well accepted as the post-capillary endothelium in the paracortex (Ford, 1975) and, in the sheep and rat, the current dogma points to an exclusive exit from the lymph node via efferent lymph. This view is supported by the observation that an immune response induced locally in a sheep lymph node can be isolated in that node by draining away all efferent lymph, resulting in no systemic memory, even though systemic reinfusion of the collected stored lymphoid cells restores an efficient immune response (Hall *et al.*, 1967). However, this route may not be exclusive, a view vigorously proposed by Saint-Marie (1975), even though the evidence in most species is not strong. Evidence for such a route has been obtained in sheep in several ingenious but somewhat artificial models, in which collected efferent lymph cells were infused into an afferent lymphatic of the same lymph node, so preventing emigration from the lymph, but not the continued entry of cells from the blood (Trevella & Morris, 1980). The authors' explanation for the observed absence of an increasing number of cells in the efferent lymph was their direct entry into the blood, but other explanations cannot be excluded, such as the prolonged retention or death of lympho-

cytes within the lymph node. Several lines of evidence have suggested that this is the predominant route of emigration from lymph nodes in the pig (Binns, 1982). These include the lack of lymphocytes in efferent lymph (Binns & Hall, 1966), the major discrepancy between intestinal afferent and efferent lymph counts (Bennell & Husband, 1981a), functional and lymphocyte tracing studies in and through lymph nodes (McFarlin & Binns, 1973; Binns, 1980; Bennell & Husband, 1981a), the failure to prevent dissemination of an intestinal immune response by cannulation and drainage of the efferent lymph of the mesenteric lymph nodes (Bennell & Husband, 1981b) and our observation that blood draining mesenteric lymph nodes labelled with FITC carries a consistently higher incidence of FITC-labelled lymphocytes than the jugular blood, and that this emigration is not prevented by complete drainage of the efferent lymph (Pabst & Binns, 1981). The present studies give direct evidence of venous emigration of labelled lymphocytes from lymph nodes in the pig, and parallel evidence from experiments using the same techniques and protocols in sheep of predominant emigration of such cells via efferent lymph. They also show that the lymphocytes which recirculate in the pig may not involve all the blood lymphocyte subpopulations.

MATERIALS AND METHODS

The animals used in these studies were Large White pigs weighing 20–90 kg body weight, and 6–9-month-old German white-face sheep weighing 30–40 kg. They were starved for 1 or 2 days before operation. The following three operations were performed.

(i) In pigs and sheep, the mesenteric lymph nodes were isolated by tying the mesenteric vascular branches between the small intestine and lymph nodes, and then cannulating the mesenteric vein and intestinal lymph duct to collect all venous and lymph drainage. All tissue and vascular connections to the animal were ligated, only retaining the arterial blood supply. A proportion (one-half to three-quarters) of the lymph node chain was then labelled by multiple microinjections of the nodes with FITC (1 mg/ml in pigs), a typical lymph node taken to estimate labelling index (normally 30–50%), and the blood and lymph collected continuously for several hours to collect and count the labelled cell emigrants. These experiments were carried out entirely under anaesthetic.

(ii) The second operation on sheep mesenteric lymph nodes was exactly as described previously for pigs (Pabst & Binns, 1981). The intestinal lymph duct and a branch of the mesenteric vein draining the small intestines were cannulated, vascular connections to other regions of the gut ligated, and the lymph nodes labelled under anaesthetic. Lymph was collected continuously in timed fractions and, at intervals, simultaneous paired samples of blood were taken from the mesenteric vein and a jugular vein cannula. After a period of collection, the abdomen was closed and the animal was allowed to recover from anaesthesia. Collections were made for up to $\sim$24 hr.

(iii) In the third operation carried out wholely under anaesthetic on the isolated superficial inguinal lymph node of large pigs, the methods were essentially as descrived previously (Binns, 1980). The node and surrounding fat was isolated carefully, retaining only the arterial supply, and the efferent vein cannulated. The lymph node was injected and draining blood collected continuously in 10-min fractions until the end of the experiments. Blood draining the superficial inguinal lymph node also drained the adjacent fat, muscle and skin served by the same vascular tree, and was locally heparinized to supplement systemic heparinization. At intervals, blood was also taken from the jugular vein to assess the systemic labelling index.

In all experiments, the blood samples were heparinized, diluted and centrifuged on Ficoll/triosil to isolate lymphocytes. FITC-labelled cells were enumerated as described previously (Binns, Blakeley & Licence, 1981). In recovery experiments, a typically labelled small lymph node was taken just after revascularization to assess the labelling index, and sometimes samples of the lymphoreticular tissues were taken when the animal was put down at the end of the experiment. The subpopulations of lymphocytes involved in emigration were assessed by washing the lymphocytes four times and including them in standard DARR and E rosette-forming assays for B and T cells (Binns, 1978, 1982; Binns *et al.*, 1979). Assays for rosette formation were read after incubation at 4° overnight, with and without addition of fluorescein diacetate, the former to obtain data on the total blood lymphocyte populations and the latter only counting rosette formation among the $FITC^+$ cells. In this way, the incidences of B, T and Null (100%−%B+%T) cells in the labelled and unlabelled cells were calculated.

RESULTS

Incidence of labelled cells emigrating in the blood and efferent lymph draining FITC-labelled pig and sheep lymph nodes

Previous studies in pig (Pabst & Binns, 1981) had shown that levels of labelled lymphocytes in the mesenteric vein blood draining mesenteric lymph nodes labelled with FITC consistently exceeded those in the general circulation (jugular vein) in several paired samples, and that the total number of labelled lymphocytes emerging in the drained intestinal lymph was very small compared to that which must be emigrating directly into efferent venules (Fig. 1). Three similar experiments were carried out in sheep. In only one of the three sheep (no. 1) did lymph flow continuously with no impedence. Figure 1 shows that the level of lymphocytes in lymph fell progressively during the collection (~24 hr) but that, in contrast to the pigs, very few labelled cells were present in the mesenteric and jugular blood. Moreover, labelled cell indices were the same or higher in jugular blood. Also, the distant lymphoid tissues contained few labelled cells; e.g. at day 1 the prescapular and unlabelled mesenteric lymph nodes in the successful sheep contained 0·03 and 0·01% $FITC^+$ cells, respectively, compared to a mean of 0·7 and 1·0% in the similar experiments in pigs. Thus, these data provided no support for the existence of an important venular emigration pathway from the lymph node in sheep. The other two sheep showed poor or intermittent lymph flow and, although levels of labelled cells fell in the same way in the lymph, numbers of cells were present in the blood ($\lesssim$1% and in the tissues at day 1), but escape of lymph via other efferent lymphatic routes or via lymphatic-venous anastamoses could not be excluded.

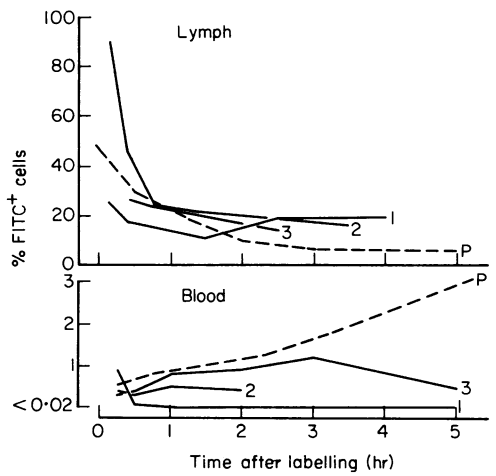

Figure 1. Incidence of FITC-labelled lymphocytes in efferent lymph and venous blood draining labelled sheep mesenteric nodes. Labelling indices in efferent lymph and blood are shown for three sheep (——), in which the intestinal lymphatic and a branch of the mesenteric vein were cannulated before half the mesenteric nodes were labelled by microinjection of FITC. Mean data from similar experiments in young pigs (Pabst & Binns, 1981) are also shown (– – –) for comparison. In sheep no. 1, lymph flowed uninterrupted and consistently and contained ~10–25% labelled lymphocytes, but levels in blood were 0·1% after the postinjection sample. In sheep 2 and 3, lymph flow was inconsistent and labelled lymphocytes reached the blood circulation. This difference in 'escape' to the blood stream due to interruption of lymph flow was reflected in the labelled cell incidence 1 day later in unlabelled mesenteric nodes, i.e. sheep 1 = 0·01%; sheep 2 = 0·04% and sheep 3 = 1·1% compared to ~1% in the pig experiments. At this time, ~15–30% of the remaining lymphocytes were $FITC^+$ in the labelled nodes.

Lymphocyte emigration from isolated FITC-labelled lymph nodes with retained arterial supply under anaesthesia

In the experiments quoted above, mesenteric blood from the labelled lymph nodes was sampled but not interrupted, and evidence for venular emigration depended both on the differences in labelled cell indices in mesenteric and jugular blood, and on the lack of effect of complete deviation of the intestinal lymph on the appearance of labelled cells in the general venous circulation. These experiments under anaesthetic, collecting the whole venous (and sometimes lymph) drainage from superficial inguinal and mesenteric lymph nodes, retaining only their arterial connection to the animal, also showed major direct imigration to veins in the pig (Fig. 2), but a predominant lymph emigration the sheep with only a minor venous emigration (Fig. 3).

Figure 2 shows the pattern of incidence of FITC-labelled cells in the blood draining the superficial inguinal lymph node and surrounding tissue following FITC-injection of the lymph node in three pigs. Labelled lymphocytes were found in the blood stream during and immediately after injection of FITC, but fell rapidly thereafter to then increase from $\lesssim$1 hr to an average $FITC^+$ cell incidence of up to ~2% at 1–3 hr. The data shown are the mean figures of sequential short collections averaged in successive 20-min periods, but indices in individual collections and in the different animals varied widely (<0·5%–>5%) for

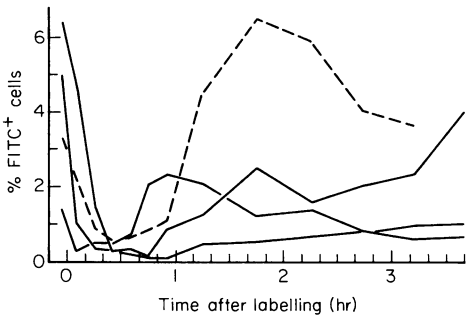

Figure 2. Incidence of FITC-labelled lymphocytes in the blood draining pig lymph nodes in which the nodes were isolated retaining only the arterial connection and all venous effluent was collected under general anaesthetic. Data are shown for superficial inguinal nodes in three pigs (——) and mesenteric nodes in one pig (– – –). Labelled cells are present during injection of FITC, but fall to a low level for > 30 min before increasing again.

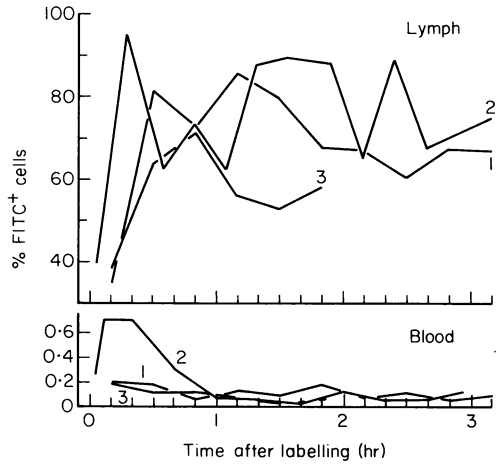

Figure 3. Incidence of FITC-labelled lymphocytes in blood and lymph draining labelled 'isolated' mesenteric nodes in the anaesthetised sheep; experiments carried out as in the pig (Fig. 2). In these experiments under general anaesthetic, retaining only the arterial connection, large numbers of lymphocytes continued to emerge in efferent lymph throughout the period of study. In the blood after the post injection samples, very low, but nevertheless consistent, numbers of labelled cells were present (~0·1–0·3%).

reasons which were not clear. Figure 2 also shows that in blood draining labelled mesenteric lymph nodes, similar changing patterns of incidence of $FITC^+$ cells were found, with indices of up to ~7% of labelled lymphocytes. At the same time, no labelled cells were found in the general circulation (< 1:10,000).

In sheep, the same experiment yielded quite different results (Fig. 3). Large numbers of labelled lymphocytes were present in the intestinal lymph (i.e. ~50–90% of cells during most of the period of collection), but in the blood < 0·2% of cells were labelled during most of the collection, constituting ~1–10 labelled cells/mm^3. Moreover, the number of labelled cells tended to be highest shortly after injection of FITC and fell thereafter, in contrast to the pig experiments in which the cell incidence increased from ~30–60 min (Fig. 2). Comparison of the estimated number of labelled cells emigrating from the mesenteric lymph nodes in the sheep showed that < 3% of the total number of cells emigrate via the blood (i.e. emigrants in lymph $= 115 \times 10^6$/hr cf. $2{\cdot}8 \times 10^6$/hr in blood), and that broadly similar numbers of cells

Table 1. The incidence (%) of lymphocyte subpopulations among unlabelled and FITC-labelled lymphocytes in splenic venous blood at various times after the *in vivo* perfusion of the spleen with FITC

		Time after opening main blood vessels					
		7 min		30 min		60 min	
Marker	Pig	FITC-labelled	Unlabelled	FITC-labelled	Unlabelled	FITC-labelled	Unlabelled
DARR	1	25·8	19·4	31·5	20·1	33·8	19·2
	2	22·7	ND	22·0	10·6	ND	ND
DS	1	74·4	41·5	70·9	28·9	70·9	32·7
	2	79·5	ND	71·6	44·6	ND	ND
Null	1	−0·2	39·1	−2·4	51·1	−4·7	48·1
	2	−2·2	ND	6·4	47·8	ND	ND

($\sim 10^8$/hr) leave the pig lymph nodes in the blood and sheep lymph nodes in the lymph.

The subpopulation selectivity of lymphocyte emigration from FITC-labelled pig spleen and lymph nodes.

Study of the labelled and unlabelled lymphocytes in blood draining FITC-labelled spleen and lymph nodes showed that Null cells are absent or much depleted among the labelled emigrating cells. Thus, as shown in Table 1, study of the labelled recirculating lymphocytes which emerge in the splenic vein blood at 7, 30 and 60 min after reestablishing normal blood flow to the labelled spleen showed that essentially no labelled Null cells were present, although there were normal large numbers of unlabelled Null cells present. Similar studies were carried out on the labelled lymphocytes emigrating from superficial inguinal and mesenteric lymph nodes by direct venular entry. Figure 4 shows that, during the phase when rising numbers of labelled lymphocytes are found in the blood, these include essentially no Null cells. Thus, the unlabelled lymphocytes in the blood include normal numbers of Null cells

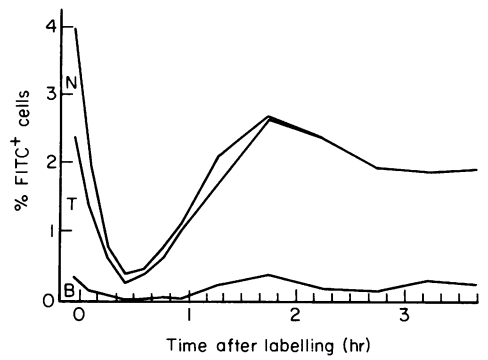

Figure 4. The changes in the draining blood in incidence of FITC-labelled lymphocytes and in their proportions of B, T and Null cells after labelling of isolated pig lymph nodes. The figures shown are mean data of three experiments on superficial and mesenteric nodes for timed samples taken during labelling and for ~ 1 hr after labelling in 10-min collections, and thereafter in 25–30 min collections. The falling numbers of Null cells after labelling are shown by plotting the $FITC^+$ indices (top line) and proportions of these accounted for by B, T and Null cells, computed by multiplying the indices by the mean percentage of each cell type among the labelled cells. The slightly higher numbers of Nulls at ~ 75 min are an artifact due to a delay in the onset of the wave of emigration of labelled T and B cells in one animal, resulting in a continuing significant proportion, but not number, of Null cells. During these experiments, the incidence of unlabelled Null cells in the effluent blood remained within normal ranges (~ 35–45%).

(~ 35–45%) and, during and after FITC-injection as the labelled cell index falls, Null cells are present but their incidence decreases progressively. However, as the labelled cell incidence increases again, the labelled Null cells disappear. The reasons for the presence of residual labelled Null cells during FITC-labelling remain obscure.

DISCUSSION

Several points emerged from this study. It confirms the value of fluorochromes for the labelling of lymphoreticular cells *in situ* in their normal microenvironment in a variety of tissues in pigs (Pabst & Binns, 1981; Pabst, Kaatz & Westermann, 1983) and sheep (Reynolds & Pabst, 1984). Moreover, cell emigration was observed from labelled lymph nodes and spleens at rates consistent with available data using other methods.

The central finding of these studies is the demonstration of direct emigration of lymphocytes into the blood draining both visceral (mesenteric) and somatic (superficial inguinal) lymph nodes in pigs. Such evidence had been provided directly in preliminary studies using ^{51}Cr-labelled cells infused into an afferent lymphatic (Binns, 1980). Also, indirect evidence was obtained by FITC-labelling mesenteric lymph nodes, and showing both the increasing incidence of labelled cells in mesenteric blood, even though the efferent lymph was being drained away, and the consistently higher labelling index in mesenteric compared to jugular blood (Pabst & Binns, 1981). However, the present direct observation of emigration under anaesthesia from lymph nodes in which only the arterial connection was maintained, occurring at rates of a similar order (i.e. up to $> 7\%$) with the emigration observed in other species ($< 25\%$, Trnka & Cahill, 1980), gives incontrovertable proof of the importance of this route. Moreover, this phenomenon and the remarkable paucity of lymph lymphocytes was shown in pigs of ages varying from < 3 months to full adulthood, showing that this route is not a characteristic of one age group. It is difficult to see how that data from previous and present experiments revealing vascular direct entry could result from artefacts induced by the experimental conditions, but the 'control' experiments of both types in sheep yielding the quite opposite, conventional conclusion that emigration is almost exclusively via the lymph show that this cannot be the case. Thus, in the more physiological experiments on sheep which recovered from anaes-

thetic after labelling the mesenteric lymph nodes, and in which the lymph was collected and mesenteric blood only sampled, very few labelled cells reached the blood stream when lymph flow continued uninterrupted. Moreover, when the experiments were carried out on isolated lymph nodes only connected to the artery, high indices of labelled cells were present in the sheep efferent lymph and very few in the blood, in contrast to the opposite result in pigs. In these experiments, broadly similar numbers of lymphocytes ($\sim 1 \times 10^8$/hr) were emerging from labelled pig and sheep mesenteric lymph nodes, but predominantly in the blood in the pig and in the efferent lymph in sheep. These observations of direct vascular reentry of lymphocytes were further confirmed in three experiments in which the blood vessels of mesenteric lymph nodes were cannulated and the nodes perfused *in vitro* in the same system as was described for spleen (Pabst & Trepel, 1975; Binns, Pabst & Licence, 1981), except that the perfusate was not recirculated, instead collecting 50 ml fractions continuously. Lymphocytes appeared in the perfusate, and continued to emerge in the 'venous drainage' throughout the $2\frac{1}{2}$ hr period of collection.

Although these experiments have confirmed that emigration is predominantly via lymph in the sheep, they do not exclude the vascular route of emigration. A mean of $\sim 2{\cdot}7 \times 10^6$ labelled lymphocytes per hour were measured in the venous blood ($< 3\%$ of the total emigration rate) recovered from mesenteric lymph nodes, in spite of the fact that afferent lymph was interrupted in the isolated node experiments. These observations could be taken to support the suggestion of Trevella & Morris (1980) that such a route exists in sheep. However, these data cannot support the view that this represents a major route of lymphocyte emigration from the lymph node in non-porcine species (Saint-Marie, 1975). While there is some evidence from scanning electron microscopy (Cho & Bruyn, 1981) and from the emigration of antibody-producing cells in the secondary response (Geldof & Van de Ende, 1984) that such a route may even exist in rodents, Sedgley & Ford (1976), studying isolated perfused rat lymph nodes, could find no evidence for such a route of emigration. At first sight, our data on sheep and those of Trevella & Morris appear to conflict with the ability to isolate an immune response in a single lymph node by total lymph drainage (Hall *et al.*, 1967; Trnka & Cahill, 1980). However, no data have been provided on the lymphocyte subpopulations involved in this migration, and the possibility cannot be excluded that only some subpopulations can use this route. Indeed, experiments in isolated lymph nodes repeatedly stimulated by antigen, in which progressive selective specific depletion of immunological responsiveness was observed (McConnell, Lachmann & Hobart, 1974), might be explained by a selective direct vascular emigration of either virgin or specific suppressor lymphocytes. The evidence on lymphocyte subpopulations obtained from pigs supports the view that recirculation is selective. Thus, in both the spleen and the superficial inguinal and mesenteric lymph nodes, both E rosette-forming T and sIg^+ B lymphocytes were present in the major phase of emigration of labelled cells, but no Null cells were involved. These data confirm our previous observations that recirculating emigrants from the spleen purfused *in vitro* include no Null cells, though the blood of the donor had contained $\sim 40\%$ of these cells, and that FITC-labelled blood lymphocytes included in the perfusate show an increasing incidence of labelled Null cells remaining in the perfusate, consistent with the hypothesis that these are not recirculating cells (Binns, Pabst & Licence, 1981). This peculiarity of the enigmatic Null cell population adds another negative property to their repertoire. These cells lack any specific markers and have not been shown to be involved in *in vitro* mitogen, antibody or cytotoxic responses, and yet appear to be a single family of cells since they disappear after thymectomy and are highly non-adherent for nylon wool (Binns, 1982). However, some Null cells did emerge in the blood during the phase of decreasing labelled cell index immediately after labelling. These first labelled cell emigrants were very similar to blood cells in population spectrum, and the possibility is raised that a pool of intravascular cells which remain in the lymph node are labelled, in a way not found in the labelled spleen. The nature of this compartment remains obscure, but it might involve closed-down static capillary beds or peripheral margination of blood leucocytes.

Thus, these studies provide strong evidence that direct vascular re-entry of lymphocytes is the main route of emigration from lymph nodes in pigs and a minor one in sheep, and raise again the possibility that such a route may exist and be of physiological importance in other species, including man.

ACKNOWLEDGMENTS

These studies were partly supported by grants from the Deutsche Forschungsgemeinschaft (Pa 240-3 to

R.P.) and the European Economic Community Agricultural Division (0172 to R.M.B). We are grateful to Marita Kaatz and Brigitte Schwenck for excellent technical assistance.

REFERENCES

BENNELL M.A. & HUSBAND A.J. (1981a) Route of lymphocyte migration in pigs. I. Lymphocyte circulation in gut-associated lymphoid tissue. *Immunology*, **42**, 469.

BENNELL M.A. & HUSBAND A.J. (1981b) Route of lymphocyte migration in pigs. II. Migration to the intestinal lamina propria of antigen-specific cells generated in response to intestinal immunization in the pig. *Immunology*, **42**, 475.

BINNS R.M. (1978) Sheep erythrocyte rosettes in pigs, sheep, cattle and goats demonstrated in the presence of dextran. *J. Immunol. Meth.* **21**, 197.

BINNS R.M. (1980) Pig lymphocytes—behaviour, distribution and classification. In: *Essays of the Anatomy and Physiology of Lymphoid Tissues* (eds Z. Trnka and R. N. P. Cahill). *Mongr. Allergy*, **16**, 19.

BINNS R.M. (1982) Organization of the lymphoreticular system and lymphocyte markers in the pig. *Vet. Immunol. Immunopathol.* **3**, 95.

BINNS R.M., BLAKELEY D. & LICENCE S.T. (1981) Migration of fluoresceinated pig lymphocytes *in vivo*: technical aspects and use in studies of autologous and homologous cell survival for up to three weeks. *Int. Archs. Allergy appl. Immun.* **66**, 341.

BINNS R.M. & HALL J.G. (1966) The paucity of lymphocytes in the lymph of unanaesthetised pigs. *Br. J. exp. Path.* **47**, 275.

BINNS R.M., LICENCE S.T., SYMONS D.B.A., GURNER B.W., COOMBS R.R.A. & WALTERS D.E. (1979) Comparison of direct antiglobulin rosetting reaction (DARR) and direct immunofluorescence (DIF) for demonstration of sIg-bearing lymphocytes in pigs, sheep and cattle. *Immunology*, **36**, 549.

BINNS R.M., PABST R. & LICENCE S.T. (1981) Classification of lymphocytes recirculating in the spleen. *Immunology*, **44**, 273.

CHO Y. & DE BRUYN P.P.H. (1981) Transcellular migration of lymphocytes through the walls of the smooth-surfaced squamous endothelial venules in the lymph node: evidence of the direct entry of lymphocytes into the blood circulation of the lymph node. *J. Ultrastruct. Res.* **74**, 259.

FORD W.L. (1975) Lymphocyte migration and immune responses. *Progr. Allergy*, **19**, 1.

GELDOF A.A. & VAN DE ENDE E. (1984) Migration of specific antibody-forming cells during the secondary immune response against horseradish peroxidase. *Virchows Arch. Path. Anat. Physiol.* **45**, 107.

HALL J.G., MORRIS B., MORENO G.D. & BESSIS M.C. (1967) The ultrastructure and function of the cells in lymph following antigenic stimulation. *J. exp. Med.* **125**, 91.

MCCONNELL I., LACHMANN P.J. & HOBART M.J. (1974) The restoration of specific immunological virginity. *Nature (Lond.)*, **250**, 113.

MCFARLIN D.E. & BINNS R.M. (1973) Lymph node function and lymphocyte circulation in the pig. In: *Microenvironmental Aspects of Immunity*, Fourth International Conference on Lymphatic Tissue and Germinal Centres in Immune Reactions. (eds B. D. Jankovic and K. Isakovic), p. 87. Plenum Publishing Co., New York.

PABST R. & BINNS R.M. (1981) *In vivo* labelling of the spleen and mesenteric lymph node with fluorescein isothiocyanate for lymphocyte migration studies. *Immunology*, **44**, 321.

PABST R., KAATZ M. & WESTERMANN J. (1983) *In situ* labelling of bone marrow lymphocytes with fluorescein isothiocyanate for lymphocyte migration studies in pigs. *Scand. J. Haematol.* **31**, 267.

PABST R. & TREPEL F. (1975) The predominant role of the spleen in lymphocyte recirculation. I. Homing of lymphocytes to and release from the isolated perfused pig spleen. *Cell Tissue Kinet.* **8**, 529.

REYNOLDS J.D. & PABST R. (1984) The emigration of lymphocytes from Peyer's patches in sheep. *Eur. J. Immunol.* **14**, 7.

SAINT-MARIE G. (1975) A critical analysis of the validity of the experimental basis of concept of the mode of lymphocyte recirculation. *Bull. Inst. Pasteur*, **73**, 255.

SEDGLEY M. & FORD W.L. (1976) The migration of lymphocytes across specialized vascular endothelium. I. The entry of lymphocytes into the isolated mesenteric lymph node of the rat. *Cell Tissue Kinet.* **9**, 231.

TREVELLA W. & MORRIS B. (1980) Reassortment of cell populations within the lymphoid apparatus of the sheep. In: *Blood Cells and Vessel Walls, Functional Interactions*, Ciba Foundation Symposium 71., p. 127. Excerpta Medica, Amsterdam, New York.

TRNKA Z & CAHILL R.N.P. (1980) Aspects of the immune response in single lymph nodes. In: *Essays on the Anatomy and Physiology of Lymphoid Tissues* (eds Z. Trnka and R. N. P. Cahill). *Mongr. Allergy*, **16**, 245.

NOTE ADDED IN PRESS

After this paper was submitted, the study of Chin & Cahill (1984) was published which also reports that, in sheep, lymphocyte emigration from lymph nodes is predominantly via lymphatics. Following FITC-labelling of mesenteric lymph nodes in which all efferent lymph was being drained away, almost no recirculating lymphocytes were found during 3 days' collection of efferent lymph from the prescapular lymph node.

CHIN G.W. & CAHILL R.N.P. (1984) The appearance of fluorescein-labelled lymphocytes in lymph following *in vitro* or *in vivo* labelling: the route of lymphocyte recirculation through mesenteric lymph nodes. *Immunology*, **52**, 341.