Clin. exp. Immunol. (1981) **43**, 435–442.

A method for following human lymphocyte traffic using indium-111 oxine labelling

J. WAGSTAFF,* C. GIBSON,† N. THATCHER,* W. L. FORD,‡ H. SHARMA,§ W. BENSON*¶ & D. CROWTHER* * *Cancer Research Campaign Department of Medical Oncology, and † Physics Department, University of Manchester and Christie Hospital & Holt Radium Institute, Manchester; ‡ Department of Experimental Pathology, and § Department of Medical Biophysics, The Medical School, University of Manchester, Manchester, UK*

(Accepted for publication 5 September 1980)

SUMMARY

A method is described whereby large numbers of human lymphocytes are separated from peripheral blood and labelled *in vitro* with indium-111 oxine. Following autologous reinjection, the distribution within the body is followed by means of serial blood samples, surface-probe counting and gamma camera imaging. The distribution of radioactivity following reinjection of heat-damaged labelled lymphocytes and free indium-111 oxine is different from that of 'normal' lymphocytes. The results suggest that the separation and labelling procedure does not cause significant physical damage to the lymphocytes. The importance of restricting the specific lymphocyte activity to 20–40 μCi per 10^8 cells in order to minimize radiation damage to the lymphocytes is emphasized. Good resolution of lymphoid structures is obtained using gamma camera imaging and the changes recorded in organ distribution correlate well with data from animal models of lymphocyte migration. Thus, indium-111 oxine labelling of human lymphocytes provides a non-invasive method whereby the migratory properties of human lymphocytes can be followed.

INTRODUCTION

The migration of lymphocytes from blood to the tissues and their return has been well established in small experimental animals using autoradiographic techniques and cannulation of lymphatics. The magnitude and importance of this phenomenon has been extensively studied and reviewed (Rannie & Ford, 1977; Ford, 1975; Sprent, 1977). It is clear that the nomadic existence of this group of cells is one of the corner-stones of the immune response and a more complete understanding of the nature of these events in man is essential. The techniques used in experimental animals are invasive and impossible to apply to humans. The few studies which have been carried out have mainly used $Na_2{}^{51}CrO_4$-labelled lymphocytes (Hersey, 1971; Scott *et al.*, 1972; Jønsson & Christensen, 1978). The physical characteristics of chromium-51 are not ideal for labelling lymphocytes and following their fate within the body. It has a low labelling efficiency, a high elution rate and poor gamma camera imaging characteristics. The physical half-life of 30 days means that unnecessary irradiation of the subjects occurs after the conclusion of the studies. In 1976, McAfee & Thakur showed that indium-111 oxine conjugate was an efficient means of labelling cells *in vitro*, and the method has subsequently been exploited to label granulocytes and platelets (reviewed by Goodwin, 1978). Rannie, Thakur & Ford (1977) compared indium-111 oxine-labelled rat lymphocytes with those

¶ Present address: Sydney Hospital, Macquarie Street, Sydney 2000, NSW, Australia.

Correspondence: Dr J. Wagstaff, Department of Medical Oncology, Christie Hospital & Holt Radium Institute, Wilmslow Road, Manchester M20 9BX, UK.

0099-9104/81/0300-0435$02.00

labelled with other isotopes and found indium-111 oxine to be a superior cell label. Indium-111 oxine is a lipid soluble complex which has a high labelling efficiency, a low elution rate and produces Auger electrons which allow autoradiography to be performed. A study of indium-111 oxine-labelled lymphocytes in sheep (Frost, Frost & Wilcox, 1979) and one in man (Lavender *et al.*, 1977) confirmed that the gamma emissions are ideal for external imaging on conventional gamma camera equipment. The initial promise has been tempered by further studies which have shown that due to the high intracellular radiation dose produced by indium-111 oxine the lymphocytes will only behave in a physiological manner provided that certain dosage restrictions are observed (Chisholm *et al.*, 1979; Issekutz, Chin & Hay, 1980; Sparshott *et al.*, 1981). It is clear, however, that labelling lymphocytes with indium-111 oxine has enormous potential as a non-invasive means of studying the migratory properties of these cells. The purpose of the present work was to develop the technique of labelling human lymphocytes with indium-111 oxine and to assess its usefulness as a tool for studying the migratory properties of human lymphocytes within the body.

METHODS

Principle. Lymphocytes were separated from the peripheral blood of the subjects by means of a cell separator. The collection was purified *in vitro* and the lymphocytes labelled with indium-111 oxine. Following reinjection the distribution of the lymphocytes was assessed using serial blood sampling, gamma camera imaging and surface-probe counting.

Subjects. Initial studies were performed on patients with neoplastic disease and a life expectancy of 2 years or less. Two normal volunteers were also studied. Informed consent was obtained from all subjects.

Preparation of lymphocytes. Lymphocytes were obtained after the method of Segel *et al.* (1976) using a Model 30 Haemonetics intermittent cell separator. A cannula (16 gauge) was inserted into a peripheral vein in each forearm. Samples were obtained for a blood count and estimation of lymphocyte surface marker characteristics. Peripheral blood was anticoagulated with acid citrate dextrose prior to passing through the cell separator. Ten millilitres of platelet-poor plasma was collected for later use. The buffy coat was collected from 1–3 litres of whole blood and a sample obtained for cell counts.

Purification of lymphocytes. All subsequent manipulations were carried out in the laboratory using strict sterile techniques.

Platelet depletion of the buffy coat suspension was performed using low-velocity centrifugation. The buffy coat was diluted 1 : 1 (v/v) with phosphate-buffered saline (PBS) and transferred to 50-ml centrifuge tubes (Falcon 2070, California). The tubes were centrifuged at 200 ***g*** for 15 min. The platelet-rich plasma was removed and the cell pellet resuspended to 40 ml with PBS. The centrifugation was repeated and the supernatant again discarded. The cells were resuspended with PBS to a total volume of 80 ml, 20-ml aliquots were layered over equal volumes of Lymphoprep (Flow Laboratories, England) and centrifuged at 18°C for 30 min (400 ***g*** at interface). The interfacial layer was harvested, washed with PBS and centrifuged at 400 ***g*** for 10 min. The cell pellet was resuspended in PBS to a volume of 5 ml. The cell suspension was counted using an improved Neubauer counting chamber (Weber, England) and a differential cell examination performed.

Indium-111 oxine labelling. Indium-111 oxine was obtained from two sources. The early experiments were carried out using indium-111 oxine (in ethanol) produced by the Department of Medical Biophysics at Manchester University Medical School (Sparshott *et al.*, 1981). Ethanol-free indium-111 oxine was latterly obtained from the Radiochemical Centre, Amersham, as a clinical trials material. Indium-111 oxine was added to the lymphocyte suspension to give between 20 and 40 μCi per 10^8 lymphocytes. Incubation was at room temperature for 15 min. The labelled cells were then diluted to 15 ml with PBS and layered over 5 ml of the plasma obtained earlier. Centrifugation was performed at 400 ***g*** for 10 min. The cell pellet was resuspended to 20 ml with PBS and centrifugation repeated. The final cell pellet was resuspended in normal saline for injection (approximately 10 ml). A 100-μl aliquot was removed and diluted in 20 ml of PBS to act as a standard for gamma counting of blood samples later. Viability was assessed using trypan blue

exclusion and phase-contrast examination. Cell counts and a differential assessment of purity was done. Smears were made and autoradiography performed using Kodak AR10 stripping film to assess the degree of uniformity of cell labelling. The film was exposed for 4 days. The labelled lymphocytes were drawn into a 10-ml syringe and the volume and activity of the injection recorded.

Serial blood sampling. This was done to characterize the rate of clearance and reappearance of the labelled cells in the blood and to assess the amount of cell-free isotope. A 5-ml blood sample was taken via an indwelling cannula prior to reinjection and then at 2, 5 and 30 min after injection of the labelled cells. Further samples were obtained at 30-min intervals up to 4 hr, 4-hr intervals to 24 hr and finally, 6-hr intervals to 48 hr. The samples were divided into two portions, one 2-ml aliquot was retained for whole blood gamma counting, the second was centrifuged at 800 ***g*** and the plasma separated for gamma counting. All the samples together with five 2-ml aliquots of the standard prepared earlier were gamma-counted at the end of the experiment.

Static gamma camera imaging. A large-field-of-view Nuclear Enterprises 8970 'Digicamera' with an 'on-line' computer (Data General Nova 3) was used. The studies were stored on cartridge disk for subsequent computer processing and analysis. A high sensitivity collimator (Nuclear Enterprises 8962) was used. The lower energy peak of indium-111 (172 KeV) was selected and a peak tracking circuit used to set the energy window. For this peak of indium, the window was approximately $\pm 12{\cdot}5\%$. The peak tracking circuit was disabled prior to acquisition of the patient images ensuring that the energy window was the same for all views. Views were taken at $\frac{1}{2}$, 4, 24 and 48 hr, and designed to encompass all lymphoid tissue and any other areas of clinical interest. The views were acquired over 200 sec. A standard indium-111 source was prepared and the count rate from it recorded prior to acquisition of the views at each time interval. These were used to correct the views for isotope decay to allow comparison. Viable lymphocytes and those damaged by heating at 50°C for 15 min were studied. The distribution of free indium-111 oxine was also assessed.

Surface-probe counting. This was performed by positioning a sodium iodide crystal at a fixed height (10 cm) above the area of interest and measuring the count rate. A lead shield ($10 \times 5 \times 1$ cm) was placed beneath the crystal and the count rate was again recorded. The count rate from the area encompassed by the shield was derived by subtraction. A standard indium-111 source was measured prior to the measurement of the count rate at each time interval and a correction made for isotope decay. Measurements were made at 2, 24 and 48 hr after injection. A recording over the upper thigh (excluding regional lymph nodes) was used to subtract for tissue and blood background.

Urine collections. These were carried out on two patients. The bladder was emptied at the beginning of the experiment. Two 24-hr collections were made and 2-ml aliquots gamma-counted at the same time as the blood samples.

Blood volume estimations. These were derived from standard tables (Nadler, Midalgo & Bloch, 1962).

RESULTS

Lymphocyte separation, purification and viability

In eight patients studied the total number of labelled cells in the final suspension varied from 0·46 to $1{\cdot}99 \times 10^9$. Ninety-five per cent or more of these were lymphocytes. Red cell contamination was minimal and there were never more than 10^4 platelets in the final suspension. In one case, the mononuclear suspension contained 16% monocytes and an additional stage was necessary to remove these but this subject had a peripheral blood monocyte count of $1{\cdot}03 \times 10^9$/l. Viability was greater than 95% as assessed by trypan blue exclusion and phase-contrast examination in all cases but one. This was a patient with chronic lymphocytic leukaemia who had 10% smear cells in a peripheral blood film and the handling of the lymphocytes lowered the viability to 90%.

Cell labelling (Table 1)

Labelling efficiencies with Manchester-prepared indium-111 oxine (mean = 61·1%) were lower than with Amersham indium-111 oxine (mean = 78·1%). Specific lymphocyte activity varied from 8·0 to

Table 1. Lymphocyte labelling efficiencies and activities per 10^8 cells following a 15-min incubation with indium-111 oxine at room temperature

	Activity added (μCi)	Activity in the cell (μCi)	Labelling efficiency (%)	Activity per 10^8 lymphocytes (μCi)
Manchester-prepared indium-111 oxine	m = 482·0 +137·2 −123·7	m = 266·5 +102·5 −71.5	m = 61·1 +14·0 −12·6	m = 24·01 +11·69 −16·01
Amersham-prepared indium-111 oxine	m = 495·4 +72·4 −78·2	m = 391·5 +57·5 −95·7	m = 78·1 +3·3 −7·2	m = 27·9 +13·2 −19·4

41·1 μCi per 10^8 cells and the total activity injected from 195 to 449 μCi. Autoradiographs of the labelled lymphocytes showed that there was little extracellular radioactivity and that the label was evenly distributed amongst the lymphocytes.

Blood disappearance curves

The percentage of the labelled lymphocytes that remained within the blood volume was calculated for each time interval, and the results expressed graphically in Fig. 1a. The normal subject depicted showed a rapid fall followed by a secondary rise beginning at 3–4 hr. There was then a plateau lasting from 4–8 hr followed by a second fall. This pattern was found in both normal subjects and several of the other subjects with disease. Heat-damaged labelled cells were removed from the circulation much more rapidly than those which were viable. After an initial fall, there was a rise in the amount of circulating radioactivity, which was cell-associated, up to 4 hr post-injection. In both the undamaged and heat-damaged lymphocyte studies the cell-free radioactivity was consistently less than 2% of the total activity in the blood.

Urinary excretion

Urinary excretion of radioactivity was approximately 1% of the total injected over the 48 hr of the studies.

Surface probe count results (Fig. 1b)

The normal subject showed a high splenic activity at 4 hr which fell by 39% over the next 44 hr. At

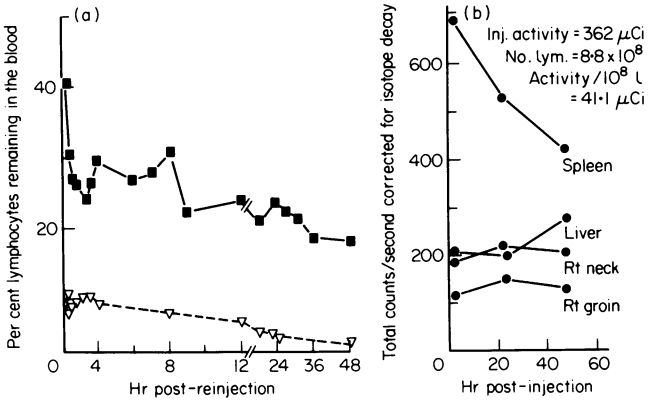

Fig. 1. (a) Blood clearance curves for indium-111 oxine-labelled lymphocytes. (▽- - -▽) Heat-damaged labelled lymphocytes, (■——■) normal lymphocytes. (b) Normal subject, surface probe counts.

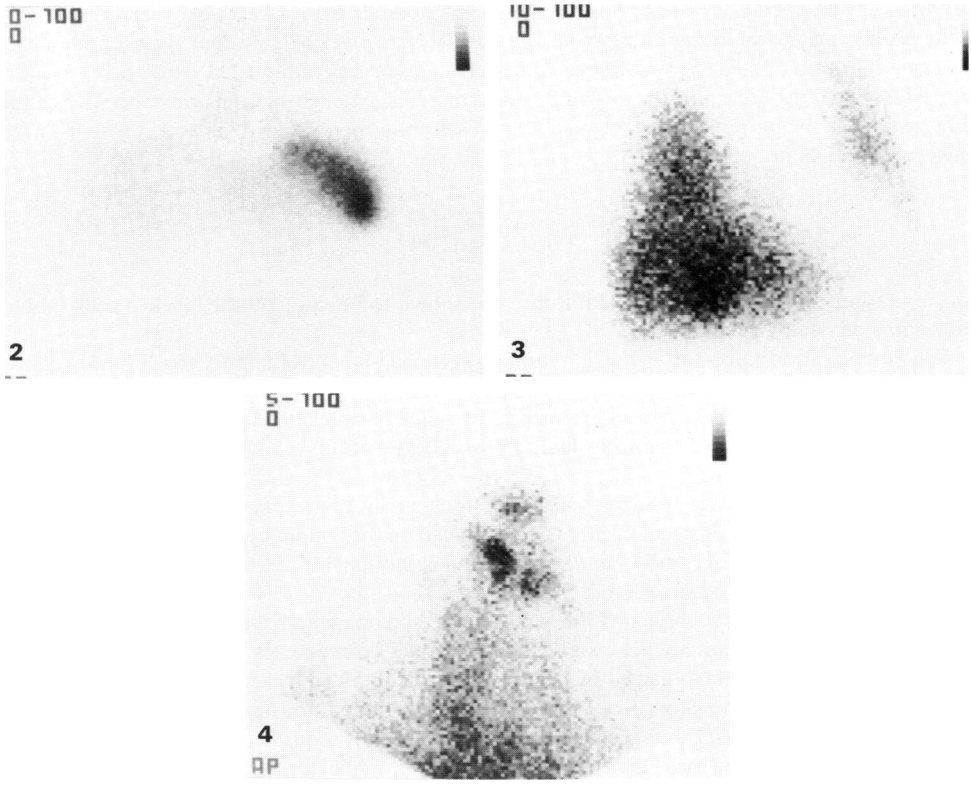

Fig. 2. An anteroposterior view of the liver and spleen 4 hr after injection of indium-111 oxine-labelled normal lymphocytes. There is marked splenic uptake but little activity in the lungs or liver.

Fig. 3. An anteroposterior view of the liver and spleen 4 hr after injection of indium-111 oxine-labelled heat-damaged lymphocytes. In contrast to Fig. 2 there is little splenic activity but marked uptake in the lungs and liver.

Fig. 4. An oblique anteroposterior view of the right neck showing imaging of normal lymph nodes 24 hr after the injection of indium-111 oxine-labelled lymphocytes.

the same time the count rate over the areas containing lymph nodes was seen to rise (approx. 10·5% at 24 hr). The liver count rate remained the same between 4 and 24 hr but rose by 30% at 48 hr. Similar results were found in the second normal subject and in a number of other patients with disease.

Gamma camera imaging

Fig. 2 shows an anteroposterior gamma camera image of the liver and spleen at 4 hr after injection of indium-111 oxine-labelled lymphocytes. The important features are the lack of lung activity, the minimal liver uptake and the marked splenic accumulation. The picture was similar at 24 hr but the splenic activity had fallen significantly. In contrast, Fig. 3 shows a similar gamma camera projection following injection of heat-damaged indium-111 oxine-labelled lymphocytes. There was marked lung and liver uptake at 4 hr post-injection but by 24 hr the lung activity had waned and the major site of localization was the liver. The splenic uptake at both time intervals was minimal. Free indium-111 oxine injected intravenously (views not shown) was visible in the liver and heart at 4 hr and by 24 hr bone marrow and liver were the predominant sites of localization. Fig. 4 is an oblique view of the neck of a patient with normal-sized lymph nodes 24 hr after the injection of viable

indium-111 oxine-labelled lymphocytes. Similar lymph node structures were also clearly discernible in the groins, mediastinum and abdomen. In contrast, imaging of equivalent lymph node areas following injection of labelled heat-damaged lymphocytes or free indium-111 oxine gave very low count rates and failed to demonstrate lymph node structures. After correction for isotope decay, the count rate over lymph nodes rose between 4 and 24 hr and then either remained constant or fell between 24 and 48 hr.

DISCUSSION

For any radioisotope to be used in man it must be shown to be safe and the labelled cells should behave in a physiological manner.

Whole-body irradiation for 500 μCi of indium-111 oxine-labelled lymphocytes has been calculated at 0·1–0·3 rads to total decay, with the dose to the spleen of 4–10 rads and the liver 0·5–0·8 rads (Lavender *et al.*, 1977). These figures are within the range of doses produced by many diagnostic isotopic and radiological investigations (Hall, 1978). The use of this cell label is therefore not likely to be particularly hazardous.

Damage to lymphocytes might be expected to impair their migratory patterns and could arise from two sources. Firstly, physical insult may be inflicted on the lymphocytes during the separation and purification process; secondly, as the intracellular indium-111 decays, radiation to the cell accumulates and damage becomes more likely.

The extent of the first of these possible sources of cell damage was assessed by comparing the migratory properties of indium-111 oxine-labelled normal lymphocytes with those labelled cells which had been deliberately damaged by heat. The results obtained by serial blood sampling (Fig. 1b) and gamma camera imaging (Figs 2 and 3) were very different for heat-damaged lymphocytes from those of 'normal' cells. Damaged lymphocytes were initially sequestered by the lungs, and later became redistributed to the liver. In contrast to 'normal' lymphocytes, few damaged cells migrated to the spleen. These findings add weight to the belief that the separation and labelling of lymphocytes as described does not cause significant physical damage.

The second possible source of lymphocyte damage, arising from radiation to the lymphocytes, has been examined in small animals by Chisholm *et al.* (1979) and Sparshott *et al.* (1981). They pointed out that for a concentration of 20 μCi of indium-111 oxine per 10^8 lymphocytes a dose of 7·6 rads per hr would be delivered to the cells, giving a total of 160 rads over the first 24 hr. Since as little as 50 rads are known to damage lymphocytes (reviewed by Anderson & Warner, 1976), it might be expected that the physiological properties of lymphocytes labelled with 20 μCi per 10^8 cells would be impaired after 24 hr. Their experiments confirmed that rat lymphocytes labelled with increasing doses of indium-111 oxine migrated normally initially but subsequently were retained by lymphoid tissue. The rapidity of onset and extent of this immobilization was dose-dependent and for 20 μCi per 10^8 cells autoradiography demonstrated radiation damage at 24 hr. Later the indium-111 became associated with non-recirculating macrophages. In our studies we accepted an upper limit of 40 μCi per 10^8 lymphocytes before the results from animal models became available, and as a consequence the interpretation of the latter parts of some of our early studies, in terms of normal lymphocyte physiology, is difficult. It is therefore clear that the dose of isotope used to label lymphocytes must be kept as low as possible, ideally 10–20 μCi per 10^8 cells for studies lasting 24 to 48 hr, and that the possibility of radiation damage is considered when the results from labelling experiments are being interpreted.

At least 100 μCi of indium-111 are necessary to obtain adequate gamma camera imaging. Therefore, in order to achieve lymphocyte-specific activities of 10–20 μCi per 10^8 lymphocytes between 0·5 and $1{\cdot}0 \times 10^9$ lymphocytes would be required. This necessitates either handling large volumes of blood (approx. 500 ml for a normal lymphocyte count) or obtaining mononuclear suspensions by using a cell separator. We have used the latter method because it is simple, speedy and gives a high yield of lymphocytes in a manageable volume. Since indium-111 oxine is a non-specific lipid soluble cell label it will label lymphocytes and contaminating cells according to either their volume or surface area. The major contaminant in these studies was platelets which have

a volume approximately 1/300th of a lymphocyte. Thus, if equal numbers of platelets and lymphocytes were present in the final suspension then 0·3% of the indium-111 oxine would be in the platelet fraction. This amount will not affect the results of these studies. The rather lower labelling efficiency with Manchester-prepared indium-111 oxine was probably due to heavy metal impurities in the isotope suspension (Sparshott *et al.*, 1981). This problem has been overcome and the Amersham isotope gives consistent labelling results.

Despite our reservations concerning the detrimental effect of indium-111 oxine on the physiology of lymphocytes, certain features in these initial studies suggest that labelled lymphocytes are migrating as would be expected. For example, the two normal subjects (only one illustrated) have surface-probe count results over the spleen (Fig. 1b) which were high initially and fell over 48 hr. Meanwhile, the blood disappearance curves (Fig. 1a) have a secondary rise and a plateau between 4 and 24 hr. Lymph node activity was seen to accumulate progressively. These observations suggest that lymphocytes were migrating initially to the spleen and were thereafter re-entering the blood to become redistributed to lymph nodes. This situation is analogous to that found in small animals with the transit time of lymphocytes through the human spleen being similar to animals (Bradfield & Born, 1978; Ford, 1969a, 1969b). Further, this work has confirmed that it is possible to image lymphoid tissue in various sites in man. Clear pictures of normal and abnormal lymph nodes have been obtained in the neck (Fig. 4), groins, abdomen and mediastinum (latter three sites not illustrated).

The time scale that we have found in our preliminary investigations for the migration into and out of lymph nodes also approximates to that found in experimental animal systems (Ford, 1975).

In conclusion, these studies have shown that it is possible to obtain sufficient numbers of lymphocytes by the method described and to label them with quantities of indium-111 oxine which do not significantly impair their migratory patterns. The total activity injected in these studies allows good images to be obtained of lymphoid organs on conventional nuclear medicine equipment. By using a combination of gamma camera imaging and surface-probe counting, it will be possible to assess, in an objective manner, the changing patterns of distribution of lymphocytes in man following reinjection. This technique, therefore, has the potential of providing important insight into the traffic of human lymphocytes. The constant patrol of recirculating memory lymphocytes and the migration of effector cells to their targets is of prime importance in the modulation of the immune response. A better understanding of this phenomenon and the factors which control it could provide a means whereby therapeutic intervention would allow alteration of the immune response in a beneficial manner.

The authors wish to thank Dr Kelly of the Radiochemicals Centre, Amersham, for supplying the indium-111 oxine. We would also like to thank the staff of the Isotope Department at the Christie Hospital for their forbearance, and Mrs Barbara Whittle for typing the manuscript.

REFERENCES

ANDERSON, R.E. & WARNER, N.L. (1976) Ionising radiation and the immune response. *Adv. Immunol.* **24**, 215.

BRADFIELD, J.W.B. & BORN, G.V.R. (1973) The migration of rat thoracic duct lymphocytes through the spleen *in vivo*. *Br. J. exp. Pathol.* **54**, 509.

CHISHOLM, P.M., DANPUVE, H.J., HEALEY, G. & OSMAN, S. (1979) Cell damage from the labelling of rat thoracic duct lymphocytes and HeLa 53 cells with Indium-111 oxine. *J. Nucl. Med.* **20**, 1308.

FORD, W.L. (1969a) The kinetics of lymphocyte recirculation through the rat spleen. *Cell Tissue Kinet.* **2**, 171.

FORD, W.L. (1969b) The immunological and migratory properties of lymphocytes recirculating through the rat spleen. *Br. J. exp. Pathol.* **50**, 257.

FORD, W.L. (1975) Lymphocyte migration and the immune response. *Prog. Allergy*, **19**, 1.

FROST, H., FROST, P. & WILCOX, C. (1979) Lymph node scanning in sheep with Indium-111 labelled lymphocytes. *Int. J. Nucl. Med. Biol.* **6**, 60.

GOODWIN, D.A. (1978) Cell labelling with oxine chelates of radioactive metal ions: techniques and clinical implications. *J. Nucl. Med.* **19**, 557.

HALL, E.J. (1978) *Radiobiology for the Radiologist.* Harper & Row, New York and London.

HERSEY, P. (1971) The separation and ^{51}Cr labelling of human lymphocytes with *in vivo* studies of survival and migration. *Blood*, **38**, 360.

ISSEKUTZ, T., CHIN, W. & HAY, J.B. (1980) Measurement of lymphocyte traffic with indium-111. *Clin. exp. Immunol.* **39**, 215.

JØNSSON, V. & CHRISTENSEN, E.B. (1978) ^{51}Cr labelling of normal human T and B lymphocytes for kinetic studies *in vivo*. *Scand. J. Haematol.* **20,** 319.

LAVENDER, J.P., GOLDMAN, J.M., ARNOT, R.B. & THAKUR, M.L. (1977) Kinetics of Indium-111 labelled lymphocytes in normal subjects and patients with Hodgkin's disease. *Br. Med. J.* **ii,** 797.

MCAFEE, J.G. & THAKUR, M.L. (1976) Survey of radioactive agents for *in vitro* labelling of phagocytic leukocytes. I. Soluble agents. *J. Nucl. Med.* **17,** 480.

NADLER, S.B., MIDALGO, J.U. & BLOCH, T. (1962) Prediction of blood volume in normal human adult. *Surgery,* **51,** 224.

RANNIE, G.H. & FORD, W.L. (1977) Physiology of lymphocyte recirculation in animal models. In *Proceedings of the IXth International Congress, Lyons*, p. 165. Excerpta Medica International Congress Series No. 423. Excerpta Medica, Amsterdam.

RANNIE, G.H., THAKUR, M.L. & FORD, W.L. (1977) Experimental comparison of radioactive labels with potential application to lymphocyte migration studies in patients. *Clin. exp. Immunol.* **29,** 509.

SCOTT, J.L., DAVIDSON, J.G., MANNO, J.V. & MACMILLAN, R. (1972) Leukocyte labelling with ^{51}Cr. III. The kinetics of normal lymphocytes. *Blood,* **40,** 276.

SEGEL, G.B., LICHTMAN, M.A., GORDON, B.R., MACPHERSON, J.L. & NUSBACHERS, J. (1976) Platelet pheresis residues: a source of large quantities of human blood lymphocytes. *Transfusion,* **16,** 455.

SPARSHOTT, S.M., SHARMA, H., KELLY, D. & FORD, W.L. (1981) Factors influencing the fate of Indium-111 labelled lymphocytes after transfer to syngeneic rates. *J. Immunol. Methods.* (In press.)

SPRENT, J. (1977) *The Lymphocyte: Structure and Function* (ed. by J. F. Marchalonis), p. 43. Marcel Dekker, New York.