Immunology 1976 **30** 135

Use of [^{75}Se]L-selenomethionine as a label for lymphoid cells

D. R. BAINBRIDGE *Department of Immunology, Institute of Pathology, London Hospital Medical College, Turner Street, London*

Received 23 *June* 1975; *accepted for publication* 6 *August* 1975

Summary. [^{75}Se]L-selenomethionine can be employed as a label for following lymphocytes *in vivo*. Its properties are broadly similar to those of ^{51}Cr with regard to uptake, release of the isotope on cell death and reutilization; thus relatively high levels of ^{75}Se in lymphoid tissue indicate the presence of viable labelled cells. It differs from ^{51}Cr in that active release of selenium occurs from the labelled cells when the ambient cell concentration is low. No significant reutilization of leaked label occurs. Leakage of ^{75}Se label occurs *in vivo*; this differs in the spleen and lymph nodes. Evidence is presented to suggest that ^{75}Se losses reflect the transit times of cells through lymphoid organs.

INTRODUCTION

Many features of the immune response hinge upon migratory properties of lymphocytes, which have been the subject of elegant studies by Gowans, Ford and their collaborators (Gowans, 1959; Gowans and Knight, 1964; Ford, 1969). Various problems are unresolved, however, such as whether circulating lymphocytes migrate in a definite direction from organ to organ, or are composed of discrete subpopulations directed to different sites. Part of the difficulty arises from the paucity of suitable markers for mature lymphoid cells.

Correspondence: Dr D. R. Bainbridge, Department of Immunology, Institute of Pathology, London Hospital Medical College, Turner Street, London E1 2AD.

Various gamma-emitting radioisotope compounds have been investigated by the author as possible lymphocyte markers. Among these, ^{75}Se (half-life 121 days) is a moderately energetic (major peaks at 140 and 270 keV) gamma-emitting isotope, and in the form of its compound [^{75}Se]L-selenomethionine, an analogue of the sulphur-containing amino acid methionine, can be incorporated into cellular proteins. The present paper reports that ^{75}Se can be used in much the same way as ^{51}Cr for following lymphocytes *in vivo*.

MATERIALS AND METHODS

Animals

Male and female CBA mice aged 3–5 months were used as donors and recipients of labelled cells.

Labelling of lymphocytes

[^{75}Se]L-selenomethionine (Radiochemical Centre, Amersham, Bucks), was made isotonic with sodium chloride before use. Preparation of cell suspensions and their labelling, carried out at a dose of 40–70 μCi/10^8 cells for $\frac{3}{4}$ hr in Tyrode's solution, followed the general method for ^{51}Cr labelling (Bainbridge, Brent and Gowland, 1966; Bainbridge and Gowland, 1971), but without addition of ascorbic acid.

In some experiments cells were doubly labelled with ^{51}Cr at 150–200 μCi/10^8 cells and ^{75}Se at 40–50 μCi/10^8 cells. Both labels were added simul-

taneously to the cells, and ascorbic acid was added at the end of incubation.

Cell transfer

Labelled cells were injected intravenously at doses of 10^6 or 10^7 cells per mouse. Recipients were killed 24 h later.

In serial transfer experiments secondary cell suspensions were made in Tyrode's solution–20 per cent foetal calf serum, washed once, and injected intravenously into further recipients as 1 : 1 transfers, i.e. one donor spleen or one set of donor peripheral lymph nodes (cervical, axillary, brachial and inguinal) per recipient mouse. The number of cells transferred was $2–3 \times 10^7$ viable lymph node cells and $7–8 \times 10^7$ viable spleen cells per recipient. Viabilities were measured by eosin exclusion.

Radioactive counting

At 24 h various organs were removed for counting. An important detail of the technique was to dissect out lymph nodes cleanly. Samples were counted in a Packard Autogamma scintillation counter and the results expressed as percentages of the dose of radioactivity injected into the animal. In some experiments whole body counts were performed on a modified EKCO scintillation counter (Type N612B).

Cell fractionation

Discontinuous density gradients of bovine serum albumin (Fraction V) (Sigma Chemical Corporation) were prepared from a nominal '50 per cent BSA' (50 g BSA plus 100 ml Tyrode's medium), diluted to make various concentrations with Tyrode's medium, without further adjustment. These were layered onto a cushion of Triosil-75 (Glaxo Laboratories, Ltd). Cells were incorporated in the '29 per cent BSA' layer at about 10^8 cells/ml.

RESULTS

Distribution of ^{75}Se label from live and dead lymph node cells

Twenty-four hours after injection of 10^6 ^{75}Se-labelled viable lymph node cells, the distribution of label in the organs of recipient mice was as shown in Table 1. Seven per cent of the radioactivity injected was recovered in the mesenteric and peripheral lymph nodes, 6 per cent in the spleen and 11 per cent in the liver. Essentially the same results were obtained with 10^5 or 10^7 cells, and with semi-allogeneic $(A \times CBA)F_1 \rightarrow CBA$ transfers over the same dose range.

Labelled cells, killed by heating at 50° for 20 min, were intact microscopically, but retained only about 54 per cent of their label (54·49 per cent and 53·34 per cent in duplicate experiments). Following injection of 10^7 cells, little radioactivity was retained in the lymphoid tissue of recipients (1·7 per cent), the activity in the liver increased slightly (14·2 as against 11·5 per cent), but the visceral label did not change (Table 1). Supernatants from cells disrupted by freezing and thawing, or killed by heating to 50°, gave identical results.

Reutilization of label from dead cells

10^8 Labelled cells were frozen in liquid nitrogen and thawed at 45–50° twice and centrifuged at 45,000 *g* for 30 min. The pelleted debris retained 17·5 per cent of the radioactivity. 10^8 Unlabelled viable lymph node cells were incubated with the supernatant for ¾ h at 37°. A negligible amount of the label, 2·17 per cent, was taken up. The washed suspension was injected into recipients at a dose equivalent to 10^7 cells per recipient. Its distribution at 24 h was the same as that of live cells.

The following experiment was carried out to determine whether reutilization of label released locally occurred in lymphoid tissue, i.e. when cells had been killed after arriving there. Semiallogeneic $(A \times CBA)F_1$ lymph node cells have been shown to arrive in equal numbers in the lymph nodes of normal CBA mice and animals maximally sensitized to A-strain antigens. In the latter the cells are destroyed and only debris remains 24 h after injection (Bainbridge and Gowland, 1971). Accordingly, mice were sensitized by intraperitoneal injection of 10^7 $(A \times CBA)F_1$ spleen cells and tested at 8 days by injection of ^{75}Se-labelled $(A \times CBA)F_1$ lymph node cells. As shown in Table 2, at 24 h the distribution of radioactivity in the lymph nodes, spleens and livers was the same for 10^5, 10^6 or 10^7 testing doses of cells, but far less radioactivity was recovered in the lymph nodes or spleens of sensitized mice. The amount of label, about 25% of that in unsensitized mice, was similar to the label of debris from cells disrupted by freezing and thawing (17·5 per cent), suggesting that little reutilization of free ^{75}Se took place.

Table 1. Distribution of ^{75}Se label at 24 h after injection of viable or heat-killed lymph node cells*

Tissues				Viable cells			Total
Lymphoid:	Peripheral nodes	Mesenteric node		Spleen	Thymus		
	4·29	3·14		5·79	0·18		13·40
	(0·33)	(0·30)		(0·32)	(0·01)		
Liver:	11·47						11·47
	(0·43)						
Other viscera:	Lung	Bone marrow†	Kidney	Salivary gland	Intestine	Blood‡	
	1·05	0·87	4·49	0·62	15·47	0·45	23·13
	(0·09)	(0·05)	(0·16)	(0·11)	(0·56)	(0·04)	
				Heat-killed cells§			
Lymphoid:	Peripheral nodes	Mesenteric node		Spleen	Thymus		
	0·16	0·42		0·96	0·14		1·67
	(0·01)	(0·03)		(0·15)	(0·01)		
Liver:	14·19						
	(0·32)						
Other viscera:	Lung	Bone marrow†	Kidney	Salivary gland	Intestine	Blood‡	14·19
	0·83	0·57	5·77	0·48	15·59	0·39	23·76
	(0·04)	(0·02)	(0·09)	(0·02)	(0·45)	(0·02)	

* Mean radioactivity in organ as a percentage of dose injected with s.e.m. in parentheses. Number of animals = 5.
† Two femora and two tibiae.
‡ 0·1 ml of blood.
§ Heated at 50° for 20 min.

Table 2. Distribution of allogeneic (A × CBA)F_1 label in sensitized mice*

Dose of (A × CBA)F_1 labelled cells:	Recipients					
	Unsensitized			Sensitized		
	Peripheral nodes	Spleen	Liver	Peripheral nodes	Spleen	Liver
10^5	3·85	6·73	14·40	1·14	1·86	17·18
$n = 3$	(0·29)	(0·81)	(0·16)	(0·51)	(0·11)	(0·60)
10^6	3·62	6·59	14·59	0·63	0·96	17·92
$n = 3$	(0·04)	(0·51)	(0·39)	(0·15)	(0·23)	(1·44)
10^7	4·03	7·11	13·17	0·94	1·64	16·85
$n = 3$	(0·30)	(0·08)	(0·14)	(0·51)	(0·72)	(0·28)

* Mean radioactivity in organ as a percentage of dose injected 24 h before, with s.e.m. given in parentheses. Number of animals is shown by *n*.

Leakage of label from viable cells *in vitro*

^{75}Se-labelled lymph node cells were incubated at 37° *in vitro* at a concentration of 10^8 cells/ml. After various time intervals aliquots were removed and centrifuged to determine the amount of label in the supernatant. As shown in Table 3, 40 per cent of the total cell label was released into the medium, the process being complete within 1 h.

No more than 6·5 per cent of the label in the medium after 2 h incubation was precipitable by 10 per cent TCA. To test if it had been released from a

Table 3. Leakage of label *in vitro*

	Length of incubation (h)								
	0	1	2	3	4	5	6	7	8
Percentage radioactivity in supernatant	15·98	40·24	41·72	41·00	40·00	40·56	38·65	39·79	42·05
Percentage viability	75·00	72·20	74·69	70·46	67·94	72·31	74·15	75·52	77·69
Total cell count ($\times 10^8$/ml)	1·33	1·12	1·41	1·14	1·31	1·21	1·04	1·20	0·98

precursor pool within the cells, its ability to label other cells was examined. In various experiments, the supernatant from a 2-h incubation of labelled lymph node cells was injected intravenously into mice; supernatant was incubated with fresh blood for 2 h before injection; and blood from mice receiving supernatants was transferred to secondary recipients. In all cases the distribution of radioactivity in the final recipients at various times was similar to that of label from dead cells, very little being retained in the lymph nodes and spleen (0·2–0·3 and 0·3–0·5 per cent of the dose respectively).

Leakage of label *in vivo*

Despite broad similarities between Table 1 and the distribution of ^{51}Cr activity after injection of viable ^{51}Cr-labelled cells (Bainbridge *et al.*, 1966) the ^{75}Se radioactivity recovered in the spleen at 24 h was much less than might have been expected. It seemed possible that ^{75}Se leakage might be occurring *in vivo*; indeed, 4 h after injection of labelled cells the amount of ^{75}Se label in the spleen was similar to the reported ^{51}Cr arrival (14·5 ± 1·56 per cent) (n = 5), but it then fell to 5·5 ± 0·77 per cent (n = 5) at 24 h.

Cells singly or doubly labelled with ^{51}Cr and ^{75}Se were found in preliminary experiments to show the same 24-h distributions of ^{51}Cr or ^{75}Se radioactivity. Lymph node cells were then labelled with both ^{51}Cr and ^{75}Se and the recipients of the cells killed at 4 h or 24 h (Table 4). The 4-h ^{75}Se radioactivity in the spleen was about two-thirds of the ^{51}Cr activity, and fell rapidly over the succeeding 20 h. The ^{51}Cr level however, altered only slightly. In the lymph nodes, by contrast, both ^{51}Cr and ^{75}Se remained fairly level. The proportion of ^{75}Se lost from the spleen was also much greater than that lost from the lymph nodes.

The different behaviour of ^{75}Se and ^{51}Cr radioactivity in the spleen could be explained if these labels were carried largely on different cell populations. Lymph node cells were doubly labelled with ^{75}Se and ^{51}Cr, washed, and fractionated on a discontinuous gradient of bovine serum albumin by centrifugation at 20,000 *g* for 70 min. Cell fractions were collected, washed and injected in a standard volume into groups of mice. Table 5B shows that substantial activity was present throughout the gradient. Significant spleen-homing activity at 24 h was found from the 29/38 fraction to the 47/50

Table 4. Leakage of label *in vivo*: distribution of ^{51}Cr and ^{75}Se label

Time after injection	Percentage of injected dose recovered in:*		
	Lymph nodes	Spleen	Liver
4 h (n = 5)			
^{51}Cr	6·56 (0·36)	16·56 (0·26)	14·79 (0·56)
^{75}Se	3·46 (0·23)	9·88 (0·30)	9·75 (0·29)
24 h (n = 5)			
^{51}Cr	7·02 (0·42)	15·14 (0·65)	15·58 (0·55)
^{75}Se	2·66 (0·15)	5·08 (0·18)	11·75 (0·68)

* Mean radioactivity in organ as a percentage of dose injected 24 h before, with s.e.m. given in parentheses. Number of animals is shown by n.

Table 5. Gradient fractionation of doubly labelled cells*

A	Fraction†	Percentage of lymph node label*		Percentage of spleen label*	
		^{75}Se	^{51}Cr	^{75}Se	^{51}Cr
	10/29	0·14	−0·01	0·52	0·60
	29/38	0·43	0·22	0·28	2·88
	38/40	4·35	3·35	9·17	8·27
	40/42	44·61	41·00	40·88	34·94
	42/44	40·47	42·29	32·91	34·70
	44/47	7·54	10·31	8·59	11·23
	47/50	1·34	1·51	1·68	2·62
	50+	1·11	1·33	3·48	4·82
	Total	100·0	100·0	100·0	100·0

B	Percentage of fraction arriving in lymph nodes		Percentage of fraction arriving in spleen		Percentage of total activity in fraction	
	^{75}Se	^{51}Cr	^{75}Se	^{51}Cr	^{75}Se	^{51}Cr
10/29	0·37	−0·03	3·15	7·49	1·36	1·61
29/38	0·31	0·41	4·58	13·36	4·98	4·35
38/40	1·56	4·23	7·54	25·96	10·02	6·41
40/42	5·10	13·90	10·71	29·55	31·43	23·90
42/44	5·01	13·28	9·34	27·18	29·00	25·80
44/47	2·25	5·77	5·88	15·70	12·02	14·47
47/50	1·99	2·89	5·72	12·52	2·42	4·23
50+	0·46	0·56	3·27	5·06	8·76	19·23

* Distribution of total ^{75}Se and ^{51}Cr radioactivity recovered in any organ amongst the recipients of different fractions of a doubly labelled lymph node suspension, 24 h after injection. Means of three mice are shown for each fraction.

† The fraction consists of the band of cells at the interface between the two named BSA concentrations, e.g. 10/29 is the band between the 10 and 29 per cent layers.

fraction. However, as can be seen in Table 5A, the total ^{75}Se activity in the spleens, and also in the lymph nodes, was distributed among the fractions in the same way as the ^{51}Cr label.

The difference in ^{75}Se leakage between the spleen and lymph nodes could be attributed to spleen-seeking cells leaking ^{75}Se more readily. To test this, serial transfers of labelled lymph nodes or spleens were carried out. Lymph node cells (L_0) were injected at a dose of 2×10^7 cells/mouse into primary recipients (R_1). Twenty-four hours later the lymph node-seeking or spleen-seeking cells in these mice were injected as 1:1 lymph node (L_1) or spleen (S_1) transfers, respectively, into secondary recipients (R_2), which were killed at 4, 24, 48 or 72 h. Parallel transfers were carried out starting from labelled spleen cells (S_0). The recipients of lymph node-seeking cells (L_1) had high lymph node activity (L_0L_1, about 10%; S_0L_1, about 13%), and the recipients of spleen-seeking cells (S_1) had much less (L_0S_1, about 6 per cent; S_0S_1, about 4%, see Table 6). On the other hand, the patterns of ^{75}Se radioactivity in the spleen were closely similar in all four groups of recipients, whether they had been given lymph node-seeking (L_0L_1, S_0L_1) or spleen-seeking (L_0S_1, S_0S_1) cells. The activity fell rapidly from 26–28 per cent to 7–8 per cent over the 48–72 h period.

Splenic ^{75}Se leakage

To test whether spleen cells themselves induced the release of ^{75}Se activity from labelled cells, 10^7 labelled lymph node cells were incubated for 2 h at

Table 6. Serial transfer of ^{75}Se-labelled lymphoid cells*

	Percentage of dose injected recovered in secondary recipients (R_2)							
	Lymph nodes (h)				Spleens (h)			
	4	24	48	72	4	24	48	72
Primary cell source: lymph node (L_0)								
Secondary transfer: lymph nodes (L_0L_1) ($n = 5$)	10·22 (1·05)	10·69 (0·24)	8·91 (0·82)	—	25·67 (2·81)	10·70 (0·41)	7·28 (0·47)	—
spleens (L_0S_1) ($n = 4$)	6·26 (0·75)	7·13 (1·07)	6·73 (0·40)	—	28·84 (2·21)	10·77 (2·48)	8·72 (0·38)	—
Primary cell source: spleen (S_0)								
Secondary transfer: lymph nodes (S_0L_1) ($n = 4$)	12·94 (2·83)	13·98 (3·26)	9·96 (1·84)	7·97 (0·67)	27·64 (1·06)	12·69 (1·47)	8·15 (1·02)	7·25 (0·69)
spleens (S_0S_1) ($n = 4$)	3·63 (0·14)	6·07 (0·19)	4·12 (0·55)	4·05 (0·21)	27·46 (1·71)	18·95 (1·20)	11·14 (0·90)	8·47 (0·48)

* Mean radioactivity in organ as a percentage of dose injected with s.e.m. given in parentheses. Number of animals is shown by *n*.

37° in the presence or absence of unlabelled spleen or lymph node cells. Table 7 shows that, far from increasing the amount of supernatant label, 4×10^8 unlabelled spleen or lymph node cells/ml halved the spontaneous release. In further experiments labelled cells themselves showed much less spontaneous release at high concentrations. The absolute values varied, but within any experiment the pattern of results was consistent (Fig. 1a). Fig. 1b is a composite figure, obtained by shifting the vertical height of graphs from several experiments, so as to superimpose them at 10^7 and 10^8 cells/ml. Although the vertical scale is arbitrary, it is clear that leakage markedly decreased above 10^8 cells/ml. Finally, labelled lymph node cells in a ratio of 1:10 with unlabelled spleen cells were incubated at various cell concentrations for 2 h, at 4° or 37°. Table 8 shows that the high spontaneous release of 3×10^6 cells/ml occurred only at 37°.

Table 7. Effect of spleen cells on ^{75}Se leakage of 10^7 labelled cells

	Unlabelled cells/ml		
	None	4×10^8 Spleen	4×10^8 Lymph node
Percentage label in supernatant	40·60	23·27	24·26

^{75}Se leakage was found to be the same in Tyrodes' solution, syngeneic mouse plasma, or 100 per cent fresh anticoagulated whole blood. Continuing leakage *in vivo*, however, could be prevented if the circulation were to become saturated with released ^{75}Se label. This was tested by injecting 10^7 labelled lymph node cells into 'saturated' mice (animals which had received 10^7 labelled cells 24 h previously). The loss of ^{75}Se label between 4 and 24 h attributable to the test injection in saturated mice was 4·78 per cent for the spleen and 0·97 per cent for the lymph nodes. By comparison, for mice receiving the same labelled cells as a first injection, the corresponding figures were 4·48 per cent and 1·05 per cent respectively.

^{75}Se leakage and cellular migration

^{75}Se leakage was investigated in BALB/c mice with Friend virus leukaemia, a disease producing gross enlargement and structural change in the spleen, which might be expected to alter cellular migration patterns in the organ. Four hours after injection of doubly labelled normal BALB/c lymph node cells the ^{51}Cr and ^{75}Se levels in the lymph nodes and spleens of recipients were unaltered by infection, even at a late stage of the disease (Table 9). The changes of ^{51}Cr level between 4 and 24 h were the

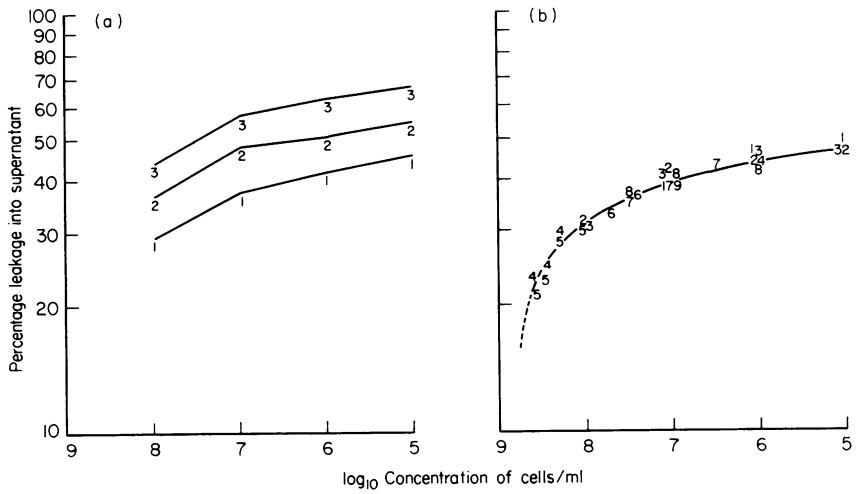

Figure 1. Percentage loss of radioactivity from ^{75}Se labelled cells after 2 h incubation at different concentrations. (a) Three experiments (1, 2, 3) showing the same curve but at different levels. (b) Composite dose–response curve of nine experiments (1–9), produced by displacing the experimental curves vertically to coincide as far as possible at 10^8 and 10^7 cells/ml.

Table 8. ^{75}Se leakage at 4° and 37°

	Cell concentration (cells/ml)		
	3×10^8	3×10^7	3×10^6
Percentage label in supernatant after 2 h incubation at:			
37°	29·98	—	47·49
4°	20·04	21·49	21·29

same throughout, and also, once infection had taken place, the ^{75}Se changes from 4 to 24 h in the lymph nodes. However, the splenic ^{75}Se loss between 4 and 24 h markedly diminished in infected mice. Table 9 shows these results summarized by an 'index of replacement':

$$IR = 100\times\log\left(\frac{^{75}Se\ 4\ h}{^{75}Se\ 24\ h}\right)\Big/\left(\frac{^{51}Cr\ 4\ h}{^{51}Cr\ 24\ h}\right),$$

representing the extent to which the ^{75}Se loss from 4 to 24 h exceeded the ^{51}Cr loss in the same time interval. The splenic index fell progressively with the course of the disease. Fig. 2 shows, further, that it was closely related to the organ weight.

DISCUSSION

[^{75}Se]L-selenomethionine, an analogue of the sulphur-containing amino acid methionine, has been widely employed as a tool for gamma-counting and scintillation scanning in radiodiagnostic work. As a label for lymphocytes many of its properties have proved to be broadly similar to those described for ^{51}Cr (Bainbridge *et al.*, 1966; Bainbridge and Gowland, 1971). Substantial amounts of ^{75}Se were recovered in lymphoid organs after injection of labelled viable, but not heat-killed, lymphoid cells (Table 1). The label released by cell death was not significantly reutilized by lymphoid tissues *in vivo* or *in vitro*. ^{75}Se-labelled cells transferred into immunized allogeneic mice and destroyed in the lymph nodes and spleens (Bainbridge *et al.*, 1971) lost most of the label (Table 2), showing that label released on cell death *in vivo* was not retained to any significant extent locally. Thus, exactly as for ^{51}Cr, for a few days after injection, a substantial amount of ^{75}Se activity in lymphoid organs indicates the presence of living cells.

There was, however, a major difference between the two labels. The level of ^{75}Se activity in the spleen at 4 h after injection of living cells was comparable to figures reported for ^{51}Cr, but at 24 h was disproportionately low, suggesting that ^{75}Se-labelled cells migrated more rapidly through the spleen than cells labelled with ^{51}Cr, or alternatively, that substantial leakage of ^{75}Se label occurs *in vivo*. In numerous short-term experiments the behaviour of ^{75}Se-labelled cells was modified at most to a minor degree by simultaneous ^{51}Cr labelling (^{51}Cr may be deleterious to cells beyond 48–72 h, however)

Table 9. Change of splenic circulation by Friend virus infection in BALB/c mice*

	Days after infection						
	0	2	4	6	8	12	14
Percentage of dose recovered in lymph nodes:							
^{51}Cr at 4 h	1·42	1·52	1·33	1·51	0·95	1·72	1·88
	(0·10)	(0·05)	(0·10)	(0·09)	(0·12)	(0·21)	(0·12)
^{75}Se at 4 h	1·61	1·87	1·61	1·83	1·33	2·21	2·21
	(0·14)	(0·11)	(0·09)	(0·18)	(0·17)	(0·21)	(0·10)
Index of replacement†	−0·67	7·76	6·34	4·47	10·94	6·22	4·33
Percentage of dose recovered in spleen:							
^{51}Cr at 4 h	9·36	10·45	10·09	11·40	8·21	7·93	8·42
	(0·23)	(0·21)	(0·39)	(0·39)	(0·31)	(0·61)	(0·62)
^{75}Se at 4 h	12·95	14·31	14·63	16·79	13·61	14·93	15·56
	(0·33)	(0·21)	(0·83)	(0·36)	(0·32)	(0·98)	(0·97)
Index of replacement	15·19	16·88	11·82	11·03	7·87	−1·50	1·07
Mean spleen wt (mg) of 24-h recipients	137·9	112·7	213·1	258·9	759·6	1864·9	1505·8

* Mean radioactivity in organ with s.e.m. given in parentheses. Number of animals was four per group.

† Calculated as $100 \times \log \left(\frac{^{75}\text{Se 4 h}}{^{75}\text{Se 24 h}}\right) / \left(\frac{^{51}\text{Cr 4 h}}{^{51}\text{Cr 24 h}}\right)$ (see text).

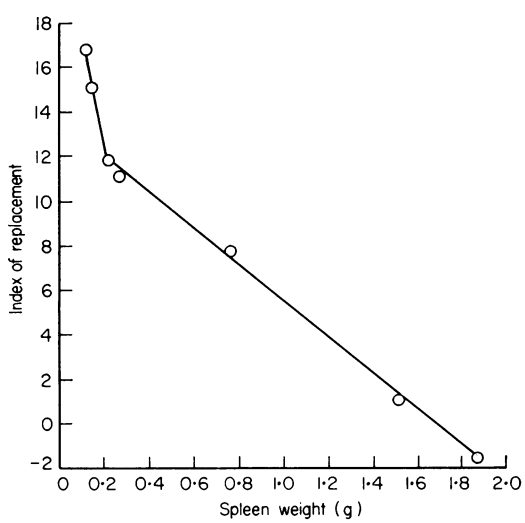

Figure 2. Index of replacement in the spleens of Friend virus infected BALB/c mice, plotted against mean 24-h spleen weight. Index = $100 \times \log \left(\frac{^{75}\text{Se 4 h}}{^{75}\text{Se 24 h}}\right) / \left(\frac{^{51}\text{Cr 4 h}}{^{51}\text{Cr 24 h}}\right)$.

(Bainbridge and Gowland, 1971). On testing with lymph node cells doubly labelled with ^{75}Se and ^{51}Cr, little change in ^{51}Cr level accompanied the substantial loss of ^{75}Se activity between 4 and 24 h in the spleen (Table 4). This would confirm the notion of splenic leakage of ^{75}Se, unless ^{75}Se and ^{51}Cr labels are attached to different populations of lymphocytes. ^{51}Cr labels small lymphocytes relatively uniformly (Howard, Hunt and Gowans, 1972). Much of the ^{75}Se label would then have to be confined to a minor subpopulation. On BSA density gradient fractionation of doubly labelled cells, radioactivity was widely spread throughout the gradient. Spleen-homing activity, which was present in most fractions (Table 5B) was distributed similarly among the fractions for ^{75}Se and ^{51}Cr (Table 5A), being concentrated in two of the denser layers. No major difference was apparent. This is perhaps not surprising if the T2 subpopulation to which the majority of unstimulated murine lymph node lymphocytes belong is relatively homogeneous.

According to the results (Tables 1, 2 and 4) the

leakage of ^{75}Se from viable cells was much greater in the spleen than in lymph nodes. Possibly spleen-seeking cells (Lance and Taub, 1969) have a greater propensity to lose label than lymph node-seeking lymphocytes. Cells with enhanced or reduced affinity for the lymph nodes of secondary recipients (R_2) were produced by serial transfer of ^{75}Se-labelled lymph node (L_0) or spleen (S_0) cells. The two types arrived equally in secondary spleens (S_2) at 4 h and showed the same loss of ^{75}Se over a 48–72-h period, the 'spleen-seeking' cells (L_0S_1, S_0S_1) at a rate slightly slower, rather than faster, than the lymph node-seeking cells (L_0L_1, S_0L_1) (Table 6). Also the amount of ^{75}Se lost from the spleen was the same in primary and secondary transfers, showing that the residual label was still freely exchangeable.

^{75}Se-labelled lymph node cells, incubated *in vitro* at 37°, lost up to 40 per cent of their total cell label within 1 h, under conditions where no loss of viability or cell number occurred (Table 3). This was not simply release of [^{75}Se]L-selenomethionine from a precursor pool, however, for the label could no longer be utilized by lymphocytes or lymphoid tissue. Leakage was an active metabolic process, inhibited at 4° (Table 8), and markedly dependent on the ambient cell concentration (Table 7, Fig. 1). There was a high spontaneous release at low cell densities, which decreased markedly above 10^8 cells/ml, whether the cell suspension was uniformly labelled (Fig. 1) or a mixture of labelled and unlabelled cells (Table 7). This incidentally indicated that spleen cells *per se* did not provoke the release of ^{75}Se *in vivo* from labelled lymphocytes.

In view of the foregoing it was not differences in the behaviour of subpopulations of lymphocytes or of compartments of the label within cells that explained the discrepancy between the ^{75}Se leakage of the spleen and lymph nodes. The *in vitro* observations suggested another possibility. If ^{75}Se leakage occurred in the circulation, i.e. at low concentrations ($0{\cdot}5$–$1{\cdot}5 \times 10^7$ cells/ml), and not in lymphoid tissue, i.e. at high cell concentrations (1–$2{\cdot}5 \times 10^9$ cells/ml), the slow transit of lymphocytes through lymph nodes (about 18 h in the rat; Ford and Simmonds, 1972), would protect labelled cells in the lymph nodes. Cells entering the spleen, however, for which the modal transit time in the rat is 5–6 h, (Ford, 1969) could re-enter the blood, leak label and perhaps repeat the cycle three to four times in 24 h; or alternatively, could pass mainly into red pulp (Hammond, 1975), and leak ^{75}Se there as if in blood.

^{75}Se leakage was examined in a situation where the cellular migration patterns might be expected to change. During the course of Friend leukaemia virus infection the spleen of BALB/c mice undergoes gross structural changes. The number of lymphocytes in the organ is two to three times normal at a relatively early stage, though the arrival of ^{51}Cr-labelled cells is normal or reduced (Bainbridge and Bendinelli, 1972). The migratory behaviour of injected cells is not affected by a sojourn of up to 48 h in the infected spleen (Bainbridge and Bendinelli, 1972), suggesting that cells accumulate because their passage through the organ is prolonged or prevented. Doubly labelled normal BALB/c lymph node cells arrived normally in the spleens and lymph nodes of infected mice. The ^{75}Se losses from the spleen however, were markedly different at different times after infection. An 'index of replacement', expressing the relative ^{75}Se loss between 4 and 24 h, decreased sharply as the disease progressed (Table 9), and was found to be closely related to the spleen weight (Fig. 2), suggesting that the length of the path taken by the cells increased *pari passu* with increasing size of the organ. This strongly suggests that the extent of ^{75}Se leakage from a lymphoid organ, appropriately corrected, reflects the transit time of lymphocytes through it, rapid release of ^{75}Se corresponding to a short transit time and slow release to a long one.

ACKNOWLEDGMENT

I should like to acknowledge the invaluable technical assistance of Mrs Julia M. Parsley.

REFERENCES

BAINBRIDGE D.R. & BENDINELLI M. (1972) Circulation of lymphoid cells in mice infected with Friend leukaemia virus. *J. nat. Cancer Inst.* **49**, 773.

BAINBRIDGE D.R., BRENT L. & GOWLAND G. (1966) Distribution of allogeneic ^{51}Cr-labelled lymph node cells in mice. *Transplantation*, **4**, 138.

BAINBRIDGE D.R. & GOWLAND G. (1971) Studies on transplantation immunity. I. The nature of the response to allogeneic ^{51}Cr-labelled lymphoid cells and its relationship to homograft immunity. *Cell. Immunol.* **2**, 115.

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

FORD W.L. & SIMMONDS S.J. (1972) The tempo of lymphocyte recirculation from blood to lymph in the rat. *Cell Tissue Kinet.* **5**, 175.

GOWANS J.L. (1959) The recirculation of small lymphocytes from blood to lymph in the rat. *J. Physiol. (Lond.)*, **146**, 54.

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

HAMMOND B.J. (1975) A compartmental analysis of circulatory lymphocytes in the spleen. *Cell Tissue Kinet.* **8**, 153.

HOWARD J.C., HUNT S.V. & GOWANS J.L. (1972) Identification of marrow-derived and thymus-derived small lymphocytes in the lymphoid tissue and thoracic duct lymph of normal rats. *J. exp. Med.* **135**, 200.

LANCE E.M. & TAUB R.N. (1969) Segregation of lymphocyte populations through differential migration. *Nature (Lond.)*, **221**, 841.