

THE RECIRCULATION OF LYMPHOCYTES FROM BLOOD TO LYMPH IN THE RAT

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In all the mammalian species which have so far been examined, large numbers of lymphocytes enter the blood each day from the main lymphatic vessels in the neck (reviewed by Yoffey & Courtice, 1956). In the rat, for example, enough lymphocytes enter the blood from the thoracic duct to replace all those in the blood about 11 times daily (Gowans, 1957*a*). This is a minimum estimate of the actual daily replacement since it ignores the contribution from the lymphatics which drain the head, neck, forelimbs and thorax; taking these into account Whaler & Widdicombe (1956) estimated that the average time spent by lymphocytes in the blood of the rat was probably less than one hour. Many theories have been advanced to explain this rapid turnover of lymphocytes in the blood but none of them has good experimental support (Yoffey & Courtice, 1956; Florey & Gowans, 1958; Trowell, 1958).

In a previous paper (Gowans, 1957*a*) it was shown that in order to maintain the output of lymphocytes from a thoracic duct fistula in an unanaesthetized rat it was necessary to re-infuse continuously into the blood all the lymphocytes which issued from the fistula. When either cell-free lymph or lymph which contained killed lymphocytes was re-infused a profound fall in the output of cells from the thoracic duct eventually occurred, similar to that described by Mann & Higgins (1950) in rats which received no intravenous replacement of either cells or fluid. The aim of the present investigation was to discover the mechanism by which the intravenous infusion of living lymphocytes influenced the output of lymphocytes from the thoracic duct. Essentially, this aim was achieved by determining the fate of thoracic duct lymphocytes after their transfusion into the blood. Preliminary accounts of these experiments have already been given (Gowans, 1957*b*, 1958).

METHODS

The methods used previously (Gowans, 1957*a*) were extended so that thoracic duct lymphocytes from a number of donor rats could be transfused continuously and for long periods into the blood of a recipient rat. An essential requirement for these experiments was that the transfused

lymphocytes should survive normally in the recipient animal. Since a transfusion of lymphocytes is, in effect, a homograft, this requirement would be met if the donor and recipient rats were genetically identical. Animals of highly inbred strains of albino and of hooded rat were used in the present experiments but the genetic uniformity of the members of each strain was not checked by skin homografting. However, the results of the experiments made it clear that the fate of the transfused lymphocytes had not been qualitatively affected by the intervention of a homograft reaction. Unless otherwise stated the donor and recipient rats were male albino litter mates weighing 200–250 g.

The thoracic duct was cannulated by the method of Bollman, Cain & Grindlay (1948) with the minor modification that the cannula was brought out through the skin of the back of the animal. The troublesome whip of the polythene tubing was overcome by bending the cannula into the correct shape by mild heat before its insertion into the duct. After operation the rats were placed in a restraining cage (Bollman, 1948) where they remained, unanaesthetized, for periods up to 11 days. Rats with thoracic duct fistulae ate and drank normally while in the restraining cages and were lively and well when released. They all, however, suffered some loss of body weight. Loss of body weight, in itself, is not associated with any change in the output of lymphocytes from the thoracic duct of rats (Mann & Higgins, 1950).

Transfusion of thoracic duct lymphocytes. The essential feature of the method was that it enabled lymphocytes and lymph to be pumped into the blood of a rat at a physiological rate for many days. The transfused lymphocytes were alive when they entered the blood of the recipient rat, since microscopic observation of samples taken from the outlet of the pump showed that the cells were motile. Continuous measurements were made of the output of lymphocytes from the thoracic duct of the recipient rat. The apparatus was, in the main, the same as that described previously (Gowans, 1957*a*). Thoracic duct lymphocytes from the donor rats were pumped from a siliconed glass reservoir into the femoral vein of the recipient rat. Lymph from the thoracic duct of the recipient rat was collected on a fraction collector which was housed in a refrigerator at 4° C. An improved reservoir was designed for the present experiments (Text-fig. 1). This reservoir with its inlet and outlet tubes could be quickly dismantled, washed out and reassembled.

Krebs–Ringer solution containing heparin at a concentration of 2 u./ml. was infused into the femoral vein of each donor rat at about 2 ml./hr; this ensured a free flow of lymph and minimized the formation of clots in the cannula during the first hours after cannulation. When prolonged transfusions were performed, it was necessary to employ a series of donor rats, since the output of lymphocytes from the thoracic duct of any one animal falls progressively after cannulation (Mann & Higgins, 1950). The lymph from the thoracic duct cannula of each successive donor rat dripped directly into the reservoir, as shown in Text-fig. 1. A solution of heparin was pumped slowly into the reservoir to prevent the lymph from clotting. In other experiments it was necessary to know the number of lymphocytes which were being transfused. For this purpose, a cell count was made on each successive 2–3 hr collection of lymph from the donor rat; each sample, containing a known number of lymphocytes, was then added to the reservoir.

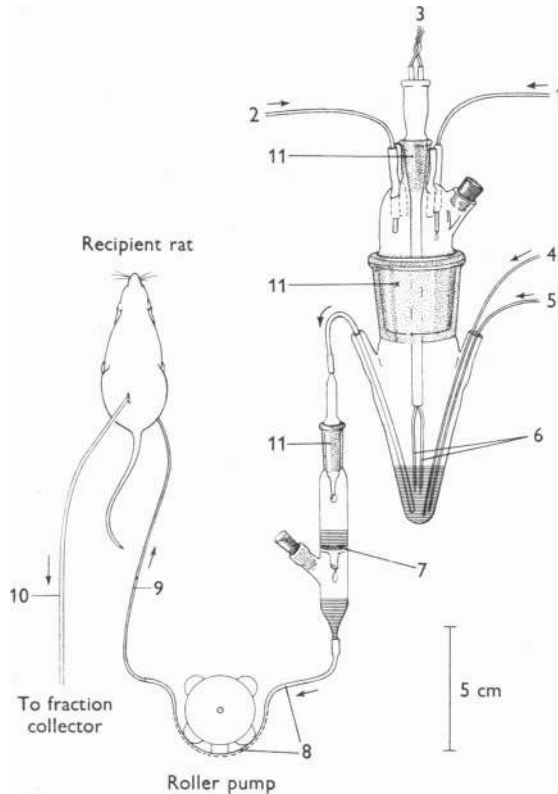
If lymph from either the donor or recipient rats was required for transfusion or re-infusion, it was collected into chilled Krebs–Ringer solution containing heparin at a concentration of 20 u./ml. When the lymph was needed for cell counts only, it was collected into a solution of 3% sodium citrate and 10% formaldehyde. The collections were made into 15 ml. centrifuge tubes each of which contained a measured volume of one of these anticoagulant solutions. The volume of anti-coagulant solution added to each tube was calculated on the basis of 0.5 ml. for each hour's collection of lymph. The Krebs–Ringer solutions contained 200 µg each of penicillin and streptomycin per millilitre.

The lymph and lymphocytes in the reservoir were pumped continuously into the femoral vein of the recipient rat at a rate of 2–4 ml./hr by means of a roller pump. The contents of the reservoir were mixed by a slow stream of oxygen bubbles. If the pumping rate exceeded the rate of inflow of lymph into the reservoir, the circuit across the electrodes (6 in Text-fig. 1) was eventually broken. A relay then switched off the pump and switched on an electrically driven syringe which

delivered fluid into the reservoir until the electrodes were again covered. In this way it was ensured that air was not pumped into the rat.

The blockage of some part of the pumping circuit by small clots was a constant problem in these experiments. A dripper with a filter (Text-fig. 1) was incorporated into the circuit to trap these clots and to allow a visual check that the pump was working efficiently. As an added precaution the whole circuit was washed through with saline each morning and evening. Rats have been successfully transfused for periods up to 7 days with thoracic duct lymph and lymphocytes from a series of donor animals.

Labelling of lymphocytes with ^{32}P . ^{32}P -orthophosphate (PBS 2 from Radiochemical Centre, Amersham) was injected subcutaneously into the donor rats. A total of $1\ \mu\text{c/g}$ body wt. was given



Text-fig. 1. Diagram of apparatus for the intravenous transfusion of thoracic duct lymph and lymphocytes into a rat. The dripper and reservoir only are drawn to scale. 1, polythene cannula (bore, 0.5 mm) from thoracic duct of donor rat; 2, polythene tube (bore, 1.0 mm) which delivers Krebs-Ringer solution containing heparin 2 u./ml. into the reservoir when the circuit across the electrodes (6) is broken by the falling fluid level; 3, leads from electrodes to relay circuit; 4 and 5, nylon tubing (bore, 0.25 mm); 4, slow oxygen stream; 5, Krebs-Ringer solution containing 20 u./ml. entering at 0.3 ml./hr; 6, platinum electrodes sheathed in polythene tubing; 7, disk of stainless steel mesh in upper compartment of siliconed glass dripper; 8, polyvinyl tubing (bore, 0.5 mm) passing through block of roller pump; 9, polyvinyl cannula (bore, 0.25 mm) entering femoral vein of recipient rat; 10, cannula from thoracic duct of recipient rat; 11, ground glass joints.

in two equal doses; the second dose was given 12 hr after the first and the thoracic duct was cannulated 12 hr later. Successive 2-3 hr samples of lymph were collected into 1 ml. lots of chilled Krebs-Ringer solution containing 20 units of heparin. The cells were separated from the lymph by centrifuging, washed once in cold Krebs-Ringer solution and then resuspended in fresh Krebs-Ringer containing heparin at a concentration of 2 u./ml. The samples of washed cells were added successively to the reservoir after small volumes had been taken for cell counts and for measurements of radioactivity.

Measurement of radioactivity. The lymphocytes were separated by centrifuging and either plated out on planchets and counted with a thin mica end-window G-M tube, or suspended in 10 ml. of the sodium citrate-formaldehyde solution and counted in a G-M liquid counter. Lipid was extracted from lymphocytes by the extraction procedure described by Davidson, Frazer & Hutchison (1951); the acid-soluble fraction was discarded and the final lipid extract dissolved in 10 ml. of chloroform and counted in a G-M liquid counter. The samples were counted to give an error of less than 3%. The usual corrections were made for radioactive decay.

Labelling of lymphocytes with tritium-labelled thymidine. Tritium-labelled thymidine is an ideal compound for studying the synthesis of deoxyribonucleic acid (DNA) in single mammalian cells since thymidine is incorporated exclusively into the DNA and, in autoradiographs, the tritium label is localized strictly over the nucleus of each cell (Pl. 1, figs. 2-5). A solution of tritium-labelled thymidine (Schwarz Laboratories Inc.; specific activity, 200-360 mc/m-mole) was infused intravenously into a rat for 12 hr at a constant rate of 20 μ c/hr. The lymphocytes were separated from the thoracic duct lymph by centrifuging, washed once in Krebs-Ringer solution and resuspended in a small volume of rabbit serum to make a thick suspension. Autoradiographs were prepared from smears of this suspension by the stripping-film technique (Pelc, 1956). The preparations were exposed for 7 days, developed, and then stained through the emulsion with Wright's stain. The proportion of lymphocytes which contained labelled DNA was then determined. During the infusion of the tritiated thymidine the number of grains per cell in the autoradiographs increased to a maximum of about 80 per large lymphocyte; the number decreased slowly after the infusion was discontinued.

RESULTS

Lymphocyte transfusion on the fifth day after thoracic duct cannulation

The earlier experiments (Gowans, 1957*a*) suggested that the output of lymphocytes from the thoracic duct was in some way determined by the number of lymphocytes which entered the blood. If this were so, a suitable transfusion of lymphocytes would be expected to restore to a high level the low output of cells which results from the prolonged drainage of lymph from the thoracic duct. The experiments illustrated in Text-fig. 2 confirmed this expectation.

A continuous intravenous transfusion of lymphocytes from a series of donor rats was started at the end of the 4th day after cannulation of the recipient rat. All the lymph from the thoracic duct of the recipient rat was collected for cell counts and then rejected. When the transfusion was between litter mates of the same inbred strain, the output of cells from the recipient rat rose progressively and levelled off at a point below the initial output, but within the range of normal values for the output in rats of this age and strain. In contrast, a transfusion from hooded into albino rats resulted in a slower rise in output which terminated abruptly on the 4th day. Despite the continued transfusion of lymphocytes, the output fell progressively to a level below that at the start of the transfusion (Text-fig. 2). Thus, the fall in the lymphocyte

output described by Mann & Higgins (1950) can be reversed by transfusing lymphocytes from one rat into another, providing the genetic disparity between the donor and the recipient animals is not too great.

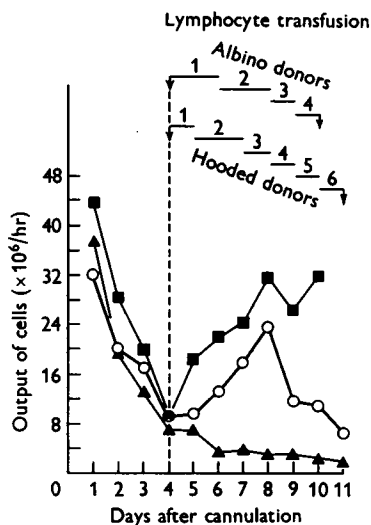


Fig. 2

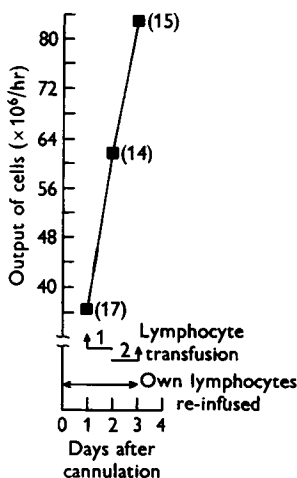


Fig. 3

Text-fig. 2. Output of lymphocytes from the thoracic duct of three albino rats; mean values per hour for each successive 24 hr collection of lymph. The rats received intravenous transfusions of lymphocytes from either successive albino donors: ■—■; or from successive hooded donors: ○—○. One rat received no transfusion: ▲—▲. 1, 2, etc. = periods of transfusion from successive donor rats.

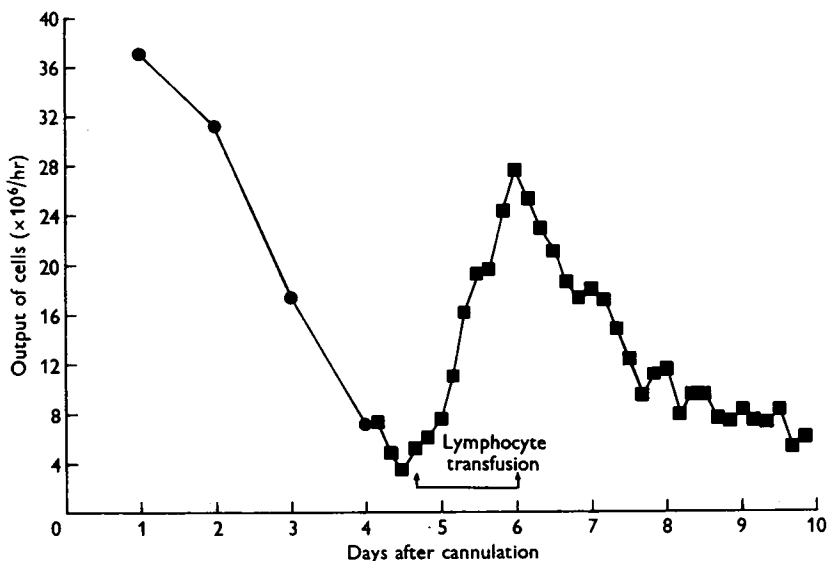
Text-fig. 3. Output of lymphocytes from the thoracic duct of a rat which received an intravenous transfusion of lymphocytes from two successive donor rats. In addition, all the lymphocytes from the recipient's own thoracic duct were continuously re-infused. (17), (14), (15) = number of hours during which samples of lymph were collected for cell counts on each day. 1, 2 = periods of transfusion from successive donor rats.

Lymphocyte transfusion on the second day after thoracic duct cannulation

Since the first day's high output of lymphocytes can be maintained or restored by the intravenous infusion of lymphocytes, it might be supposed that there is a more or less constant physiological level to which the output always adjusts itself, given a sufficient input. Text-fig. 3 shows that this is not so. A vigorous transfusion of lymphocytes, together with the continuous re-infusion of all the lymphocytes from the recipient's own thoracic duct, increased the output to a value more than double that of the first day.

Relation between input and output of lymphocytes. The relation between the number of lymphocytes transfused and the number emerging from the thoracic duct was studied in rats whose lymphocyte output had fallen to a low level following the drainage of thoracic duct lymph for 5 days. The output of

lymphocytes from such rats provided an adequate base line for quantitative studies. Text-fig. 4 illustrates an experiment in which a known number of lymphocytes was transfused and the output of lymphocytes from the thoracic duct was measured in successive 4 hr samples of lymph. A continuous rise in the output of lymphocytes began during the first 4 hr after the start of the transfusion and a fall occurred during the 4 hr following the end of the transfusion. In a series of such experiments a delay of up to 12 hr sometimes occurred before a rise in the output was detected (e.g. Text-fig. 5) and the output sometimes continued to rise for 8 hr after the end of the transfusion



Text-fig. 4. Output of lymphocytes from the thoracic duct of a rat which received an intravenous transfusion of lymphocytes during the 5th and 6th days after cannulation. Mean values per hour for successive 24 hr (●—●) and 4 hr (■—■) collections of lymph.

TABLE 1. Effect of the intravenous transfusion of lymphocytes, on the output of lymphocytes from the thoracic duct

Rat no.	Number of cells transfused ($\times 10^6$)	Duration of transfusion (hr)	Duration of experiment (hr)	*From start of transfusion		
				Estimated base-line output of cells ($\times 10^6$ /hr)	Total output of cells in excess of base-line production	
				As % of number of cells transfused		
				$\times 10^6$		
143	1180	32	124	6	895	76
151	720	24	104	5	578	80
157	496	15	112	6	428	86

* Lymph was drained from the thoracic duct of each recipient rat for 4-5 days before the start of the transfusion.

(e.g. Text-fig. 8). The total output of lymphocytes in excess of the estimated base-line output amounted to about 80% of the number of cells transfused (Table 1).

Mechanism by which the input of lymphocytes into the blood increases their output from the thoracic duct

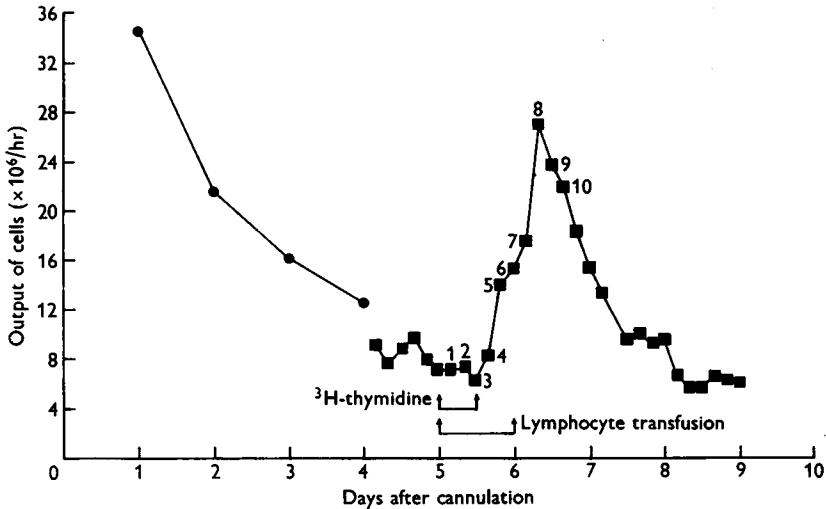
The experiments described so far show that the output of lymphocytes from the thoracic duct varies directly with the number of lymphocytes transfused into the blood. The transfused cells could cause an increase in the output from the thoracic duct by three possible mechanisms: (1) Chemical substances derived from the transfused lymphocytes might be essential for the formation of new lymphocytes in the lymph nodes of the recipient animal. (2) The transfused cells might repopulate the lymph nodes of the recipient animal and, by successive cell divisions, give rise to a progeny of new lymphocytes. Or (3), the transfused lymphocytes might recirculate from the blood back into the thoracic duct lymph, i.e. the transfused cells and the extra cells emerging from the thoracic duct are identical. Mechanisms (1) and (2) would both operate by initiating a burst of new lymphocyte production in the recipient animal.

Formation of new lymphocytes. The possibility that the intravenous transfusion of lymphocytes had initiated the production of new lymphocytes in the recipient animal was tested by infusing tritium-labelled thymidine into one femoral vein of a rat while lymphocytes were transfused simultaneously into the other. Autoradiographs were prepared from successive 4 hr samples of lymphocytes collected from the thoracic duct of the recipient. If the extra lymphocytes which emerged from the thoracic duct had been formed after the start of the transfusion, their DNA would have become labelled. The results of the experiment are shown in Text-fig. 5 and Table 2. During the rising phase of the output (collections 4–8) more than 90% of the cells in the lymph were small lymphocytes; 2% or less of these contained labelled DNA. This very low proportion of labelled small lymphocytes was not due to an inadequate level of tritium-labelled thymidine in the animal, since, during the same period, more than 90% of the larger lymphocytes, which are known to divide in the thoracic duct lymph, became labelled. It was concluded that almost all the small lymphocytes from the thoracic duct of the recipient rat were 'old' cells, in the sense that their DNA had been formed before the start of the transfusion. This experiment rules out mechanisms (1) and (2) unless it is held that 'new' lymphocytes can be produced by some process that does not involve the synthesis of new DNA at any stage.

Recirculation of lymphocytes. The exclusion of mechanisms (1) and (2) strongly suggested that the lymphocytes which were transfused into the blood found their way back into the thoracic duct lymph. This hypothesis was

tested directly by transfusing ³²P-labelled lymphocytes into a rat and following the appearance of radioactivity in the cells emerging from the thoracic duct.

The transfusions were begun at the end of either the first or the 5th day after cannulation of the recipient rat (Text-fig. 6, 8). In each case a continuous rise in the radioactivity in the cells issuing from the thoracic duct started during the first 4 hr. The radioactivity curves had a time course which was similar to that of the cell output curves illustrated in Text-figs. 4 and 5.



Text-fig. 5. Output of lymphocytes from the thoracic duct of a rat which received an intravenous transfusion of lymphocytes during the 6th day after cannulation. Tritium-labelled thymidine was infused intravenously at 20 μc/hr during the first 12 hr of the transfusion. Mean output of cells per hour for successive 24 hr (●—●) and 4 hr (■—■) collections of lymph. 1-10: successive 4 hr collections of lymphocytes from which autoradiographs were prepared, see Table 2.

TABLE 2. Proportion of small lymphocytes and of tritium-labelled lymphocytes in autoradiographs prepared from the collections of lymphocytes in Fig. 5

Collection no.	Small lymphocytes (%)	Labelled lymphocytes*	
		Small (%)	Medium and large (%)
1	88.8	0	40
2	88.8	0.1	66
3	92.4	0.4	88
4	93.0	2.0	93
5	94.3	0.9	97
6	96.7	1.2	97
7	94.9	1.6	93
8	97.0	0.4	91
9	95.6	1.1	91
10	94.3	1.0	77

* Percentage derived from counts on 1000 small lymphocytes and 100 large and medium lymphocytes in each collection.

Table 3 shows the relation between the radioactivity in the lymphocytes entering the blood and that in the cells from the thoracic duct. About 20% of the total radioactivity and about 40% of the radioactively labelled lipid in the transfused cells were recovered in the lymphocytes from the thoracic duct.

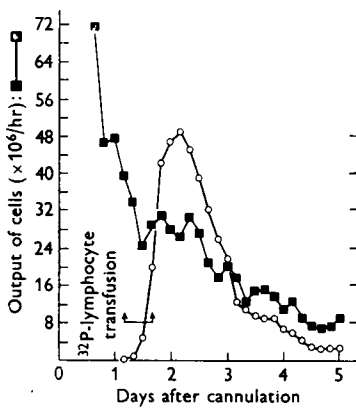


Fig. 6

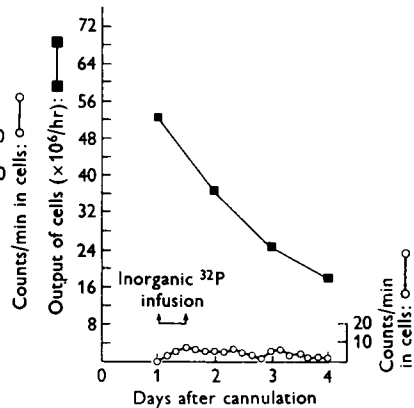


Fig. 7

Text-fig. 6. Output and radioactivity of lymphocytes from the thoracic duct of a rat which received an intravenous transfusion of ^{32}P -labelled lymphocytes. Total counts/min in transfused cells, 47,500. Mean output of cells per hour (■—■) and total radioactivity in cells (○—○) for each successive 4 hr collection of lymph. In this experiment the number of cells transfused was insufficient to cause a very noticeable change in the output of lymphocytes from the thoracic duct, for the recipient's own output was falling rapidly. When the transfusion was begun later (Fig. 8) the radioactivity curves and the cell-output curves followed each other closely in the same animal.

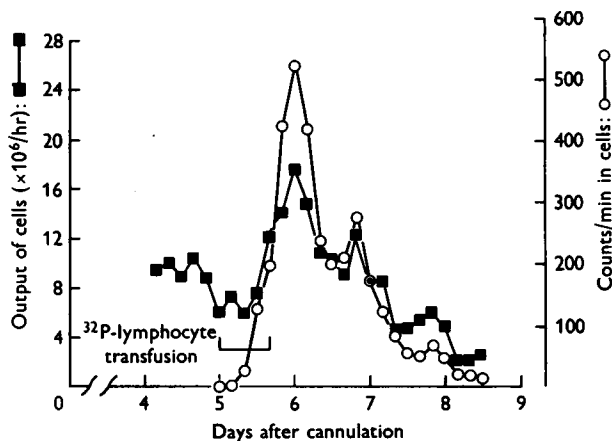
Text-fig. 7. Output and radioactivity of lymphocytes from the thoracic duct of a rat which received an intravenous infusion of ^{32}P as inorganic phosphate. Total counts/min in infused phosphate, 48,800. ■—■ = mean output of cells per hour for each successive 24 hr collection of lymph. ○—○ = total radioactivity in lymphocytes from each successive 4 hr collection of lymph.

TABLE 3. Recovery of radioactivity in lymphocytes from thoracic duct after the intravenous transfusion of ^{32}P -labelled lymphocytes

Rat no.	Period of lymph drainage before start of transfusion (days)	Number of cells transfused ($\times 10^6$)	Duration of transfusion (hr)	Duration of experiment from start of transfusion (hr)	Radioactivity recovered in cells from thoracic duct					
					Counts/min in transfused cells		Counts/min		As % of counts/min transfused	
					Total	Lipid	Total	Lipid	Total	Lipid
149	1	708	14	108	82,800	—	16,680	—	20.1	—*
153	1	444	12	92	47,400	—	10,000	—	21.0	—*
157	5	496	15	120	14,700	1,730	3,310	656	22.5	38.0†
169	5	604	16	84	18,800	1,210	3,270	456	17.4	37.7†

* Radioactive assays, on planchets; † in liquid counter.

It could be argued that the radioactivity in the thoracic duct lymphocytes was due to leakage of ^{32}P from the transfused cells and its uptake by the recipient rat's own lymphocytes. However, when the amount of radioactivity which had been transfused into a rat as ^{32}P -labelled lymphocytes (Text-fig. 6), was infused into another rat as inorganic phosphate (Text-fig. 7), the cells from the thoracic duct contained a negligible amount of radioactivity.



Text-fig. 8. Output and radioactivity of lymphocytes from the thoracic duct of a rat which received an intravenous transfusion of ^{32}P -labelled lymphocytes during the 6th day after cannulation. Mean output of cells per hour (■—■) and total radioactivity in cells (○—○) for each successive 4 hr collection of lymph.

The proportion of newly formed lymphocytes normally present in thoracic duct lymph

The idea that lymphocytes continuously recirculate from the blood to the lymph would provide a satisfactory explanation of the apparently high turnover of lymphocytes in the blood of the normal animal. This explanation would become even more plausible if it could be shown that the number of new lymphocytes formed each day was only a small fraction of the total number which normally issues from the thoracic duct.

Mann & Higgins (1950) showed that the output of lymphocytes from the intestinal lymph duct in the rat was almost equal to that from the thoracic duct. Thoracic duct lymphocytes in the rat, therefore, are gathered from the lymphatic bed drained by the intestinal duct. The lymphoid tissue in this bed comprises the mesenteric, caecal and possibly the post-gastric and portal lymph nodes together with the Peyer's patches (nomenclature of lymph nodes from Sanders & Florey, 1940). Plate I, fig. 1 shows that the volume of a 24 hr collection of lymphocytes from the thoracic duct exceeds the combined bulk of the mesenteric, caecal, portal and post-gastric lymph nodes; it would probably rival that of all the lymphoid tissue in the bed drained by the

intestinal duct. It seemed inconceivable that the observed output of lymphocytes from the thoracic duct could be produced each day by the relatively small amount of lymphoid tissues in the intestinal lymphatic bed. Accordingly, an estimate was made of the proportion of newly formed lymphocytes in the thoracic duct of a freshly cannulated rat. Tritium-labelled thymidine was infused intravenously into a rat for the first 12 hr after the cannulation of its thoracic duct. Successive 24 hr samples of lymph were collected for a total period of 4 days. The rat received no transfusion or re-infusion of lymphocytes. Autoradiographs were prepared of the cells in each sample of lymph and of histological sections of the mesenteric and caecal lymph nodes at the

TABLE 4. Proportion of tritium-labelled lymphocytes in autoradiographs prepared from successive 24 hr collections of lymphocytes from thoracic duct. Tritiated thymidine was infused intravenously at 20 $\mu\text{C/hr}$ during the first 12 hr after cannulation

Day after thoracic duct cannulation	Labelled lymphocytes*	
	Small (%)	Medium and large (%)
1	0.8	94
2	1.3	92
3	1.5	79
4	0.6	22

* Percentage derived from counts on 2500 small lymphocytes and 200 large and medium lymphocytes in each day's collection.

end of the experiment. The results are shown in Table 4. During the first 2 days, when sufficient tritiated thymidine was present to label over 90% of the large and medium lymphocytes, only about 1% of the small lymphocytes contained labelled DNA.

The very small proportion of labelled small lymphocytes detected in this experiment would still be consistent with a high rate of formation of new lymphocytes if the newly formed cells remained in the lymph nodes for some days before their release into the lymph. However, the autoradiographs of the lymph nodes taken from the rat at the end of the experiment again showed extremely few labelled small lymphocytes.

DISCUSSION

It has been shown under a variety of conditions that the transfusion of lymphocytes into the blood of a rat results in an increase in the output of lymphocytes from its thoracic duct. The increase was observed whether the transfusion was begun 1 day or 5 days after the thoracic duct of the recipient rat had been cannulated; in the latter case it was possible to estimate that the total increase in the output amounted to about 80% of the number of cells transfused. Virtually all the small lymphocytes appearing in the duct contained DNA which had been synthesized before the start of the transfusion;

the increased output, therefore, did not result from any process involving the formation of new cells by mitosis. These experiments strongly suggested that the transfused cells had recirculated from the blood back into the lymph.

When ^{32}P -labelled lymphocytes were transfused, radioactive lymphocytes appeared rapidly in the thoracic duct; about 20% of the total radioactivity and 40% of the total ^{32}P -labelled lipid in the transfused lymphocytes were recovered in the cells from the thoracic duct. This result is regarded as crucial evidence that at least some of the transfused cells had recirculated. The figures for the recovery of radioactivity in the thoracic duct lymphocytes do not conflict with the estimate that 80% of the transfused cells had recirculated. The bulk of the initial radioactivity in the transfused lymphocytes is in the non-DNA phosphorus fractions. A considerable loss of ^{32}P from these fractions would be expected to occur during the 4–5 days of the experiment. No particular emphasis is placed on the numerical recovery of 80% of the transfused cells; it is only inferred that the great majority of them could be accounted for. An improvement in the technique for transfusing lymphocytes might well allow even higher recoveries to be achieved.

These experimental findings immediately suggest that, in the intact animal, the great majority of the small lymphocytes in the thoracic duct are cells which have recirculated from the blood back into the lymph, and that this circuit is traversed repeatedly by lymphocytes during their life-time. The rapid physiological turnover of the blood lymphocytes would thus be more apparent than real, since the same cells would be continually disappearing from the blood and reappearing in it.

The existence of a recirculation of lymphocytes in the normal animal is strongly supported by the demonstration that the rate of formation of new lymphocytes does not keep pace with the rate at which lymphocytes are discharged from the thoracic duct. The experiments with tritiated thymidine showed that a very small proportion of the cells in the thoracic duct were newly formed and that no store of new small lymphocytes remained in the lymph nodes. Similarly, the daily production of new lymphocytes, as calculated from mitotic counts (Kindred, 1942) and from DNA turnover (Andreasen & Ottesen, 1945) in the intestinal lymphoid tissue of rats, amounts at the most to about 1/10 of the actual output from the thoracic duct as determined by the technique of Bollman *et al.* (1948) (author's calculation). This fraction is an upper limit, for the assumption that mitotic counts and DNA turnover are measures of new lymphocyte production requires independent proof; many cells may be produced and die within the lymph nodes without ever leaving them. If then, the output of lymphocytes from the thoracic duct is not maintained by the formation of new cells, it can only be maintained by the continuous recycling of old cells from the blood back into the lymph.

Ottesen (1954) and Hamilton (1956), from studies on the very prolonged

retention of radioactivity in the DNA of lymphocytes in human blood, have suggested that the life span of at least some lymphocytes may be considerably more than 100 days. It is not possible to make any reliable estimate of the life span of the rat lymphocyte from the present experiments, but the low rate at which new small lymphocytes are formed again suggests that it is probably very long. The long life of the lymphocyte would not, by itself, necessarily indicate that a recirculation occurs; the cells might spend most of their existence in the lymph nodes and then be released, as old cells, into the blood. But this would require both an enormous store of pre-formed lymphocytes, and a rate of new lymphocyte formation equal to the daily output from the thoracic duct. Neither of these requirements is met by the experimental data. There is a low rate of new lymphocyte formation, and the number of pre-formed lymphocytes which could be accommodated in the intestinal lymphoid tissue would be equal, at the most, to a very few days' output from the thoracic duct (Pl. 1, fig. 1).

If it is accepted that the majority of the small lymphocytes in the thoracic duct have recirculated from the blood, then the small proportion of tritium-labelled lymphocytes which was found in the lymph from the thoracic duct must also imply that no other lymph duct had contributed large numbers of newly formed lymphocytes to the blood. Certainly, no large store of pre-formed lymphocytes can be contained in the nodes whose bed is drained by the cervical and right lymph ducts (Pl. 1, fig. 1). It is reasonable to conclude therefore, that the output of lymphocytes from *all* the main lymph ducts is maintained by a recirculation of cells from the blood.

The route taken by lymphocytes in passing from the blood into the lymph is not known. Sjövall (1936) argued from circumstantial evidence that lymphocytes were normally formed at a low rate in the lymph nodes and that, in order to maintain the output of lymphocytes from the lymph ducts, they must recirculate from the blood to the lymph. He proposed that lymphocytes entered the tissue spaces and returned to the blood via the peripheral lymphatics. A slow recirculation of this kind probably does take place through most of the tissues of the body, but it is not large enough to account for more than a fraction of the thoracic duct output. Thus Yoffey & Drinker (1939) found only a few lymphocytes in the peripheral lymph from the hind limbs of dogs and cats, and Baker (1933) noted small numbers in the peripheral lymphatics of the cat intestine. Since large numbers of lymphocytes are added to lymph as it passes through a lymph node (Baker, 1933), and new lymphocytes are formed at a low rate in the nodes, it is possible that the main channel of recirculation is through the lymph nodes.

The demonstration that small lymphocytes recirculate leaves the problem of their ultimate fate unsolved. However, if they have a long life, and the number normally dying each day or being removed by any other process is

relatively small, then it is not unreasonable to suppose that they may meet a conventional end in the cells of the reticulo-endothelial system. There is, as yet, no convincing evidence that a special mechanism is reserved for the disposal of effete small lymphocytes.

The fate of the large and medium lymphocytes, which normally make up less than 5% of the cells in rat thoracic duct lymph, cannot be deduced from the present experiments. These cells are known to be capable of cell division (Gowans, 1957*a*) and in the experiments with tritiated thymidine almost all of them became heavily labelled. It is likely that the large and medium lymphocytes are newly formed in the lymph nodes, since their output on successive days after thoracic duct cannulation falls only slightly (Gowans, unpublished observations), whereas that of the small lymphocytes falls precipitously. When living preparations of lymphocytes are observed *in vitro*, size and ability to divide are the only features which distinguish the large and medium lymphocytes from the small; in all other respects their appearance and behaviour are identical (Harris, 1953; Gowans, 1957*a*). However, it has never been demonstrated that the larger lymphocytes are the precursors of the small, and it is possible that the life histories of the two groups are quite distinct.

SUMMARY

1. A method was developed for transfusing thoracic duct lymphocytes from one or more rats into the blood of an unanaesthetized rat with a thoracic duct fistula. Recipient rats were continuously transfused at a physiological rate for periods up to 7 days.

2. The low output of lymphocytes which resulted from the prolonged drainage of lymph from a thoracic duct fistula could be restored to a normal level by the intravenous transfusion of lymphocytes, providing the genetic disparity between the donor and recipient rats was not too great. Similarly, the normal high output of lymphocytes from the thoracic duct of a freshly cannulated rat could be more than doubled by a vigorous lymphocyte transfusion.

3. The increase in the output of cells from the thoracic duct which followed a transfusion of lymphocytes amounted to at least 80% of the number of cells transfused. Experiments with tritium-labelled thymidine showed that this increase in output was not due to the formation of new lymphocytes in the recipient rat.

4. The intravenous transfusion of ^{32}P -labelled lymphocytes into a rat resulted in the rapid appearance of radioactive lymphocytes in its thoracic duct lymph. It was concluded that lymphocytes which are transfused into the blood can be largely recovered in the thoracic duct lymph.

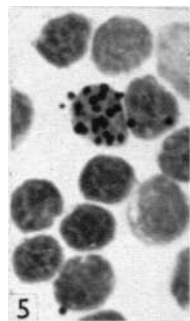
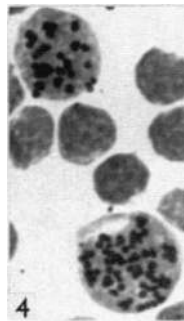
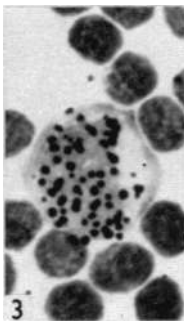
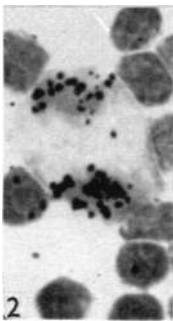
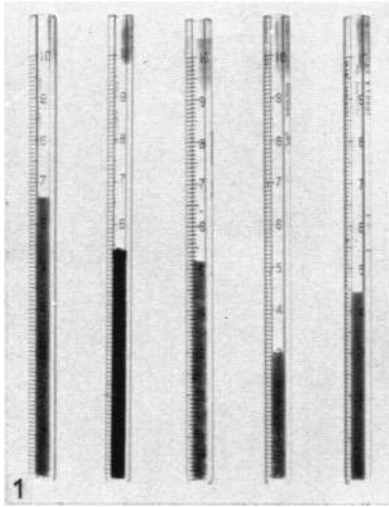
5. Further experiments with tritium-labelled thymidine showed that the number of new lymphocytes formed each day in a rat amounts to only a small fraction of the normal output of lymphocytes from the thoracic duct. It was

concluded that in the normal rat the high turnover of the blood lymphocytes is due to a continuous recirculation of lymphocytes from the blood back into the lymph.

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(Facing p. 69)

EXPLANATION OF PLATE

Fig. 1. The volume occupied by various lymphoid organs of a rat compared with the volume of a 24 hr collection of lymphocytes from the thoracic duct of another rat of the same age, weight and strain. The lymphoid organs were teased under saline and their connective tissue was included in each sample. All the tubes were then centrifuged together. From left to right: thymus; spleen; cervical, submaxillary and thoracic lymph nodes; mesenteric, caecal, portal and post-gastric lymph nodes; output of lymphocytes from the thoracic duct during the first 24 hr after cannulation.

Figs. 2-5. Autoradiographs of thoracic duct lymphocytes from rats which had received intravenous infusions of tritium-labelled thymidine ($\times 1500$).

Fig. 2. Labelled large lymphocyte in telophase.

Fig. 3. Labelled large lymphocyte.

Fig. 4. Two labelled medium lymphocytes.

Fig. 5. A heavily labelled small lymphocyte is above the centre of the photograph.