AUTORADIOGRAPHIC ANALYSIS OF LYMPHOPOIESIS AND LYMPHOCYTE MIGRATION IN MICE BEARING MULTIPLE THYMUS GRAFTS*

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It has been shown that the thymus influences the proliferation of lymphoid cells (1, 2) and antibody-forming cells (3, 4) in the body. There is evidence from thymus extract experiments and from experiments using thymus grafts in cell-impermeable millipore diffusion chambers that, in part at least, the thymus exerts this influence via a humoral factor (5, 6).

In previous studies it was shown that when more than one thymus is grafted to the same recipient animal, the weight attained by each thymus graft is the same as that attained by single grafts in normal or thymectomized recipients (7). In these earlier observations it was found that the effect of multiple thymus grafts on the lymphoid organs of the host animal was remarkably small. Such animals showed a slight lymphocytosis and a slight increase in subcutaneous lymph node weights but these increases were not as high as would have been expected from the very large mass of lymphopoietic tissue present in the animals, if significant cell migration occurred from the thymus graft tissue.

However it has been tacitly assumed by many, since the thymus exhibits intense lymphopoiesis, yet maintains a constant weight over long periods, that most lymphoid cells produced in the thymus leave the organ and seed in host tissues, particularly lymphoid tissues.

In the present experiments we have used in vivo labeling with tritiated thymidine (H^3-Tdr) to examine in more detail lymphocyte production in animals bearing multiple thymus grafts and have used this type of animal to investigate the question of whether or not lymphoid cells produced in thymus tissue migrate from this tissue and seed in host lymphoid organs.

Materials and Methods

Mice.—Mice used were male and female mice of the C57Bl strain maintained in an inbred state in this Institute. Mice were housed in metal boxes in animal rooms maintained at 75°F and were fed Barastoc dog pellets, greens, and water ad lib.

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Thymus Grafting.—Donor mice aged less than 24 hr were killed with chloroform and the thymus glands removed aseptically and immersed in a small volume of sterile normal saline. Recipient C57Bl mice aged 2 months were anesthetized with intraperitoneal nembutal and an incision made in the midline over the back. Each recipient mouse was grafted subcutaneously over the thoracic and abdominal walls with 12, 24, or 48 1-day-old C57Bl thymus glands. These grafts were placed in a random fashion along subcutaneous paths radiating outwards from the skin incision. Skin incisions were closed with skin clips.

Tritiated Thymidine Labeling.—1 month after grafting the thymus-grafted mice and 3-month-old control C57Bl mice were injected intravenously with 5 μc/g tritiated thymidine (H^3-Tdr)(14.8 or 5.0 c/mmole; Radiochemical Centre, Amersham, England) diluted in saline to give an injected volume between 0.2 and 0.3 ml. Totals of 40 thymus-grafted and 35 control mice were injected and mice of both groups were killed in parallel at intervals from 1 hr to 14 days after injection. All 1 hr time point mice were injected and killed between 10 a.m. and 12 p.m.

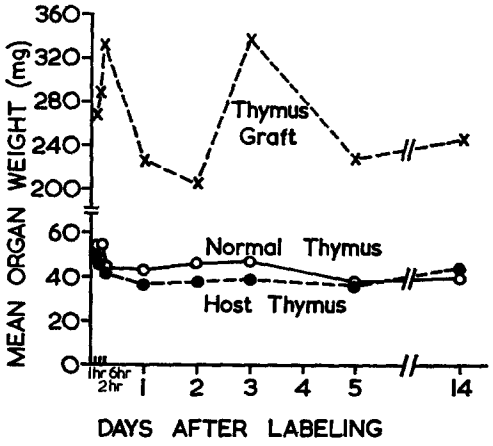
Autoradiographic Procedure.—Thymus-grafted and control mice were killed with ether and the lymphoid organs removed, dissected free of fat, and weighed on a torsion balance. Pieces of thymus, thymus graft tissue, subcutaneous lymph node, and mesenteric node were teased apart with needles in fetal calf serum. Drops of these cell suspensions were transferred to gelatin-coated microscope slides and smeared. Smears were fixed in freshly prepared methanol/acetic acid (89% methanol, Merck, A. G. Darnstadt, Germany, 1% glacial acetic acid, 10% distilled water). Kodak AR10 autoradiographic stripping film (Kodak Ltd., London, England) was floated on, and the slides stored at 4°C in light tight boxes with copper sulphate as dessicant. Multiple smears were made of each tissue examined. At intervals, smears were developed and stained with Giemsa in phosphate citrate–buffered saline pH 5.75. Exposure times of the smears were varied from 3 to 200 days, according to the individual requirements of each time point after labeling. For every time point, smears were exposed until the uncorrected mean grain count of the cells had reached 10 to 20 gr per cell. Where different cell types in the same smear had widely different grain counts, duplicate smears were exposed for different periods to give grain counts within the above limits for each cell type. In addition to the grain count smears a second set of smears was exposed for three times the exposure time required for satisfactory grain counting on the most lightly labeled cell type in the thymic smears from each animal. This latter set of autoradiographic smears was used to establish percentage labeling data for the various cell types in the different organ smears. This procedure was followed, using thymic cell smears as the reference point, because thymic cells were more lightly labeled than lymph node cells and the primary purpose of the investigation was to detect the possible presence of migrant cells from the thymus in the other lymphoid tissues.

Cell Typing.—Lymphoid cells from the various lymphoid organs examined were typed into three categories; large, medium, and small (Figs. 1, 2, and 4). Typing was based on mean cell diameters, each cell being measured on an ocular grid as the smear was scanned. Size categories for total cell diameters were: large, greater than 11 μ; medium, 7 to 11 μ; and small less than 7 μ. In addition to cell size, account was taken also of general morphology, nuclear/cytoplasmic ratios, and cytoplasmic basophilia. In this analysis of cell populations from the various lymphoid organs, plasma cells and reticulum cells were excluded from the cell typing. The data to follow therefore refer only to percentages of the total lymphoid population present.

Analysis Procedure.—Each set of cell smears from the various lymphoid organs was subjected to the following analysis; the percentage distribution of cell types and the overall percentage labeling (of all lymphoid cells combined) were each determined by counts on 2000 consecutive cells; the percentage labeling of large and medium lymphocytes was determined by counts on 200 consecutive cells of each type; and the percentage labeling of small lympho-

cytes on 500 cells. Mean grain counts were determined by counts on 50 cells of each type. Cells were scored as labeled if they had 3 or more grains over the nucleus. Background grain counts in these preparations were less than 0.1 gr per 100 μ^2. For analysis, all grain counts were corrected to a 10 μc/g dose and a 20 day exposure time. In all cases, data from male and female animals were similar and the results were pooled.

Histological Analysis.—Pieces of the various lymphoid organs from the thymus-grafted and control mice were fixed in 10% formalin. In addition, sections were also taken of host liver, kidney, and gut. These sections were dehydrated, embedded in paraffin, and sectioned at 5 μ. Sections were placed on gelatin-coated slides, layered with AR10 stripping film, and exposed for autoradiographic analysis using a routine exposure time of 3 months. When developed, these autoradiographic sections were stained with Unna Pappenheim.



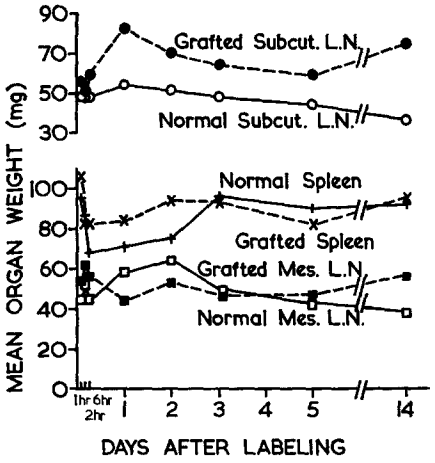
TEXT-FIG. 1. Total thymus graft and host thymus weights in multiple thymus-grafted mice.

RESULTS

General Results.—Text-figs. 1 and 2 show the weights of thymus grafts and host lymphoid organs in the animals used in the present study. There was some variation between individual animals but the mean total weight of the thymus graft tissue in mice with 12 thymus grafts varied between 210 and 350 mg. In animals grafted with 24 thymus grafts the mean total thymus graft weight was 609 mg and in animals grafted with 48 thymus grafts the mean thymus weight was 1160 mg.

It will be noted that the presence of this large amount of thymus graft tissue had no effect on the host thymus weight or on host spleen and mesenteric node weights. In agreement with previous observations (7), the total weight of the six subcutaneous lymph nodes in thymus-grafted mice was slightly (20 to 40%) heavier than that of subcutaneous lymph nodes in control mice. It was found however that the thymus grafts had no significant effect on the percentage distribution of large and medium lymphocytes in either the host subcutaneous or

mesenteric lymph nodes (Table I). Absolute blood lymphocyte levels were not determined in thymus-grafted or control mice but a study of the blood films from these mice revealed no obvious change from control mice in the total



TEXT-FIG. 2. Effect of multiple thymus grafts on host lymphoid organ weights.

TABLE I

Effect of Thymus Grafts in C57Bl Mice on Host Mesenteric and Subcutaneous Lymph Node Weights and Cytology

Organ	Type of mouse	No. of mice	Mean organ weight	Mean cell distribution		
				Large lymphocyte	Medium lymphocyte	Small lymphocyte
			mg	%	%	%
Mesenteric lymph node	Normal	35	48±13*	0.8±0.3	3.8±1.2	95.4±1.3
	Thymus-grafted	36	51±14	0.8±0.4	3.6±1.3	95.6±1.5
Subcutaneous lymph node	Normal	35	47±9	0.5±0.2	2.8±1.0	96.7±1.2
	Thymus-grafted	36	62±16	0.4±0.2	2.6±1.3	97.0±1.5

* Standard deviation.

number of lymphocytes or in the frequency of medium and small lymphocytes in the blood.

The dose of H^3-Tdr used in the present experiments (5μc/g) was higher than that used by most workers (1 μc/g) and theoretically exposed the labeled cells to what might have been a significant degree of radiation damage. This question was closely investigated in these mice and in a similar study in AKR mice

reported previously (8). It may be seen from Text-figs. 1 and 2 that there appeared to be a slight weight loss in the thymus and the spleen in the first 6 hr following labeling but this weight loss was minor in degree and was not progressive with time. In view of the small number of mice at each time point

TABLE II

Percentage of Various Types of Lymphoid Cell in Thymus and Thymus Tissue in C57Bl Mice at Various Intervals after Labeling with H^3-Tdr

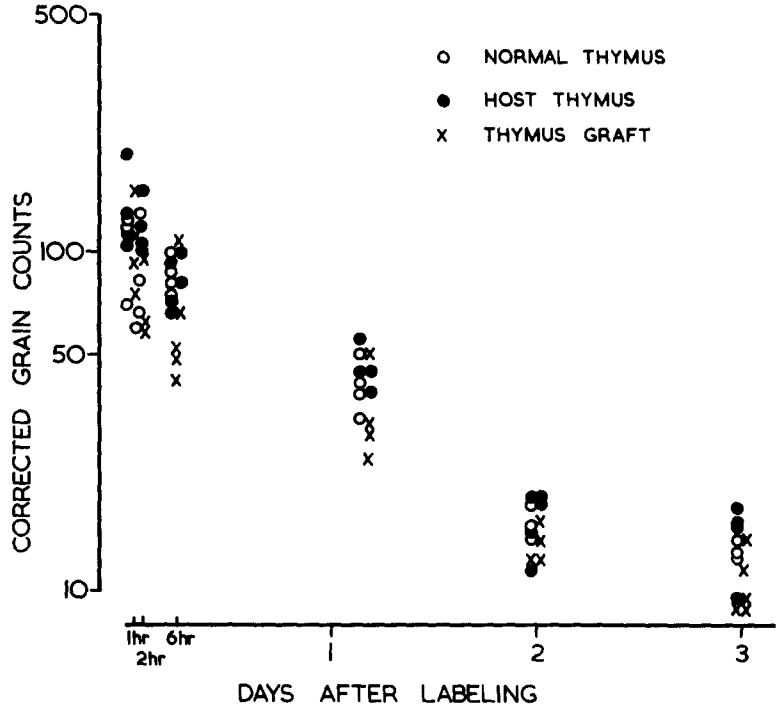
Organ	Type of mouse	Time after labeling	No. of mice	Mean weight of thymus and grafts	Mean cell distribution		
					Large lymphocyte	Medium lymphocyte	Small lymphocyte
				mg	%	%	%
Thymus	Normal	1 hr	4	54±5*	1.5±0.5	7.9±1.9	90.6±2.4
	Thymus-grafted		4	50±7	1.4±0.4	8.3±1.8	90.3±1.9
	Normal	3 days	4	47±14	1.3±0.3	8.5±1.8	90.3±1.6
	Thymus-grafted		5	39±3	1.2±0.2	7.0±0.4	91.8±0.3
	Normal	5 days	6	38±6	1.3±0.3	7.7±0.4	91.1±0.3
	Thymus-grafted		6	37±6	1.5±0.6	7.2±1.9	91.4±2.2
	Normal	14 days	2	40±17	1.3±0.0	7.4±0.6	91.3±0.6
	Thymus-grafted		3	43±8	1.5±0.2	7.1±0.1	91.5±0.6
Thymus graft	Thymus grafted	1 hr	4	268±118	1.5±0.3	8.3±1.4	90.2±1.7
	Thymus-grafted	3 days	5	339±100	1.2±0.3	8.0±1.0	90.8±0.9
	Thymus-grafted	5 days	6	227±23	1.5±0.8	9.0±2.0	89.5±2.8
	Thymus-grafted	14 days	3	244±39	1.3±0.8	7.0±1.2	91.8±2.0

* ± Standard deviation.

(4–6) this initial apparent weight loss may even have been due to individual variation in the normal organ weights of the mice being sampled. An analysis of the percentage distribution of large, medium, and small lymphocytes in the various lymphoid organs at intervals after labeling with H^3-Tdr (Table II) gave

no indication of any significant shift in the percentage distribution of these cell types with increasing time after labeling.

These findings reinforce previous data from AKR mice labeled with 5 μc/g H^3-Tdr and have led us to conclude that whilst a minor degree of radiation damage to lymphoid cells may have occurred because of the high dose of H^3-



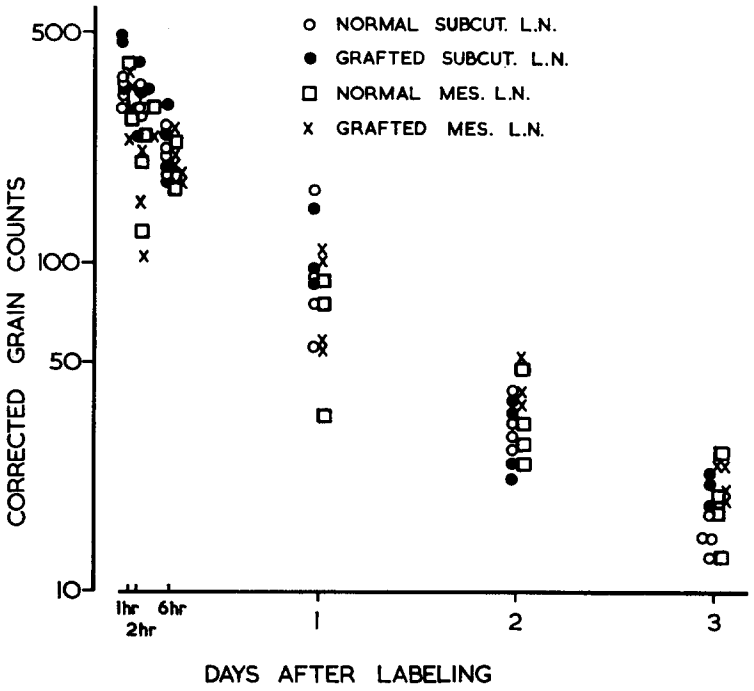
TEXT-FIG. 3. Fall with time in corrected mean grain counts of medium lymphocytes in thymus graft, host thymus, and control thymus tissue (Counts corrected to 10 μc/g dose and 20 days exposure time).

Tdr used, such damage was not sufficiently great to interfere with the interpretation of the data obtained.

Indeed we have found the high dose to be necessary at the longer time intervals after labeling to ensure the development of grains over all labeled cells, even when exposure times as long as 150 to 200 days were used. When control autoradiographs were prepared using 1 μc/g dosage, and the routine 30 days exposure time employed by other workers, it was obvious that not all labeled cells could be detected.

Thymus Graft Lymphopoiesis.—Lymphopoiesis in thymus graft tissue was compared with that in the host thymus and control thymus tissue by analyzing

the fall with time in mean grain counts of large, medium, mitotic, and small lymphocytes. In all three types of thymus tissue, 1 hr grain counts were similar (Text-fig. 3) although thymus graft cells tended to have a slightly lower mean grain count than cells from host or control thymus tissue. The grain counts at 1 hr, of thymus graft lymphoid cells were significantly lower than those of the



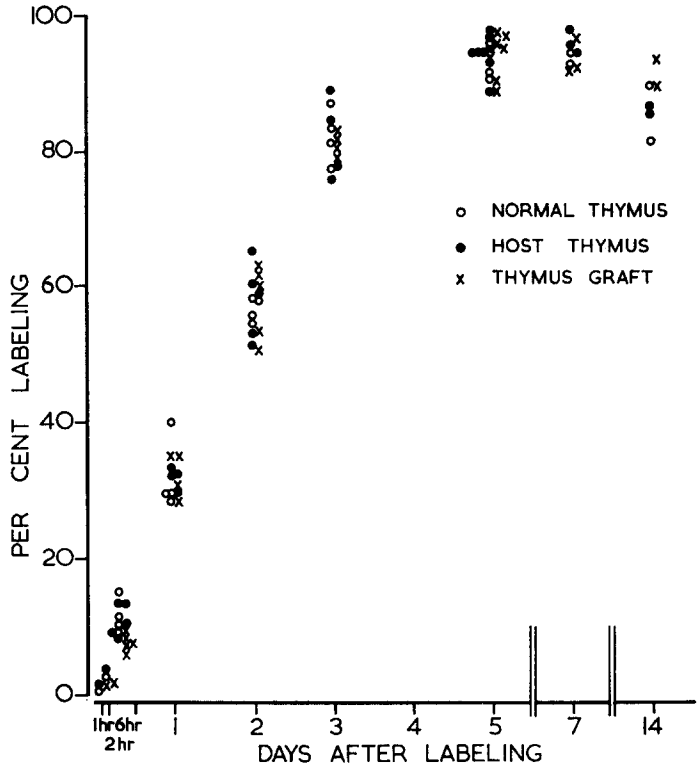
TEXT-FIG. 4. Fall with time in corrected mean grain counts of medium lymphocytes in subcutaneous and mesenteric lymph nodes of thymus-grafted and control mice (Counts corrected to 10 μc/g dose and 20 days exposure time).

corresponding large and medium lymphocytes in the subcutaneous or mesenteric lymph nodes (Text-fig. 4).

The rates of fall in the mean grain counts of the large and medium lymphoid cells were identical in all three types of thymus tissue. As an example of this similarity, in Text-fig. 3, the data are plotted for the corrected grain counts of medium lymphocytes, from the three types of thymus tissue.

These mice were not studied in sufficient detail to establish cell cycle times for large and medium lymphocytes but the data from the time points examined are similar to more complete data obtained previously from normal 2-month-old AKR mice (8) in which it was established that thymus large lymphocytes divide every 6 hr and medium lymphocytes every 8 hr.

In Text-fig. 5 are plotted the data obtained on the accumulation of labeled small lymphocytes in thymus graft tissue compared with the accumulation in host thymus and in control thymus tissue. It is quite clear that during the first 3 days after labeling, the accumulation of labeled small lymphocytes was linear and that the rates of accumulation of labeled cells were identical in the



TEXT-FIG. 5. Rise with time in percentage labeled small lymphocytes in thymus graft, host thymus, and control thymus tissue.

three types of thymus tissue. It should be noted that by day 3 after labeling, between 80 and 90% of thymus and thymus graft small lymphocytes were labeled (Fig. 3), indicating a mean intrathymic life span of 3 to 4 days for most thymus small lymphocytes in C57Bl mice. However the percentage labeling of thymic small lymphocytes still had not reached 100% by day 5 after labeling, which suggests that there may be a small population (possibly 5%) of thymic small lymphocytes which have a considerably longer intrathymic life span. The autoradiographs of C57Bl thymus sections at days 3 and 5 after labeling suggested that these long lived lymphocytes may be located in the thymus

TABLE III

Percentage Cell Distribution and Labeling of Lymphoid Cells in Thymus, Thymus Graft, Mesenteric, and Subcutaneous Lymph Node Tissue, and Blood in C57Bl Mice 1 Hr after Injection of H^3 Tdr

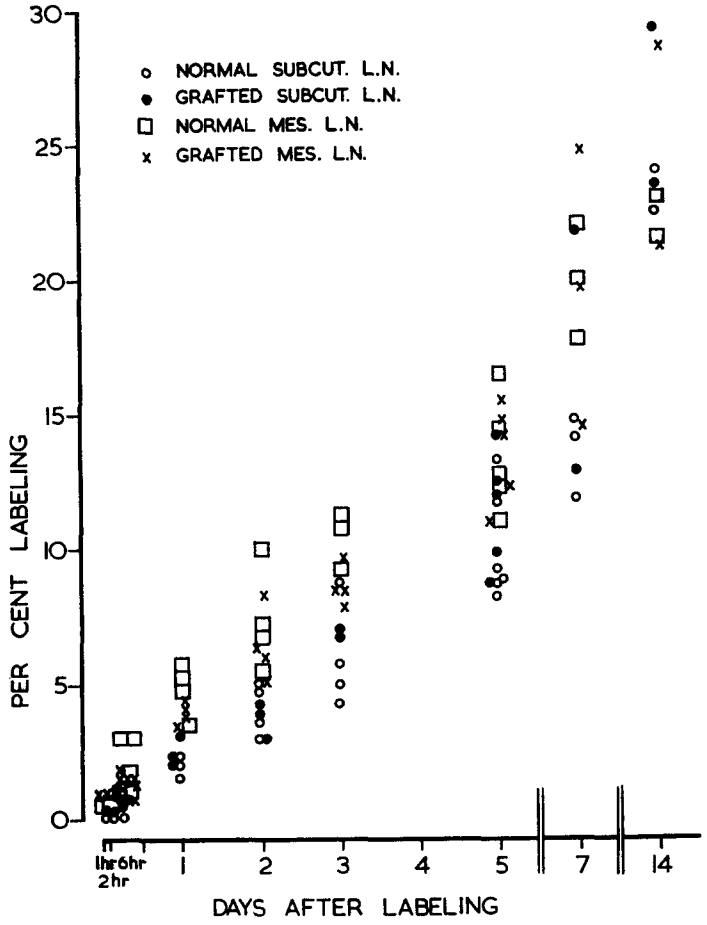
Organ	Type of mouse	No. of mice	Mean cell distribution			Mean labeling in			
			Large lymphocyte	Medium lymphocyte	Small lymphocyte	Total cells*	Large lymphocyte	Medium lymphocyte	Small lymphocyte
			%	%	%	%	%	%	%
Host thymus	Normal	4	1.5±0.5‡	7.9±1.9	90.6±2.4	11.5±1.7	87.8±1.4	69.4±8.2	0.9±0.2
	Thymus-grafted	4	1.4±0.4	8.3±1.8	90.3±1.9	10.7±1.4	86.7±3.9	71.6±6.1	1.1±0.3
Thymus graft	Thymus-grafted	4	1.5±0.3	8.3±1.4	90.2±1.7	10.6±0.9	86.3±2.4	71.4±5.9	1.1±0.2
Mesenteric lymph node	Normal	4	0.8±0.2	3.3±0.6	95.9±0.7	3.0±0.6	79.1±6.7	54.1±24.5	0.4±0.2
	Thymus-grafted	4	0.9±0.2	3.9±1.0	95.3±1.1	3.4±0.6	80.3±6.5	42.4±4.8	0.3±0.3
Subcutaneous lymph node	Normal	4	0.4±0.2	2.9±0.6	96.8±0.5	1.9±0.8	80.8±5.9	39.6±5.8	0.1±0.1
	Thymus-grafted	3	0.6±0.5	3.1±1.5	96.3±2.0	1.9±1.0	79.6±5.0	32.7±12.7	0.3±0.3
Blood	Normal	4							0.3±0.3§
	Thymus-grafted	4							0.2±0.2§

* Including reticulum cells, immature plasma cells, and monocytes but excluding mature plasma cells.

‡ Standard deviation.

§ All blood lymphocytes classified together as "small lymphocytes" but this includes a small number of medium lymphocytes.

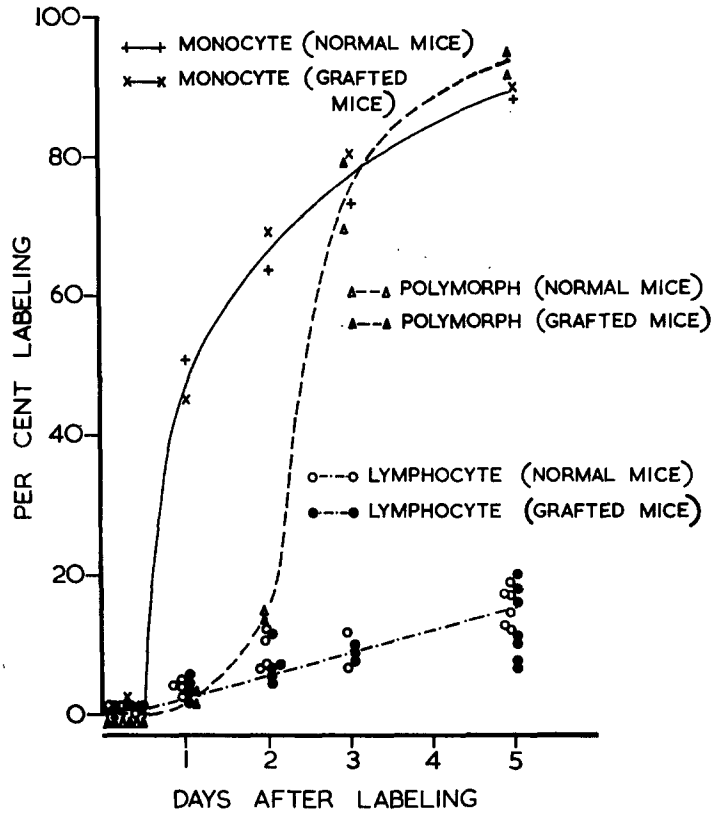
medulla. At 3 and 5 days after labeling, virtually all lymphoid cells in the thymus cortex were labeled but many unlabeled small lymphocytes were seen in the medulla.



TEXT-FIG. 6. Rise with time in percentage labeled small lymphocytes in subcutaneous and mesenteric lymph nodes in thymus-grafted and control mice.

Lymph Node and Blood Lymphocyte Kinetics in Thymus-Grafted Mice.— In Table III are listed the percentages of various lymph node lymphoid cells observed to incorporate tritiated thymidine directly (1 hr uptake data). Approximately 80% of lymph node large lymphocytes incorporated H^3-Tdr in both mesenteric and subcutaneous lymph nodes. However only 50% of mesenteric node and only 35% of subcutaneous lymph node medium lymphocytes

incorporated isotope. These percentage uptake data for lymph node medium lymphocytes were significantly lower than for thymic medium lymphocytes and suggest that medium lymphocytes divide more slowly in lymph nodes (particularly in the subcutaneous lymph nodes) than in the thymus.

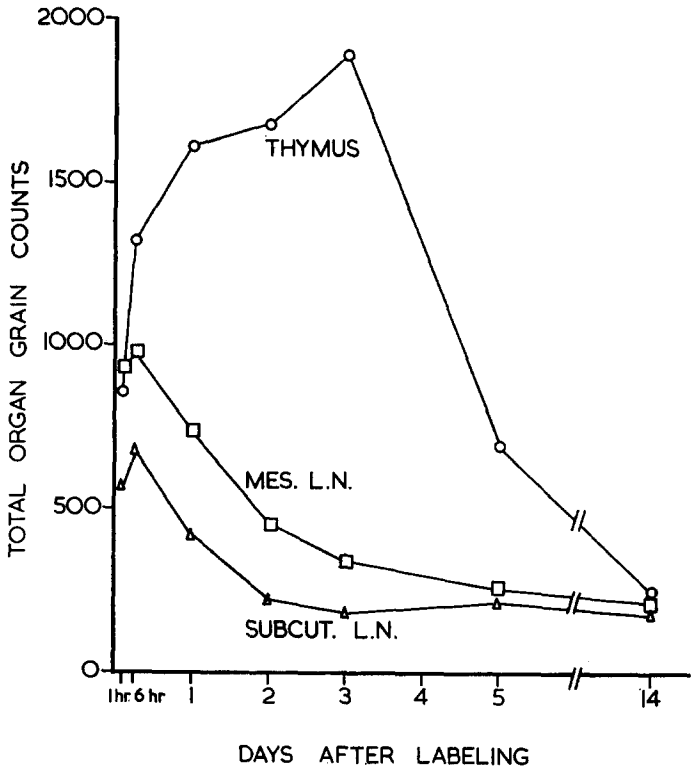


TEXT-FIG. 7. Rise with time in percentage labeled blood lymphocytes and polymorphs and monocytes in thymus-grafted and control mice.

It will be seen from Table III that the presence of thymus grafts did not affect the percentage of either large or medium lymphocytes in the lymph nodes which incorporated isotope directly. The presence of thymus grafts also did not affect the minute percentage of the lymph node small lymphocytes which appeared capable of direct H^3-Tdr incorporation. A similar lack of effect of thymus grafting on lymphocyte uptake of H^3-Tdr was observed when blood lymphocyte labeling patterns were studied.

An analysis of the fall in mean grain counts with time in all lymph node lymphoid cells failed to reveal any difference in the rate of fall of grain counts

in lymph nodes from thymus-grafted animals when compared with lymph nodes from control animals. An example of this is shown in Text-fig. 4, which depicts the fall in grain count in medium lymphocytes in the subcutaneous and mesenteric lymph nodes from both types of animal.

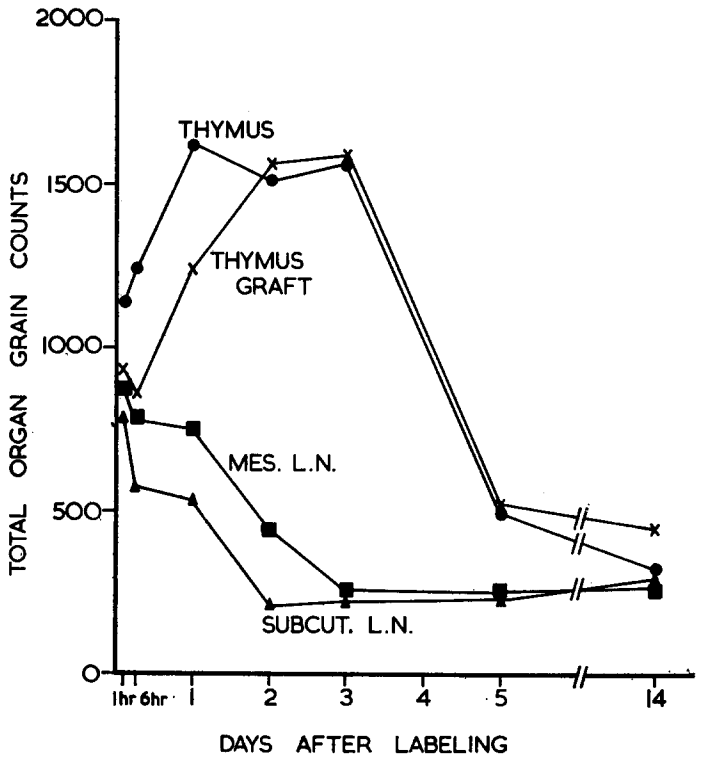


TEXT-FIG. 8. Fall with time in total organ grain counts in the thymus, mesenteric, and subcutaneous lymph nodes in control C57Bl mice.

When the accumulation with time of labeled small lymphocytes was studied in the lymph nodes and blood, it was observed, as for thymus tissue, that labeled cells accumulated in a linear fashion when plotted arithmetically against time. The rate of accumulation of labeled cells was not significantly different in the mesenteric and subcutaneous lymph nodes and the rates in lymph node tissue from thymus-grafted animals were not significantly different from those in control animals, even as late as 14 days after labeling (Fig. 4) (Text-fig. 6). Included in the data for day 5 in Text-fig. 6 are results from four animals carrying 24 and 48 thymus grafts each.

In the blood a similar situation was found to that in the lymph nodes.

Again the accumulation of labeled cells was linear and no difference was observed between thymus-grafted and control mice (Text-fig. 7). It was also observed that blood polymorph and monocyte labeling patterns were identical in both types of animal.



TEXT-FIG. 9. Fall with time in total organ grain counts in thymus grafts, host thymus and host mesenteric and subcutaneous lymph nodes in multiple thymus-grafted C57Bl mice.

Total Organ Grain Counts.—It was reported by Craddock et al. (9), who labeled rats with H^3-Tdr, that the initial level of H^3-DNA attained by the thymus remained constant for 3 days after labeling before commencing to fall. In contrast the total lymph node content of H^3-DNA commenced falling immediately after initial labeling. In the present experiments, estimates were made of total organ content of H^3-Tdr (presumably incorporated in high molecular weight molecules) at various intervals after labeling using the following method; total organ grain count = sum of total grain counts of the various lymphoid cell types in the organ (the percentages of these cell types and the size of the organ were assumed to remain constant throughout the observation period,

as was in fact indicated by the data). The total grain counts for each cell type were computed by multiplying the percentage of the cell type × the percentage labeling × the corrected mean grain count (corrected to 10 μc/g dose and 20 days exposure time). The figures obtained were only relative and gave no estimate of the absolute amount of H^3-Tdr in the organs but they did allow a comparison of the fate of total organ label between different organs. The data obtained are shown in Text-figs. 8 and 9. In confirmation of the results of Craddock et al. (9), in the normal thymus H^3-Tdr levels did not fall in the first 3 days after labeling. In fact there may have been a slight rise in organ H^3-Tdr over this period. The host thymus of thymus-grafted animals showed a similar pattern as did thymus graft tissue. After 3 days, total H^3-Tdr content in all three types of thymus tissue fell sharply.

In the lymph nodes, there was an immediate fall in organ H^3-Tdr content after initial labeling, and this fall appeared to be identical in lymph nodes from thymus-grafted and control animals.

DISCUSSION

The present autoradiographic data indicate that thymus graft lymphopoiesis is identical with that of the normal thymus. In all parameters studied, percentage of primitive lymphoid cells, percentage of various lymphoid cells taking up isotope, grain count fall, rate of appearance of labeled small lymphocytes and total organ H^3-Tdr content, no differences were observed between the two types of thymus tissue. The present data indicate that, despite its subcutaneous location, thymus graft tissue differs sharply from lymph node tissue in all aspects of lymphopoiesis.

The thymus has been demonstrated to regulate lymphopoiesis in the nonthymic lymphoid organs via a humoral factor (6), and thymus extracts, at least in baby mice (5) and thymectomized irradiated adult mice (11), have been demonstrated to influence blood lymphocyte levels. However the present data on multiple thymus-grafted animals have confirmed earlier observations (7, 10) that, apart from slightly increasing the weights of regional lymph nodes of the host, even massive amounts of thymus graft tissue in normal adult animals have no other observable effects on lymphopoiesis in host lymphoid organs.

The present experiments were initiated primarily to determine whether or not lymphocytes migrate from thymus tissue to nonthymic lymphoid organs. Numerous reports have been made on the fate of intravenously injected suspensions of thymic lymphoid cells (12). However such experiments are only suggestive and do not prove that lymphoid cells normally leave the thymus. More recently Nossal (13), and Murray and Woods (14) have reported studies in guinea pigs on the fate of thymic lymphoid cells, labeled by direct intraorgan injection of H^3-Tdr. Both groups reported that some heavily labeled lymphoid cells, presumably thymic in origin, were subsequently to be found in the lymph nodes of the animal. However the small number of migrant cells observed by

these authors suggests that very few of the lymphoid cells produced in the thymus actually migrate from the organ. Other studies using chromosome markers have indicated that when thymus grafts are placed in neonatally thymectomized animals, mitotic cells of thymus graft origin can be demonstrated in the lymph nodes and spleen of the host animal (4, 15, 16). However the techniques used do not establish the time at which such migration occurs. At grafting, thymus tissue is mechanically disrupted, and immediately following grafting there is extensive anoxic death of much thymus tissue and a probable disruption of thymic architecture (17). At this time lymphoid cells may be free to escape from the thymus tissue and seed and proliferate in host lymphoid organs, particularly if the host is in a lymphocyte depleted state. However, once the thymus graft tissue has regenerated and the normal architecture of epithelial and reticulum cells has been restored, such migration may no longer occur.

In the present experiments animals were grafted with multiple thymus grafts and at the time of testing (when thymus graft architecture was fully restored) up to 1100 mg of thymus graft tissue was present in animals whose total host lymphoid organ mass was approximately 250 mg. As indicated by the H^3-Tdr labeling data, by day 3 after labeling up to 90% of thymus graft lymphoid cells were labeled whereas at the same time only 10% of lymph node lymphocytes and blood lymphocytes were labeled. The data suggest that the population turnover time of small lymphocytes in thymus graft and normal thymus tissue in these mice approximates 3 to 4 days. Thus in the 3 to 4 day period following the first 3 days after labeling it is necessary for the existing population of labeled thymic cells either to migrate from the organ, or to die in the organ, to make way for the next generation of cells produced in the next thymic cell cycle. If this is achieved solely by cell migration from the thymus then in these multiple thymus-grafted animals approximately 1000 mg of labeled thymic lymphocytes must migrate from the thymus graft tissue in the interval between days 3 and 8 after labeling. If these labeled lymphocytes seed in the host lymphoid organs (totalling less than 250 mg in weight) then it can be calculated what increase in the total percentage labeling of the population of such lymph nodes will result from such migration. In the present studies, with the variation encountered in individual mice, it appeared that an increase in percentage of labeled cells in the lymph nodes of as little as 2% could readily have been detected but no such increase was detected. If it is assumed that a very small increase had occurred but was not detectable (the upper limit of this increase being less than 2%) it can be calculated that fewer than 0.5% of cells produced in the host thymus and thymus graft tissue could have seeded in the host lymphoid organs over the time intervals studied.

Histological examination of autoradiographs of the kidney, bowel, and lung from thymus-grafted mice revealed no evidence of the accumulation of unusual numbers of labeled lymphocytes in these organs. It seems doubtful therefore

whether massive numbers of labeled thymic lymphocytes were being eliminated from the host animals via these normal routes of elimination of lymphoid cells. The present data have led us to conclude that whilst some cell migration may be possible from the thymus and thymus graft tissue, such migration is the fate of only an extremely small percentage of the total number of thymic cells produced in the organ.

It may be argued that the body has a mechanism for preventing the entry into the animal of unwanted excessive numbers of thymic lymphocytes from thymus graft tissue. Such a mechanism is quite conceivable but it would demand the existence of an effective mechanism for destroying such unwanted excess thymic lymphocytes *in situ* in the thymus graft tissue. As was seen in the data no differences whatsoever were observed between thymic-labeling patterns in thymus graft tissue and normal thymus tissue. If such a mechanism exists for destroying thymic lymphoid cells *in situ* in thymus grafts, and yet results in no deviation in labeling patterns from those of the normal thymus, then it seems likely that a similar process of massive destruction of thymic small lymphocytes occurs normally in the normal thymus.

It should not be concluded from these comments that such cell migration as may occur from the thymus is necessarily of little importance for lymphocyte homeostasis in the host lymphoid organs. It is quite conceivable, even if only a small number of thymic cells migrates from the thymus to the lymph nodes and spleen, that the population size of these migrants could quickly be built up to form a sizeable fraction of the population of these organs particularly if they are stimulated specifically by antigens or they are more long lived than lymph node lymphoid cells.

The present data leave as an unresolved problem the purpose of the major portion of the extensive lymphopoiesis occurring in the thymus, for if the present conclusions are correct, very few of these cells ever leave the organ and yet there is little morphological evidence of them undergoing any functional activity within the organ itself. It may be that the excessive lymphopoiesis represents reserve function of the thymus, or it may be as proposed by Burnet (18) that excessive numbers of lymphocyte mitoses need to occur in the thymus in order to generate on a random basis lymphocytes with all the needed antigenic reactivity patterns. Alternatively, lymphocyte breakdown products derived from the lysis of thymic lymphocytes may be a valuable source of building blocks for other tissues in the body (19).

SUMMARY

Lymphopoiesis was studied in 3-month-old normal C57Bl mice and in 3-month-old C57Bl mice carrying from 12 to 48 C57Bl thymus grafts using tritiated thymidine labeling.

Thymus graft lymphopoiesis was found to be identical with that of normal

thymus tissue and the presence of thymus grafts was found to have no influence on host thymus lymphopoiesis.

No evidence was found that the massive amounts of thymus graft tissue in the mice affected any parameter of host lymph node lymphopoiesis nor was any evidence detected for the migration of thymic lymphocytes from these massive deposits of thymus graft tissue either to host lymph nodes and blood or to other organs in the host animal.

It is concluded that the majority of small lymphocytes produced in the thymus and thymus graft tissue do not migrate from these tissues but die locally at the end of their intrathymic life span of 3 to 4 days.

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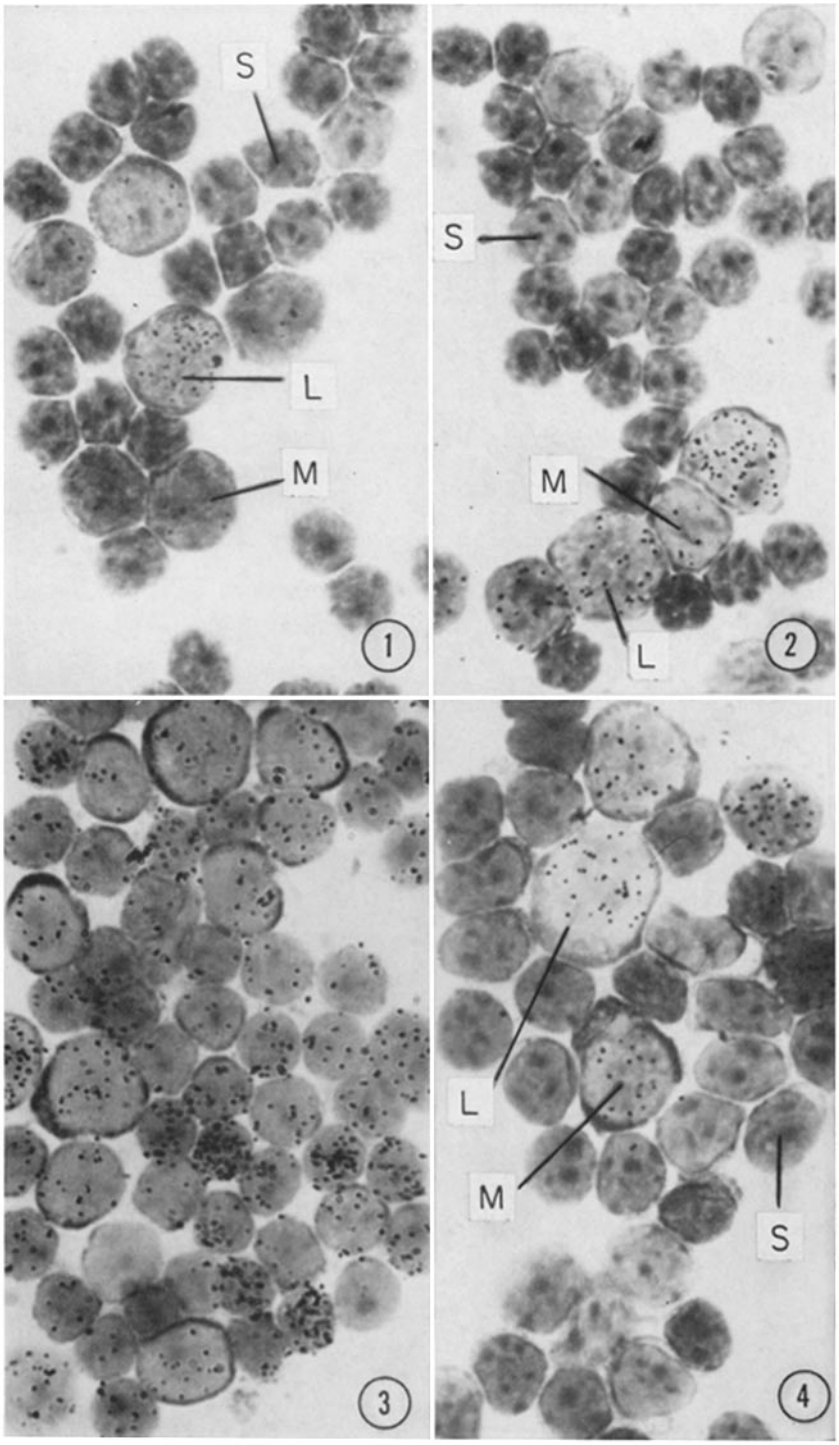
EXPLANATION OF PLATE 70

FIG. 1. Autoradiograph of thymus cell smear from a control C57Bl mouse 1 hr after labeling with H^3-Tdr. Note large (*L*), medium (*M*), and small (*S*) lymphocytes. Exposure time 13 days. Giemsa, × 1300.

FIG. 2. Autoradiograph of thymus graft cell smear 1 hr after labeling with H^3-Tdr. Note large (*L*), medium (*M*), and small (*S*) lymphocytes and the similarity of cell morphology to that of normal thymus. Exposure time 19 days. Giemsa, × 1300.

FIG. 3. Autoradiograph of thymus graft cell smear 3 days after labeling with H^3-Tdr. Note labeling of majority of small lymphocytes in preparation. Exposure time 44 days. Giemsa, × 1300.

FIG. 4. Autoradiograph of subcutaneous lymph node cell smear from an animal carrying multiple thymus grafts 3 days after labeling with H^3-Tdr. Note large (*L*), medium (*M*), and small (*S*) lymphocytes and the fact that few of the small lymphocytes are labeled. Exposure time 44 days. Giemsa, × 1300.



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