Mechanisms of Leukocyte Production and Release
IX. Kinetics of Leukocyte Release in Leukocytapheresed Rats* (33970)

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Previous studies have clearly demonstrated the ability of laboratory animals to mobilize large numbers of leukocytes into the peritoneal cavity following intraperitoneal injections of bacterial pyrogen (1, 2). This leukocytapheresis (LAP) procedure, permitting extensive removal of leukocytes from the body, has proved a useful method of investigating problems of leukocyte release and its humoral regulation by the leukocytosis-inducing factor, LIF (2–8). Although considerable data are available on the *in vivo* effects of the LIF, there is little information regarding leukocyte production and release in the LAP animal which produces this factor. Therefore, the present study was undertaken to compare leukocyte release in normal rats, to release in rats subjected to LAP, using standard techniques of tritiated thymidine autoradiography (9).

Methods. Tritiated thymidine (^{3}HT)[2] was administered intraperitoneally (1 μCi/g of body wt) to prelabel 40 normal male rats (200–250 g) of a modified Long-Evans strain. Peripheral blood samples for differential leukocyte counts and autoradiography were taken prior to isotope administration and at 24-hr intervals for 5 days thereafter. Following the 24-hr sampling, 25 of these rats ("LAP rats") were subjected to the first of a series of peritoneal lavages (2, 8) performed every 8 hr for 4 days in order to mobilize and then to remove approximately 1 billion leukocytes.

All blood smears were fixed in methanol, dipped in Kodak NTB-3 nuclear track emulsion, exposed 14 days (in lightproof boxes at 5°) and developed in Kodak D-19. The smears were then stained with Giemsa according to a modification of the technique of Gude *et al.* (10, 11). Differential labeling indices were determined by counting 500 consecutive cells/slide. Smears made from blood of uninjected rats were processed together with experimental preparations for background correction. Only rarely were grains found overlying cells in these control autoradiograms, however, an arbitrary labeling threshold of 3 or more grains was used. All slides for a given experiment were processed simultaneously so that autoradiographic and staining techniques were identical for both experimental and control rats.

Results. No significant alterations (Table I) in the total WBC counts of normal and LAP animals were noted during the 5-day period following isotope injection. In Table I the emergence times for labeled neutrophils into the peripheral blood, presumably from the marrow, are also given for normal and LAP rats. The emergence time is defined (12) as the interval between injection of ^{3}HT and the first appearance of any labeled peripheral granulocytes. The first labeled neutrophilic granulocytes ($\cong$3.5%) appeared in the blood of normal animals at the 24-hr sampling interval after isotope administration. An increase in the neutrophil labeling index occurred on day 2, attaining an average value of 12.5% with peak neutrophil labeling ($\cong$50–60%) by 3–4 days after injection. Examination of the data further revealed a significant shortening in the emergence time for neutrophils of LAP animals. Maximal neutrophil labeling indices, although similar to those for normal rats, were obtained about

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[2] Schwarz Bio Research, Inc. (sp. act 6.6 Ci/m mole).

TABLE I. Neutrophil (PMN) Cell Counts and Labeling in Normal and LAP Rats Following Administration of Tritiated Thymidine (^{3}HT).

Group	After ^{3}HT (days)	WBC/mm^3 ($\times 10^{-3}$)	PMN/mm^3 ($\times 10^{-3}$)	PMN labeling index (%)	Absolute PMN labeled ($\times 10^{-3}$)
Normal (40)[a]	0	19.3 ± 1.4[b]	5.8 ± 0.7		
Normal[c] (40)	1	17.7 ± 2.8	5.9 ± 0.5	3.5 ± 0.9	0.18 ± 0.1
LAP[c] (25)	2	18.9 ± 1.1	6.6 ± 1.5	58.8 ± 3.3[d]	3.9 ± 0.4[d]
Normal (15)	2	18.4 ± 1.4	3.4 ± 0.5	12.5 ± 2.1	0.4 ± 0.1
LAP	3	20.1 ± 0.9	6.2 ± 0.5	56.9 ± 3.2	3.7 ± 0.4
Normal	3	20.6 ± 1.0	5.0 ± 0.4	49.9 ± 2.8	2.6 ± 0.3
LAP	4	18.1 ± 2.7	7.7 ± 1.2	47.4 ± 3.9[d]	3.8 ± 0.8
Normal	4	20.9 ± 1.1	4.3 ± 0.5	60.1 ± 4.5	2.5 ± 0.3
LAP	5	20.0 ± 1.1	7.8 ± 1.0	32.5 ± 4.6	2.6 ± 0.5
Normal	5	21.4 ± 1.7	4.6 ± 1.0	39.1 ± 5.6	1.9 ± 0.1

[a] Number of rats given in parentheses.

[b] Mean ± standard error of mean.

[c] LAP procedure initiated on 25 of original 40 normal rats after day 1 blood samples were taken.

[d] ≤ 0.05 when compared to normal rats.

2 days earlier. By day 5, values for both groups had decreased to approximately the same level.

The absolute numbers of labeled neutrophils appearing in the peripheral blood of LAP and normal rats are also given in Table I. In general, these data conform with those obtained for labeling indices. Large numbers of labeled neutrophils ($\cong$4000/mm^3) were present in LAP animals by day 2 postinjection, attaining values which exceeded those found for control animals during the same sampling interval (371/mm^3). However, the maximum numbers of labeled cells appearing in normal animals on days 3 and 4 postinjection (2615 ± 290/mm^3 and 2516 ± 323/mm^3, respectively) were not appreciably different from those found in LAP animals on day 2 (3929 ± 384/mm^3). Thus, these results again established that large numbers of neutrophils entered the peripheral blood of LAP animals 1–2 days earlier than in control rats.

For LAP rats, the entry rate of labeled neutrophils was 56%/day for the interval: days 1–2. This would indicate a maximal turnover time of about 2 days for peripheral granulocytes in these rats. In controls, the entry rate for labeled neutrophils between days 2–3 was about 37%, indicating a maximal turnover time for these cells of about 2.5 days. This small difference might indicate a slightly more rapid peripheral turnover of granulocytes in LAP rats.

Figure 1 gives the changes in labeling indices for mononuclear cells in normal and LAP rats with time after flash labeling with tritiated thymidine. No difference was found in the emergence time of mononuclear cells in LAP and normal animals.

Discussion. Previous studies in which neutrophil kinetics in normal rats were examined

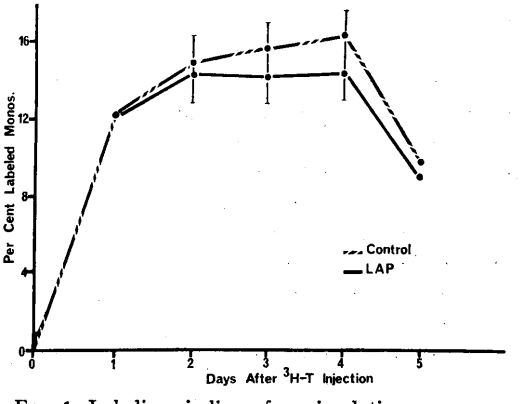

FIG. 1. Labeling indices for circulating mononuclear cells (monos) in normal and LAP rats: vertical lines indicate ±1 standard error of the mean.

showed that labeled neutrophils first appeared in the circulation 1–2 days after isotope administration and that peak neutrophil labeling occurred at 3–4 days (6, 13, 14). The results obtained in the present study confirmed these findings and, in addition, indicate that an earlier release of neutrophils into the peripheral blood occurred in LAP than in normal rats; emergence time being 1–2 days shorter. Analyses of the alterations in labeling indices suggested that the population of cells which normally entered the circulation 3–4 days after isotope injection, appeared on day 2 in LAP animals. This interpretation was also borne out by the observation of a striking similarity between grain count distributions on day 2 for blood from LAP rats and on days 3–4 for blood from normal rats. The higher values for both neutrophil labeling indices and numbers of labeled cells found in LAP rats on the second day following isotope administration implied that removal of cells from the peritoneal cavity somehow stimulated the early release of granulocytes from the bone marrow into the peripheral circulation.

In the present study, 2 lavages and the removal of approximately 175 million cells were sufficient to evoke the early release of labeled granulocytes from the marrow into the circulation resulting in the shortened emergence time observed. One reason for this early appearance might be that marginated (unlabeled) cells entered the circulation to compensate for the loss of cells removed by the LAP procedure. Such "demargination" might then have induced the premature release of newly formed (labeled) cells from the bone marrow into the circulation. The shifts of large numbers of cells from marginal sites to the peripheral circulation are not normally accompanied by a change in the total numbers of cells in the blood, or an alteration in production (15). This might account for the fact that there were no significant changes in the total white blood cell counts in the LAP animal. Therefore, if it were not for the fact that a cohort of releasable cells was labeled by ^{3}HT, the observed early emergence would have gone undetected.

No apparent difference was found in the emergence time of mononuclear cells in LAP and normal animals. Since there are numerous sites of lymphocyte production and storage in addition to the bone marrow, it is improbable that 2 lavages would be sufficient to stimulate an early release of these cells.

Summary. Leukocyte release was studied in normal rats and in rats subjected to massive leukocyte withdrawal via peritoneal lavage (leukocytapheresis, LAP) using ^{3}HT autoradiography. A significant difference in the emergence time of neutrophils in LAP rats, when compared to normal rats, was observed after flash labeling with ^{3}HT. Peak neutrophil labeling in LAP animals occurred on day 2 following isotope injection, whereas in normal (non-LAP) animals the peak was seen on days 3–4. Thus, large numbers of neutrophils entered the peripheral circulation of LAP animals 1–2 days earlier than normal.

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The Induction of Neurogenic Hypercholesteremia* (33971)

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A central nervous influence on plasma cholesterol concentration was suggested by our observations (1) on accountants and other individuals subjected to time–pressure stress, and by our study in collaboration with Dr. C. G. Gunn (2) in which we observed plasma cholesterol rises in rabbits receiving electrical stimulation in the diencephalon—a finding confirmed by Gutstein *et al.* (3). However, in our study (2), the area of the hypothalamus responsible for the observed plasma cholesterol rise was not determined because stimulation of various hypothalamic loci (*e.g.*, the anterior hypothalamic area, the supraoptic nuclear area, the ventral medial nucleus and the lateral hypothalamic area) all induced the observed rise in cholesterol. These results suggested either that many hypothalamic nuclei and specific areas were concerned with the plasma cholesterol level or that only one or more specific areas were being stimulated despite the anatomical location of the inserted electrode. In the present study relatively discrete electrolytic lesions were induced in the hypothalamus of the rat in the attempt to determine first whether such lesions could alter the plasma cholesterol of this species and secondly whether such areas could be more securely identified.

Methods. In the first study, hypothalamic lesions were placed in male Long-Evans rats weighing approximately 300 g each. Bilateral lesions were induced in the first group of 12 rats by insertion of electrodes 0.04–0.08 mm posterior to bregma, 0.075 lateral to midline (*L* coordinate) and 0.95 mm below the brain surface (*D* coordinate). Bilateral lesions were induced in the second group of 15 rats by insertion of electrodes at the same lateral and vertical coordinates but 0.12 to 0.16 mm posterior to bregma. A dc current of 2 mA was applied for 20 sec in all rats. Eighteen control rats were subjected to similar electrode insertions but no current was applied. The animals were fed a low cholesterol diet of vegetable origin (containing 43 mg of digitonin precipitable Liebermann-Burchard positive sterol per 100 g of diet). Two weeks after operation, all rats were bled and the samples were analyzed for plasma cholesterol concentration (4).

In the second study, 4 groups of 15 rats each were subjected to two electrode insertions on each side (0.14 and 0.18 mm posterior to bregma) but the lateral and vertical coordinates employed varied (see Table I) with each group in an attempt to damage specific nuclear areas or tracts. Two weeks after operation, these rats were bled and the samples were analyzed for cholesterol.

In the third study, 2 groups of 15 rats each received 2 electrolytic lesions (2 mA for 10 sec) on each side (0.14 and 0.18 mm, respectively posterior to bregma, 0.075 mm lateral to the midline and 0.95 mm beneath the surface of the brain). The first group was fed the low cholesterol diet and bled 3, 7, 14, 21, and 28 days for plasma cholesterol determinations. In addition, 14 days after operation, following a prior fast of 15 hr they were given 3 ml of cottonseed oil by stomach tube. Blood sample were obtained before and 6 hr after this feeding. The fasting samples were analyzed for cholesterol, triglyceride (5), and phospholipid (6), and the postpran-

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