The Intravascular Survival of Neutrophils Labeled In Vivo

By Paul C. Vincent, Arjun D. Chanana, Eugene P. Cronkite, and Darrel D. Joel

The survival of blood neutrophils labeled in vivo was studied in the calf. Disappearance of labeled neutrophils from the blood of calves was followed after a period of cross circulation with their chimeric, immunologically tolerant twins, which had been given tritiated thymidine 6½ days previously. Under these conditions, neutrophils were shown to leave the blood in a random exponential fashion, with half-disappearance times of between 6.4 and 7.5 hr. Hydrocortisone given to one calf 48 hr after cross circulation caused a neutrophilic leukocytosis, during which substantial numbers of labeled neutrophils reappeared in the blood.

THE INTRAVASCULAR survival of neutrophils has been extensively studied in man[1-9] and dogs[10] using methods in which autologous neutrophils were labeled in vitro with either diisopropylfluorophosphate ($DF^{32}P$)[1-5,7,9-11] or sodium chromate-^{51}Cr,[6,8] and reinfused. It is generally agreed that neutrophils labeled in this way disappear randomly from the blood, so that the peripheral blood radioactivity declines exponentially. Neutrophils labeled by these in vitro techniques have been shown to exclude vital dyes and to phagocytose bacteria normally.[12] However, it is still possible that in vitro handling might alter the cells sufficiently to modify their survival after reinfusion. Variable degrees of damage of this type could explain some of the differences in reported blood neutrophil half-times, which range from 3.8 hr[5] to as long as 18.9,[8] although the most commonly observed values are in the range of 4 10 hr.[1-3,6,7,9] In vivo labeling of neutrophils might be expected to overcome this problem, but available compounds which label blood neutrophils also label their precursors in the marrow.

This paper reports experiments in which neutrophils were labeled in vivo with tritiated thymidine (^{3}HTdR) in one member of a chimeric calf twin pair and their disappearance followed from the blood of the other twin after a period of cross circulation. Shortening of neutrophil survival of the type seen

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Table 1. Details of Cross-Circulation Studies

Donor No.	206	414
Recipient No.	207	413
Donor weight	118 kg	65 kg
^{3}HTdR given to donor: First dose	0 hr	0 hr
Second dose	10 hr	10 hr
A–V shunt installed	106 hr	106 hr
Heparin (5 mg/kg)	153 hr	155 hr
Cross-circulation: Started	153.7 hr	155.3 hr
Ended	156 hr	156.5 hr
Blood volumes* of donor exchanged	15.5	14.7

*Blood volume of 69 ml/kg, based on our unpublished data obtained by ^{51}Cr studies in calves of different ages and weights.

when heterologous cells are infused[13] was not a problem, since the twin pairs were immunologically tolerant of each other.

MATERIALS AND METHODS

Two pairs of chimeric twin Holstein-Fresian calves were studied (Table 1). Cross skin grafts between members of each pair[14] showed no evidence of rejection during follow-up periods of 95 days (pair 206-207) and 180 days (pair 413-414), respectively. Calves 206 and 207 were also shown to have the same blood groups, hemoglobin types and "J" blood factors (we thank Professor W. Stone of the Department of Genetics, University of Wisconsin, Madison for these studies). Similar blood group data were not obtained in the pair 413-414. Preliminary studies had shown that cross circulation in other chimeric twin calves for as long as 72 hr was free of any immediate or delayed adverse effects, while cross-circulation between nontwin calves for 14 hr caused anemia, lymphopenia, and death 17-27 days later in both donor and recipient. No adverse effects were seen in any of the members of the chimeric twin pairs 206-207 or 413-414 over periods of observation between 4 and 6 mo following cross-circulation.

A preliminary study of one member of the first pair 2 mo previously had established that the maximum labeling index of neutrophils in the blood could be expected about 150 hr following injection of tritiated thymidine (^{3}HTdR) (Fig. 1). In the definitive study, one member of each pair was given two intravenous doses of ^{3}HTdR (Schwarz Bio Research, Orangeburg, N.Y., spec. act. 1.9 Ci/mmole), each of 0.25 μCi/g body weight, 10 hr apart. Blood samples for radioautography were collected at intervals up to 154 hr from the time of the first injection. At 106 hr after the first injection, carotid-jugular arteriovenous shunts were established in both calves of the pair.[15] Arteriovenous cross-circulation between members of each pair was established at 153 and 155 hr and continued long enough for a measured exchange of between 14.7 and 15.9 blood volumes (Table 1). Disappearance of labeled neutrophils from the blood of the recipient twin was followed by radioautographic analysis of blood samples. Hydrocortisone (Solucortef, Upjohn, Kalamazoo, Mich.) was given to one recipient calf in a dose of 4.2 mg/kg intravenously 48 hr after the end of cross-circulation, and blood samples collected for an additional 24 hr. Leukocyte counts were made using a Coulter Model F Cell Counter (Coulter Electronics, Hialeah, Fla.) and radioautographs, prepared from methanol-fixed blood films using Kodak NTB-2 liquid emulsion (Eastman Kodak, Rochester, N.Y.), were exposed in the dark for 56 days. After developing and fixing, slides were stained with Giemsa 1:20, pH 5.75. Differential counts and labeling indices were performed on at least 1000 leukocytes. Background was corrected for by Stillström's method.[16] Labeling indices of blood neutrophils and the absolute numbers of circulating labeled neutrophils in recipient calves were plotted against time on semilogarithmic paper.[17]

RESULTS

Labeled neutrophils appeared in the blood of donor animals between 100 and 130 hr after the first injection of ^{3}HTdR, and increased progressively to between

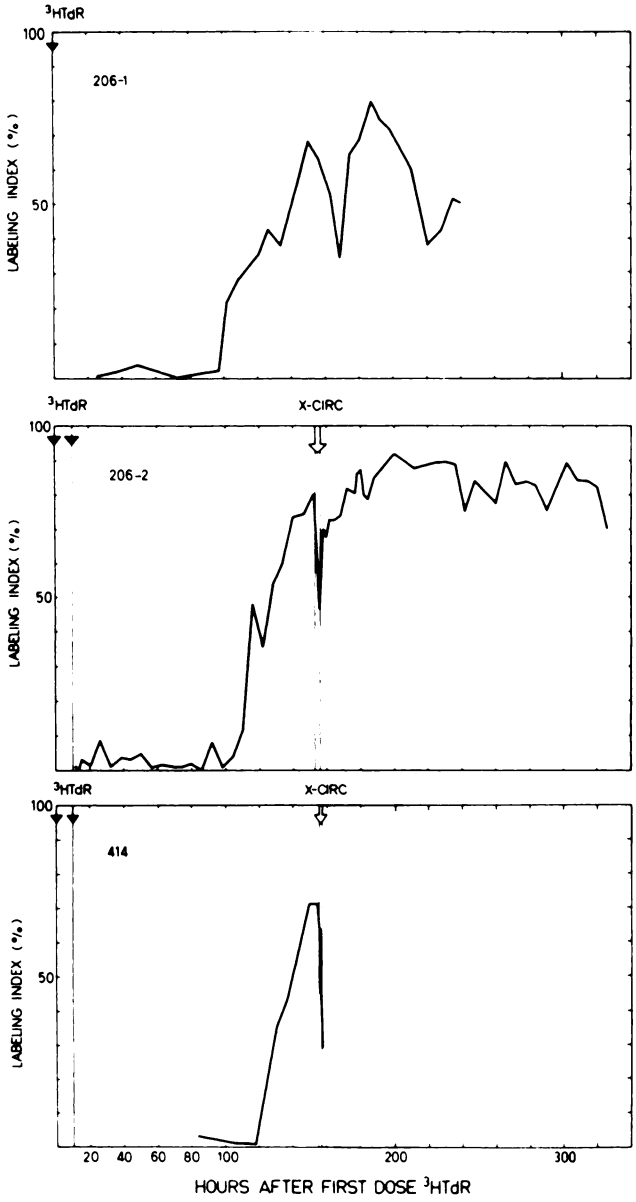

Fig. 1. Labeling indices of blood neutrophils in donor calves following ^{3}HTdR injection (0.25 μCi/g body weight) (closed arrows). Top graph: First study in calf 206. Center graph: Second study in calf 206. Lower graph: Study in calf 414. In the second study in calf 206, and in calf 414, 2 doses of ^{3}HTdR were given at 0 and 10 hr, and cross-circulation with calves 207 and 413, respectively, was carried out at the time shown.

64% and 80% by the time cross-circulation with the recipients was established (Fig. 1). The labeling indices fell by approximately one-half during the period of cross-circulation, but rose again promptly at the end of the procedure.

The disappearance of labeled neutrophils from the blood of the recipient animals following the completion of cross-circulation is shown in Figs. 2 and 3. The decline was exponential for both the labeling indices and for the absolute number of labeled neutrophils per cubic millimeter, i.e., neutrophils count times labeling index (Figs. 2 and 3). For both calculations the linear correlation coefficients were highly significant ($p < 0.001$). The half-times calculated from

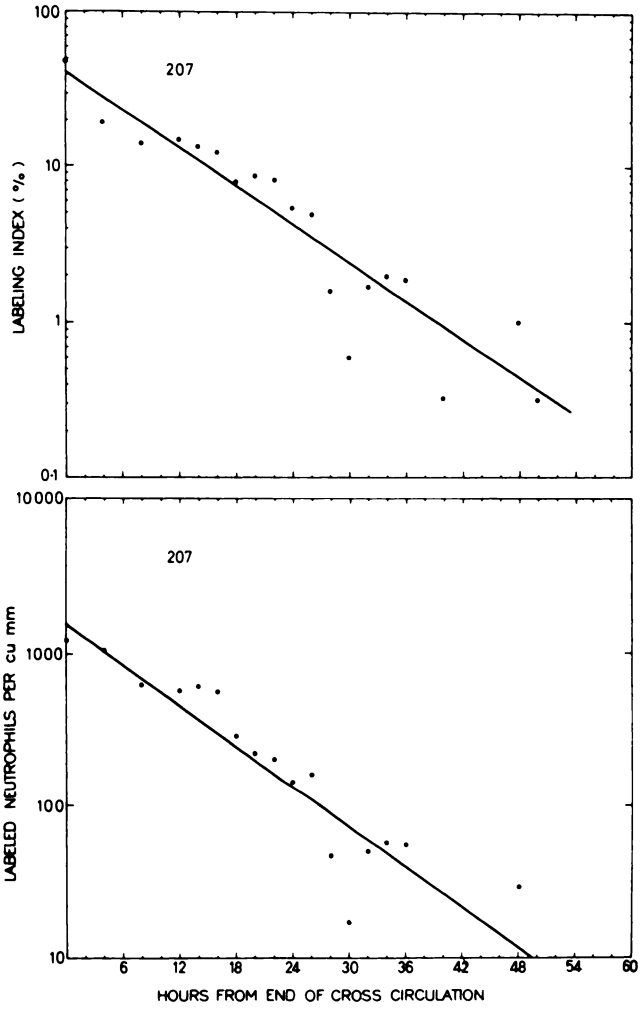

Fig. 2. Disappearance of labeled neutrophils from the blood of calf 207 following cross circulation with its immunologically tolerant chimeric twin (206) which had been labeled with ^{3}HTdR 153 hr previously. Top graph: Labeling indices of blood neutrophils (line of best fit; log y = 1.61 − 0.041 *T*; *r* = −0.93, T½ = 7.3 hr). Lower graph: Number of labeled neutrophils per cu mm (line of best fit; log y = 3.20 − 0.044 *T*; *r* = −0.93, T½ = 6.8 hr).

the labeling indices were 7.3 and 7.5 hr, and those calculated from the absolute numbers of labeled cells were 6.8 and 6.4 hr, respectively. The mean grain count of labeled neutrophils in the recipient calves did not alter appreciably throughout the study.

Forty-eight hours after cross-circulation the labeling index of neutrophils in the blood and the absolute numbers of labeled cells present had fallen to very low levels. The neutrophil leukocytosis induced by hydrocortisone in calf 413, however, was associated with increases in both the number of labeled cells in the blood, and the neutrophil labeling index (Figs. 2 and 3).

DISCUSSION

Results obtained in the donor calves of each pair following injection of ^{3}HTdR showed the emergence of labeled neutrophils at much the same time and in much the same pattern as seen in man[9,18,19] or in dogs.[20] Use of two consecutive doses of ^{3}HTdR 10 hr apart led to a prolonged maintenance of labeled

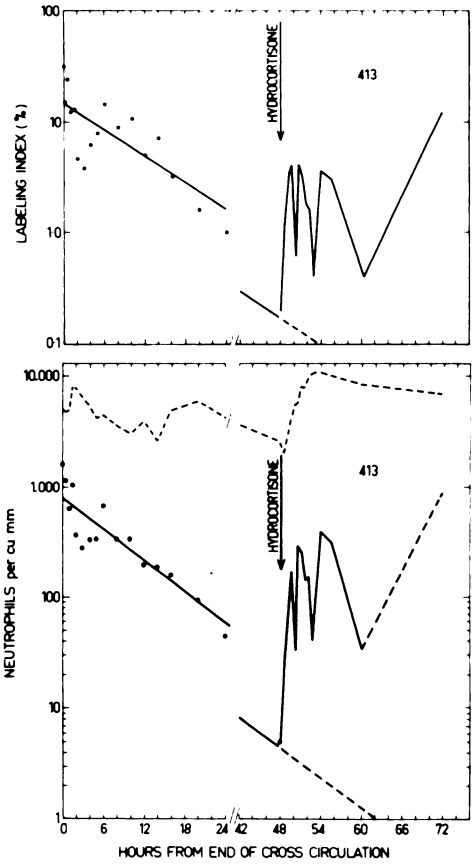

Fig. 3. Disappearance of labeled neutrophils from the blood of calf 413 following cross-circulation with its immunologically tolerant chimeric twin (414) which had been labeled with ^{3}HTdR 155 hr previously. Top graph: Labeling indices of blood neutrophils (line of best fit over first 48 hr; log $y = 1.17 - 0.040\ T$; $r = 0.90$, $T½ = 7.5$ hr). Lower graph: Blood neutrophil concentration (dotted line) and number of labeled neutrophils per cu mm (line of best fit over first 48 hr; log $y = 2.90 - 0.047\ T$ ($r = -0.96$, $T½ = 6.4$ hr). Note the break in the time scale; the continuation of each linear regression from 42 to 48 hr is shown, together with their extrapolations past 48 hr as dashed lines. Following hydrocortisone injection, there was a neutrophil leukocytosis associated with increases in both the labeling index of blood neutrophils, and in the absolute concentration of circulating labeled neutrophils.

cells in the blood, and the almost exact halving of labeling indices during cross-circulation was evidence of a virtually complete equilibration of the circulating blood pools between each animal of a pair. Since the marginated pool is known to exchange rapidly and completely with the circulating pool,[2] it is probable that the marginated pools were also equilibrated by the end of the period of cross-circulation.

The random disappearance of neutrophils from the blood, well documented by the use of cells labeled in vitro, was also seen in the present experiments where cells were labeled in vivo and then transferred to an immunologically tolerant twin. Furthermore, the problems of possible cell damage due to the label,[8] degradation of the compound (found with ^{3}H-DFP[21]) and elution[6,8] were all avoided by using ^{3}HTdR, which by the time it is found in the DNA of non-dividing cells is biologically inert and nonexchangeable until the cell dies.[22]

The similarity between the half-times found for the calf and those generally found in man[1-3,6,7,9] or dogs[10] using DF^{32}P or ^{51}Cr is quite striking. Indeed, if this similarity is accepted as being valid, our findings suggest that a blood neutrophil half-time of about 4 10 hr reported by others in humans[1-3,6,7,9] is correct, despite suggestions that it is erroneously short.[8] Neutrophils transferred at the time of cross-circulation would have varied in age, and senescent loss of

the type reported in man[23] would have been occurring continuously following the cessation of cross-circulation. For this reason, labeled pyknotic cells were not specifically looked for in the present studies.

The neutrophil leukocytosis which occurs following a single large dose of corticosteroid in normal man has been shown to be due both to an increased inflow of cells, probably from the marrow, and to a decreased egress of cells from the blood, with increases in the total, circulating, and marginated blood neutrophil pools.[24] In the present study, hydrocortisone was given when only 0.5% of the labeled cells originally present were still in the blood. The rise in the number of labeled blood neutrophils which occurred during the hydrocortisone-induced neutrophil leukocytosis was therefore quite surprising. The fact that the labeling index as well as the absolute number of labeled cells increased suggests that the leukocytosis was not due to mobilization of the marginated pool, which should have the same labeling index as the circulating pool. In addition, studies in man have ruled out mobilization of marginated neutrophils as a cause of steroid leukocytosis.[24] A decreased egress of neutrophils from the blood, while it may have occurred, would not have increased the absolute number of labeled cells present. The possibility that the labeled neutrophils came from the marrow (having lodged there earlier) despite the generally held view that blood neutrophils do not return to the marrow,[3,25] is being investigated.

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