Study of Leukocyte Dynamics by Means of Cross-Circulation Between Normal and Leukopenic Rats

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UTILIZING a method of temporary cross-circulation in rats, we studied the dynamics of leukocyte mobilization and destruction during circulatory mixing between leukopenic and normal animals. These results, along with the attempts to modify the changes observed, are the subject of the present report.

Materials and Methods

Sprague-Dawley strain male rats obtained from a local breeder, weighing from 250–400 Gm., were used in all experiments.

Leukopenia in most instances was induced by x-irradiation. A dosage of 800 roentgens (250 KV, 15 ma, Thoreus filter, 50 cm. tube distance) was found regularly to produce arterial leukocyte counts of less than 400 cu. mm. 72 hours after irradiation, when all experiments were performed.

The cross-circulation apparatus, devised by Dr. Alvin Brodish of the Department of Physiology at Yale University School of Medicine, has been described in detail.[1] Briefly, the technic consists of placing polyethylene cannulae in the femoral artery and vein of the animals to be studied. Arterial blood flows freely from one animal through plastic tubes into the top of a small plastic cup. A measured volume (1–2 ml.) is allowed to flow into the cup, and by means of air pressure is expressed from the bottom of the cup through polyethylene tubing into the vein of the opposite animal. Simultaneously, blood flows from the opposite animal in like manner. All experiments were performed with the animals anesthetized with nembutal, 12 mg. intraperitoneally and supplemented as necessary. To prevent clotting, both animals received 2 mg. heparin in the venous catheter at the beginning of the cross-circulation.

Radio-iron mixing curves, reported by Brodish and Long,[1] show that complete mixing of the two circulations in rats of this size occurs after 25–30 ml. of blood has passed from each animal. One complete circulatory mixing (30 ml.) requires 15–20 minutes. In most experiments described here 60–150 ml. (2–5 mixings) were used, although we have found it possible to continue the cross-circulation for as long as 10 total mixings (300 ml.). The data presented in this paper are accumulated from approximately 40 experiments of this type.

At suitable intervals during cross-circulation blood was obtained from the arterial cannulae for total and differential leukocyte counts. After the cross-circulation was discontinued, the arterial cannulae were retained in the artery for blood sampling.

Blood from the tail was found to give a markedly variable leukocyte count, and was not suitable for our studies. In normal rats the tail counts were found to be 1.5 to 3 times higher than simultaneous arterial samples. In the irradiated animals, tail counts were found to be as high as 2,000/cu. mm., while arterial counts were invariably below 400/ cu.mm. Arterial and venous counts were essentially equal.

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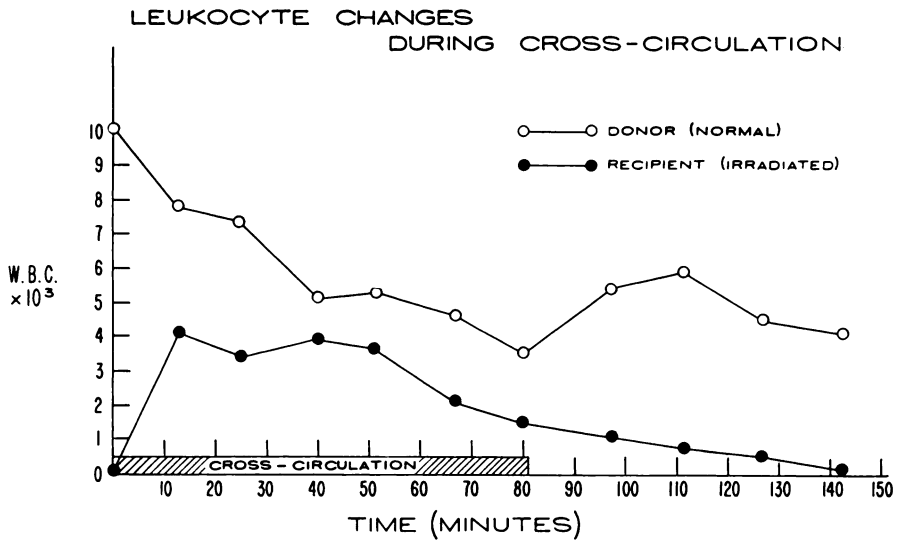

FIG. 1.—Arterial blood leukocyte counts during and after a typical cross-circulation experiment.

RESULTS

A typical experiment is shown in figure 1. The blood counts taken during the cross-circulation at intervals of one mixing time (30 ml.) show that the leukocyte counts of the animals never reach equilibrium, although in all animals the count in the leukopenic animals rose to moderate levels (never exceeding 4,000/cu. mm.) and the count of the donor animals fell. It is obvious, then, that the leukocytes transfused into the leukopenic animal rapidly were sequestered or destroyed. Concomitantly the normal donors promptly mobilized additional leukocytes from their tissues, and failed to become as leukopenic as would be expected from dilution alone. After the cross-circulation was discontinued, the arterial blood counts of the leukopenic animals fell progressively, reaching the baseline in 1–2 hours, while those of the normal donor animals showed varying degrees of recovery.

The results obtained in experiments such as the one illustrated (fig. 1) might best be presented in sections: the changes during the cross-circulation, and the response of both the irradiated and the donor animal after cross-circulation was discontinued.

A. *Changes during cross-circulation*

Representative examples of the changes observed during cross-circulation are shown in table 1. In this table the actual leukocyte counts following each period of total mixing (30 ml.) are plotted, along with a calculation of the minimal number of leukocytes lost from the circulation of the irradiated animal during that particular cross-circulation. The calculation is a simple one, and is based solely on presumed mixing. For example, if at the start of cross-circulation the donor animal (normal or splenectomized) had a leukocyte count of 10,000/cu. mm. and the recipient animal (irradiated) had a leukocyte count of 0/cu. mm.,

TABLE 1.—*Changes in Leukocyte Count During Cross-Circulation Between Irradiated Recipient and Normal or Splenectomized Donor Rats*

Leukocyte counts (× 10^3) of donor and recipient animals are tabulated during continuous cross-circulation. The deficit observed during each circulatory mixing (30 ml.) is calculated by the method described in the text.

Exp. #	Number of Circulatory Mixings																					Total Deficit
	0			1			2			3			4			5						
	Recipient	Donor	Deficit	Recipient	Donor	Deficit	Recipient	Donor	Deficit	Recipient	Donor	Deficit	Recipient	Donor	Deficit	Recipient	Donor	Deficit				
Normal Donors																						
1*	.35	7.55		.62	5.45	2.7	.8	4.1	2.7	.58	3.0	1.5	.71	3.8	0.9	.76	3.3	1.2				9.0
2*	0	6.4		.75	5.15	2.0	1.15	4.65	1.4	1.55	5.4	1.1										4.5
3	.05	10.0		4.15	7.80	.85	3.45	7.35	2.55	3.95	5.15	1.4	3.7	5.30	.9	2.15	4.60	2.3				8.1
4	.2	22.5		1.15	6.6	10.1	3.5	12.5	.35	4.15	7.0	3.85										14.3
Splenectomized Donors																						
5	0	16.65	—	.0	4.4	7.7	1.2	5.25	1.3	2.5	3.85	.7	.85	6.65	2.4							12.1
6	.10	15.1	—	1.7	4.9	5.9	1.9	3.7	1.4	1.85	3.4	1.0	1.65	4.3	1.0							9.3
7	.30	12.3	—	1.15	3.8	5.2	1.9	5.3	.6	2.65	4.15	1.0	2.75	3.4	.7							7.5
8	.05	15.25	—	1.45	4.1	6.2	2.3	4.1	.4	2.9	6.1	.3										6.9
9	.3	31.8	—	3.0	5.35	13.0	3.6	6.9	.5	2.75	5.65	2.5	2.7	4.55	1.5							17.5

* Only 20 ml. transferred during each period and calculations based on 80% mixing.

the count in the recipient after complete mixing should be half the total of the counts observed in each animal or (10,000 + 0)/2 = 5,000/cu. mm. in the recipient animal. However, a count of only 1,000/cu. mm. was found, leaving a deficit of 4,000/cu. mm. (5,000 − 1,000). The leukocyte deficit for each period of mixing was calculated in this manner. The sum total of these, as shown in the last column of the chart, represents the total leukocyte deficit during the entire period of cross-circulation. This type calculation is somewhat erroneous, since the donor animal continued to bring forth cells during the period of mixing, so that the total deficit actually is larger than that calculated.

It can be seen from table 1 that the normal donor animals showed a progressively falling white cell count during the period of cross-circulation, although of course, cells were being produced by the donors in quantities at least equivalent to the observed deficit during the period. Since each period of total mixing required 15–20 minutes, the total time of the experiments plotted was 1–1½ hours.

The splenectomized donors plotted in table 1 were subjected to splenectomy from 15 minutes to 9 days prior to cross-circulation with the irradiated recipient animals. These animals all showed a similar change. During the first period of cross-circulation the leukocyte counts decreased more than could be accounted for by mixing alone. For example, splenectomized donor No. 9 had a leukocyte fall from 31,800/cu. mm. to 5,350/cu. mm. during the first mixing period, although the calculated fall in an adynamic system would be half the total count of the two animals, or approximately 16,000/cu. mm. After the immediate fall, the splenectomized donors produced cells at a low rate, generally maintaining a

count of 3,000–6,000/cu. mm. In some experiments the recipient and donor counts became almost equilibrated.

This rapid fall in leukocyte count can be accounted for only by sequestration within the splenectomized donor, and the same phenomenon rarely was noted in normal animals (note animal no. 4). We have no ready explanation for the changes observed in the splenectomized animal, but it is apparent that these animals tended to retain their leukocytes. Since the splenectomized animals did, however, maintain a positive balance during the experiments, it is evident that the spleen was not the sole contributing source of the readily available leukocytes in the donors, and probably was not a major source of these cells.

The results plotted in figure 1 and table 1 are representative of many similar experiments. Attempts to modify the responses were made in the following ways, and all were negative: (1) splenectomy of the irradiated recipient, (2) cortisone treatment (5 mg. subcutaneously daily for 1–5 days) of either donor or recipient, (3) thorotrast blockade of the irradiated recipient animal (0.5 ml./100 Gm. 24 hours before cross-circulation), and (4) leukopenia induced by other methods (aminopterin, nitrogen mustard).

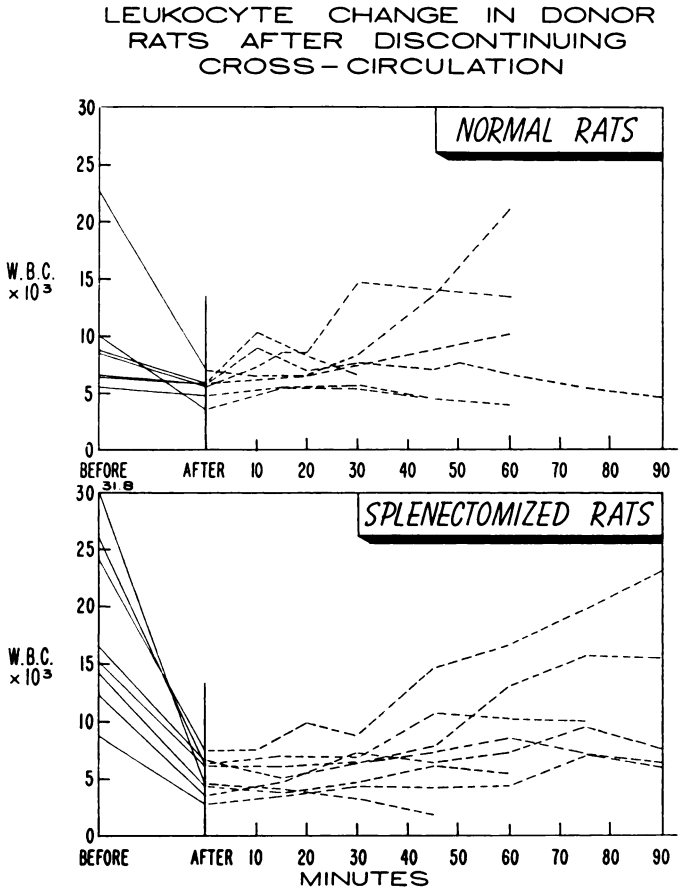

FIG. 2.—Changes in arterial blood leukocyte count of normal and splenectomized donor rats.

In the experiments described, the term "leukocytes" has been used exclusively, but since the normal rat has 80–90 per cent lymphocytes most of the data really refer to the lymphocytes. Stained films for differential counts were obtained in all experiments, and in some the number of granulocytes was higher, either spontaneously or due to the experimental procedure used. In these animals there was no apparent difference in behavior of the granulocytes or lymphocytes. We have, however, failed in attempts to produced marked granulocytic leukocytosis in rats to serve as donors in the cross-circulation experiments.

When two normal animals underwent cross-circulation, there was no essential change in total leukocyte counts during the procedure.

B. Leukocyte recovery of the donor animals after cross-circulation

In figure 2 the changes in leukocyte counts of normal and splenectomized donor animals after cessation of cross-circulation with the irradiated recipients are expressed in graphic form. The leukocyte counts of normal animals in most nstances tended to remain at low normal levels during the period of observa-

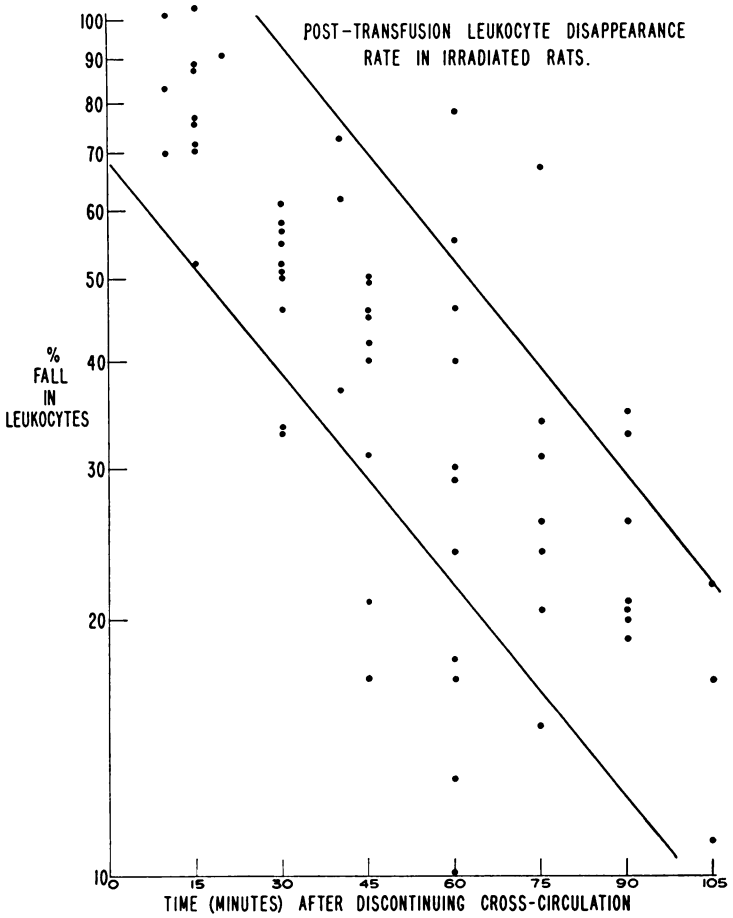

FIG. 3.—Fall of arterial blood leukocyte counts of irradiated rats after cessation of cross-circulation.

tion, although some animals showed a gradual rise in count. The splenectomized animals, as pointed out previously, showed a marked fall in count apparently due to sequestration of leukocytes during the early phases of the cross-circulation, and during recovery only two of the eight animals returned to the level of leukocytosis noted at the beginning of the experiments.

C. Disappearance of leukocytes from the irradiated recipients after cross-circulation

After stopping cross-circulation, the counts of the irradiated animals fell progressively in an exponential fashion, with an apparent biologic half-life of 30–60 minutes as indicated in figure 3. After 90–120 minutes, the counts were near the low levels found before cross-circulation. As mentioned previously, cortisone therapy, splenectomy, or blockade of the reticulo-endothelial system with thorotrast did not affect the disappearance rate.

Discussion

The results of these studies of leukocyte dynamics during and after cross-circulation between normal and irradiated rats confirms the observations of previous investigators. Continued cross-circulation caused only moderate depression of the leukocytes of the normal animals, yet large numbers of cells were removed by the irradiated animals without bringing their leukocyte count to normal levels. Lawrence et al.[2] in cross-circulation of normal and irradiated cats noted that the irradiated animal remained leukopenic, while the normal cat maintained a normal or even increased leukocyte count. The rapid disappearance of transfused leukocytes has been found by many investigators using a variety of different methods.[3-5]

Our cross-circulation technic, however, offers certain advantages over previous methods of study. Albino rats demonstrate no erythrocyte incompatibility, and this perhaps may lessen the probability of any leukocyte group incompatibility. In addition the rates of leukocyte flow between animals could be controlled, so that semiquantitative estimations of leukocyte mobilization and disappearance rates could be made.

Our studies offer certain observations on two important aspects of leukocyte physiology: (1) the size of the readily available leukocyte reserve, and (2) the fate of leukocytes transfused from one animal to another.

Concerning the size of the leukocyte pool, Osgood[6] has suggested that in the normal animal the circulating blood leukocytes probably comprise less than 1 per cent of the body total of viable, mature leukocytes. The actual location of the leukocyte tissue pools has been investigated only superficially. It is known from microscopy studies that granulocytes migrate through capillaries in and out of tissues. Dr. George P. Fulton's excellent motion pictures of capillary beds reveal that large numbers of granulocytes remain sessile on capillary endothelial walls. Mature lymphocytes are widely distributed in lymphatic tissues, and circulate in lymphatic channels. Quantitative estimates of leukocytes in these various sites have been difficult to determine. Little is known about the factors controlling leukocyte equilibrium between the "pools" and the circulating blood.

The normal donor animals in our experiments, during approximately an

hour of cross-circulation, lost at least one blood volume of white cells (table 1). There appeared to be a progressive decrease in the number of cells entering the blood as the cross-circulation progressed. The slow recovery of the white counts of the animals after cross-circulation suggests either depletion of the readily available reserve, or possibly simply lack of a specific stimulus to restore the circulating white cell volume to normal. It should also be borne in mind that these animals, under prolonged anesthesia and cross-circulated with a severely irradiated animal, may have been incapable of a truly physiologic response. Craddock et al.,[7] however, using a direct method of leukocyte removal in dogs noted a similar leukocyte depression and slow recovery. In our animals there was no evidence of bone marrow production playing a role in the responses, since no immature cells were seen in the stained blood films of the animals.

Concerning the fate of the cells transfused into the irradiated animals, there is much evidence to suggest that a large percentage of transfused leukocytes are immediately trapped in the lung.[8] There is not, however, convincing evidence that these trapped leukocytes are destroyed. Brecher, Wilbur, and Cronkite,[9] transfusing carefully prepared leukocyte suspensions into irradiated dogs, have demonstrated morphologic evidence that these cells do remain viable in tissues even though they largely disappear from the blood. On the other hand, Leahy and his co-workers[10] collected P^{32} labeled leukocytes from peritoneal exudates in rabbits, and found that these cells after saline washing actually were lysed when transfused to normal animals.

Our studies of cross-circulation between two normal animals bear on this question of viability of transfused leukocytes. If the leukocytes of the two normal animals were mutually incompatible and were destroyed by the opposite animal, one would expect progressive fall in the leukocyte count of both animals as cross-circulation was continued. Incompatibility, then, should result in the same type of depletion that occurs when the normal animal donates leukocytes to the irradiated partner. Absence of a fall in leukocyte count during cross-circulation of normal rats suggests that the leukocytes are viable. An alternative explanation is possible, however. It may be that the irradiated animal not only removes leukocytes from the normal donor, but also exerts a depressant action on leukocyte mobilization in the normal animal.

From the foregoing discussion, it is evident that the interpretation of the mechanism of the slow increase in circulating cells observed in the leukopenic animal during cross-circulation and the slow fall afterward, depends on whether the cells are viable. The disappearance of cells in the irradiated recipient almost certainly does not represent leukocyte lifespan. It does, presumably, measure transit time of leukocytes from the blood stream to a final partition within the leukocyte "pools", or transit time to their site of destruction.

Summary

(1) Certain aspects of leukocyte dynamics in rats were studied by means of a mechanical cross-circulation technic.

(2) Cross-circulation between two normal rats caused little change in the leukocyte counts.

(3) When irradiated leukopenic animals were cross-circulated with normals,

the leukocyte counts of the normal animals were moderately decreased, while the level of the irradiated animals rose. Prolonged mixing of the circulations, however, failed to bring the counts of the two animals into equilibrium.

(4) After cessation of cross-circulation, the leukocyte counts of the normal donors usually rose slowly, while those of the irradiated animals fell progressively with a half-time disappearance of 30–60 minutes.

(5) The possible significance of these observations in terms of normal leukocyte mobilization, viability and destruction was discussed.

Summario in Interlingua

1. Certe aspectos del dynamica leucocytic in rattos esseva studiate per medio de un technica de circulation crucial mechanic.

2. Circulation crucial inter duo rattos normal causava pauc alterationes in le conto leucocytic.

3. Quando le circulation crucial esseva establite inter irradiate rattos leucopenic e rattos normal, le contos leucocytic del animales normal esseva levemente reducite, durante que le contos del animales irradiate se augmentava. Tamen, le prolongate mixtion del circulationes non arrivava a establir contos equilibriate in le duo animales.

4. Post cessation del circulation crucial, le contos leucocytic del donatores normal ascendeva lentemente in le majoritate del casos, durante que le contos del animales irradiate se reduceva progressivemente con un apparente medio vita biologic del leucocytos de inter 30 e 60 minutas.

5. Es discutite le possibile signification de iste observationes con referentia al mobilisation, viabilitate, e destruction de leucocytos normal.

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