

Viability and Distribution of Leukocytes Following Cross-Circulation Experiments Between Leukemic and Normal or Irradiated Rats

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IN PREVIOUS STUDIES involving acute cross-circulation experiments in rats, evidence was obtained that leukocytes transfused by this method were not immediately destroyed.^{1,2} It was postulated that disappearance of transfused leukocytes from the arterial blood of irradiated leukopenic animals represented redistribution of these cells into a depleted body leukocyte "pool," rather than their destruction and lysis. Indirect evidence for functional viability of these cells was derived from the fact that irradiated rats, after cross-circulation with normal animals, demonstrated increased ability to remove bacteria from their blood, probably through the mechanism of phagocytosis by the transfused leukocytes.³

Morphologic identification and localization of transfused leukocytes after they had disappeared from the circulation should reveal their sites of redistribution. It has been postulated that noncirculating transfused leukocytes might remain viable either in the tissues or within sluggish vascular channels. Although the method of cross-circulation permitted transfer of 400,000,000 normal leukocytes and even more leukemic cells into irradiated recipients, Osgood's calculation⁴ would indicate that this quantity probably represents only a small fraction of the total rat leukocyte "pool." It seemed unlikely, then, that microscopic examination of the tissues would permit the identification and localization of the transfused cells. The unique circulation of the rodent tail suggested that the capillaries and venules of the tail might be a site of intravascular leukocyte aggregation. Therefore, certain studies of the normal rat tail were undertaken, and tail blood leukocyte counts were compared with arterial blood leukocyte counts during and after various cross-circulation experiments.

In addition to the observations relating to the viability of transfused leukocytes, some interesting aspects concerned with the transmission of the Shay chloroleukemia and with the ability of leukemic leukocytes to reverse radiation damage were encountered.

MATERIALS AND METHODS

The cross-circulation methods and the strain of rat chloroleukemia used have been previously described.^{1,2} Arterial blood leukocyte counts, obtained from polyethylene cannulae in the femoral arteries, were compared with counts obtained from a small incision at the tip of the tail.

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RESULTS

A. Studies of the normal rat tail

Simultaneous artery and tail counts in ten normal animals revealed a mean arterial leukocyte count of 14,990/cu. mm., with a mean tail count of 22,580/cu. mm. Samples obtained from the femoral vein were essentially identical with those from the femoral artery.

Further studies were undertaken of the relative leukocytosis of the normal rat tail blood. A small cut was made in the tail of normal animals under nembutal anesthesia. The cut required vigorous "milking" of the tail to obtain small drops of blood, and serial leukocyte counts were obtained from five successive drops of blood expressed in this way. Probably no more than 0.1–0.2 ml. of blood was removed during the experiments. As can be seen in figure 1, there was a precipitous fall in white cell count, with mean values in four experiments decreasing to approximately half the count of the initial droplet. When more free-flowing tail cuts were made, the same phenomenon was observed, but the magnitude was somewhat less striking.

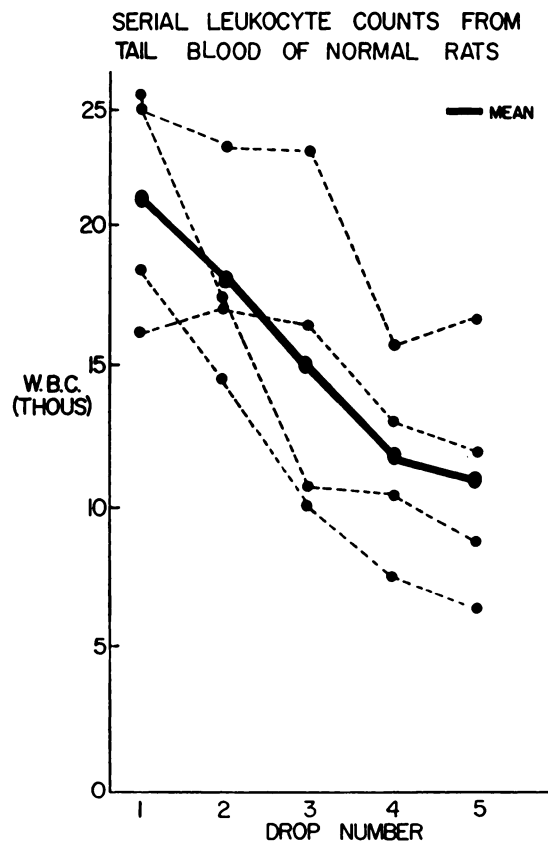


FIG. 1.—Each set of solid dots connected by a broken line represents successive leukocyte counts of blood "milked" from the tail of a normal rat.

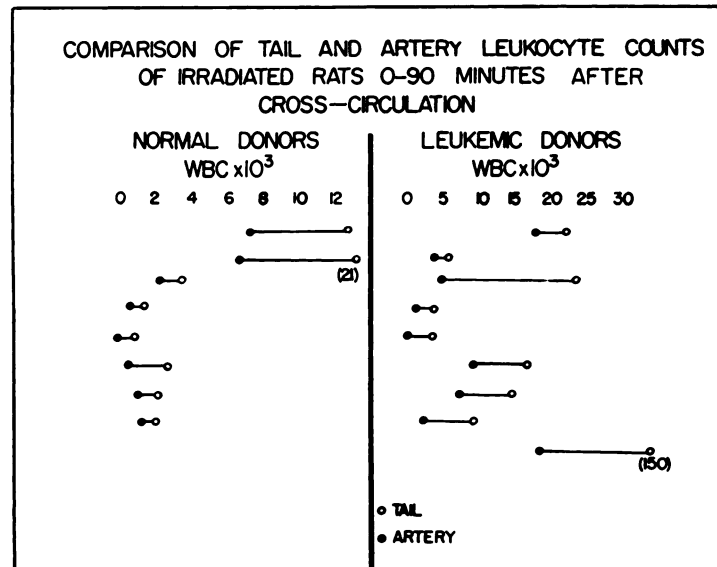


FIG. 2.—Each solid line above connects the tail and arterial leukocyte values obtained from an irradiated rat at some particular time following the cross-circulation period. In all instances a greater concentration of leukocytes was found in the tail blood.

B. Simultaneous arterial and tail leukocyte counts after cross-circulation

In a number of experiments tail counts were performed simultaneously with arterial counts during the 90 minutes following cross-circulation between an irradiated leukopenic rat and either normal or leukemic donor animals. It was evident that, although the arterial counts were raised temporarily as previously reported,^{1, 2} the tail counts were inevitably higher (fig. 2).

C. Tail counts after cross-circulation

Following many cross-circulation experiments, the arterial and venous cannulae were removed, the vessels ligated, and the skin incision closed. Total and differential leukocyte counts were obtained daily thereafter for 3-6 days. Many of the irradiated rats died shortly after the procedure, but the tail counts of those that survived are shown in figure 3. During the three days of observation it was evident that the tail counts of the irradiated rats after cross-circulation with leukemic rats were markedly elevated as compared to that of irradiated untreated rats. Although the number of irradiated animals surviving after circulatory mixing with a normal donor rat is relatively small, it seems probable that these animals also had elevations of the tail leukocyte count (fig. 3).

The leukemic cells were evident in the tail blood of normal recipient rats after circulatory mixing with leukemic donors. In "blind" study of the blood films of these rats, compared with those of normal rats after cross-circulation with other normals, definite leukemic cells were detected in progressively smaller numbers over the three days following the procedure.

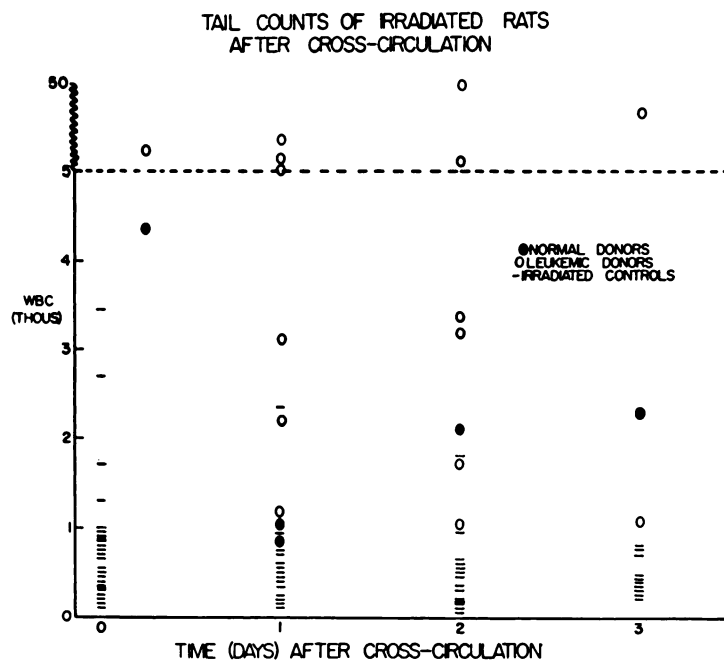


FIG. 3.—Tail blood leukocyte counts of irradiated rats after cross-circulation with either normal (solid dots) or leukemic (open circles) donor rats almost invariably were higher than those of the irradiated control rats during this period of observation.

D. Prolonged observations on recipient rats after cross-circulation with leukemic donors

Two irradiated recipients recovered from the severe radiation dosage (800 roentgens), but developed progressively fatal leukemia during the 2–4 weeks following cross-circulation with a leukemic donor. Both irradiated rats were of Sprague-Dawley strain, and the leukemic donors were of Sherman strain. In one animal the blood findings of leukemia were verified by autopsy which revealed typical hepatic and splenic infiltration by the malignant leukocytes.

Ten normal recipients of blood from leukemic cross-circulation partners were observed for periods of two months or longer after cross-circulation. Five animals became leukemic, and five have remained normal. Of the five rats that developed leukemia, three retained high tail-blood leukocyte counts continually from the time of cross-circulation and died within two weeks; one had a few days with normal blood count; and another had a 3–4 week hiatus before the leukemia became evident.

DISCUSSION

These observations on the tail leukocyte counts of rats following cross-circulation procedures clearly indicate that white blood cells transfused by this method are not immediately destroyed in the recipient. In the irradiated recipient rat, the total tail leukocyte count was increased for periods of at least three days, and in normal recipients of leukemic blood definite leukemic leukocytes were

detected in tail blood for a similar period following the procedure. After cross-circulation with normal rats, the tail counts of the irradiated recipients were increased both during and after the arterial blood changes had subsided. These changes probably were not due to new leukocyte production by the irradiated recipient rat, since the radiation dosage used was quite excessive. Also it has been shown that the bone marrow maturation time for granulocytes takes longer than one or two days.⁵ It was concluded then, that the rapid disappearance of transfused leukocytes from the arterial blood of irradiated leukopenic animals represents redistribution in the total body "pool" of leukocytes.

Our observations on rat tail blood indicate that certain vascular channels, probably small venules or capillaries, represent one portion of the noncirculating leukocyte "pool." The progressively decreasing leukocyte count of the tail after vigorous "milking" suggests that the rodent tail, with its sluggish capillary and venous network, serves normally as a leukocyte reservoir. The best explanation for the progressively falling tail count of the normal rat is that the vascular channels near the site of the cut are actually "milked" of their surplus leukocytes both granulocytes and lymphocytes. Consistent with this hypothesis are the observations of Quimby and Goff⁶ that vasoconstriction during fatal injury causes marked retention of leukocytes in the tail of rats. These authors also emphasize that tail "milking" does not cause dilution from tissue fluid. Veglens⁷ has observed a similar phenomenon in man, with the highest leukocyte count from a finger prick obtained with the first droplet of blood expressed. Even more marked leukocyte accumulation in small vessels was noted by Lucey⁸ in studies of human ear lobe blood. An opposite effect (i.e., increasing leukocyte count) would be expected if leukocyte aggregation occurred at the site of endothelial damage of the incision, and this effect is seen in repeated sampling from rabbit ear incisions.⁹ In rats, also, leukocyte adhesion does occur when repeated daily samples are obtained from the same tail site, giving an apparent progressive leukocytosis.

The concept of immediate destruction of transfused leukocytes is not supported by several experimental observations. In general, homologous tissue transplantations are not immediately rejected unless the individual has been previously sensitized, and the leukocyte would probably behave in a manner similar to tissue cells. Homologous transfer of rabbit lymph node cells has been performed, with clear evidence that the transplanted cells continue to produce specific antibody in the new host.¹⁰

The prolonged observations on rats that underwent circulatory mixing with leukemic donors were of interest. Two irradiated rats survived the acute radiation damage, after obtaining many leukemic cells by cross-circulation, although the radiation dosage used has been reported over 90 per cent lethal.¹¹ Although we have not determined the lethal rate of our irradiation procedure, these were the only rats in many experiments that did survive both the irradiation and cross-circulation procedure. This would suggest, then, that the leukemic cells protect against radiation damage. Congdon et al.¹² have demonstrated radio-protective effects of blood from mice with extreme leukocytosis. Although these two rats survived the radiation, both developed leukemia. This transmission of the disease from the leukemic Sherman strain rats to the recipient Sprague-Dawley strain irradiated recipients was not unexpected since severely irradiated animals have been shown to have accepted markedly heterogeneous transplants.¹³

The high incidence of chloroleukemia (50 per cent) transmitted from adult Sherman strain leukemic rats to other adult Sherman strain normal rats was unexpected. Adult rats are markedly resistant when inoculated with leukemic cell suspensions by the usual methods.¹⁴ The success achieved in transmitting the disease by the cross-circulation technique was probably related to the large number of leukemic cells transferred or to the minimal cell trauma incurred during cross-circulation.

SUMMARY

1. Recipient rats were observed following periods of circulatory mixing with normal or leukemic donor animals.

2. The normal rat tail appears to store leukocytes in its vascular channels, as evidenced by a progressively falling leukocyte count from a tail incision as the tail is "milked."

3. Comparison of irradiated rat tail and arterial blood leukocyte counts during and after cross-circulation revealed the tail values to be consistently higher than the simultaneous arterial levels. This observation excludes a major removal and destructive mechanism by some organ such as the lung as a major cause of the rapid decrease in arterial blood leukocyte levels noted in irradiated rats after the cross-circulation procedure.

4. Tail blood leukocyte counts of irradiated, cross-transfused rats remained higher than those of control irradiated rats for at least three days.

5. After cross-circulation with leukemic donor rats, leukemic cells were identified in the tail blood of normal rats for at least three days.

6. Prolonged observations revealed that 2 irradiated Sprague-Dawley strain recipients of leukemic cells from Sherman strain leukemic donors survived the severe radiation damage but developed leukemia. This observation suggests that transfused leukemic white blood cells protect against radiation damage.

7. Five normal Sherman strain rats developed leukemia after cross-circulation with leukemic Sherman donors. This incidence (50 per cent) of transmission to adult animals is much higher than has been reported by conventional methods of transfer.

8. The observations reported support the concept of viability of leukocytes transfused in rats by this method, and suggest that at least one site of non-circulating transfused leukocytes is within sluggish vascular channels.

SUMMARIO IN INTERLINGUA

1. Rattos recipiente esseva observate post mixtion de lor sanguine per circulation cruciate con altere rattos normal o leucemic.

2. Il pare que le cauda de rattos normal accumula leucocytos in su canales vascular. Iste postulato es supportate per le observation de un progressive reduction del numeration leucocytic in specimens de sanguine obtenite successivemente per pression ab un incision del cauda.

3. Le comparation de numerationes leucocytic in sanguine caudal e arterial de rattos irradiate, tanto durante como etiam post circulation cruciate, revelava que le valores ab le caudas esseva uniformemente plus alte que le simultanee valores arterial. Iste observation elimina le possibilitate que un significative mecanismo de abferimento e destruction per alicun organo, per exemplo le pulmones, pote esser considerate como un causa major del rapide reduction in le

nivellos leucocytic de sanguine arterial que es a observar in rattos irradiate post le manovra del circulation cruciate.

4. Le numerationes leucocytic del sanguine caudal in rattos irradiate e subjicite a transfusion cruciate remaneva plus alte, durante al minus tres dies, que illos de rattos de controlo irradiate sed non subjicite a transfusion cruciate.

5. Post circulation cruciate con rattos leucemic como donatores, cellulas leucemic esseva identificate durante un periodo de al minus tres dies in le sanguine caudal de normal rattos recipiente.

6. Observationes prolongate revelava que 2 irradiate rattos del racia Sprague-Dawley, que recipeva cellulas leucemic ab donatores leucemic del racia Sherman, superviveva al sever lesiones radiational sed disveloppava leucemia. Iste observation suggere que le transfusion de leucemic leucocytos effectua un protection contra lesiones radiational.

7. Cinque normal rattos del racia Sherman disveloppava leucemia post circulation cruciate con leucemic donatores del mesme racia. Iste incidentia de transmission de leucemia a animales adulte amontava a 50 pro cento es esseva multo plus alte que illo reportate super le base del uso de methodos conventional de transferimento.

8. Le observationes hic reportate supporta le conclusion que leucocytos transfundite in rattos per iste methodo es viable. Illos suggere que al minus un sito de non-circulante leucocytos transfundite es le ralentate canales vascular.

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