

Leukocyte Changes During Acute Cross-Circulation Experiments Between Leukemic and Normal or Irradiated Rats

By J. W. HOLLINGSWORTH AND S. C. FINCH

With the technical assistance of MARY CHALTAS

THE viability and fate of circulating and transfused mammalian leukocytes are poorly understood. In order to investigate some of these problems more completely high leukocyte count (leukemic) donor rats were cross-circulated with normal and leukopenic recipients. Post cross-circulation hematologic observations in these animals indicate that donor-recipient leukocyte concentration relationships may be an important determinant of blood leukocyte survival.

Leukopenic rats retain leukocytes in their peripheral blood for very short periods of time following cross-circulation with normal animals.¹ There is good evidence, however, that the cross-transfused leukocytes remain viable in non-circulating sites for much longer periods of time.^{1, 2} These data suggest that the blood survival time of transfused leukocytes might be increased if the "pool" of tissue leukocytes was normally saturated, and acted to impede circulatory loss. In order to test this hypothesis attempts first were made to "saturate" leukopenic animals with large volumes of leukocytes in order to slow the rate of peripheral leukocyte removal. Secondly, the peripheral leukocyte counts of normal rats were elevated greatly through cross-circulation with leukemic rats in order to demonstrate the ability of normally saturated reserves to affect more prolonged circulation of the transfused cells.

METHODS

Cross circulation experiments were performed between Sherman rats with the Shay granulocytic chloroleukemic and normal rats of the same strain. A similar number of experiments between irradiated and chloroleukemic rats were performed. Most of the irradiated recipient rats were of the Sprague-Dawley strain. All donor animals weighed from 200-300 grams and each was matched with a recipient of similar size. The irradiated rats received an 800 roentgen total body air dose of x-ray. Each was subjected to the cross circulation procedure 3 days post irradiation. In all of the studies cross-circulation between the femoral artery and vein was established by the method of Brodish and Long.³ Cross circulation was continued until 200-300 per cent circulatory mixing had occurred (60-90 ml.). Total and differential leukocyte counts from femoral arterial blood were performed before, during and following each cross-circulation study in both animals. In some selected instances tail blood samples were obtained and followed for much longer periods of time.

In order to obtain adult animals with leukemia, suckling rats were inoculated intraperitoneally with a splenic cell suspension from a leukemic donor animal.* Most of the animals developed leukemia at a time when they weighed 100-150 Gm. Since they were too small for

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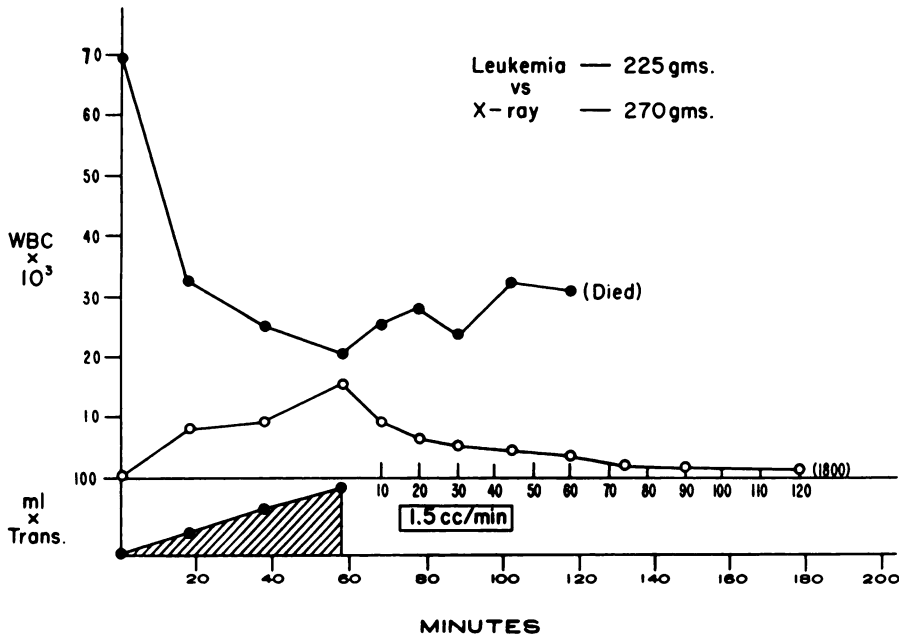


FIG. 1.—Leukocyte changes during and after cross-circulation between a leukemic donor and an irradiated recipient rat. The period of circulatory mixing is indicated by the diagonally striped area. The leukocyte counts of the leukemic donor animal are designated by solid dots and those of the irradiated recipient by open circles.

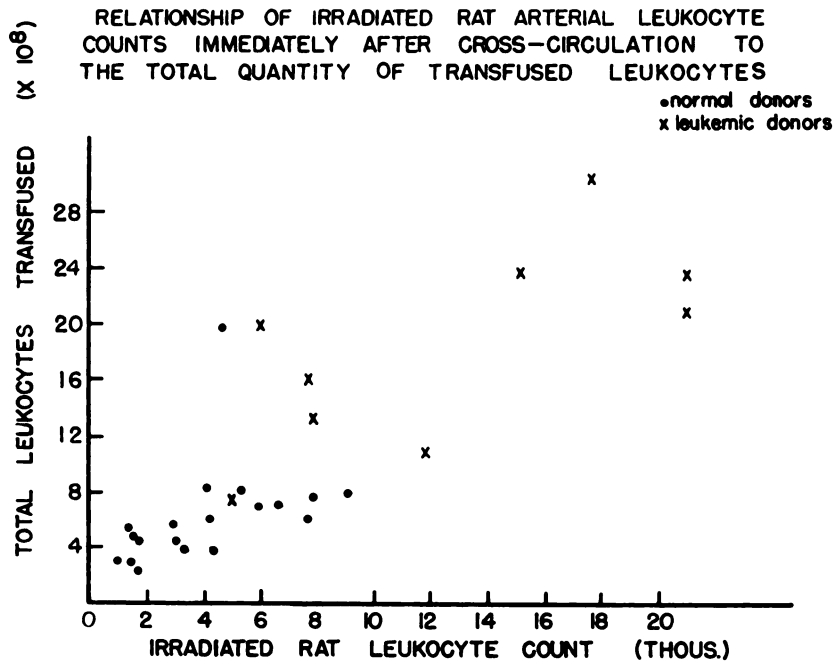


FIG. 2.—The leukocyte levels attained in irradiated recipient rats were roughly proportional to the total number of transfused leukocytes. This relationship appears to have been maintained with both leukemic and normal leukocyte populations.

the cross-circulation studies, the leukemia was temporarily suppressed with triethylene thiophosphoramide (Thio-Tepa)⁴ and allowed to relapse when the rats were considerably larger. From one to three intraperitoneal injections (10 mg./Kg.) of Thio-Tepa usually was sufficient to cause these temporary remissions. Arterial leukocyte counts of the leukemic rats in relapse occasionally went as high as 550,000/cu. mm. This type of leukemia closely resembles human subacute or chronic granulocytic leukemia in its clinical and morphologic aspects.⁵

RESULTS

When irradiated leukopenic rats were cross-circulated with leukemic animals, the arterial blood leukocyte counts of the irradiated animals were similar to those obtained when normal rats served as the leukocyte donors. In a typical experiment (fig. 1) it is evident that the cross-circulation resulted in a marked drop in the leukocyte count of the leukemic donor, while the count of the irradiated rat was increased moderately. Equilibration of leukocyte counts did not occur with continued cross-circulation. After the procedure was terminated the irradiated rat count fell rapidly. Arterial blood leukocyte levels of irradiated rats at the cessation of circulatory mixing appeared directly proportional to the total number of leukocytes transfused, regardless of whether the cells were normal or leukemic

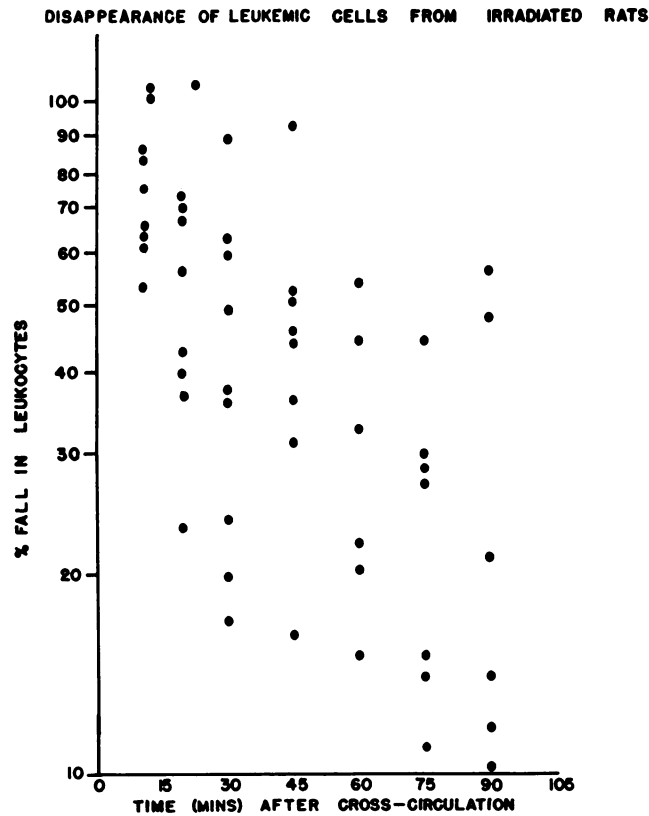


FIG. 3.—The arterial blood leukocyte levels of irradiated rats after cross-circulation with leukemic donors decreased rapidly in an exponential fashion.

(fig. 2). After cross-circulation was discontinued, arterial blood leukocyte levels decreased rapidly during the 90 minute period of observation. The rate of decrease of these leukemic cells in the irradiated rat (fig. 3) appeared almost identical with the disappearance rate of transfused normal leukocytes.¹

When normal rats were cross-circulated with leukemic animals the arterial blood leukocyte counts of the normal recipients were increased greatly. Immediately after cross-circulation, the leukocyte levels of the recipients studied in 9 separate experiments were increased from 240–1980 per cent with a mean of 530 per cent. A high level of leukocytosis was maintained during the entire 90 minute period of observation following cross-circulation. At the end of the observation period, definite leukemic cells (8–50%) were identified in all animals. The mean arterial leukocyte counts of the 9 normal rats before and after circulatory mixing with the leukemic animals are compared in figure 4 with the mean arterial leukocyte counts of three pairs of normal rats subjected to similar periods of circulatory mixing. The cross-circulation procedure *per se* induced no leukocytosis in the normal animals.

The leukemic donor animals, during cross-circulation, demonstrated a marked fall in leukocyte levels with only a moderate increase during the 90 minute observation period after the procedure. The mean changes of arterial leukocyte levels of leukemic rats after cross-circulation with either normal or irradiated recipient rats are shown in figure 5. As compared to the normal donors, the leu-

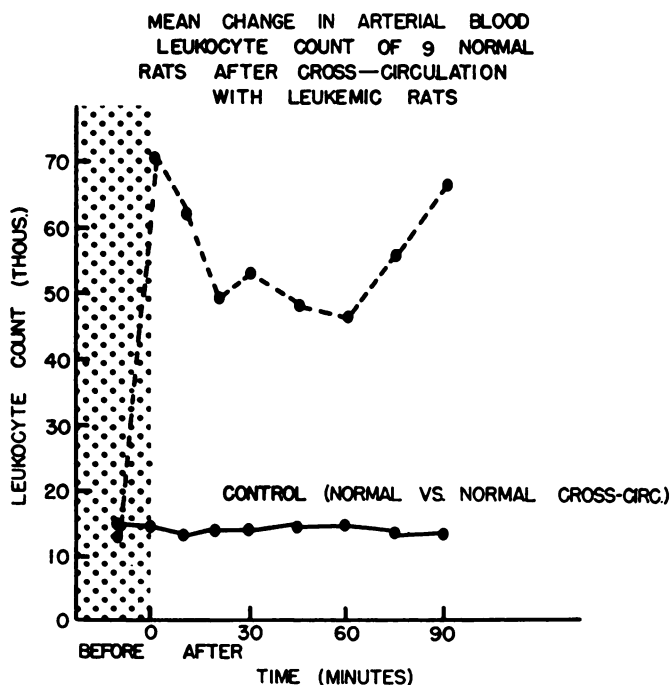


FIG. 4.—The leukocyte count of normal rats after cross-circulation with leukemic donors (interrupted line) remained markedly elevated after the procedure was discontinued. The procedure itself caused no leukocytosis, as indicated by the results of cross-circulation between normal animals (solid line).

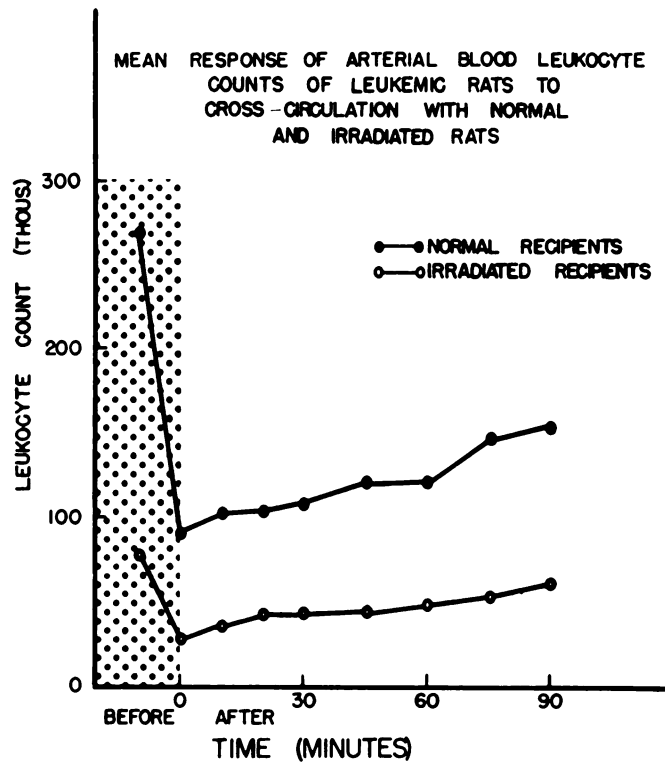


FIG. 5.—At the completion of cross-circulation the leukocyte counts of the leukemic rats had fallen from the initial levels shown in the stippled area to the levels designated at time 0. Thereafter, the replacement rate of leukocytes into the circulation was comparable in the two leukemic groups studied.

kocyte counts of the leukemic donor rats showed greater depression and less tendency to recover after cross-circulation. Systematic observations of arterial leukocyte levels at later times were not performed, but the cross-circulation procedure appeared to have no effect on the mortality rate of the leukemic animals.

DISCUSSION

Leukemic animals were used in our studies for the sole purpose of providing a large donor leukocyte population. The study was not designed to compare the peripheral blood survival of leukemic leukocytes with that of normal leukocytes. It is of vital importance to note that the post cross-circulation survival rates of leukemic and normal leukocytes in x-ray leukopenic rats were identical. This indicates that under these experimental conditions leukemic cells of this type behave and are handled in much the same way as are normal leukocytes. With this in mind a valid comparison can be made between the disappearance rates of normal and leukemic leukocytes introduced into the peripheral circulation of normal and irradiated leukopenic rats. In our studies the transfused leukemic leukocytes disappeared from the circulation of normal rat partners at a much

slower rate than from the circulation of irradiated rat partners. The most likely explanation for these differences in clearance rates lies in the size of the total body "pools" of leukocytes. It seems probable that the irradiated rats were depleted of a large volume of noncirculating leukocytes so that a relatively small volume of leukocytes entering their circulation would be removed rapidly from the peripheral circulation in the process of filling the vacant reserve areas. The introduction of 5-10 times the normal number of leukocytes in the form of leukemic cells was insufficient to alter this equilibration rate. On the other hand, the normally saturated leukocyte "pools" of the nonirradiated recipients allowed the entering leukocytes to remain in circulation for much longer periods of time. The results of these cross circulation studies are summarized in figure 6.

It is possible that the more rapid rate of removal of the transfused leukocytes from the circulation of the irradiated rats might be explained by some enhanced removal mechanism. It seems highly improbable that increased reticulo-endothelial system activity could account for the accelerated leukocyte disappearance rate since the functional capacity of this system is either unaltered or impaired by this amount of irradiation.^{6,7} Local vascular factors might play a role, but the removal rate of infused blood platelets⁸ and particulate material^{6,7} is not

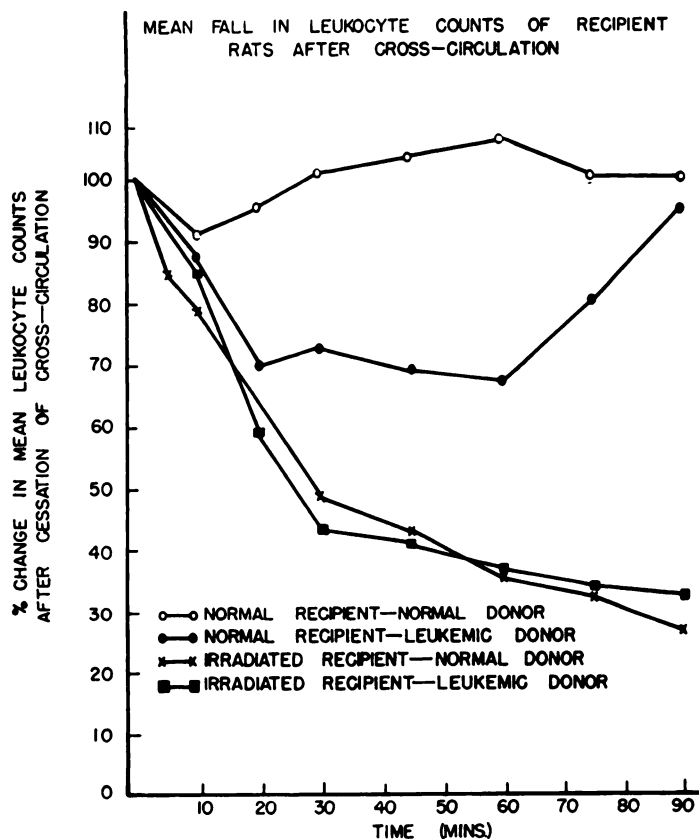


Fig. 6.— Mean percentage change in leukocyte levels of various recipient rats during 90 minutes observation after cross-circulation.

abnormally rapid in irradiated animals. In previous experiments¹ the rate of decrease in transfused leukocytes was found to be rapid when the leukopenia was induced by drugs, rather than by irradiation.

The possibility must be considered that the large numbers of leukocytes in the peripheral blood of the normal recipients following cross-circulation with leukemic donors were the result of an induced leukocytosis. That this was not the case is emphasized by the fact that the peripheral blood of the cross circulated recipients retained leukemic leukocytes during the period of observation in numbers which were about equal to those of the equilibrated leukemic donors. This observation lends additional support to the contention that the added cells are completely responsible for the persistent leukocytosis of the normal recipient animals.

The normal rat contains a lower percentage of granulocytes in his peripheral blood than does either man or many other species. However, the peripheral granulocytes of the rat presumably are in equilibrium with the total body granulocytic reserves in a fashion similar to that of other species. Although the total number of cells involved may not be identical the same factors which play a role in maintaining the balanced state should apply to any granulocyte system. Our observations do not differentiate the effects of a normal lymphocyte population from those of a normal granulocyte population in regulating the outflow rate of added granulocytes. However, they do point out the importance of the leukocyte "pool" in the regulation of peripheral leukocyte levels.

Most observers who have studied the fate of transfused leukocytes have noted the rapid disappearance of these cells from the circulation after transfusion into leukopenic recipients.⁹⁻¹² The thesis has been advanced that the capillary bed of the lung represents a major removal site of normal and transfused leukocytes. Bierman et al. in man¹³ and Ambrus et al. in dogs¹⁴ have found that normal lung removes leukocytes, as indicated by a decreased leukocyte count in blood leaving the lung. If the leukocytes removed by the normal lung were destroyed, the calculated leukocyte lifespan in man would be only a few minutes, while leukocyte lifespan estimated by many other more physiologic methods¹⁵ indicate a lifespan of days to weeks. Only saline washed leukocytes obtained from peritoneal exudates have been shown to be definitely lysed.¹⁶

It seems very logical that pulmonary endothelium should remove from circulation transfused leukocytes which have been damaged either through physical trauma or immunologic incompatibility. The lungs represent the first major capillary network to which intravenously injected cells come into contact. In many of the previous experiments either physical or immunologic donor leukocyte damage may have played a major role. Moeschlin¹⁷ has emphasized the importance of the pulmonary capillaries in the removal of immunologically altered leukocytes from the circulation. Our studies indicate that in the absence of leukocyte damage the reticulo-endothelial tissue or pulmonary vascular bed *per se* does not rapidly remove transfused leukocytes from circulation. If a vigorous and active pulmonary endothelial system had been clearing large numbers of transfused leukocytes from circulation one would have expected a more rapid rate of removal of these cells from the circulation of the normal recipient than from that of the irradiated leukopenic. Just the reverse was observed.

The existence of a large noncirculating leukocyte pool as described by Osgood¹⁸

is most compatible with our observations. Osgood points out that circulating leukocytes of the normal animal constitute a small proportion (probably less than 1 per cent) of the total number of viable leukocytes in the body. Recent studies by Craddock et al.¹⁹ appear to confirm this impression and suggest that a major site of leukocyte storage is in the bone marrow. Additional evidence consistent with the existence of a leukocyte "pool" which bears on the viability of transfused leukocytes has been obtained by other investigators. Ambrus et al.²⁰ have demonstrated leukocyte filtration by capillary beds other than the pulmonary bed, and have shown that continued perfusion eventually satiated the leukocyte filtering activity of a capillary network.²¹ Brecher, Wilbur and Cronkite²² observed that carefully collected leukocytes disappeared rapidly from the circulation after infusion into irradiated dogs, but were capable of migration to sites of infections. Ambrus et al.²³ observed that injection of epinephrine caused the reappearance in the circulation of transfused atabrine-labeled leukocytes. Bierman and co-workers²⁴ performed cross-circulation studies between a patient with chronic granulocytic leukemia with a high white cell count and another with lymphocytic leukemia with a low white cell count. The conditions of this human cross-transfusion study were very similar to those of our rat experiments, and it is interesting to note that the granulocytic cells were not removed rapidly from the blood of the recipient.

The problem now exists as to the location of the leukocyte "pools," and the fate of the transfused leukocytes which so rapidly leave the peripheral circulation of the irradiated recipients. The post-circulation arterial leukocyte observations in this study were short because of technical reasons. However, additional studies indicate functional viability for transfused leukocytes in leukopenic recipients up to several days or more, and demonstrate at least one collection site for these cells.²⁵

SUMMARY AND CONCLUSIONS

1. Normal and irradiated rats underwent periods of cross-circulation with Shay granulocytic chloroleukemic rats.
2. The leukemic white blood cells disappeared rapidly from the arterial blood of the irradiated rat at a rate approximately identical with that of the removal of cross-transfused normal leukocytes.
3. Many leukemic cells remained in the arterial blood of normal recipient rats during the 90 minute observation time after cross-circulation.
4. It was postulated that leukocytes transfused in rats by this method remain viable. In the irradiated leukopenic recipient, the rapid arterial blood removal rate of transfused cells may represent distribution in a depleted total body leukocyte "pool." Similarly, distribution may occur more slowly in normal animals with normally saturated tissue stores of leukocytes.
5. There was no indication that a large dose of total body irradiation increased the capacity of the reticulo-endothelial system to remove transfused leukocytes from circulation.

SUMMARIO IN INTERLINGUA

1. Rattos normal e rattos irradiate esseva subiecte a periodos de circulation cruciate con rattos chloroleucemic granulocytic (Shay).

2. Le leucemic cellulas blanc dispaveva rapidamente ab le sanguine arterial de rattos irradiate. Le rapiditate de lor disparition esseva approximativement identic con illo del elimination de transfundite leucocytos normal.

3. Multe cellulas leucemic remaneva in le sanguine arterial de recipientes normal durante le 90 minutas de observation post le circulation cruciate.

4. Esseva postulate que leucocytos transfundite in rattos per iste methodo remane viabile. In le irradiate recipiente leucopenic, le rapide elimination de cellulas transfundite ab le sanguine arterial indica possibilmente que illos es distribuite in le deplete "reservoirs" leucocytic del corpore total. Similente, il es possibile que le distribution occorre plus lentamente in animales normal con normalmente saturate reservoirs de leucocytos in le textos.

5. Esseva notate nulle indication que un grande dose de irradiation del corpore total augmenta le capacitate del systema reticulo-endothelial de eliminar transfundite leucocytos ab le circulation.

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