In Vitro Studies on the Danger of Use of Mixed Bloods for Open Heart Surgery *

ROBERT SCHREK, M.D., WILLIAM E. NEVILLE, M.D.

From the Tumor Research and Surgical Research Laboratories, Surgical and Research Services, Veterans Administration Hospital, Hines, Illinois

POSTOPERATIVE complications in open heart surgery have been attributed to homologous blood syndrome,[5] to the presence of vasotonins in the donor blood,[3] and to red cell destruction[7] or denaturation of plasma protein[6] by the oxygenator. The question arose whether leukocytes might be injured by the oxygenator and whether the injured cells might lead to postoperative complications. By *in vitro* methods we have found that both neutrophils and lymphocytes in the blood used for priming the oxygenator were injured or altered. However, the observed changes in the leukocytes were not due to the oxygenator but were the result of the mixture of bloods of different individuals.

Methods

During eight open heart operations, a rotating disc oxygenator with a Gebauer heat exchanger was primed with four to six units of blood drawn by a commercial laboratory about 20 hours before an operation. The blood was heparinized and refrigerated overnight in accordance with standard practices.

Shortly before the operation, samples of about 20 ml. were obtained from one to three blood units to be used for the oxygenator. The oxygenator was then filled with four to six blood units. Samples were obtained of the mixed bloods before the machine was turned on and immediately after the surgical procedures. After decannulation, the remaining blood was recirculated through the pump oxygenator for an hour and an additional sample was obtained.

To control the experiments on the commercial blood, samples of blood were drawn directly from patients in the hospital. The patients selected were ambulatory and had fairly normal hemograms.

The blood was allowed to sediment at 37° C. for 60 to 90 minutes. The supernatant was removed and centrifuged and the precipitated cells washed and resuspended in equal parts of TC 199 and a compatible human serum to give a concentration of about 3,000 leukocytes per mm^3. The suspensions were distributed in 1 and 3 milliliter amounts in small test tubes (75×13 mm.) and incubated without shaking at 37° C. for two or more days. Aliquots of 3 milliliters were used for neutrophil studies and 1 milliliter, for lymphocytes.

To study and count viable cells, the incubated suspension was transferred to slides which had chambers 38 millimeters in diameter and 0.9 millimeter in height. The cells were studied with an inverted phase microscope and the viable cells were counted in an area 10×0.04 millimeters.[10] The number of viable cells after incubation was expressed as a percentage of the original number before incubation.

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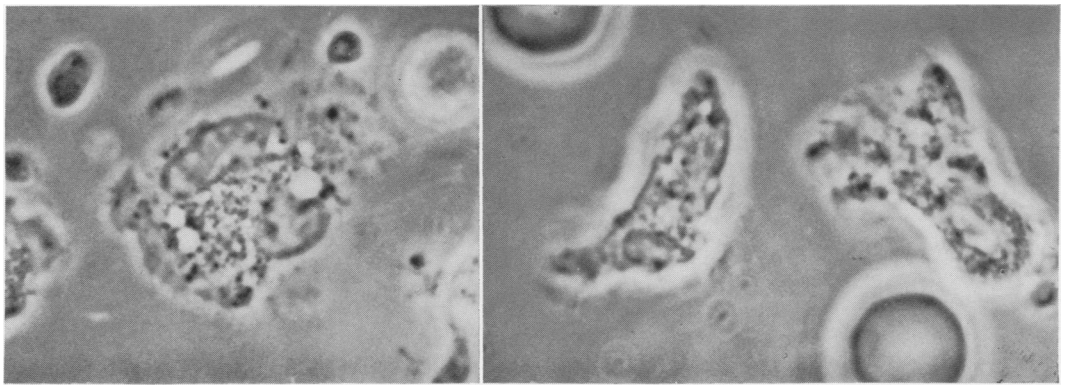

FIG. 1. (left) Viable neutrophil that has flattened itself on glass slide. The cell has a two-lobed nucleus, an endoplasm filled with fine granules and a few small vacuoles, and a clear, almost invisible ectoplasm (Magnification 2,000×).

FIG. 2. (right) Two non-flattened, motile neutrophils filled with fine granules. The cell at the right has pseudopods extending upward and a smooth posterior end. The nuclei are not visualized (Magnification 2,000 ×).

Observations

In vitro survival of neutrophils. The *in vitro* survival of neutrophils was determined with freshly drawn blood from 19 individuals. Morphologic characteristics were used as criteria of the viability of neutrophils. The neutrophil in a freshly prepared suspension with fresh serum was seen to flatten and spread on the glass slide, apparently in an attempt to phagocytize the glass (Fig. 1). The polymorphic nucleus was distinctly seen, usually with a small central dark chromatin mass in each lobe of the nucleus. The cytoplasm had clearly defined ectoplasm and endoplasm with many fine dark granules which showed cytoplasmic streaming. The cell wall was usually clearly outlined and showed slow undulating movements.

After a few hours of incubation in the slide chamber, the neutrophil lost to a considerable extent its tendency to flatten (Fig. 2). The non-flattened neutrophil was elongated and frequently actively motile.

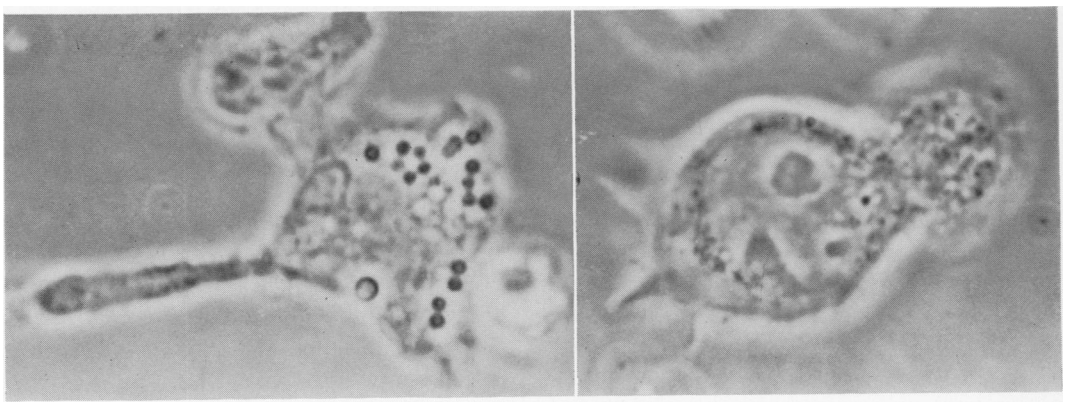

FIG. 3. (left) Viable neutrophil incubated at 37° C. for 5 days. The cell has a few large lipid granules. Above the neutrophil is a motile lymphocyte which is out of focus.

FIG. 4. (right) Lymphoblastoid cell in 6 day culture of mixed blood, removed from pump oxygenator before surgery. The cell has a large nucleus with 2 nucleoli, one of which is attached to the nuclear membrane (Magnification 2,000 ×).

TABLE 1. *Effects of a Rotating Disc Oxygenator and of Mixing of Bloods on the Survival of Neutrophils Incubated in Test Tubes for Two to Five days*

	Average Percentage of Neutrophils Surviving Incubation at 37° C. for			
	2 days	3 days	4 days	5 days
Fresh blood	56 (2)	44 (19)	26 (5)	16 (6)
Individual blood units used for oxygenator	34 (6)	33 (1)	10 (2)	—
Blood from oxygenator				
1) Before operation	24 (7)	7 (5)	0.1(3)	—
2) After operation	75 (7)	76 (6)	49 (4)	—
3) 1 hour after operation	64 (4)	66 (3)	63 (2)	—
Mixture of 2 leukocyte suspensions derived from 2 freshly drawn bloods	18 (1)	15 (5)	3 (2)	1 (2)
Mixture of leukocyte suspension and purified lymphocyte suspension derived from 2 freshly drawn bloods	18 (2)	4 (4)	—	—

Number of observations are shown in parentheses.

The anterior end consisted of a granular-free pseudopod with a soft irregular outline. The posterior end had a rounded, firm, dark outline. The cell was filled with fine granules and the nucleus could be seen only occasionally during the movement of the cell.

After incubation for two to five days in test tubes, many cells were seen to have the morphologic characteristics of non-flattened viable neutrophils as seen in the original suspension. In many suspensions, the surviving neutrophil had a few large brownish granules which were located near the upper surface of the cell and were presumably lipid granules (Fig. 3). Dead neutrophils were perfectly spherical with relatively few granules and a pyknotic or lysed nucleus. The morphologic characteristics that were considered to indicate viable neutrophils were irregular shape, anterior pseudopod, closely packed fine granules and streaming of the cytoplasm.

The average percentage of neutrophils that survived in suspensions from freshly drawn normal blood was 44 per cent after three days and 16 per cent after five days of incubation (Table 1).

Blood samples obtained from the oxygenator after the completion of the surgical procedures consisted largely of the patient's own blood. Examination of suspensions from these blood samples showed an unusually high percentage of neutrophils, presumably as a result of leukocytosis that developed during operation. The neutrophils that survived in the sample obtained after operation were 76 and 49 per cent after three and four days incubation, respectively. These percentages are significantly higher than those obtained with freshly drawn blood posssibly due to the presence of young leukocytes released during operation.

Four samples of blood obtained one hour after operation occasionally showed evidence of slight hemolysis in the sera due to damage to red blood cells during one hour in the oxygenator. In these samples, 66 and 63 per cent of the neutrophils survived three and four days of incubation. These percentages are not significantly different from those for samples obtained immediately after operation. In other words, the tests did not show any significant damage to neutrophils circulated through the oxygenator for one hour.

TABLE 2. *Effect of a Rotating Disc Oxygenator on the Survival of Lymphocytes Incubated in Test Tubes for Two to 15 Days*

	Average Percentage of Lymphocytes Surviving Incubation at 37° C. for			
	2 days	5 days	6 days	15 days
Fresh blood	81 (3)		48 (5)	28 (3)
Individual blood units used for oxygenator	66 (2)		58 (2)	32 (1)
Blood from oxygenator				
1) Before operation	63 (1)		36*(1)	3 (1)
2) After operation	78 (1)		64 (1)	
Mixture of 2 purified lymphocyte suspensions		82.2*(2)		

Number of observations are shown in parentheses.
* The incubated suspension had a few large lymphoblastoid cells and an occasional cell in mitotic division.

Of particular interest were the samples obtained from the machine before operation. These samples represented a mixture of four to six compatible blood units about 20 hours after bleeding. The percentages of neutrophils surviving three and four days of incubation were relatively low (7 and 0.1 per cent, respectively). It was concluded that the neutrophils in the mixed blood sample were physiologically injured as evidenced by a decreased capacity to survive *in vitro*.

The question then arose whether the poor condition of the neutrophils was due to mixing of the bloods or to other factors, such as refrigeration for 20 hours before testing. Samples of the individual blood units were, therefore, tested. The survival percentages of the neutrophils in suspensions from these samples was slightly less than the neutrophils from freshly drawn blood but was greater than in the mixed blood. These findings indicate that mixing of bloods of different individuals had deleterious effects on the neutrophils so that the cells showed reduced capacity to survive *in vitro*.

It seemed to us surprising that a mixture of bloods should affect the survival of the neutrophils *in vitro*. Therefore, in five experiments two freshly drawn bloods of the same blood group were processed to give two cell suspensions which were mixed and the survival of neutrophils in the mixture was studied. The neutrophils in four mixed samples showed an average of only 15 per cent survival after three days incubation as compared to 44 per cent in the control blood samples. These experiments confirmed the findings with the mixed blood samples obtained before heart surgery.

The next question that developed was whether the neutrophils of one person were reacting to neutrophils, lymphocytes, or other cells of a second person. To study this problem, cell suspensions from bloods of two individuals were prepared. One of the cell suspensions was purified by methods described elsewhere[14] to eliminate most of the granulocytes and monocytes. In the resulting suspension, 90 per cent or more of the nucleated cells were lymphocytes. In a mixture of the purified and non-purified suspensions, only 4 per cent of the neutrophils survived three days (Table 1). It would appear from this result that mixture of neutrophils of one person with lymphocytes of another resulted in injury to neutrophils as evidenced by decreased *in vitro* survival.

Reactions of lymphocytes. Counts were also made of the number of viable lymphocytes to determine the *in vitro* survival capacity of lymphocytes in the various samples of blood. Criteria of viability of lymphocytes have been described previously[5] and include morphologic integrity of the nucleus and irregularities in the shape of nuclear or cellular walls.

It is seen in Table 2 that control, freshly drawn blood showed high percentages of surviving lymphocytes even after six and

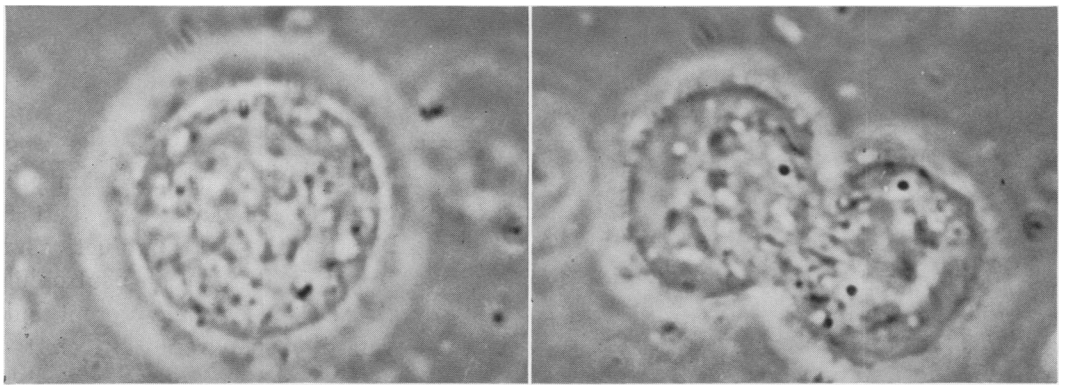

FIG. 5a, b. Cell in mitotic division in six day culture of mixed blood removed from pump oxygenator before surgery. Figure 5b was taken six minutes after Figure 5a (Magnification 2,000 ×).

15 days of incubation. Comparison of Table 1 and 2 shows that normal human lymphocytes survived longer *in vitro* than neutrophils. In the blood samples obtained during heart surgery, the lymphocytes in the various samples showed approximately the same survival percentages as in the control, freshly drawn blood. It was evident that *in vitro* survival capacity of lympocytes was not appreciably affected by the various procedures use in refrigeration and mixing of bloods before operation.

Microscopic observations of the cells of the mixed blood obtained before operation showed peculiar morphologic changes in cells. After three to six days of incubation, the suspensions from these samples were found to contain a small number of large lymphoblastoid cells (Fig. 4). The cells had large clear, gray nuclei with one or more large irregularly shaped nucleoli which were frequently adherent to the nuclear wall. These cells were observed in the samples of mixed bloods obtained during each of eight operations but not in the samples of the individual blood units nor in the blood obtained after operation.

In addition, a few cells in mitotic division (Fig. 5a, b) were observed in suspensions derived from the mixed blood. The dividing cells were usually large and were presumably lymphoblastoid cells. A few dividing

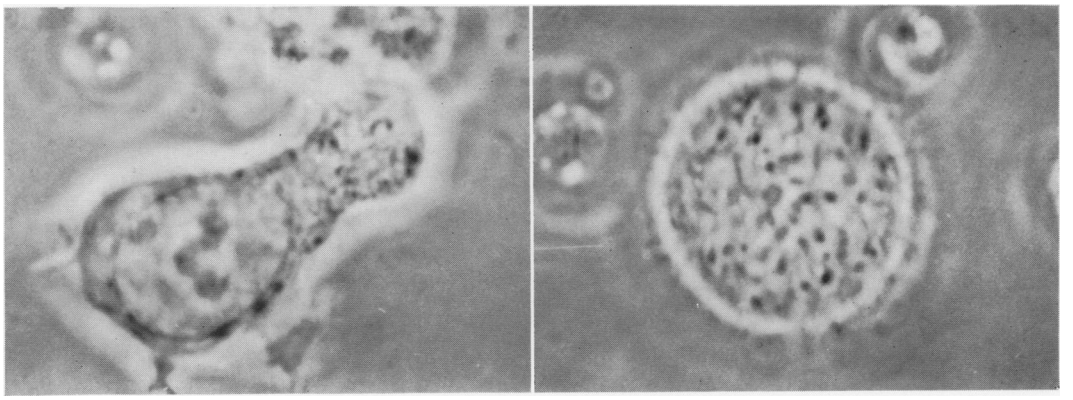

FIG. 6. (left) Lymphoblastoid cell in six day culture of a mixture of 2 lymphocyte suspensions (Magnification 2,000 ×).

FIG. 7. (right) Cell in mitosis in five day culture of a mixture of 2 lymphocyte suspensions (Magnification 2,000 ×).

cells were followed and gave rise to fairly large daughter cells which were seen to develop nucleoli in about two hours.

To determine the origin of lymphoblastoid and mitotic cells, blood cell suspensions of two freshly drawn bloods were purified. Incubation of a mixture of two lymphocyte suspensions produced a small number of lymphoblastoid and dividing cells similar to those described previously (Fig. 6, 7). The findings indicate that mixture of blood from two individuals resulted, after five days of incubation *in vitro*, in the transformation of a few lymphocytes into lymphoblastoid cells, some of which underwent cell division.

Discussion

During open heart surgery, the pump oxygenator is frequently primed with several units of blood which is then perfused into the patient. The danger of an immunologic, graft versus host, reaction as a result of this massive transfusion has been pointed out by Petrakis and Politis.[8] A possible graft versus host reaction may have been observed by Seaman and Starr[15] in nine post-cardiotomy patients.

The present study gives cytologic evidence of the possibilities of homograft reactions following mixing of bloods of two or more individuals. The lymphoblastoid and mitotic cells observed in suspensions derived from the mixed blood in the oxygenator were similar to cells that developed after exposure of lymphocytes to phytohemagglutinin,[12] tuberculin,[9] diphtheria and tetanus toxoids and other antigens.[4] It seems that lymphoblastoid and mitotic cells are an indication of an immunologic reaction by lymphocytes to an antigen.[4, 13]

Lymphoblastoid cells in mixed bloods have been observed previously in this laboratory.[11] An extensive study of the development of blast cells and mitoses in mixed blood was made by Bain *et al.*[2] As they failed to find these cells in mixtures of blood from identical twins, they concluded that this finding is a sign of homograft reaction *in vitro* in mixed bloods from two non-related individuals.

Another problem in open heart surgery is the development of post perfusion pulmonary vasculitis. Histologic examinations of lung biopsies showed margination of leukocytes and infiltration of neutrophils into interalveolar spaces.[7] The present findings on survival of neutrophils suggest a possible etiologic factor in the production of pulmonary vasculitis. It was seen that neutrophils in mixed bloods have a decreased capacity to survive *in vitro*. This shortened survival time showed that neutrophils in mixed bloods underwent cell damage or injury. The nature of the injury is not known. However, it would seem that the *in vitro* homograft reaction that occurs on mixing of bloods affects not only lymphocytes but also neutrophils. The homograft reaction produced mitoses in lymphocytes, but caused cell injury and early *in vitro* death in neutrophils.

Extrapolating from the *in vitro* findings, it may be assumed that the neutrophils in the mixed blood have a shortened survival time *in vivo*. These injured cells would be expected to be rapidly eliminated from the circulating blood. The margination of the neutrophils and the infiltration of interalveolar walls may be one means of removal of damaged neutrophils. The infiltration of neutrophils in the lungs may signify not injury to the lung tissue but rather injury of the neutrophils. It is concluded that one of the complications in open heart surgery is due to the transfusions of large amounts of mixed blood in which the neutrophils of the different individuals react to homologous blood cells.

In any transfusion, there is mixing of bloods. Therefore, in all transfusions one might expect, to a greater or lesser extent, the homograft reaction of neutrophils and a shortened survival time of the injured

neutrophils *in vivo*. If this hypothesis is correct, it is necessary to use caution in evaluating the physiology and survival time of neutrophils by experiments involving the transfusion of blood of one animal into another. Furthermore, such transfusion experiments may not give valid findings on the role of the lung in the regulation of the white blood cell level.[1]

Finally, our present findings of the effect of mixing bloods on leukocytes point to the danger of the use of mixed blood for priming oxygenators in open heart surgery. If blood is to be used for this purpose, it would seen best first to remove, by some means, the leukocytes in the blood.

Summary and Conclusions

The effects of a rotating disc oxygenator and of mixing of bloods of two or more individuals on neutrophils and lymphocytes were studied by *in vitro* methods. The oxygenator itself was found to have no appreciable effect on the morphology or the *in vitro* survival of the cells.

Mixture of bloods of two or more individuals produced *in vitro* 1) reduced survival time of neutrophils and 2) the transformation of a few lymphocytes into large lymphoblastoid cells, some of which underwent mitotic division. Both of these effects were considered to be homograft reactions of the lymphocytes of one individual against the lymphocytes and neutrophils of another.

The reduced survival capacity of neutrophils in the mixed blood used for priming oxygenators was considered to be an etiologic factor in post perfusion pulmonary vasculitis which sometimes develop after open heart surgery.

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