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EFFECTS OF EXTRACORPOREAL IRRADIATION OF BLOOD AND LYMPH ON HOMOTRANSPLANTATION*

by

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INTRODUCTION

Extensive investigations have been carried out to determine the role of the small lymphocyte in the process of homograft rejection. Such investigations have included study of the reaction in the regional lymph nodes (1-4), the cellular reaction at the site of transplantation (5-9), and the transfer of transplantation immunity by lymphoid cells (10-11). Studies on animals in which normal development of lymphoid tissue has been prevented (12), small lymphocytes removed from the animal (13), or lymphocytes destroyed by means of anti-lymphocyte serum (14) have yielded circumstantial evidence regarding the role of the lymphocyte in homograft rejection. Evidence regarding the role of a humoral antibody in first set skin homograft rejection is controversial (15-17).

A state of immunologic deficiency can be achieved by lymphocyte depletion, but the methods used to depress the lymphoid immune system, including irradiation (18) and cytotoxic drugs (19,20), suffer from the lack of selectivity. This results in indiscriminate damage to all rapidly dividing cells, including those of bone marrow and intestinal mucosa.

Extensive research is being conducted to discover ways in which selective depression of lymphoid cells can be achieved. The techniques that have been tried include irradiation of lymphoid tissue (21), chronic drainage of thoracic duct (13,22,23), administration of antilymphocyte serum (14) and attempts at selective chemical depression of lymphocytes (24).

It has been shown that immunologically competent small lymphocytes are constantly recirculating through the blood (25). In view of this recirculation, it is perhaps not unreasonable to divert a portion of the blood stream through an extracorporeal shunt in order to destroy these relatively

radiosensitive lymphocytes in a suitable radiation field. Procedures for extracorporeal irradiation of blood (ECIB) and lymph (ECIL) were perfected in our laboratory. Although the original description of such a procedure dates back to 1939 (26), it is only recently that the procedure has been effectively used. To date Cronkite and his colleagues have described the production of prolonged and sustained lymphopenia with ECIB (27), the mathematical considerations of the dose distribution to the blood (28), the combined effect of thoracic duct drainage and extracorporeal irradiation of blood upon the concentration of lymphocytes in the lymph and blood (29), the histologic picture of lymphoreticular tissue after prolonged extracorporeal irradiation of the blood (30), the construction and operation of a ^{60}Co irradiator (31), the influence of varying dose and repetitive short sessions of extracorporeal irradiation of blood upon the production of lymphopenia (32), the tolerance of red cells to radiation (33), and semi-permanent arterio-venous and thoracic duct shunts in calves (34). This communication deals with the influence of ECIB and ECIL upon the skin homograft rejection process in calves and discusses the role of immunologically activated small lymphocytes in this process (35).

MATERIALS AND METHODS

A. Skin Grafts: The technique, along with the histological and clinical observations, has been described in detail elsewhere (36). Our method is a slight modification of the technique of Anderson et al. (37). Full thickness pinch grafts are removed from the dorsum of the ear and grafted either on to the withers (anterior grafts), or posterior to the iliac crest (posterior grafts). Unless specifically defined, the word skin graft means anterior graft. Autografts for comparison are utilized

in every case. Biopsies for histological studies are taken at regular intervals. The rejection time for skin homografts in untreated animals is from 8 to 10 days. The criteria for graft rejection are edema, hardening, discoloration and separation of graft and host margins. Actual separation of graft from host bed is not taken into ^{spacing} consideration as this varies considerably from animal to animal.

B. Irradiator: A 11000 curie ⁶⁰Co irradiator has been used. The irradiation coil is wound around the source. The transit dose to elements of lymph or blood depends upon the dose rate of the source, the volume in the radiation coil and the flow rate. Flow rate of the blood is measured by injecting a bubble of air at the arterial end and noting the time it takes to arrive at the venous end. Lymph is intermittently pumped through the irradiation coil at a fixed rate.

C. Arterio-Venous (A-V) and Thoracic Duct Shunts: The shunts, which have been described in detail elsewhere (34), consist of Teflon cannulae and Silastic tubing.¹ For ECIB, A-V shunts are used which function for several weeks. Whenever ECIB is desired, the carotid-jugular shunt is connected to the irradiation coil, and the blood circulates around the irradiation source because of differences in arterial and venous pressures. Heparinization is used during ECIB in order to prevent the blood from clotting in the long irradiation coil. The thoracic duct is cannulated in the neck using smaller sized Silastic and Teflon tubing and lymph is returned via the left inferior cervical vein. These shunts also function for several weeks. Lymph is collected by gravity and returned by pump to the animal². The details of the technique have been described elsewhere (38). The lymph reservoir is continually heparinized. Lymph has been treated in three ways: 1) ECIL,

2) Centrifugation of lymph and return of cell-free lymph to the animals (for this purpose an IEC PR-2 refrigerated centrifuge equipped with a elixtractor was employed which removes virtually all the cells)^{3, 9)} *pumping* of untreated lymph to the rumen through a Foley catheter ruminostomy.

Figures 1 and 2 depict the general arrangements for ECIB and for preparation of cell-free lymph respectively.

D. Antibiotics: Penicillin and Streptomycin are administered I.M. for five days after shunt surgery. Cannulae tracks are flushed out daily with 7-8 ml of saline containing 20 mgm of Terramycin.

E. Animals: Fifty-two calves ranging in weight from 175 to 712 pounds have been used. Eighteen calves served as non-treated comparison animals, 25 calves received ECIB, 5 calves received ECIL, 2 calves had centrifugation of lymph, 1 calf received ECIL and centrifugation of lymph, and 1 calf had lymph pumped to the rumen.

RESULTS:

A. ECIB: (Table 1) Varying schedules and graded transit doses of ECIB were tried. Six calves (the upper part of Fig. 3) received continuous ECIB for 24 hours, each and skin homografts were applied five days later. Transit doses ranged from 15 to 60 rads, and the number of blood volumes irradiated ranged from 90-240. The average pretreatment small lymphocyte count was $2600/\text{mm}^3$. It dropped to $500/\text{mm}^3$ at the termination of ECIB and rose to $1800/\text{mm}^3$ ten days later. Average homograft survival in this group was 10 days.

Three animals received repetitive pregraft ECIB. Results in one of these animals (#113) are depicted in the middle of Figure 3. Two animals received a transit dose of 50 rads each, and 325 and 160 blood volumes

were irradiated. The duration of ECIB was 98 and 112 hours spread over 13 and 19 days respectively. The average pretreatment small lymphocyte count was $4100/\text{mm}^3$ which dropped to $200/\text{mm}^3$ at the termination of ECIB and ranged from 100 to $500/\text{mm}^3$ during the next 15 days. Skin homografts which were applied at the termination of ECIB survived for 11 and 14 days respectively while in non-treated twins of these animals survival time was 9 and 10 days respectively. In the third animal, the transit dose varied from 10 to 1000 rads, and 210 blood volumes were irradiated during 114 hours spread over 57 days. The small lymphocyte count dropped from $4000/\text{mm}^3$ to $700/\text{mm}^3$ at the termination of ECIB at which time skin homografts were applied. Skin homografts survived for 13 days, and at that time small lymphocytes had risen to a level of $1000/\text{mm}^3$.

A group of five animals received pre- and post-graft, repetitive ECIB. Transit doses in this group ranged from 215 to 800 rads, and the number of blood volumes irradiated ranged from 122 to 547. The duration of ECIB varied from 54-118 hours spread over periods of 13 to 30 days. The average pretreatment small lymphocyte count was $2300/\text{mm}^3$ which dropped to a level of $760/\text{mm}^3$ at the time of skin grafting and was $740/\text{mm}^3$ at the time of graft rejection. Skin homografts in this group survived from 12 to 20 days. Fig. 3 (lower part) depicts the animal (#136) which received irradiation to 547 blood volumes at transit doses of 215-770 rads.

B. ECIB AND IMURAN (AZATHIOPRINE):⁴ (Table I) Imuran has been used as an immunosuppressive agent alone or in combination with other drugs, particularly to prolong renal homotransplant survival (39,40). We planned some experiments to determine if a combination of ECIB and small non-toxic dosage of Imuran would yield better results than ECIB alone. Three animals received 1 to 1.5 mgm/kg body weight of Imuran I.V./day after an initial dose of twice this amount. There was no significant change in the level of lymphocytes during administration of Imuran. No prolongation of skin homograft survival was observed in any of these animals, but the one animal on 1.5 mgm/kg of Imuran developed granulocytopenia, the granulocytes dropping from pretreatment level of $2800/\text{mm}^3$ to $400/\text{mm}^3$ by the ninth day. In all subsequent experiments a dose of 1 mgm/kg I.V. daily was employed.

Two animals received repetitive pregraft ECIB and postgraft Imuran. The transit doses were 750 and 2000 rads, and 106 and 49 blood volumes were irradiated. The duration of ECIB was 70 and 49 hours over 8 and 5 days respectively. The average lymphocyte level prior to commencement of ECIB was $3450/\text{mm}^3$, which dropped to $500/\text{mm}^3$ at the end of ECIB. Skin grafts were applied, Imuran started, and ECIB discontinued. Homografts survived for 16 and 15 days respectively. At the time of rejection the average lymphocyte level was $1300/\text{mm}^3$.

In the next experiment, six animals received pre- and post-graft repetitive ECIB and Imuran. The transit dose ranged from 500 to 800 rads, and number of blood volumes irradiated varied from 67 to 152. The

duration of ECIB was from 54 to 78 hours spread over periods of 7 to 17 days. Imuran was continued, however, until the time of skin graft rejection in each animal. The average preexperimental level of small lymphocytes of $3050/\text{mm}^3$ dropped to $500/\text{mm}^3$ at the time of skin grafting and rose to a level of $750/\text{mm}^3$ by the time of skin graft rejection. Skin homografts survived from 12 to 29 days.

C. THORACIC DUCT LYMPH TREATMENT: (Table II) Calves #165 and #185 (Fig. 4) had centrifugation and return of cell-free lymph for 10 and 24 days respectively. The cumulative output of lymphocytes during these periods was 9.3×10^{11} and 14.1×10^{11} respectively. Skin homografts were applied at the end of treatment periods and survived for 10 and 18 days respectively. Pretreatment levels of small lymphocytes in the blood fell from $3400/\text{mm}^3$ and $3000/\text{mm}^3$ to $1800/\text{mm}^3$ and $1200/\text{mm}^3$ at the end of treatment period and rose to $3300/\text{mm}^3$ and $2100/\text{mm}^3$ by the time of graft rejection. Calf #186 (Fig. 4), the twin of #185, had ECIL for 22 days, and the cumulative output of lymphocytes that received irradiation was 13.8×10^{11} . Skin homografts were applied at the end of this treatment and survived for 22 days. The pretreatment level of small lymphocytes fell from $3400/\text{mm}^3$ to $1100/\text{mm}^3$ and returned to $2100/\text{mm}^3$ by the time of graft rejection.

Calf #151 had cell-free lymph returned for 8 days, return of untreated lymph for two days and ECIL for 4 days. During the first 8 days 8.2×10^{11} cells were removed by centrifugation. The number of small lymphocytes in the

peripheral blood dropped from $5000/\text{mm}^3$ to $1900/\text{mm}^3$. A further drop to $800/\text{mm}^3$ occurred during ECIL. Skin homografts, which were applied at the end of treatment, survived for 40 days. The small lymphocyte count in the blood at the time of graft rejection was $1400/\text{mm}^3$. The untreated twin of this animal received skin homografts from the same donor. These grafts were rejected at 10 days.

In order to find an explanation of our failure to achieve long term survival of skin homografts with chronic treatment of thoracic duct lymph, it was decided to study the behavior of anterior and posterior homografts. The anterior grafts, grafted onto the withers, are not usually in the drainage bed of the thoracic duct, but the posterior homografts are completely within its drainage bed. Unrelated donors were used for anterior and posterior homografts. Calves #200 (Fig. 5) and #202 had ECIL for 34 and 68 days. During this period the cumulative output of lymphocytes was 33.5×10^{11} and 28.0×10^{11} respectively. Anterior and posterior skin homografts were applied 7 days after ECIL started. In calf #200 small lymphocytes in blood had dropped from a pretreatment level of $3000/\text{mm}^3$ to $500/\text{mm}^3$ at the time of grafting. Anterior grafts were rejected in 22 days while the ECIL was still in progress. Posterior grafts survived the duration of ECIL. Rejection as evidenced by edema of the homograft commenced 2 days following discontinuation of ECIL and proceeded slowly to completion, the survival time of posterior grafts being 39 days. In calf #202, small lymphocytes in blood had dropped from a pretreatment level of $2400/\text{mm}^3$ to $600/\text{mm}^3$ at the time of grafting. Anterior grafts were rejected on day 27 while ECIL was still in progress. The animal died suddenly on the 68th day of ECIL due to septicemia. At this time posterior homografts were still in good condition, i.e., 61 days after grafting. Calf #203, the twin of #202, acted as an untreated comparison.

Anterior and posterior grafts were rejected at 10 days.

Calf #239 (Fig. 5) received ECIL for 10 days; the cumulative output of lymphocytes was 6.5×10^{11} . Small lymphocytes in the blood dropped from a pretreatment level of $2200/\text{mm}^3$ to $800/\text{mm}^3$. At the termination of ECIL anterior and posterior homografts were applied, which survived for 18 days.

Calf #206 (Fig. 5) received ECIL for 25 days, and the cumulative output of lymphocytes during this time was 15.6×10^{11} . The blood lymphocyte level dropped from $3500/\text{mm}^3$ to $300/\text{mm}^3$ by the 15th day of ECIL but rose up to $900/\text{mm}^3$ by the last day of ECIL. Anterior and posterior skin homografts were applied just before the commencement of ECIL. Anterior grafts were rejected on day 13 while ECIL was still in progress. Posterior grafts survived the duration of ECIL. Evidence of graft rejection was noticed within 24 hours of discontinuation of ECIL. Total survival time for posterior grafts was 28 days.

In order to exclude any effect of irradiation on cell-free lymph itself, a thoracic duct-rumen fistula was established in calf #209. Lymph was pumped from thoracic duct to rumen for 26 days. During this period, 22×10^{11} lymphocytes entered the rumen from the thoracic duct and were presumably destroyed. Blood lymphocytes dropped from a pretreatment level of $3200/\text{mm}^3$ to $700/\text{mm}^3$. Anterior and posterior skin homografts were applied 7 days after thoracic duct-rumen fistula was established. Four days later the thoracic duct cannula was partially clamped to reduce the lymph output from 3 liters/hour to 1.5 liters/hour, in order to prevent hypoproteinemia and diarrhea (41). Anterior and posterior grafts survived for 14 and 18 days respectively.

DISCUSSION:

ECIB modified the normally acute and violent homograft rejection process to a mild and slow one in all the cases at all transit doses. There was,

however, no significant increase in skin homograft survival with pregraft ECIB alone at transit doses varying from 15 to 1000 rads. Somewhat better results were obtained in animals which received repetitive pre- and post-graft ECIB at transit dose range of 200-800 rads. In one animal the skin graft survival time was prolonged to 20 days from the normal of 8-10 days. Combination of ECIB and Imuran resulted in a very slight improvement in results. The maximal skin graft survival time was 29 days in this group. Failure of Imuran to contribute significantly to results was not surprising since we used rather small non-toxic doses. In addition, although Imuran has contributed significantly in the management of patients receiving renal homotransplants (42) it was found to be of little value in prolonging the survival of skin homografts in dogs (43). The failure of ECIB to markedly prolong the skin homograft survival, though disappointing, can be explained by the fact that ECIB kills only a fraction of the recycling immunologically competent and immunologically activated small lymphocytes by shunting only a portion of the blood through the irradiator (Fig. 7). Even a doubling of the normal skin graft rejection time, however, cannot be completely ignored as better results may be possible in other organs, such as the kidney, in which rejection is less violent. Preliminary studies to evaluate the role of ECIB in combination with immunosuppressive therapy to prolong the life of renal homotransplant in patients are in progress in collaboration with Dr. J. P. Merrill's group of Peter Bent Brigham Hospital, Boston (44). ECIB may also condition closely related recipient-donor pairs either alone or in combination with ECIL to postpone the ^{spacing} institution of cytotoxic drugs with all their undesirable side effects.

THORACIC DUCT LYMPH: We had the following three questions in mind when treatment of thoracic duct lymph was contemplated in order to prolong

skin homograft survival:

1) Do the products of cell death or cell bound antibodies that are returned to the animal as a result of ECIB have a deleterious effect on homograft survival?

2) Is there any difference between blood and thoracic duct lymphocytes?

3) Will there be a better survival of those skin grafts which are placed on those parts of the body that are drained solely by the thoracic duct?

To answer the first question, twin pair #185 and #186 was studied (Fig. 4). Calf #185 had centrifugation and return of cell-free lymph for 24 days. Calf #186 had ECIL for 22 days with return of all irradiated cells and any products of cell death to the animal. There was, however, very close approximation in the cumulative output of lymphocytes, blood lymphopenia and skin graft survival time in these two calves. This experiment did not show any deleterious effects of products of cell death on homograft survival.

The answer to the second question is still not clear. The lowest levels of lymphocytes were obtained with prolonged ECIB, yet the longest anterior homograft survival followed ECIL. The observed differences between mean survival were not statistically significant, however. We are not inclined to believe that there are fundamental differences between the blood and thoracic duct lymphocytes.

It is clear from Figure 7 that whatever the mode of sensitization, central at the site of the graft or in the regional lymph node due to the arrival of the graft antigen via lymph vessels (45), the immunologically activated small lymphocyte (25) can get at the posteriorly placed graft only by reaching the blood stream via the thoracic duct. If one prevented these

activated cells from reaching the blood stream by cannulating the thoracic duct and removing or destroying these cells, then the posteriorly placed graft would be expected to survive for as long as these activated cells were prevented from reaching the graft. This was the case in calves #200 and #202. These calves received skin grafts 7 days after ECIL was started. Anterior grafts on the withers on the right side were rejected during ECIL. Posterior grafts in the drainage bed of thoracic duct were not rejected during ECIL. Further indirect support for this hypothesis was provided by the next set of experiments. If our reasoning in regard to the different behavior of anterior and posterior skin grafts is correct, then pregraft lymphocyte depletion of the animal is not required for prolongation of survival of posterior grafts. By the same token, if an animal is depleted before grafting but treatment is discontinued after grafting, the survival of anterior and posterior grafts should not differ. Both of these expectations were borne out by experiments described below. Calf #206 received anterior and posterior skin homografts just prior to commencement of ECIL. As expected, the anterior grafts were rejected during ECIL while posterior grafts were rejected three days after ECIL was discontinued. Calf #239 received ECIL for 10 days. One day after termination of ECIL, anterior and posterior grafts were applied, all surviving for 18 days. The next experiment was designed to rule out any influence of irradiation on cell-free lymph itself in prolonging the homograft survival. A thoracic duct-rumen fistula was established in calf #209. Previous work (46) had shown that in such preparations the degree of blood lymphopenia produced is comparable to that produced by ECIL or by centrifugation and return of cell-free lymph. This lymphopenia is presumably due to the destruction of thoracic duct lymphocytes in the rumen.

The only problem in these animals is progressive hypoproteinemia followed by severe diarrhoea and death after 2-3 weeks. The ideal experiment would be to remove the cells mechanically but we are not aware of the existence of any centrifuge that will work continuously for weeks and remove 100% of cells from 50-80 litres of lymph/day. Even a small number of escaping activated lymphocytes may be sufficient to destroy the graft. Anterior and posterior skin homografts were applied seven days after the thoracic duct-rumen fistula was established, and these grafts survived for 14 and 18 days respectively. The degree of blood lymphopenia and cumulative output of lymphocytes was comparable to animals treated with ECIL or centrifugation (Fig. 4). Rejection of posterior grafts occurred while the thoracic duct-rumen fistula was still functioning. We believe that this was due to partial deliberate obstruction of thoracic duct as described above and subsequent opening up of collaterals, or lymphatico-venous channels. A few activated lymphocytes might have reached the blood stream and attacked these grafts.

Under the conditions of our experiments, there is no definite evidence that the regional lymph node response to first set posterior skin homografts spreads to distant nodes if the effector limb of the rejection process is interrupted at the thoracic duct. The results support the hypothesis that viable activated lymphocytes from the sensitized regional lymph nodes are essential for the first set skin homograft rejection. It is a possibility that these activated cells may require further processing during their transit through other body tissues before these cells can react against the graft. In view of the rapid onset of posterior homograft rejection after discontinuation of ECIL this seems unlikely. The procedure makes possible further studies on the basic phenomenology of homograft rejection. One

could further investigate effector and effector limbs of the rejection process utilizing regional ECIB and ECIL. Studies utilizing these techniques are in progress to investigate the role, if any, of a humoral antibody in the first set skin homograft rejection.

SUMMARY:

Effects of extracorporeal irradiation of the blood (ECIB) upon skin homograft survival in calves have been described. In all ECIB-treated calves, the normally acute and biolent skin homograft rejection process was modified to a mild and slow one. Prolongation of skin homograft survival ranged from 1-11 days. Combination of ECIB and Imuran given in small non-toxic doses improved results to a slight degree. Prolongation of skin homograft survival in this group ranged from 3 to 20 days. The possible reasons for these results are discussed.

Experiments involving thoracic duct lymph drainage were conducted in nine calves. The procedures for handling the lymph included 1) extracorporeal irradiation of lymph (ECIL), 2) centrifugation of lymph and return of cell-free lymph to the animal, 3) pumping of untreated lymph to the rumen in one animal with a thoracic duct-rumen fistula. Only one animal, which was treated by centrifugation and return of cell-free lymph for 10 days, failed to show any prolongation of skin graft survival. Prolongation of survival time of anteriorly placed grafts ranged from 4-31 days in 8 calves. Posteriorly placed grafts survived the duration of treatment which ranged from 25-68 days in three animals. Implications of the results with particular reference to the different behavior of anterior and posterior grafts are discussed.

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FOOTNOTES

- ¹ Available from Extracorporeal and Medical Specialties Co., Inc.,
Medford, New Jersey.
- ² Variable Speed Peristaltic Pump, Model 505-1200, Harvard Appliance
Company, Dover, Massachusetts.
- ³ International Equipment Company, 302 Avenue, Needham Heights,
Massachusetts, 02194.
- ⁴ Kindly supplied by Dr. Donald S. Searle, Medical Director, Burroughs
Wellcome and Co., (USA) Inc., 1 Scarsdale Road, Tuckahoe, N.Y. 10707.

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FIGURE LEGENDS

Figure 1. Shows arrangement for ECIB.

- A → arterial end of shunt connected to irradiation coil.
- V → venous end of shunt connected to irradiation coil.
- I → Cobalt-60 irradiator.
- H → Heparin infusion pump.
- W → Water bath for pumping water at 40°C around that portion of irradiation coil which is outside the irradiation chamber.
- T → thermometer.

Figure 2. Shows arrangement for centrifugation of lymph and return of cell-free lymph to the animal.

- T → thoracic duct cannula.
- V → venous cannula.
- H → heparin infusion pump.
- R → lymph-collecting reservoir.
- P → pump to lift lymph to top reservoir R.
- C → continuous flow refrigerated centrifuge equipped with helixtractor.

Figure 3. Shows influence of ECIB on number of lymphocytes in peripheral blood.

Top	24 hours continuous ECIB. Average of six calves.
Middle
(#113)	Pregraft repetitive ECIB.

(continued)

Figure 3, continued

Bottom
(#136) Pre- and post-graft repetitive ECIB.
 G → Day of skin homografting.
 R → Day of skin homograft rejection.

Figure 4. Shows influence of various treatments of thoracic duct lymph on number of lymphocytes in peripheral blood and cumulative output of lymphocytes from thoracic duct.

Top (#209) Thoracic duct - Rumen fistula.
Middle (#185) Extracorporeal Irradiation of Lymph (ECIL).
Bottom (#186) Centrifugation and return of cell-free lymph (C-L) to the animal.

T-D. C. → Thoracic duct cannulation.
T-D. R. → Thoracic duct-rumen fistula.

G → Day of skin homografting.

R- and R-A → Day of rejection of anterior skin grafts.
R-P → Day of rejection of posterior skin grafts.

Figure 5. Shows influence of ECIL on number of lymphocytes in peripheral blood.

Top (#206) → Pre-ECIL anterior and posterior skin grafting.
Middle (#239) → Post-ECIL anterior and posterior skin grafting.
Bottom (#200) → Anterior and posterior skin grafting one week after commencement of ECIL. Calf #200 also received ECIB for 2-4 hours a day for 9 days to study the influence of ECIB on output of cells from thoracic duct.

Figure 5, Continued

T-D.C. → Thoracic duct cannulation.

G. → Day of skin homografting.

R-A → Day of rejection of anterior skin graft.

R-P → Day of rejection of posterior skin graft.

Figure 6. Shows the appearance of skin grafts in animal #206, 24 days after grafting.

H. → Posterior skin homografts. 4 healthy grafts are shown.

A → Posterior skin autografts. 2 healthy grafts are shown.

Figure 7. This diagrammatic sketch shows the possible roles of immunologically competent small lymphocytes and immunologically activated small lymphocytes in the posterior skin homograft rejection. As an arterio-venous shunt diverts only a portion of the blood stream through the irradiator, ECIB can destroy only a portion of immunologically competent and activated small lymphocytes. Cannulation of thoracic duct removes only a portion of immunologically competent small lymphocytes as re-circulation from blood to other lymph channels remains uninterrupted. Cannulation of thoracic duct removes all the activated small lymphocytes in case of posteriorly placed homograft and thus prevents the destruction of homograft.

This sketch also shows the possible mechanisms of sensitization:

- 1) Central sensitization due to interaction of immunologically competent small lymphocyte and graft antigen.
- 2) Sensitization in the regional lymph node by free or macrophage borne graft antigen.

TABLE I

ECIB AND IMURAN VS SKIN HOMOGRAFT REJECTION IN CALVES

Treatment	No. of Calves	Transit Dose (Rads)	Hours of ECIB ^a	Blood Volumes Irradiated	Day of Grafts Rejection
--	18	--	--	--	8 (2) 9 (12) 10 (4)
Pregraft Continuous ECIB	6	15-60	24	90-240	10
Pregraft Repetitive ECIB	3	50(2) ^d 10-1000	98, 112 114	325, 160 210	11, 14 13
Pre & Postgraft Repetitive ECIB	5	215-770 500-800 (4)	105 54-118	547 122-192	20 12, 12, 14, 15 ^c
Pregraft Repetitive ECIB & POSTGRAFT IMURAN ^b	2	700 2000	70 49	106 49	16 15
Pre & Postgraft Repetitive ECIB and IMURAN	6	684 500-800 (5)	57 54-78	124 67-152	29 12, 12, 13 ^c , 14, 16
IMURAN	3	--	--	--	8, 10 10

^a ECIB = Extracorporeal irradiation of blood. ^b IMURAN = Azathioprine.

^c Skin grafts intact at the time of accidental death of the animals.

^d Figures in parenthesis refer to the numbers of calves.

Weight Range of Calves - 240-712 lbs.

TABLE II

THORACIC DUCT LYMPH VS SKIN HOMOGRAFT REJECTION IN CALVES

Calf No.	Treatment of Lymph	Days of Treatment	Lymph Output Per Minute $\times 10^8$		Cumulative Lymph Output $\times 10^{11}$	Day Grafted	Graft Rejection Time (Days)	
			Min.	Max.			Anterior ^a	Posterior ^a
151	CENT. ^b & ECIL ^c	14 ^e	0.2	4.3	8.2	14	40	--
165	CENT.	10	0.25	2	9.3	10	10	--
185 ^f	CENT.	24	0.24	1.04	14.1	24	18	--
186 ^f	ECIL	22	0.22	1.18	13.8	22	17	--
200	ECIL	34	0.28	2.05	33.5	7	22	39
202	ECIL	68	0.14	1.4	28.0	7	27	61
206	ECIL	25	0.13	2	15.6	1	13	28
239	ECIL	10	0.3	2.3	6.5	10	18	18
209	TD-R ^d	26	0.12	4.9	22	7	14	18

^a For details see text.

^b CENT. = Centrifugation of lymph and return of cell free lymph to animal.

^c ECIL = Extracorporeal irradiation of lymph (Transit Dose Range 450-5000 rads).

^d TD-R = Thoracic Duct - Rumen Fistula.

^e CENT. from Day 1-8 ECIL from Day 10-14. Cumulative Output from Day 1-8 only.

^f Twins with common donor.

All Animals — Holstein

Weight Range 174-475 lbs.

FIG. 1

FIG. 2

FIG. 3

FIG. 4

11-964-65-

FIG 5

11-6163-65

Fig. 6

I.C.S.L. = IMMUNOLOGICALLY COMPETENT SMALL LYMPHOCYTES
 L.P.C. = LARGE PYRINOPHILIC CELL
 I.A.S.L. = IMMUNOLOGICALLY ACTIVATED SMALL LYMPHOCYTES

Fig. 7

5-2-58-11