that man can tolerate relatively large amounts of $Mg^{++}$ for prolonged periods without ill effect(14). It should be mentioned that other than an initial weight loss and early diarrhea no gross clinical effects were observed in these rats. However, once radioactive strontium is fixed in the skeleton, the possibility of enhancing its turnover rate is small.

Although it would appear obvious, it should be emphasized that, although agents may cause significantly greater urinary and fecal excretion of skeletally bound isotope, unless a major portion of the skeleton turns over, this increased excretion will result in only a minor change in the skeletal burden of strontium.

*Summary.* Administration of $Mg^{++}$ concurrently with radioactive strontium decreased significantly skeletal retention of the isotope. $Mg^{++}$ administration had a small but significant lowering effect on the retention of radioactive strontium when administered after the isotope was well fixed in bone.

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Received April 22, 1964. P.S.E.B.M., 1964, v116.

## Antigen Pretreatment and Mechanisms of the Resulting Temporary Tolerance.* (29428)

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In previously reported studies antigen pretreatment was evaluated as a technique for prolonging the survival of homografted kidneys in dogs(1,2). Two cc donor blood given 10 and 5 days before transplantation, was found to be of moderate effectiveness, allowing a functioning survival of 29.2 ± 10.9 days (controls 9.1 ± 1.3 days). Recipients thus conditioned demonstrated a clinical "wasting syndrome" characterized by anorexia, vomiting, diarrhea, weight loss, anemia and a persistent leukocytosis beginning long before renal function deteriorated. At autopsy they all showed severe depletion of lymphocytes in the spleen and lymph nodes. The thymus, however, was uniformly spared. The present studies were an attempt to answer several questions arising from the above work: 1. Is a graft-*vs*-host reaction involved in the genesis of this lymphoid depletion and delayed rejection? 2. Is the spared thymus a source of, or stimulus to, lymphocytes re-populating these depleted animals? 3. Does the source or bulk of the transplanted allogeneic tissue affect lymphoid depletion?

* Supported in part by U.S.P.H.S. grant.

*Methods.* Adult mongrel dogs ranging in weight between 12 and 18 kg were maintained on *ad libitum* feedings of dog chow and supplementary meat and bones. No transplants were made from males to females, and phenotypic similarities between donors and recipients were avoided.

Conditioning consisted of administration to the recipient of 2 cc of blood from the future kidney donor 10 and 5 days before transplantation. This was drawn into plastic syringes without anti-coagulant and injected subcutaneously.

Kidney Transplants: The kidneys were flushed and cooled with 100 cc heparin-saline at 4°C after removal from the donor. They were transplanted into the opposite iliac fossa of the recipient, using end-to-end arterial and end-to-side venous anastomoses to the external iliac artery and common iliac vein respectively. Ureteroneocystostomy was done by the method of Paquin(3). The period of renal ischemia ranged from 18 to 33 minutes. Both kidneys of the recipient were removed at time of transplantation.

Urinary output was measured daily; blood urea nitrogen, blood creatinine and endogenous creatinine clearance (Cc) were determined bi-weekly. Complete blood counts were done weekly. Recipients were sacrificed after 2 successive days of oliguria (output $< 50$ cc) unless they died before this time. Complete post mortem examinations were done on all animals.

Skin Transplants: Full thickness grafts measuring $15 \times 15$ cm were used. These were taken from and placed on the flanks, carefully preserving the underlying panniculus carnosus and its vasculature. The grafts were sutured in place with a continuous 0000 silk suture, covered with tulle gras, and protected by a snug plaster belt. Rejection was considered to have occurred when one-half or more of the graft had desquamated, induration had occurred and no capillary filling was present(4). The grafts uniformly began to separate within 48 hours of this stage.

Irradiation: Blood to be used for conditioning was exposed to 5,000 r; the excised kidneys, prior to transplantation, to 500 r. These doses were delivered by a 2 meV linear accelerator.

Thymectomy: A median sternotomy or left thoracotomy approach was used. Both phrenic nerves were freed and reflected. Mediastinal contents were removed *en bloc* from the pericardium to the thyroid glands, sacrificing the internal mammary vessels and stripping the great vessels.

*Results.* Ten control kidney homografts in non-pretreated recipients survived $9.1 \pm 1.3$ days. Seven dogs were pretreated with blood from dogs other than the prospective kidney donors. Allogeneic renal transplants in these animals survived $8.7 \pm 1.8$ days, and none of these dogs showed a clinical or pathologic "wasting syndrome." Eight kidney homografts in animals conditioned with blood from the donor of the kidney survived $25.9 \pm 9.7$ days. These recipients uniformly showed clinical wasting and lymphocytic depletion of their lymphoid tissues.

The sites of injection of the blood used for conditioning were inspected in all the dogs at time of autopsy. Occasionally slight residual staining was seen, but in none were abscesses or other signs of infection or inflammation found. Histologic studies revealed only pigment-laden macrophages; no pyroninophilic cells were demonstrable.

1. Irradiated-Conditioned Group (11 dogs): The mean functioning survival of the allogeneic kidneys in this group of dogs was $20.9 \pm 5.4$ days (range 15-26 days). The animals were anorectic, had intermittent diarrhea, and lost weight during the entire postoperative course. Renal function showed deterioration beginning several days prior to frank rejection with rising BUN and diminishing Cc; urinary output was often maintained longer. At post mortem examination the kidneys showed cortical congestion and hemorrhage; microscopically perivascular round cell infiltration characteristic of primary rejection was present. Lymphocytic depletion of the nodes and spleen was moderate to severe in these animals, and was comparable to that found in non-irradiated conditioned recipients.

2. Thymectomized-Conditioned Group (14 dogs): Mean kidney survival time in these

dogs was 19.9 ± 6.1 days (range 12-25 days). The clinical "wasting syndrome" of anorexia, vomiting, weight loss and anemia was noted in all. Leukocytosis of 20,000-30,000 cells/mm$^3$ was consistently present. At autopsy they all showed typical gross and microscopic signs of homograft rejection as well as lymphoid depletion.

3. Massive Skin Grafts (6 dogs): Six control grafts survived 11.3 ± 2.6 days. The 6 conditioned dogs bearing these large skin homografts were well clinically. They showed none of the anorexia, vomiting, diarrhea, or weight loss which were seen in the conditioned animals bearing kidney transplants. Mean survival time of these massive grafts in conditioned dogs was 24.1 ± 4.3 days. At post mortem examination no evidence of lymphoid depletion was present in any of the 6 recipients, grossly or microscopically.

*Discussion.* The effectiveness of irradiated blood in producing temporary tolerance has recently been demonstrated by Kinsky and Mitchison(5). This has been confirmed in the present studies. The question regarding the occurrence of a graft-*vs*-host reaction in conditioned recipients of kidney homografts cannot be answered completely. For fear of injuring the kidney to be transplanted the X-ray dosage was limited to 500 r. This dose is not adequate to destroy lymphocytes left in the kidney after flushing, but even a lower dose has been shown to prevent the production of runting by adult spleen cells in 98-99% of mouse neonates(6). In view of this fact, and the convincing demonstration by Siskind and Thomas(7) that adult renal tissue does not produce runting even in the newborn mouse, it would appear safe to assume that graft-*vs*-host reactions did not occur in the Group I recipients. Since lymphoid depletion occurred in these dogs much the same as in non-irradiated conditioned recipients, this phenomenon must result from causes other than runting, and the temporary tolerance cannot be explained as being secondary to a graft-*vs*-host reaction(8).

Thymectomy did not increase homograft survival. Therefore repopulation of the lymphoid system from the thymus or stimulation of the recipient's own lymphoid system by the thymus probably plays no significant role in the recovery of normal immunologic potential when a conditioned recipient rejects a graft. In the case of tumor homografts, the build-up for a second-set response proceeds at a normal pace, even while the first-set (enhanced) graft shows prolonged survival(9). In the case of vascularized whole organ transplants, lymphoid depletion is added to the picture of enhancement but apparently contributes litttle to the actual prolongation of homograft survival.

The curious nature of the lymphoid depletion prompted the massive skin graft studies. Conditioned animals bearing small skin grafts show no such changes(4). Using skin grafts comparable in antigenic bulk to kidney transplants, again no evidence of the clinical syndrome of wasting or of lymphoid depletion was found. Since there are no qualitative antigenic differences between skin and kidney(10), immediate vascular contact (present in kidney transplants but not in skin grafts) between the allogeneic tissue and the conditioned recipients remains as the only differentiating factor. It must be assumed therefore, that the "wasting syndrome" and lymphoid depletion are not necessarily dependent on the bulk of a large homograft, but on the early intimate contact provided by primary vascular anastomosis between graft and conditioned (= sensitized) recipient.

*Summary.* Three factors were studied in animals showing prolonged homograft survival as a result of donor antigen pretreatment. The possible contribution of a graft-*vs*-host reaction to prolonged homograft survival, wasting, or lymphoid depletion was eliminated by using irradiated donor blood for conditioning, and irradiated transplants, and finding no significant change in homograft survival or in degree of lymphoid depletion. Any role the thymus may play as a source of lymphoid repopulation when conditioned animals reject homografts was ruled out by finding no increase in homograft survival in animals conditioned and transplanted after thymectomy. The importance of primary vascular contact between conditioned

recipient and transplant was shown by the absence of wasting and lymphoid depletion on conditioned animals which receive massive skin homografts, rather than kidney transplants.

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Received April 22, 1964. P.S.E.B.M., 1964, v116.

## Studies on Preservation by Freezing of Human Diploid Cell Strains. (29429)

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Low temperature preservation of cells and tissues is an important recent development in biological research. In a review by Smith(1) and from later reports(2-13), it is apparent that procedures for optimal preservation by freezing may vary with individual neoplasms and tissue cell types. Although good results have been obtained in low-temperature preservation of heteroploid cell lines in tissue culture, fewer reports have described successful results in freezing cells from primary cultures or unaltered diploid strains. In addition, it has been shown that dissociated cells from fresh tissues, primary cultures or unaltered cell strains are more sensitive than continuous cell lines to freezing injury(4,5,7, 8).

Growing interest in viral vaccine development and administration of such vaccines to man has focused attention on the use of human diploid cell strains for virus vaccine production(14,15). As these strains survive only a limited number of passages *in vitro*, their proposed use in commercial vaccine production requires that they be readily and effectively preserved.

Methods for preservation by freezing of various passages of human diploid cell strains have been explored in this laboratory, and results are reported here.

*Materials and methods. Tissue cultures.* Used in this study were human diploid cell cultures (strains Led-L94, Led-L106, Led-L166, Led-L130 and Led-S95), established from human fetal or newborn tissues. These fibroblast strains were derived from lung tissues, with the exception of S-95, which originated from newborn full-thickness skin. All strains are capable of vigorous growth and can be subcultured through a limited number of *in vitro* passages before eventual death. They are characterized by the properties described in detail by Hayflick(14).

Cells were propagated in 8 oz Neutraglas bottles in "LAPAGT" medium comprised of 0.4% lactalbumin hydrolysate in Earle's saline(16) containing vitamins and glutamine as suggested by Eagle(17), and further supplemented per liter with: l-tyrosine 40 mg, sodium pyruvate 100 mg, l-asparagine 30 mg, adenylic acid 50 mg, ascorbic acid 50 mg, glucose 1.0 g and cow serum 150 ml. The antibiotics penicillin 100 U, streptomycin 50 $\gamma$ and neomycin 20 $\gamma$, were incorporated into each ml of culture medium only upon primary explantation of tissues