Secondary Rejection Phenomenon Elicited by Primary Homograft in Pretreated Rats. (24490)

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The graft rejection phenomenon has been considered explicable on an immunologic basis, in part due to the chronology of primary and secondary homograft rejections. Simonsen(1) has shown that prior immunization with skin from a kidney donor would sensitize the recipient so that accelerated kidney rejection would then occur. Dempster (2) showed that not only would skin grafting immunize against kidney from the same donor, but also that kidney grafts immunized against skin. Essentially the same findings were demonstrated by Billingham *et al.*(3) when it was shown that injections of an homogenate of lyophilized donor whole kidney would delay primary skin homograft rejection in pretreated neonatal rats. Based upon such reports indicating an antigenic relationship between skin and kidney, we evaluated a distinct anatomical component of the kidney in this relationship. The work to be reported here involved pretreating adult rats with rat glomerular basement membrane and subsequently performing an orthotopic skin homograft. In view of the report by Billingham *et al.*(4) concerning the role of nucleoproteins in graft rejection, RNA and DNA determinations were performed on our material.

Methods. A pool of several hundred rat kidneys was obtained from 5 different laboratories, all purchasing from different vendors. Glomeruli were isolated and basement membrane obtained from them by Greenspon's method(5) with the slight modification that no attempt at sterility procedures was attempted. Recipient and donor rats (of the skin grafts) were chosen from different lots so as to be as genetically disparate as is possible when purchasing from a single vendor. Two intraperitoneal injections of 40 mg (wet weight) of glomerular basement membrane in physiologic saline were given to 24 white, female, Holtzman strain rats (150 g) at an interval of one week. Seven days after the last injection, the orthotopic homograft was performed. One square centimeter of full-thickness skin was removed, trimmed of underlying fat and set into place on the prepared recipient area. The graft was secured with 8 to 10 stitches, and a pressure dressing of gauze was applied. Five days later dressing and stitches were removed. Numerous control autografts had previously validated the surgical technic. A group of 24 untreated homografted rats were given a second homograft from the same donor 2 weeks after rejection of the first graft.

RNA and DNA determinations on rat glomerular basement membrane were performed by the methods of Ceriott(6) and Webb(7) respectively. We are indebted to Dr. Max Rafelson, Dept. of Biochemistry for these determinations.

Results. Using rejection time criterion as complete breakdown of the graft, the base-line value of 13.2 $\pm$ 0.7 days was obtained for the primary homograft in the untreated rats. The pretreated rats rejected their primary homograft in about the same time, 7.3 $\pm$ 0.4 days, as the secondary homografts were rejected in the untreated rats, 7.8 $\pm$ 0.6 days. RNA and DNA values of 0.09 mg% and 0.05 mg% respectively were obtained.

Discussion. This preliminary report thus seems to substantiate the working hypothesis that there may be an immunologic relationship between a component of glomerular basement membrane and skin. This is not to imply that only the glomerular basement membrane is involved in the skin-kidney relationship. Currently other kidney structures are being investigated.

Aside from relating an anatomical component seemingly involved in the skin-kidney relationship, it would seem to us that greater significance may be attached to the fact that,

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as opposed to the works cited(1-3), the material employed for pretreatment was from a pooled source and not from the skin donors.

The low nucleic acid content of our material limits coordination of our findings with the hypothesis presented by Billingham *et al.* (4). Experiments are underway to evaluate the role of a polysaccharide material isolated from rat glomerular basement membrane by a method reported elsewhere(8) in enhancing takes of skin grafts.

Summary. Intraperitoneal injections of pooled rat glomerular basement membrane preparations into rats subsequently homografted with a primary orthotopic skin graft facilitated a secondary rejection phenomenon on the part of such treated animals. The low nucleic acid content of the preparations limited correlation with the hypothesis of nucleoproteins as the transplantation antigens.

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Occurrence and Enzymatic Degradation of Phosphoethanolamine.* (24491)

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The biological occurrence of phosphoethanolamine was first observed in bovine tumor tissue by Outhouse(1), who failed to find it present in normal tissues. Colowick and Cori(2) demonstrated the ester to be present in rabbit and pig intestine. Lately it has been shown to occur in a variety of normal tissues and often in quite large amounts(3,4,5,6). Thus far, the major biochemical interest of the compound lies in its possible role as an intermediary in the metabolism of phospholipids. Little is known of other possible functions, or of the enzymology of phosphoethanolamine metabolism. Roche and Bouchilloux (7) have shown that dog liver, human prostate, and beef kidney split phosphoethanolamine at pH 5.6, although at a lower rate than glycerophosphate. Strickland *et al.*(8) reported that alkaline phosphatase of various tissues readily split phosphoethanolamine, but that there was a relative inactivity toward this phosphate ester by acid phosphatase of these same tissues. This paper reports information about distribution of the compound in various tissues, and its enzymatic degradation by tissue homogenates under different conditions, *i.e.*, pH, buffer, metal ions, etc.

Methods. Phosphoethanolamine was synthesized by the method of Outhouse(9) and phosphate determinations were made by the method of Gomori(10). As soon as animals were sacrificed, samples of tissue were immediately homogenized with Teflon pestle in distilled water. Aliquots of the homogenate were treated with 10% trichloroacetic acid to remove proteins, and after removal of the acid with ether the resulting solution was concentrated *in vacuo*. Quantitative determination of phosphoethanolamine was then made by Dowex-50 chromatography as previously described(5). The incubation system contained in final volume of 11 ml, 9 ml of buffer (veronal-acetate, pH 4.5, or veronal, pH 9.0), 1 ml of substrate (15 x 10^{-5} moles of either phosphoethanolamine or beta-glycerophosphate, and 5 x 10^{-3} moles of $MgCl_2$), and 1 ml homogenate. Incubations were carried out in small Erlenmeyer flasks with shaking in

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