Parabiosis in Rabbits as Preliminary to Homotransplantation of Tissues. (24518)

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Homotransplantation of specialized tissues in higher animals and in man is unsuccessful because of adverse immunologic reactions that result in necrosis of the transplant. Homograft rejection is probably an antigen-antibody reaction between host tissues and transplant. Demonstration of tissue antibodies by fluorescein technics confirms this concept(1, 2). Medawar(3,4) using skin grafts and Dempster(5) working with kidney transplants have also demonstrated that homologous transplantation sensitized the host to future transplants. Secondary transplants necrotized in a shorter time than primary grafts, and tertiary transplants had even a lesser life span. Renewed interest in homotransplantation has been stimulated by the work of the Kamrins(6) and a clinical study of Merrill, Murray, Harrison and Guild(7) who reported transplantation of a kidney from one identical twin to another. This demonstrates that a kidney can function after temporary cessation of its blood supply and denervation. But transferring a kidney from one identical twin to another does not constitute true homotransplantation. The Kamrins, working with rats, reported that parabiosis was a means of overcoming immune reactions that retard homotransplantation of tissue. After litter-mate rats were maintained in parabiosis for 25 days kidney slices were exchanged between the parabiotic rats. Pieces of renal tissue survived as permanent homografts as long as the rats remained in parabiosis. Kamrin later reported that the transplanted kidney slices remained viable after separating successful parabionts as skeletonized parenchymal structures, but with a well vascularized stroma. Evidently separating the parabionts abrogated the neutralizing mechanism slowly with enough protection remaining to prevent sloughing and necrosis of the homograft. Our experiments consisted of placing young litter-mate rabbits of the same sex in parabiosis as a preliminary to homotransplantation of tissues, to repeat Kamrin's work in an animal with a more complex immunologic system. The rabbit has 4 blood types, whereas the rat has a single type.

Methods. The method of parabiotic union used was a modification of the procedure of Sauerbruck and Heyde(8,9). Forty-six rabbits were placed in parabiosis successfully. Prior to surgery the rabbits were anesthetized with intravenous sodium pentobarbital. The dose used was 0.5 cc/kg of body weight, given into ear veins. As a rabbit can be killed by overdose of pentobarbital (or any other anesthetic agent) extreme caution was used in administering the anesthetic agent. Rabbits were then placed parallel to each other on an operating board and the skin prepared for operation. Thirty of the 46 rabbits were placed in parabiosis as follows: Parallel incisions were made in adjoining mid axillary line of each animal from last rib to the inguinal area. Abdominal fascia, muscles, and peritoneal layers were then incised in line of the incision. The corresponding layers of each animal were then anastomosed with 4-0 silk sutures placed on atraumatic needles. In the remaining 16 rabbits intact peritoneal layers were placed in adjacent positions sutured to each other but not opened. Skin and muscle layers were then anastomosed as previously. With the later method it was anticipated that there would be less rapid cross circulation between the 2 animals and internal herniation of the intestines would be prevented. At termination of operations, all animals were given appropriate doses of penicillin and streptomycin for the first 3 post-operative days. None of the post-operative animals struggled to free themselves from parabiotic union. All were docile and behaved normally. In some animals, agents that modify immune processes were administered at time of opera-

tion. The majority of this group were injected with a solution of Evans Blue dye(10, 11,12). Four animals received an immunologic paralyzing dose of pneumococcous polysaccharide. Evans Blue dye (T-1824), an isomer to trypan blue (T-1835) is of low toxicity. After parenteral administration it combines mainly with serum albumin, and to a lesser degree, globulin, and so exerts a modifying effect on the immune system. The other method to obtain alteration of immune processes was parenteral injection of a polysaccharide obtained from pneumococcus according to the method of Felton(13.14.15). Felton found that intraperitoneal injection of 0.002 mg/kilo of pneumococcus polysaccharide in mice will act as antigen and stimulate creation of antibodies. However, increasing the dose to 0.5 mg/kilo created an immunologic paralysis, so that an animal thus treated was unable to develop antibodies to a later immunizing injection during most of its life span. These mice were hypersusceptible to infection. This effect can be reached by giving a single large dose or by repeated small doses as long as the total is 0.5 mg/kilo. Injected polysaccharides are fixed in the reticulo-endothelial cells and in fibroblasts throughout the body, thus exerting an effect on the animal's immune processes.

Results. In the 30 parabiotic animals with common peritoneal cavities, 7 of the parabiotic pairs did not receive medication that could modify immune processes. In 6 of these pairs one of the partners died within 24 hours after operation while its mate remained apparently unaffected. In only one of the pairs did both parabionts maintain themselves until the tenth day.

In 4 pairs with common peritoneal cavity both rabbits were injected with 5 cc of Evans Blue dye. The injection was given prior to placing the animals in parabiotic union. Of these, one pair lived 3 days, one 9 days, and one 12 days, and one 21 days before one of the parabionts died. In 2 additional pairs only one of the parabionts were injected with Evans Blue and no dye given to the partner. In both of these pairs the rabbits without the Evans Blue died within 24 hours.

In the remaining 2 pairs of parabiotic animals with common peritoneal cavity, both parabionts were injected with an immunologic paralyzing dose of pneumococcus polysaccharide. Dr. L. D. Felton generously sent the material, and it was prepared for use by Dr. M. Tremaine, Immunology, State University of N. Y. In one pair, death of one of the rabbits occurred on 6th postoperative day because of a strangulated small bowel hernia through the peritoneal cavity connection which was made smaller than usual. The other pair survived until the twenty-first postoperative day when one of the animals died.

As survival was so short when a common peritoneal cavity method of parabiosis was used, modification of the method was done in 16 animals or 8 pairs of rabbits. Also 8 survivors were placed into parabiosis making an additional 4 pairs. No substances that effect immunity were administered to 6 of the original 8 pairs. Three pairs separated on the 14th postoperative day because of necrosis at site of operation. In the remaining 3 pairs, one of the partners in one pair died in 24 hours, in another pair in 12 days, in the 3rd pair on the 21st day.

The remaining 2 pairs of parabiotic rabbits with intact peritoneal cavities had Evans Blue dye injected into the only one of the parabionts of each set. Necrosis of the operative site and separation occurred in one pair on 7th postoperative day. In the second set the parabiont without Evans Blue died on the 7th day.

Two of the pairs that did not receive drugs and that had separated on the 14th day were then rejoined. This time separation of one pair occurred in 6 days, and one of the rabbits of the other pair died on tenth day.

The rabbits that had been placed in parabiosis on 2 occasions, then separated after 6 days were joined for the third time. Now the rabbits separated after only the third day.

Two further pairs were then made, joining an Evans Blue survivor with a non-Evans Blue survivor. One pair separated on the 7th day and the Non-Evans Blue rabbit of the second pair died on the sixth day.

Discussion. Various methods have been

tried to obviate the immune reactions that preclude homotransplantation. Of these, whole animal x-ray radiation, administration of cortisone, large particle dyes or vital donor cells(16) led to a delay in immune reactions that destroy homografted tissues. The modifying effect is probably accomplished by action of these substances on white blood cells, reticuloendothelial system, and plasma proteins, suppressing production of the host's antibodies and delaying rejection of a homograft. Egdahl and Hume(17,18) attempted to modify this reaction by artificially maintained cross transfusion between donor and host prior to renal transplantation. However, they found that blood from a donor can also sensitize a host since the subsequent kidney transplant behaved as a secondary transplant after cross transfusion. Nevertheless the cross circulation afforded protection to the transplanted kidney for the period that cross circulation was allowed to continue. The protective effect of cross circulation is similar to that demonstrated by experiments of Kamrins. However, in our experiments a prolonged self-sustained cross circulation as demonstrated by parabiosis could not be maintained in the rabbit for more than 14 days.

Our results indicate that administration of Felton's polysaccharide antigen did not afford prolonged protection from homotransplant rejection. However the antigen was used in only 2 experiments so that further work will have to be done with this agent before definite conclusions can be drawn.

Summary. 1. Prolonged parabiosis in rabbits, which have a complex and sensitive immunologic mechanism, has been unsuccessful. 2. Using a common peritoneal cavity method of parabiosis, rapid absorption of an antigen that is lethal to one of the parabiotic animals occurred in one day. 3. Administration of Evans Blue and pneumococcic polysaccharide prolonged duration of parabiotic union in rabbits by their effect on animals' immune reactions. 4. Secondary and tertiary attempts at parabiosis are rejected more rapidly than the initial parabiosis, again confirming the sensitivity theory of homograft rejection.

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