Mixed Aggutination with Cell Cultures in Rabbit Homotransplantation.* (29855)

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The mixed agglutination technique with cell cultures(1,2) has recently been studied in this laboratory. Reactions between antiviral antibodies and cell cultures infected by measles virus were examined(3). In testing cell cultures of various species origin against heteroimmune sera to organ suspensions, species-specific antigens were described which are thermolabile and non-extractable in saline (4). The mixed agglutination procedure has been also successfully used for detection of antibodies accompanying homograft rejection in mice(5).

It appeared interesting to explore the possibility of employing the mixed agglutination technique for studies on transplantation in other animal species. Rabbits were selected for these studies since in this species relatively little work on humoral transplantation antibodies has been done. In addition, in previous experiments the antiglobulin consumption test was successfully used to demonstrate humoral antibodies appearing in response to skin homotransplantation in rabbits(6). It was of some interest to compare results obtained by these two techniques.

Materials and methods. Rabbits were prepared as previously described(6). In brief, orthotopic full thickness skin grafts were carried out on adult rabbits of several varieties. Usually each rabbit in a pair served as both donor and recipient to its partner. Second skin grafts from the same donor were performed 4-7 weeks after the first graft. Two to three weeks later the right kidney was removed extraperitoneally under local anesthesia, and used for preparation of cell cultures. Ultimately, the animals were sacrificed, cell cultures were prepared from lung tissue, and the remainder of the organs were stored at —20°C.

The kidney and lung cultures were prepared as described by Barron and Karzon(7) and were studied when the monolayer was complete, usually after 7 to 9 days of incubation.

In performing the mixed agglutination test, a previously described procedure was followed(4). In principle, recipient serum was incubated with cultures of donor cells. The antigen-antibody combination was demonstrated by exposing the monolayer to an indicator consisting of sheep erythrocytes sensitized by a subagglutinating dilution of rabbit anti-sheep erythrocyte serum, and agglutinated by goat antiserum against rabbit serum (Coombs serum). In this situation, antibodies of the Coombs serum, which were bound to sensitized erythrocytes had available reactive sites that could attach to rabbit antibody on the monolayer. Thus, in positive reactions, when the monolayer was inverted and examined under the microscope, red blood cells were found adhering to the monolayer. The positive results were graded from 1+ to 4+ according to the proportion of the area of the monolayer which was covered by erythrocytes. The reagents used for the indicator system had been adjusted in preliminary titrations and then they were kept constant throughout this study.

Absorption of transplantation sera with dried and pulverized organs was performed as outlined previously(4).

Results. Table I shows the results of a typical experiment in which sequential samples of the recipient serum were tested against cell cultures of donor lung. First positive reactions were observed with the serum sample obtained during the fifth week after the first graft. However, all the reactions recorded after the first graft were weak, and

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TABLE I. Mixed Agglutination Test with Cell Cultures of Donor Lung and Sequential Serum Samples of Recipient 1829.

Serum dilution 1 to:	Samples of recipient serum collected X days after — First graft: 0	9	17	26	33	47	Second graft: 11	17	24	Control with donor serum
50	—	—	—	±	1+	1+	3+	3+	4+	—
250	—	—	—	±	1+	1+	3+	3+	3+	—
1,250	—	—	—	—	—	—	2+	3+	2+	—
6,250	—	—	—	—	—	—	1+	2+	2+	—
31,250	—	—	—	—	—	—	1+	2+	1+	—
156,250	—	—	—	—	—	—	—	—	—	—

TABLE II. Mixed Agglutination Test with Cell Cultures of Donor Lung and Recipient Serum 1829. Effect of absorption of the serum by lung and liver powder.

Serum dilution 1 to:	Unabsorbed serum	Serum absorbed with powder of: Donor lung	Donor liver	Recipient lung	Recipient liver
50	2+	—	—	3+	3+
250	2+	—	—	2+	2+
1250	2+	—	—	1+	1+
6250	1+	—	—	—	—

strong reactions were observed only with the sera taken after the second graft.

In testing sera of 8 recipients against cell cultures derived from their respective donors, positive results were obtained in all instances. The pattern of reactions obtained with sequential sera from 5 animals was similar to the one presented in Table I, *i.e.*, weak reactions were found after the first graft and strong reactions after the second graft. In one animal, strong reactions were already present 13 days after the first graft. In 2 remaining animals, consistently negative results were obtained after the first graft and only the serum samples taken after the second graft were positive.

In comparing the reactions on kidney and lung cultures, no significant difference was noted.

Table II shows the results of an absorption study. It is seen that the donor organ powder prepared from either lung or liver did absorb antibodies detectable in the reaction with cell cultures of donor lung. On the other hand, the powder of the recipient organs failed to absorb antibodies.

Three animal pairs were tested in which each animal served as both donor and recipient to its partner. Transplantation sera and cell cultures originated from the same animals. It may be noted in Table III that consistently negative results were obtained whenever the transplantation serum was tested against the animal's own cell cultures. In contrast, the reactions with the donor cells were always positive. In addition to donor cell cultures, the transplantation sera also combined with cell cultures originating from other, unrelated rabbits. This may be seen in tracing the results observed in testing sera originating from the 6 exchange-graft partners, as well as in reactions produced by 4 additional transplantation sera which were included in this experiment. Significantly, the titer of reactions with the donor cell culture was in general higher than the titer with cell cultures originating from unrelated rabbits.

Discussion. These results showed that rabbits exposed to skin homografts form antibodies combining with cell cultures of rabbit organs. The isoantibody nature of the antibodies under investigation was proved by demonstrating that the transplantation sera never combined with the recipient's own cells, whereas they always combined with donor cells and with cells originating from some other rabbits.

In comparing the results obtained by mixed agglutination test and by antiglobulin con-

TABLE III. Mixed Agglutination Test with Lung Cell Cultures and Transplantation Sera Obtained After Second Skin Homograft.

		Cell cultures					
		2192	1829	2128	2140	1831	2136
Sera of exchange-graft partners	2192 anti-2136	<50	1,250			1,250	6,000
	1829 anti-1831		<50	250		30,000	
	2128 anti-2140	250		<50	30,000		
	2140 anti-2128			150,000	<50		
	1831 anti-1829		30,000	1,250		<50	
	2136 anti-2192	30,000	6,000				<50
Sera of other recipients	2159	6,000	1,250	1,250		1,250	6,000
	2182	<50	1,250	<50		6,000	
	2184		6,000	250		30,000	
	2178	250					<50

sumption test, it was surprising to find a considerable difference. In general, positive mixed agglutination reactions did not occur until 4 to 5 weeks following the first skin graft. These reactions were weak but once present persisted throughout the study period. In contrast, positive results by antiglobulin consumption were almost always obtained 14-21 days after the first graft; however, the reaction rapidly weakened and usually disappeared within 6 weeks. No explanation for the different results obtained by these two procedures can be offered.

Summary. Mixed agglutination tests using cell cultures have been applied to the study of antibodies accompanying skin homograft in rabbits. Transplantation sera were incubated with lung or kidney cell cultures. Binding of antibody was demonstrated by an indicator consisting of sheep erythrocytes sensitized by rabbit anti-sheep erythrocyte serum and agglutinated by goat antiserum against rabbit serum. Positive reactions were obtained with sera of all recipients tested. The reactions usually became positive five weeks after the first graft and increased in strength after the second graft. Transplantation sera combined with cell cultures originating from the donor animal and some other unrelated rabbits, but they fail to act upon the recipient's own cell cultures.

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Fat Utilization in Rats Fed Cholestyramine, a Bile Acid Sequestrant. (29856)

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The anion exchange resin cholestyramine is used clinically in treatment of pruritus due to partial biliary obstruction(1) and for lowering plasma cholesterol levels(2,3). Cholestyramine binds bile acids in the intestines and prevents their reabsorption *via* the enterohepatic cycle(1). Little has been done to determine whether this loss of bile acids interferes with absorption of dietary fat and fat soluble nutrients.