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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