sufficient to bind about 60 percent of 0.01 μg (nitrogen) of I^{131}-labeled BSA. To 0.5 ml portions of this dilution of antiserum to BSA (in 10 percent normal rabbit serum) was added 0.5 ml of increasing amounts of unlabeled RSA or BSA, as a blocking antigen. After 18 hours at 0°C, 0.5 ml of buffer solution containing I^{131}-BSA (0.01 μg N) was added to each tube, followed by another 18 hours at 0°C. Then 1.5 ml of saturated ammonium sulfate was added to each tube, and the resulting precipitate was removed by centrifugation, washed, and the I^{131} content was determined. As can be seen in Fig. 2, the reaction between labeled BSA and antiserum to BSA was completely blocked by a sufficient quantity of either BSA or commercial RSA. The slopes of all the curves are quite similar, and from the amount of RSA required to produce 50 percent inhibition (0.49 to 0.75 μg of RSA nitrogen as compared with 0.00265 μg of BSA nitrogen), it could be calculated that the three RSA preparations examined contained from 0.35 to 0.54 percent BSA. When inhibition was related to the calculated amount of BSA in each increment of commercial RSA, all four curves were superimposed (Fig. 3).

The data strongly indicate that all of seven commercial RSA preparations, both crystalline and fraction V, from two different sources, contained appreciable quantities of BSA. It is most unlikely that the denaturation processes during preparation could have altered the rabbit albumin so greatly that it would cross-react 100 percent with BSA, which appears to be the only alternative explanation of the data presented. The extent to which the commercial RSA samples might also have contained proteins from species other than the bovine was not investigated. The contamination observed could probably have resulted from inadequate cleaning of fractionating equipment, and it appears to be serious enough to warrant this note of caution (3).

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Homograft Rejection in the Fetal Lamb:
The Role of Circulating Antibody

Abstract. Specific rejection of sterile orthotopic skin homografts by the fetal lamb in utero was unaccompanied by the presence of plasma cells in either the graft and its bed, or in the reactive draining lymph node. The grafts appeared not to stimulate the production of circulating immunoglobulins. The presence in the fetal circulation of rabbit anti-sheep 7S γ-globulin and anti-β_{2M}-globulin did not inhibit the normal course of homograft rejection. These data support the contention that conventional circulating antibody is not an obligatory participant in the rejection of solid-tissue homografts.

The nature of the immunologic mechanisms responsible for the rejection of orthotopic skin homografts has not yet been fully resolved (1). While most investigators favor the view that graft rejection involves the mechanism of delayed hypersensitivity mediated by sensitized mononuclear cells (2), the obligatory participation in this process of conventional circulating antibody has proved difficult to exclude (3). This report describes an experimental model that may provide a suitable approach to this problem. The method takes advantage of the ability of the fetal lamb *in utero* to reject skin grafts specifically (4), and of the fact that in this unique environment the fetus is deficient or completely lacking in immunoglobulins (5). This deficiency results from the limited production of these proteins by the fetus, and also from the inability of maternal antibodies to cross the ovine placenta.

Employing a modification of the technique of Schinkel and Ferguson (4), orthotopic fetal skin grafts were applied to the fetal lamb *in utero* between the 120th and the 139th day of the normal 150-day gestation, without interruption of pregnancy. At appropriate times thereafter the fetuses were delivered by Caesarian section and bled from the umbilical cord for serologic studies, and the grafts and representative lymphoid tissues were collected for histopathologic examination. Ten fetal lambs were grafted for the purposes of the present study; nine of these proved to be useful in that a primary take of the graft was evident. Initial healing and subsequent graft rejection could be evaluated in the usual manner. The typical pattern of epithelial junction and proliferation and vascular anastomosis was followed by mononuclear infiltration, vascular stasis, hemorrhage, epithelial death, polymorphonuclear invasion, and ultimately by necrosis and hyalinization of donor collagen. Autograft survival served as a further control of the technique.

The ability of the homograft to stimulate plasma cell and immunoglobulin formation in the fetus was studied by employing fetal skin as donor material. Full-thickness skin was taken from an aseptically delivered fetus immediately prior to use, care being taken to maintain strict surgical technique and to avoid the introduction of other exogenous antigens. The fetuses were delivered 7, 10, and 14 days later. Histologically, each of the grafts was found to have taken well, and all were in the process of typical rejection. In no instance were mature or immature plasma cells found either in the infiltrate in the graft or graft bed, or in the lymph node draining the graft site. Pyroninophilia was limited to large lymphocytes in the reactive areas of the draining lymph node. The sera of these animals revealed no detectable change from the normal electrophoretic pattern, in that the β_{2M}-globulin did not appear to be increased above the normally observed amount, and the typical 7S γ-globulin and β_{2A}-globulin arcs were still lacking (5).

These data suggest that the fetal lamb, demonstrably competent to form antibodies against a number of different antigens (6), did not respond to a sterile skin homograft with the formation either of those cells normally implicated in the production or those proteins involved in the action of circulating antibody.

In order to test more critically the suggestion in the foregoing data that circulating antibody plays no role in skin homograft rejection in the fetal lamb, four additional fetuses were grafted. In this experiment, however, the fetuses (weighing some 1500 to 2000 g) were injected intraperitoneally or intracardially with 10 ml each of rabbit anti-sheep 7S γ-globulin and rabbit anti-sheep β_{2M}-globulin at the time of the grafting procedure. In addition, two fetuses were similarly grafted, but injected with 20 ml of normal rabbit serum as an experimental control.

Ten days later the fetuses were delivered, bled, and autopsied.

The rationale behind this experiment was as follows: If the fetus could be shown to reject a skin homograft in the normal way in the presence of persisting rabbit anti-sheep globulins, then the conclusion would be almost inevitable that circulating antibody does not play an obligatory role in this process. Under these conditions, fetal antibody globulins, even if produced, should be unable to reach the graft site. If, on the other hand, graft rejection were inhibited or altered by the rabbit anti-sheep globulins, then circulating anti-graft antibody must be implicated in the rejection process according to a similar line of reasoning.

In every instance, the grafts were rejected in an apparently normal fashion. In addition, each fetus showed a persisting level of circulating rabbit γ-globulin at the end of the 10-day period. Of the animals that had received rabbit anti-sheep globulins, only one showed persistence of detectable anti-sheep 7S γ-globulin and anti-sheep β_{2M}-globulin activity in its serum by tanned cell hemagglutination assay. Although the titers were only moderate (1 : 8 in each case), satisfactory negative controls and standards render the data significant. It became apparent that at this point (10 days after injection) the rabbit anti-sheep globulins were being cleared rapidly from the fetal circulation, and that we were fortunate in finding persisting anti-globulin activity even in the one fetus. In addition to the normal catabolism of rabbit protein in the lamb, some of the rabbit anti-sheep globulins were disappearing due to their binding by the continuous production of low levels of β_{2M}-globulin normally found in the fetal lamb (5). Perhaps the chief reason for the rapid disappearance of the rabbit anti-sheep globulins, however, was initiation of antibody production by the fetus against one or more of the rabbit protein antigens injected, since each of these animals had plasma cells in its lymph nodes and spleen. Such antibody globulin response on the part of the fetus would tend to deplete its circulation more rapidly of rabbit anti-sheep globulin antibodies. These considerations suggest that during the earlier stages of graft rejection, circulating anti-sheep globulins were probably present in all four animals. In future experiments, more highly purified rabbit globulin preparations should be employed to diminish the

number of antigens to which the fetus may respond.

Despite these drawbacks, it is felt that this experimental model has provided useful data and may be capable of further improvement and broader application. Thus far, it has furnished evidence that the formation of plasma cells and immunoglobulins is not a necessary accompaniment of the homograft rejection process, and that the orthotopic skin graft in the fetal lamb is rejected normally even in the presence of circulating anti-sheep globulins. These data are offered as evidence that conventional circulating antibody may not be an *obligatory* participant in the specific rejection of solid-tissue homografts (7).

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X-ray Diffraction Pattern of Nerve Myelin: A Method for Determining the Phases

Abstract. *The x-ray diffraction patterns of normal myelin, and of myelin expanded by soaking in hypotonic solutions, have been compared quantitatively. Application of the "shrinkage stage" method for determining the course of a real Fourier transform allows all but 2 of the 32 possible sign combinations to be eliminated.*

The small-angle diffraction pattern of nerve myelin consists of five orders of a fundamental repeat of 150 to 180 Å (depending on the type of nerve and species). Electron microscopy shows that this repeating structure consists of two adhering plasma membranes, the surface of adhesion presumably forming a mirror plane at low resolution. Since there are two surfaces of adhesion for each repeat, there will also be two such mirror planes, respectively, between the adhering inner (cytoplasmic) and outer surfaces of the membranes. Although these mirror planes strictly apply only locally and at low resolution to the complete myelin structure, they should apply exactly to the projection of myelin's electron density onto a radial line. It is precisely this projected electron density about which the equatorial diffraction pattern provides information. From the intensity of each order h, the magnitude $|F(h)|$ of the corresponding structure factor can be calculated, but its phase is indeterminate. Because of the mirror planes, each $|F(h)|$ can have only two possible

phases, which are equivalent to plus or minus sign. If these signs could be determined, the relative average electron density in successive planes of a cell membrane could be calculated, as a Fourier synthesis, to a resolution of about 30 Å.

Additional information is needed to decide between the 2^5 possible Fourier synthesis. Preliminary results, from a quantitative comparison of the diffraction patterns of normal nerves and of nerves modified by soaking in hypotonic media, are reported here. Soaking causes the myelin membranes to separate at their outer surfaces while the inner surfaces remain attached (1). At the same time the diffraction pattern completely changes to give successive orders of a new repeating unit of 250 to 270 Å, but this change can be reversed by reimmersion in normal Ringer solution (2). This reversibility suggests that the structure of the membranes is not much altered during the swelling process, and that the change in the diffraction pattern could be accounted for by the separation of the lamellae.