Production in Rabbits of Immune Tolerance to a Mixture of Antigens.* (27186)

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An important problem in immunology has been the means of production of antibodies in high concentrations, to an abnormal or unusual antigen mixed with many normal human antigens. The solution to this problem would probably be of considerable importance in diagnosing, and understanding the pathogenesis and relationships of various diseases. Diagnostic antisera which are of practical value in many conditions today can be produced because it is possible to get pure cultures of pathogens for injection into animals. However, when the abnormal antigen can only be found in a mixture of normal human antigens, the problem becomes more difficult. Several approaches are possible. Attempts have been made by biochemical means to obtain from human material relatively pure preparations of abnormal antigens. Another approach is a form of subtraction. An animal is immunized with material from a patient with a particular disease. Subsequently, the animal's antiserum is adsorbed or neutralized with normal human serum or tissues, and the filtrate examined for antibodies to the abnormal antigen. There are, however, serious limitations to this approach, as discussed below. A third method involves that of immunologic tolerance. If an animal can be made adequately tolerant to all antigens normally present in human blood and subsequently immunized with a mixture of normal human and other antigens, it should in theory, produce antibodies only to the other antigen(s).

However, there is a question as to whether it is possible to produce in experimental animals, adequate immune tolerance to the many antigens normally present in human blood. Most of the past studies have dealt with single purified antigens, and it was found that substantial amounts of an antigen are needed to induce tolerance(2,3). The following study was designed to find out whether or not adequate immune tolerance to the many antigens of human blood can be produced in rabbits.

Methods and materials. Three litters of New Zealand White Rabbits were used. One litter comprising 5 animals was designated a control litter, and was untreated during the neonatal period. Two litters, totalling 9 animals, were treated during the neonatal period, in an attempt to induce immunological tolerance to the antigens in normal human blood. On the day of birth, each experimental animal received, intraperitoneally, 1 ml of normal human blood constituents. This injection was repeated twice more during the next 17 days, so that each of the experimental animals received a total of 3 ml of normal human blood constituents during the first 17 days of life.

The human material injected was obtained from healthy, normal blood donors. In essence, it was whole blood, fortified with extra buffy coat. Some of the human blood was centrifuged at 2500 rpm for 30 minutes, and the buffy coat, some red cells, and plasma removed and mixed together. The injected mixture was approximately 50% plasma, 25% buffy coat, and 25% red cells. Leukocyte counts of the mixture ranged from 100,000 to 150,000 per mm^3.

After the age of 17 days, both control and treated litters received identical treatment. On reaching the age of 3 months, each rabbit received a series of immunizing injections of human, pig, and lamb serum. The human serum was obtained by pooling serum of normal donors; the pig and lamb serum were purchased from Difco Labs. The immunizing series consisted of injections of each of the 3 species' serum, every 7 days for a total of 5 injections of each kind of serum. Ten

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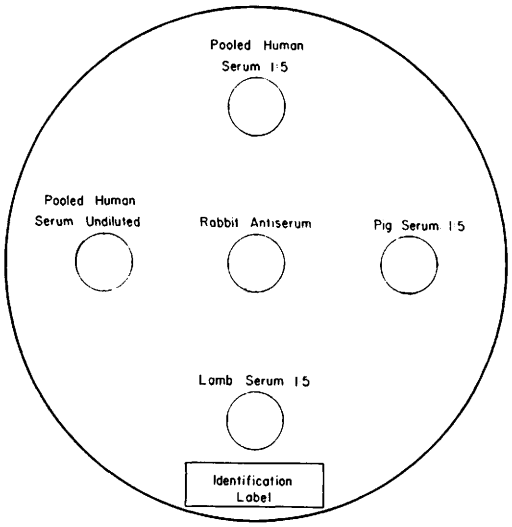

FIG. 1. Arrangement of Ouchterlony plates.

days after the last set of immunizing injections, blood was drawn from the ear vein of each rabbit, and the serum separated.

The rabbit anti-serum was then tested using the Ouchterlony plate technic essentially as described by Korngold(1). A center well was surrounded by 4 peripheral wells. The distance from the center of the interior well to the center of each peripheral well was 1.8 cm. The wells were filled only once. The center well contained 0.2 ml of the anti-serum of the rabbit tested, and the peripheral wells contained respectively, human serum undiluted, human serum diluted 1:5, pig serum diluted 1:5, and lamb serum diluted 1:5. Fig. 1 illustrates the general arrangement of the Ouchterlony plates.

In this study, "adequate" immune *tolerance* to human antigens was considered to be a degree of tolerance which would so prevent development of antibodies to human serum, that in the Ouchterlony plate, the rabbit antiserum would not produce any precipitation lines against the human serum, either diluted 1:5, or undiluted.

Results. The sera of each of the 5 control rabbits gave similar patterns. There were multiple dense precipitation lines between the rabbit antiserum and each of the 4 peripheral wells. The precipitation lines between rabbit antiserum and normal human serum diluted 1:5 were of approximately the same density of precipitation lines between the rabbit antiserum and the pig and lamb serum diluted 1:5. Precipitation lines between the rabbit antiserum and the undiluted human serum were denser than the others (Fig. 2).

The Ouchterlony plate of one of the 9 experimental rabbits which had presumably been made immunologically tolerant to normal human serum at birth, gave the same pattern as the control rabbits. This animal can therefore be considered a failure in tolerance induction. In 8 of the 9 experimental animals, however, an entirely different pattern was seen. The precipitation lines between rabbit antiserum and pig and lamb serum, 1:5 were of approximately the same density as seen in the control animals. However, there were no lines between the rabbit antiserum and the normal human serum either diluted 1:5 or undiluted (Fig. 3).

It may also be of interest that in the control animals, there were usually 2, and occasionally 3 precipitation lines between the normal rabbit serum and the pig and lamb. In the case of the experimental animals, however, there was usually one additional precipitation line between rabbit and pig or lamb serum.

Discussion. Inasmuch as the results in each of the 5 control animals showed dense precipitation lines against normal human serum, it appears that the human serum used is no less antigenic for rabbits than the pig and lamb serum and also that the immunization technic used was adequate to produce reasonably high antibody titers in rabbits to the sera of all 3 species tested. Since the antisera of the experimental rabbits produced distinct dense precipitation lines against pig and lamb serum, but not against human serum, even when the human serum was 5 times as concentrated as the pig or lamb, it appears that the tolerance-inducing injections given in the neonatal period were effective in producing a substantial degree of immunologic tolerance. This does not, of course, mean that the immunologic tolerance produced was 100% complete. However, for most purposes 100% complete immunologic tolerance would not be necessary.

It is noteworthy that a substantial degree

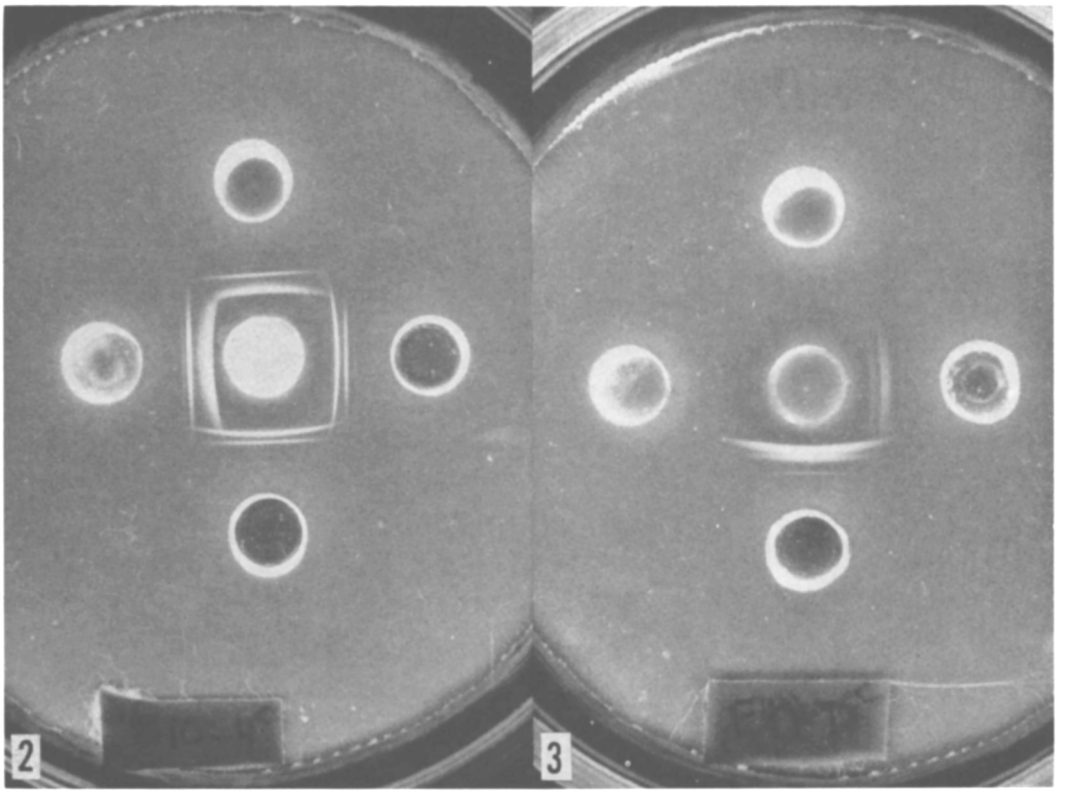

FIG. 2. Example of an Ouchterlony plate of anti-serum from a control rabbit. Note precipitation lines against human, pig, and lamb serum.

FIG. 3. Example of an Ouchterlony plate of anti-serum from an experimental rabbit. Note precipitation lines against pig and lamb serum, but not against human serum.

of immune tolerance to normal human serum was produced in these rabbits by injection of only 3 ml of human blood during the neonatal period. On the basis of other reports of immunologic tolerance(2,3) it is probable that a greater degree of tolerance can be obtained by increasing the amount of human blood given to the neonatal animals. In this connection, in a later study not yet completed, we were able to administer as much as 30 ml of human blood to neonatal rabbits with no appreciable mortality. Presumably, therefore, it should be possible to produce in rabbits an even greater degree of tolerance than has been demonstrated in this study.

In comparing these findings with those reported by others, it is essential to keep in mind the importance of species differences. In many animals, such as the guinea pig and sheep, it is not usually possible to produce immunologic tolerance in the neonatal animal. To be effective, tolerance-inducing injections must be given to the fetus, often in an early stage of development. In other animals even when tolerance can be induced in the neonatal animal, it does not persist for more than 2 or 3 weeks unless a series of reinforcing injections are given at frequent intervals. Furthermore, even when the rabbit is used as the animal to be made tolerant, there are differences in the effectiveness with which tolerance can be induced to different species. It is possible to give very large amounts of human blood to neonatal rabbits without an appreciable mortality. As much as 5 ml of human blood can be given on the day of birth to a rabbit which only has 2.5 ml of rabbit blood, and if carefully done, this procedure results in a negligible mortality rate, and as mentioned above, it has been possible to administer as much as 30 ml of human blood in the 14-day neonatal period without appreci-

able mortality. On the other hand, mouse blood and mouse tissues appear to be relatively toxic to neonatal rabbits. On the basis of the reported literature, it appears that the maximum amount of mouse blood or mouse tissue homogenate which can safely be given to neonatal rabbits in the first 14-21 days of life is about 2 ml(4,5). We have observed much the same thing, since our attempts to give more than 2 ml of mouse blood to neonatal rabbits resulted in close to 100% mortality and administration of 1.5 ml to the neonatal rabbits produced about 50% mortality.

Since this study has demonstrated the feasibility of producing in neonatal rabbits a substantial degree of tolerance to the antigens in human blood, this procedure may be found useful in solving other immunologic problems, such as the elucidation of the antigenic relationships in cancer, and other conditions in which presence of an abnormal antigen is suspected.

Conclusion. In neonatal rabbits, a substantial degree of immunologic tolerance has been produced to all the antigens in normal human serum.

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In vitro Degradation of I^{131} Labeled Angiotensin II by Normotensive and Hypertensive Human Serum.* (27187)

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The rate of degradation of intravenously administered angiotensin II-I^{131} has recently been demonstrated to be slower in patients with untreated primary benign hypertension than in normotensive subjects(1-5). The angiotensin II-I^{131} degradation rate, however, in 2 patients with secondary renal hypertension was in the hypertensive range and in one patient was in the normotensive range(2). Biological assays of the blood of hypertensive and normotensive patients indicated that greater quantities of angiotensin were present in the former than in the latter group of patients(6-8). These observations prompted investigation of the effect of normotensive, untreated primary benign hypertensive and untreated secondary renal hypertensive human sera on the degradation of angiotensin II-I^{131} *in vitro*.

Materials and methods. Iodination of angiotensin II (val-5-angiotensin II and ileu-5-angiotensin II)†‡ was accomplished by the Newerly modification of the method of Pressman and Eisen(2). Complete removal of the iodide and iodate remaining after iodination was effected by passage through resin columns.

Sera were obtained from hospitalized normotensive ward patients and from untreated primary benign hypertensive and untreated

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