EVIDENCE FOR ACTIVELY ACQUIRED TOLERANCE TO Rh ANTIGENS

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Billingham *et al.*[1] reported that mice and chickens injected as embryos with cells from other strains acquired a persistent tolerance to later skin grafts from the donor strains. It seemed possible to us that a similar, but naturally acquired, tolerance might be in part responsible for the variation in response of Rh-negative persons exposed to the Rh antigens.[2] The hypothesis to be tested was that Rh-negative children of Rh-positive mothers might, as a result of their exposure as embryos to the maternal Rh antigen, acquire a degree of persistent tolerance toward the antigen. Rh-negative children of Rh-negative mothers, on the other hand, could have had no similar embryonic exposure and might therefore be expected to react more easily upon encountering an Rh-positive antigen in later life.

We have collected data on the mothers of two groups of Rh-negative women: first, those in whom there is no evidence of Rh sensitization within three Rh-positive pregnancies and, second, those who have developed evidence of Rh sensitization during or before their third Rh-positive pregnancy. The first group we classify as relatively "tolerant" and the second as relatively "intolerant" to Rh antigens. Table 1 lists the Rh types of the mothers of women in these two groups. A simple χ^2 test for homogeneity yields a probability of less than 0.01 that the two samples of mothers could have been drawn by chance from the same population. In other words, these data seem to offer good reason to believe that the degree of Rh tolerance displayed by Rh-negative women is indeed related to their mothers' Rh types.

A number of cases included in the study have been omitted from Table 1 because of uncertainties in their classification. For example, stillbirths or miscarriages of unknown Rh type made it impossible to classify some of them according to the criteria of tolerance we have employed. Some of the data that are included in Table 1 are open to question on other grounds. Ten of the women were given transfusions or injections of blood at some time prior to their third Rh-positive pregnancy. Some of these transfusions were with Rh-positive blood, others with blood of unknown Rh type, and others probably with Rh-negative blood. Two additional women believed that they might have received injections or transfusions of blood but were not certain that they had. It is difficult to evaluate the number of exposures of these twelve women to Rh antigens or to weigh the sensitizing significance of blood injections or transfusions relative to the importance of an Rh-positive pregnancy. Table 2 lists the data after the exclusion of all cases in which there is a record suggesting that blood may have been transfused or injected. A χ^2 test for homogeneity on the data of Table 2 yields a probability of less than 0.001. It should be noted that transfusions or injections with Rh-positive blood appear to provide a very effective stimulus to Rh sensitization and that a considerable number of the Rh-negative daughters of Rh-positive women who fall in the "intolerant" class have been stimulated by this route.

The data in Table 2 are drawn mainly from two rather different sources. Fifty-one cases were studied through the Pasadena Rh Testing Laboratory.[3] The records of this organization provide unusual opportunities for studies of this sort, because over a period of years the sera of Rh-negative women married to Rh-positive men have been routinely checked for the development of Rh-antibodies during successive pregnancies, and histories have been kept. We can say with certainty that the thirty-four Rh-negative women listed as tolerant in this group failed to develop Rh antibodies detectable by the most sensitive modern tests. All of them had at least three Rh-positive pregnancies. Similarly, the seventeen women listed as "intolerant" in this group are known to have developed Rh antibodies in three or less Rh-positive pregnancies; the types and titers of these antibodies are known, and the Rh types of the grandmother, the mother and her husband, and the children

TABLE 1

RH TYPES OF MOTHERS OF RH-NEGATIVE WOMEN

WOMAN'S CLASSIFICATION	MOTHER'S TYPE		TOTAL
	Positive	Negative	
Tolerant	32	9	41
Intolerant	27	29	56
Total	59	38	97

TABLE 2

RH TYPES OF THE MOTHERS OF RH-NEGATIVE WOMEN, EXCLUDING WOMEN TRANSFUSED OR INJECTED WITH BLOOD

WOMAN'S CLASSIFICATION	MOTHER'S TYPE		TOTAL
	Positive	Negative	
Tolerant	32	9	41
Intolerant	18	26	44
Total	50	35	85

TABLE 3

RH TYPES OF THE MOTHERS OF RH-NEGATIVE WOMEN (PASADENA DATA, BASED ON ANTIBODY DETERMINATIONS ONLY)

WOMAN'S CLASSIFICATION	MOTHER'S TYPE		TOTAL
	Positive	Negative	
Tolerant	25	9	34
Intolerant	4	13	17
Total	29	22	51

are known from tests at the same laboratory. There is no record of transfusion or injection of blood in any of the cases listed in Table 3, which classifies the data from this well-defined group. The numbers are rather small, and Yates's correction for continuity has been applied as an element of conservatism in computing the χ^2 value, which yields a probability of less than 0.01 that the "tolerant" and "intolerant" groups could have been drawn by chance from a population homogeneous with respect to the grandmothers' Rh types.

The other major source of data for this study has been the Hematology Clinic of the Los Angeles Childrens Hospital. Infants are referred to this clinic when Rh difficulties are known or suspected; the occurrence of maternal Rh sensitization is assumed with the finding of a positive antiglobulin test in an infant born of known Rh-incompatible parents. The basis for the selection of cases for "tolerance"

classification in this group is therefore somewhat different from that in the group described above. In the Pasadena group, "tolerance" is classified according to the development of detectable Rh antibody, regardless of evidence of effects of such antibody on an infant. In the Childrens Hospital group, only women who have given birth to erythroblastotic babies have come to the attention of the study. Twenty-one such cases remained after uncertainties regarding transfusions or injections had been excluded from the data. All of these delivered infants having a positive antiglobulin test, and all but two of them had delivered such an infant within three Rh-positive pregnancies. Twelve of these women were the daughters of Rh-positive mothers, and seven had Rh-negative mothers. The two who had shown evidence of Rh sensitization only in their fourth Rh-positive pregnancies were daughters of Rh-positive mothers. The Childrens Hospital group offers no indication of an association between the production of an erythroblastotic child by an Rh-negative woman and the Rh type of the mother of that woman.

After our study had been under way for some time, our attention was called to a prior publication[4] reporting data similar in nature and origin to our Childrens Hospital group and conceived as a test of an idea similar to that upon which our study has been based.[5] Booth *et al.*[4] found that Rh types of the maternal grandmothers of one hundred and thirteen erythroblastotic babies were distributed according to the population incidence expected of the mothers of Rh-negative individuals, and concluded that "there is therefore no evidence to be found in these figures that the chance of an Rh-negative woman making anti-D is influenced by the Rh group of her mother." We must observe that the data of Booth *et al.* do not test the "chance of making anti-D" directly but depend only upon the appearance of diagnosed erythroblastosis. Furthermore, they do not test for the ease of sensitization of the mother, in terms of the Rh-positive pregnancy in which sensitization occurred and no consideration is given to transfusions or other complicating elements in such a classification. Nevertheless, the comparable section of our data is consistent with this previous study. There appears at present to be no reason to believe that the occurrence of erythroblastosis in a child is related to the Rh type of the child's maternal grandmother.

We therefore face a puzzling situation in interpreting our over-all data, and particularly the careful and critical data derived from our Pasadena group. There seems to be rather strong statistical evidence that the tendency to develop Rh antibody in an early Rh-positive pregnancy is greater among the Rh-negative daughters of Rh-negative women than among the Rh-negative daughters of Rh-positive women. But there is no evidence that the occurrence of erythroblastosis is similarly related to the grandmother's Rh type. It is possible that these data are misleading and that a larger study would contest them. If, however, they may be accepted as sufficiently strong to justify an attempt at explanation, we are at present inclined toward the following possible interpretation: The Rh-negative daughters of Rh-positive women appear to enjoy a degree of tolerance toward the Rh-positive antigens. This tolerance is sometimes overridden in an early Rh-positive pregnancy, perhaps by greater or more extended exposure to the antigen, and under these circumstances erythroblastosis is likely to occur. The Rh-negative daughters of Rh-negative women show no similar initial tolerance and are more likely to develop antibody on slight provocation. Under these circumstances, however,

the kind or amount of antibody produced in an early Rh-positive pregnancy is such as to permit a considerable proportion of the infants to escape without diagnosed erythroblastosis. Thus, when tolerance is classified in terms of antibody development, the relationship to the mother's type is evident, but when one classifies in terms of erythroblastosis, the relationship is obscured.

We have attempted to test this possibility by checking the hospital records of the Pasadena mothers that had been classified as intolerant only on the basis of antibody tests. All four of the daughters of Rh-positive mothers who developed antibodies in three or less Rh-positive pregnancies gave birth to severely erythroblastotic babies. Only six of the thirteen daughters of Rh-negative mothers who developed antibodies had babies, in these early sensitized pregnancies, that would surely have been classified as erythroblastotic in the absence of preliminary antibody tests. Four gave birth to babies without evidence of erythroblastosis, two were only very mildly affected, and one pregnancy is still in progress. These data are therefore consistent with the interpretation offered above, but they are so restricted in scope as to be acceptable only as slight and tentative evidence that the interpretation may indeed be sound. We suspect that other factors, which we shall not discuss here, may also be involved.

The mechanism for the acquisition of the postulated tolerance, if it exists, remains unknown. In multiple births of cattle[6] an apparently similar tolerance is associated with the establishment of intact cells, interchanged between embryos. If the present hypothesis stands, it would be of considerable interest to determine whether the maternal Rh antigen or intact maternal cells mediate a similar acquired tolerance.

Studies of the responses of human volunteers to injected blood may provide important sources of data bearing on this subject. For example, Wiener[7] has noted marked variation in the ease of sensitization of Rh-negative persons to Rh antigen. It appears that about four-tenths of the volunteers may be described as easily sensitized. This figure is in remarkable agreement with the prediction from the present hypothesis, because about four-tenths of the Rh-negative persons in our population should have Rh-negative mothers. The determination of the maternal Rh types for volunteers classified in terms of kind and degree of antibody response might be most pertinent.

Summary.—Data are presented suggesting that the probability of development of Rh antibody in an early Rh-positive pregnancy is related to the Rh type of the mother of the Rh-negative woman involved. Rh-negative daughters of Rh-positive women appear to enjoy some relative "tolerance" to the Rh antigen, compared with the Rh-negative daughters of Rh-negative women, when antibody test on their sera are used as the criteria of detection and classification of "tolerance." This relationship to the maternal type, however, does not appear to hold when the appearance of erythroblastosis is used as the criterion for the detection of "intolerance." A possible explanation for this apparent inconsistency is suggested. The hypothesis is advanced that a degree of "actively acquired tolerance" may be conferred upon an Rh-negative person by prenatal exposure to Rh antigens or Rh-positive cells derived from the mother. Presentation of this hypothesis here, on the basis of admittedly limited data, is justified by the hope that others in a position to test it will be encouraged to do so.

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[1] R. E. Billingham, L. Brent, and P. B. Medawar, " 'Actively Acquired Tolerance' of Foreign Cells," *Nature*, **172**, 603–606, 1953.

[2] Throughout this paper, the terms "Rh-negative" and "Rh-positive" refer only to reactions with anti-Rh_0 reagents, and no distinctions are made of further subdivisions within the Rh complex. The use of the term "exposure" is a matter of convenience; we do not, in fact, know the frequency, kind, or amount of true serological exposure involved in the maternal-fetal relationship.

[3] W. C. Rogers, A. G. Foord, L. G. Baldwin, and J. P. Kieffer, "The Pasadena Rh Testing Laboratory, 1947–1950," *Western J. Surg., Obstet. Gynecol.*, **60**, 345–352, 1952.

[4] P. B. Booth, I. Dunsford, J. Grant, and S. Murray, "Haemolytic Disease in First-born Infants," *Brit. Med. J.*, ii, 41, 1953.

[5] We are indebted to Dr. N. A. Mitchison for calling this prior note to our attention.

[6] R. D. Owen, "Immunogenetic Consequences of Vascular Anastomoses between Bovine Twins," *Science*, **102**, 400–401, 1945; R. D. Owen, H. P. Davis, and R. F. Morgan, "Quintuplet Calves and Erythrocyte Mosaicism," *J. Heredity*, **37**, 290–297, 1946.

[7] A. S. Wiener, "Further Observations on Isosensitization to the Rh Factor," *Proc. Soc. Exper. Biol. Med.*, **70**, 576–578, 1949.

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*ON THE NATURE OF THE EMBRYO INHIBITOR IN OVULAR TUMORS OF DATURA**

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Most interspecific crosses in the genus *Datura*, and some crosses within certain *Datura* species which have extra chromosomes, fail to produce viable seeds. It has been shown by McLean[1] and Sachet,[2] for example, that generally fertilization does take place but that the embryos do not reach maturity. The endosperm and embryo deteriorate, and in many cases a proliferation of the endothelium, the innermost layer of the integument, fills the embryo sac. Rappaport *et al.*[3] found that these outgrowths, ovular tumors or tumoral tissues, contain a water-soluble, thermostable substance, capable of inhibiting growth of *D. stramonium* embryos in vitro. It is the purpose of this paper to present data on the nature of this substance and its possible role in the mechanism of embryo abortion in vivo.

Material.—The investigation was carried out with ovular tumors from the incompatible cross *D. inoxia* × *D. discolor*. This cross yields a large number of abortive seeds in each capsule, the seeds containing an ovular tumor which often envelops a small embryo. Four to five weeks after pollination the capsules were harvested and the ovular tumors dissected from the ovules. This combination has the advantage over the cross *D. inoxia* $4n \times 2n$ used by Rappaport *et al.*,[3] in that the capsules contain a greater number of seeds, the great majority of them containing a tumoral tissue. The swollen seeds with the jelly- or cheeselike substances