Studies of Immediate Wheal Reactions and of Reaginic Antibodies in Pregnancy and in the Newborn Infant. (29848)

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Immediate wheal reactions (IWR) are generally assumed to mirror the presence of allergic reagins which are considered to be pathogenic agents in atopic diseases such as hayfever and asthma(1,2). Reaginic antibodies are unusual because of their ability to fix firmly to tissues, in contradistinction to ordinary antibodies(3). A possibility to account for this is that reagins contain a tissue fixing globulin which becomes readily attached to a similar or complementary material present in the skin or mucous membrane of most persons(4,5). At least 2 requirements would have to be met in order for this to occur (a) immunological competence of a peculiar sort must be present which can eventuate in reagin production, (b) skin and mucous membranes must contain sufficient reagin fixing material to confer the ability to fix skin sensitizing antibodies when they are present. The second mechanism may function independently of the immune response or it may be poorly operative despite adequate antibody forming facilities. Thus agammaglobulinemic patients may be successfully utilized as passive transfer recipients, a finding which indicates wheal forming competence in these persons despite an inability to form antibodies(6). On the other hand, some apparently otherwise normal persons are completely unreactive when utilized as passive transfer recipients(7,8). The possibility has been raised that such variations in the ability to form IWR may occur neonatally because of corresponding variations in placental transmission of a skin fixing factor distinct from reaginic antibodies. It has already been demonstrated that reagins when present during the course of pregnancy are poorly transmitted from mother to child(9, 10,11,12,13). The partitioning of reagins between mother and child may be evaluated separately from substances which confer wheal forming competence upon both members if direct skin tests used to determine reagins are accompanied by passive transfer studies to determine the suitability of both persons as recipients. Evidence from such experiments would be of importance in determining whether cutaneous sensitization *in utero* is a common or uncommon event since the presence of reagins may be assessed in relation to wheal forming capacity. A review of the literature would seem to indicate that the newborn infant is capable of producing wheal responses(14-18) although conflicting evidence has been reported(19). The experimental approach reported herein may be used to evaluate the possibility that offspring of atopic mothers possess differences in skin reactivity from the normal. Such a finding, if substantiated, would possess significance in relation to heritable or transmissible factors affecting the allergic state.

In the present studies 3 pregnant women with no history of allergy and 5 women with a positive history of allergy were immunized with diphtheria toxoid in accordance with schedules described earlier(20). Diphtheria antitoxin was measured in the mothers sera antenatally and postnatally and in the sera from the corresponding newborn infants. The results were correlated with those of immediate skin reactions against diphtheria toxoid, and of passive transfer tests using this antigen at sites sensitized with serum containing reaginic antibodies.

Materials and methods. Clinical material. The patients were women in the first or second trimester of pregnancy.† All were normal uncomplicated pregnancies. Five of the 8 patients were allergic and were being simultaneously followed for this reason. At time

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† Some of the patients in this study were referred by Dr. John Weber and Dr. Macy Levine of Pittsburgh, Pa.

of delivery, all patients gave birth to normal full term infants whose early neonatal course was uneventful.

Schick tests. Highly purified diphtheria toxin‡ (Massachusetts Antitoxin Batch No. Asc 77) diluted in buffered heated serum albumin(21) to 0.2 guinea pig M.L.D./ml was used in the Schick test. Highly purified toxoid containing 0.08 Lf/ml was used as the control. The volume injected intradermally was 0.1 ml. The Schick tests were read at 48 and 96 hours.

Immunizations. All of the pregnant women selected for this test were Schick negative at the time that immunization was started. All received purified fluid diphtheria toxoid (Massachusetts Antitoxin Laboratory Batch KP59A) periodically up to the time of delivery in small doses. Toxoid was administered intracutaneously in 0.1 cc amounts (2.5 Lf = 1.3 μg in the upper arm. Typical immunization schedules are shown in Fig. 1 and 2.

Bleeding and serum specimens. Whole blood was obtained from each person by the intravenous route before the start and at the end of each experiment. Additional specimens were obtained at periodic intervals during the experiments. When multiple injections of antigen were given, a blood sample was obtained at time of each injection. Sera were removed by centrifugation after blood samples had clotted, and were stored at −20°C until tested, then were rapidly brought to room temperature. Cord blood was used to obtain serum from the newborn for antitoxin determinations.

Immediate wheal reactions. Reactions were read 15 and 30 minutes after performing Schick tests at the Schick control site and prior to and after immunization with toxoid. The diameter of both the wheal and erythema were measured and the presence of pseudopodia, pruritis and color intensity noted. On the basis of these criteria, reactions were rated as negative, +, ++, +++, or ++++(22). All positive reactors to Schick control had no reactions when tested with 0.1 ml of the buffered human albumin Schick diluent.

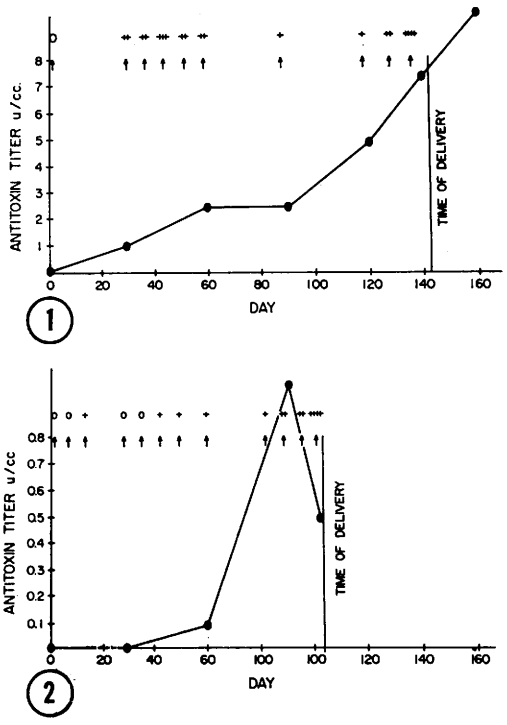

FIG. 1. Schedule of diphtheria toxoid immunization and antitoxin and immediate wheal responses in Schick negative pregnant mother (MLM). Patient MLM. ↑ = Intradermal dose of diphtheria toxoid. Plus signs signify extent of wheal reaction caused by toxoid injection.

FIG. 2. Schedule of diphtheria toxoid immunization and antitoxin and immediate wheal responses in Schick negative pregnant mother (MC). Patient MC. ↑ = Intradermal dose of diphtheria toxoid. Plus signs signify extent of wheal reaction caused by toxoid injection.

Passive transfer (P.K.) tests. The tests were carried out as follows: 0.1 ml of undiluted immune serum was injected into duplicate skin sites. The serum was obtained from an individual (Hu) who possessed antitoxic reagin(23). Each site was challenged 24 hours later with 0.02 ml of the toxoid preparation used in immunizing the donor.

Sites of skin testing. Skin tests in the mothers were carried out in the arm. Tests in the newborn were carried out in the back. The time of tests after delivery ranged from one to 4 days.

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TABLE I. Comparison Between Immediate Wheal Reactions Caused by Diphtheria Toxiod in Term Mothers and in the Newborn.

			Immediate Wheal reactions					
					Passive transfer tests			
	Antitoxin titers (units/cc)		Schick toxoid (0.1 cc i.d.)		Patient serum in normal adult recipient (24 hr toxoid challenge)		Antitoxin Hu in patient's skin (24 hr toxoid challenge)	
Name	Mother	Newborn	M	N	M	N	M	N
MN*	1.0	3.3	++++	—	+	—	++++	++
TR	<.01	<.01	—	—	—	—	++++	++++
MM	.3	.3	++++	—	—	—	++++	+++
DM	10	25	++	—	—	—	++++	+++
MZ*	.02	.01	++	—	—	—	ND	ND
MF*	.8	.8	+++	—	—	—	ND	ND
MM*	7.2	12	++++	—	+	—	++++	++++
MC*	.5	.7	++++	—	—	—	+++	+++

* Positive history of allergy.

Antitoxin determination. The *in vivo* antitoxin titer was determined by the rabbit neutralization technique described by Fraser (24). Unknown specimens were titrated simultaneously with standard antitoxin obtained from Nat. Inst. of Health.

Results. Antitoxin responses of patients to diphtheria toxoid. All persons responded to toxoid by an increase in antitoxin. Seven of eight patients formed wheal reactions during the toxoid injections (Table I). The serum of 2 patients contained small amounts of reagins as judged by IWR following sensitization of passive transfer recipients with undiluted serum and challenge with toxoid.

Comparison between diphtheria antitoxin titers and immediate wheal reactions in immunized mothers and their newborn. The antitoxin titer at term was mirrored by a similar level of antitoxin in the cord blood in 5 cases. In 3 cases the level of antitoxin was somewhat higher than observed in the mother (Table I). No wheal responses to toxoid were elicited in skin of the newborn despite the occurrence of these reactions in 7 of the 8 mothers at term.

Undiluted serum Hu which contained antitoxin reagins was used to sensitize skin of the 6 mothers and 6 infants, who thus served as passive transfer recipients. Challenge with toxoid at sensitization sites was carried out at 24 hours. Wheal responses were obtained at all sites although in 3 cases responses in the infants were smaller than those demonstrated in the skin of the corresponding parents.

Relationship between allergy history and skin responses in the newborn. The P.K. reaction differed in intensity and size between mother and newborn offspring in 3 of 6 pairs studied. However, there was no correspondence between this difference and a history of allergy in the mother (Table I).

Discussion. These studies confirm our earlier findings that frequent administration of fluid diphtheria toxoid to Schick negative persons causes a high incidence of immediate wheal reactions following challenge with intradermal toxoid(1). The findings of other workers that reaginic antibodies are not transmitted across the placenta are also confirmed. The same results are observed in offspring from allergic and non-allergic mothers. The results are not definitive since it is conceivable that the placenta is sensitive to certain threshold levels of reagins which could possibly be established by quantitative studies.

Further investigation may benefit from the use of isotopic labelled proteins late in pregnancy. This approach was adopted by Gitlin *et al*(25) who studied placental transfer of several labelled proteins, including 7S γ_2 globulin in the intact and fragmented form. Reaginic antibodies are demonstrated in γ_1 or β_{2A} electrophoretic fractions(26) whose distribution between mother and fetus has not yet been quantitated. The method of passive transfer reflects the presence of these antibodies, and therefore of at least a portion

of the corresponding electrophoretic fractions. Thus, it is possible that the placental transmission of these materials may be conveniently studied using the P.K. reaction as a screening device, particularly if measured amounts of reagins are administered late in pregnancy, and their subsequent distribution determined. Quantitative passive transfer techniques for investigation of reagins have been described (27).

It is apparent from these investigations that the skin in early infancy is able to mediate immediate wheal reactions when passive transfer tests are carried out. Similar findings have been reported by Sulzberger and Baer(18) and Dancis *et al*(28) who obtained positive reactions following histamine challenge in the skin of infants. The presence of a history of allergy in the mother seems not to be related to the extent of this ability in their newborn children. Whether the material responsible for reagin fixation in the skin at or shortly after the time of birth is derived from the mother by placental transfer, or whether it is produced in the infant is not answered by these experiments. Further studies seem indicated, particularly in allergic families and in families of persons whose skins are unreactive.

Summary. Eight Schick negative pregnant women were given repeated injections of fluid diphtheria toxoid during pregnancy to stimulate formation of reaginic antibodies as judged by IWR. Although this occurred in most cases, it was not mirrored in the cord bloods of the corresponding infants which were examined for reagins at term. In addition, the skin of all infants was unreactive in the presence of intradermal toxoid. On the other hand, IWR were elicited in these infants and their mothers when they served as recipients for passive transfer (P.K.) tests using reaginic antibodies derived from an alternate source.

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