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Received May 8, 1964. P.S.E.B.M., 1964, v116.

Maternal-Fetal Mortality in Mice with Isoantibodies to Paternal γ-Globulin Allotypes. (29454)

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A situation analogous to the Rh maternal-fetal incompatability in humans leading to erythroblastosis fetalis was developed experimentally in rabbits; this involved a maternal-fetal interaction in mothers immunized with paternal γ-globulin allotype(1). Maternal isoantibodies to the paternal γ-globulin allotype were shown to inhibit substantially the ability of the young heterozygote to develop the paternal allotype; at the same time a compensatory increase of the γ-globulin allotype controlled by the allelic gene also occurred (1). In addition, a first litter of 3 offspring from one of the mothers immunized with paternal allotype was stillborn, suggesting that fetal mortality also might be induced; subsequent litters from the same doe were viable and exhibited the "suppression-compensation" phenomenon described above(2). The high fetal mortality as well as other breeding problems in our rabbit colony precluded further evaluation of the question of fetal mortality.

The recent identification of the mouse γ-globulin allotypes and the knowledge of their genetic control(3-7) have made it possible to determine whether the "suppression-compensation" phenomenon first seen in rabbits could also be produced in mice. In addition, the low fetal mortality occurring in some strains of inbred mice favored the determination of whether isoantibodies to γ-globulin allotypes do in fact contribute to fetal and possibly maternal mortality in these strains.

Thus C57BL/6 mice with γ-globulin Asa2 were immunized with the allelic Asa1 γ-globulin of BALB/c mice to produce anti-Asa1 and conversely BALB/c mice were selected and immunized with γ-globulin of C57BL/6 mice to produce anti-Asa2 isoantibodies(6). Since γ-globulin is known to cross maternal-fetal barriers, the effect of anti-Asa2 antibody in immunized BALB/c mice, and of anti-Asa1 antibody in immunized C57BL/6 mice, on the developing heterozygous offspring which are genetically predestined to synthesize both the Asa1 and Asa2 γ-globulins could be studied.

This communication is concerned with the first phase of a more extensive investigation, and reports findings on the maternal and fetal effects of isoantibody in pregnant mice.

Methods. Four-week-old BALB/c female mice were divided into 3 groups (Ia, Ib and Ic). Group Ia was immunized with C57BL/6 γ-globulin (allotype Asa2); Group Ib was injected in the same manner with BALB/c γ-globulin (allotype Asa1); and Group Ic was not treated. At 14 weeks of age when the immunized mice (Group Ia) were producing precipitating antibody to C57BL/6 γ-globulin (allotype Asa2), all the mice in the 3 groups were mated with C57BL/6 males of the same age by placing 2 females and one male in the same cage. Thereafter, all mice were observed daily.

The mice in Group Ia were immunized by injections of washed agglutinates containing C57BL/6 γ-globulin prepared by mixing 0.1 ml C57BL/6 anti-*Proteus mirabilis* ascitic fluid with packed 10^9 *P. mirabilis* cells. The agglutinates were mixed with either complete or incomplete Freund's adjuvant or with physiologic saline and .05 ml of the mixture

was injected subcutaneously into each of the 4 foot pads, and into the inguinal and the axillary regions. The immunization schedule for each mouse was as follows: first, 2 injections in complete adjuvant at 4-day intervals; then 4 weekly injections in incomplete adjuvant and finally 4 weekly injections of the saline suspension.

After 9-10 weeks of immunization, the sera of all the mice in Group Ia exhibited precipitating antibody (anti-Asa2) to the C57BL/6 γ-globulin in Ouchterlony tests. The precipitin bands were very dense indicating the presence of a relatively high concentration of anti-allotype antibody. Except during pregnancy, additional monthly injections of the saline suspension of the agglutinates were given to the remaining mice to provide further antigenic stimuli.

The BALB/c mice in Group Ib were subjected to the same immunization procedure as those in Group Ia except that the washed agglutinates contained BALB/c γ-globulin instead of C57BL/6 γ-globulin. Ouchterlony tests of the sera of these mice failed to demonstrate antibody to the BALB/c γ-globulin.

In another experiment, the role of the 2 inbred strains was reversed. Four-week-old C57BL/6 female mice were similarly divided into 3 groups (IIa, IIb, IIc) and essentially the same procedures were followed as used in the first experiment (Ia, Ib, Ic).

Group IIa was immunized with BALB/c γ-globulin; Group IIb was injected in the same manner with C57BL/6 γ-globulin; and Group IIc was not treated. To produce demonstrable precipitating antibody to BALB/c γ-globulin (anti-Asa1) in all the immunized C57BL/6 mice (IIa), 6 additional injections of washed agglutinates in saline suspension were given over an additional 3-week period compared to the procedure used for the BALB/c mice in group Ia. The precipitin bands in the Ouchterlony tests were less dense than those obtained with the sera of the BALB/c mice in Group Ia. At 17 weeks of age (3 weeks later than in Experiment I) all mice in the 3 groups were mated with BALB/c males of the same age by placing 2 females and one male in the same cage.

For immunization of the C57BL/6 mice in Group IIb, the washed agglutinates contained C57BL/6 γ-globulin instead of BALB/c γ-globulin. Ouchterlony tests failed to demonstrate the precipitating antibody to the C57BL/6 γ-globulin.

In experiments Ia and IIa, the males were permitted to remain with the surviving females 3-4 months following the first pregnancies so as to obtain second pregnancies (Exp. Ia′ and IIa′).

Results. Table I presents the data on maternal-fetal mortality of BALB/c and C57BL/6 mothers immunized with paternal γ-globulin (experiments Ia and IIa). Precipitating antibody to paternal allotype was present in all the immunized female mice before they were mated. The two control groups for each experiment consisted of female mice injected with γ-globulin of their own (maternal) strain (Exp. Ib, IIb) and untreated female mice (Exp. Ic, IIc). None of the female

TABLE I. Maternal-Fetal Mortality in Pregnant Mice with Isoantibodies to Paternal γ-Globulin Allotypes.

				Mothers			Progeny			
Exp	Allotype & strain of γ-globulin inj into ♀ mice	Allotype & strain of male	Allotype & strain of female	No. pregnant	No. dead	No. with live progeny	No. dead 1st day	No. alive 1st day	No. weaned 4 wk later	No. weaned per pregnant mouse
Ia	a2-B6	a2-B6	a1-C	10	5	4	18	24	15	1.5
Ib	a1-C	a2-B6	a1-C	7	0	7	2	44	35	5.0
Ic	Untreated	a2-B6	a1-C	9	0	9	0	52	50	5.6
IIa	a1-C	a1-C	a2-B6	7	0	0	5	0	0	0
IIb	a2-B6	a1-C	a2-B6	8	1	7	0	42	36	4.5
IIc	Untreated	a1-C	a2-B6	9	0	9	0	42	40	4.5
Ia′	a2-B6	a2-B6	a1-C	3	0	3	2	23	19	6.3
IIa′	a1-C	a1-C	a2-B6	3	0	2	1	4	2	.7

B6 = C57BL/6; C = BALB/c; a1 = allotype Asa1; a2 = allotype Asa2.
Ia′ and IIa′ are results obtained from second pregnancies of mice in Exp Ia and IIa, respectively.

mice injected with γ-globulin of their own strain produced demonstrable precipitating antibody to the γ-globulin which had been injected.

Five of the 10 immunized BALB/c pregnant mice (Exp. Ia) died at term of pregnancy or shortly thereafter. There were no deaths among the 7 mothers injected with γ-globulin of the maternal strain (Exp. Ib), or among the 9 untreated mothers (Exp. Ic).

Among the C57BL/6 pregnant mice (Exp. IIa, IIb, IIc), only 1 death occurred and this mouse was one of the controls which had been injected with C57BL/6 γ-globulin (Exp. IIb).

Eighteen progeny of the 10 immunized BALB/c mice (Exp. Ia) were found dead on the day of birth; 10 from the 5 deceased mothers, 4 from 1 surviving female with no live progeny, and the remaining four from the 4 surviving mothers with live progeny. In this experiment, 15 of the 24 live progeny survived until weaning for an average production of 1.5 offspring per pregnant mouse.

In the control group of 7 BALB/c mice injected with BALB/c γ-globulin, there were 2 dead and 44 live progeny on the first day; 35 survived until weaning for an average production of 5.0 offspring per pregnant mouse (Exp. Ib). In the control group of untreated BALB/c mice, there were 52 live progeny on the first day; 50 were weaned for an average production of 5.6 offspring per pregnant mouse (Exp. Ic).

No live progeny were obtained from the 7 immunized C57BL/6 mice (Exp. IIa); of the 5 progeny found, all were dead. In contrast, all 42 offspring of the control group of 8 C57BL/6 mice injected with C57BL/6 γ-globulin (Exp. IIb) were alive at birth; 36 were weaned for an average production of 4.5 offspring per pregnant mouse. Similarly, in the control group of untreated C57BL/6 mice, all 42 offspring were alive at birth and 40 were weaned for an average production of 4.5 offspring per pregnant mouse.

Three of the 5 surviving immunized BALB/c mice (Ia) became pregnant a second time producing 6.3 offspring per pregnant mouse (Ia′). Second pregnancies of 3 of the 7 surviving immunized C57BL/6 mice (IIa) produced only 0.7 offspring per pregnant mouse (IIa′).

Table II presents additional information concerning the pregnant mice that failed to produce viable progeny. Four of the immunized BALB/c mothers (758, 761, 763, 764) delivered 1-2 dead progeny and then each succumbed before delivering the next fetus which remained wedged in and partially extruding from the birth canal. Palpation or autopsy of these mothers revealed the presence of additional fetuses that had been retained. One mouse (757) was found dead with 4 dead progeny which were badly bruised, misshapen and appeared to be elongated; no fetuses were retained. One mouse (765) produced 4 badly bruised and misshapen progeny but did not succumb. This mouse (765) remained caged with the male for 3 months postpartum but did not become pregnant again.

The 7 immunized C57BL/6 mothers (Exp. IIa) all survived but produced no viable progeny in their first pregnancy. Of these, 769, 770 and 771 delivered no progeny but the fetuses were retained and still present when sacrificed 3 months later. Three mothers (767, 772 and 776) delivered dead progeny; no retained fetuses were found, and subsequently these became pregnant again. One mother (768) delivered one dead offspring; no retained fetuses were found, and she did not become pregnant again during the 3 months postpartum.

The C57BL/6 control mouse (1352) succumbed at term and autopsy revealed 5 incompletely developed fetuses.

Discussion. These findings make it clear that a substantial increase occurs in the incidence of maternal and fetal deaths when male mice are mated to female mice with anti-allotype antibodies to paternal γ-globulin. It seems reasonable to conclude that isoantibodies produced this mortality, and that these phenomena involve antigen-antibody reactions. The question then arises as to the origin of the antigen which reacts with this antibody, as well as the location of the sites where the antigen-antibody reaction produces its effect.

The obvious source of the paternal allo-

TABLE II. Individual History of Pregnant Mice Without Live Progeny.

Exp	Mouse No.	Dead progeny at birth	Time of maternal death	Remarks
Ia BALB/c immunized with paternal (C57BL/6) γ-globulin	757	4	1 day postpartum	Badly bruised and misshapen elongated fetuses
	758	1	*Idem*	Retained fetuses (palpated); 1 in birth canal
	761	2	"	4 retained fetuses; 1 wedged in birth canal (autopsy)
	763	2	15 days postpartum	Retained fetuses (palpated); 1 in birth canal. Progressive blanching and weakness of mother
	764	1	At term	5 retained fetuses (autopsy)
	765	4	—	Badly bruised and misshapen elongated fetuses; no 2nd pregnancy 3 mo postpartum
IIa C57BL/6 immunized with paternal (BALB/c) γ-globulin	767	2	—	2nd pregnancy
	768	1	—	No 2nd pregnancy 3 mo postpartum
	769	0	—	Fetuses palpated at term. Fetuses retained 3 mo after pregnancy with pockets of white calcified material in uterus (autopsy). No 2nd pregnancies within 3 mo.
	770	0	—	
	771	0	—	
	772	1	—	2nd pregnancy
	776	1	—	" "
IIb C57BL/6 inj with C57BL/6 γ-globulin	1352	0	At term	Incomplete development of 5 fetuses (autopsy)

type antigen is from the developing heterozygous fetuses which are genetically constituted to produce the same allotype as that of the father. However, allotype with the same specificity as that of the father is not found in the sera of normal heterozygotes until 24-32 days after birth when tested by Ouchterlony precipitin tests. This allotype may nonetheless be present in serum in amounts undetectable by the precipitin method; furthermore, the allotype may be also present in the allotype-synthesizing cells of the fetuses. Indeed, in the fetuses these cells may first function in producing allotype antigen shortly before birth since, in our experiments, maternal-fetal mortality occurred at term or shortly thereafter.

Since the mother's antibody to paternal allotype can cross the maternal-fetal barrier, the allotype synthesizing cells of the heterozygous fetus develop in the presence of antibody to one of the allotypes it will synthesize. Thus, the postulated antigen-antibody reactions could occur in the region of the cells which synthesize the allotype antigen; this might very well lead to cell destruction and ensuing fetal death. Should this actually occur, the maternal mortality in the immunized BALB/c mice (Exp. Ia) would be interpreted as a consequence of fetal death. The absence of maternal deaths in the immunized C57BL/6 mice (Exp. IIa) might be attributable to the small litter (compared to that of the BALB/c mice) or it may be a consequence of some other strain difference presently unidentified. On the other hand, although the antigen-antibody reaction can occur at the site of the source of antigen in the fetuses, this reaction may not be toxic to the allotype-synthesizing cells in the fetus, but may exert its effect indirectly on other fetal tissues or even maternal tissues. Thus the possibility should not be excluded that maternal pathology occurs first, making for difficult parturition with resulting fetal death.

The antigen depots present in the mother because of the numerous injections of antigen-adjuvant mixtures may represent another source of antigen. A prolonged period of con-

tinuous release of antigen from these depots may occur favoring the formation of antigen-antibody complexes. Antigen-antibody complexes are known to cause tissue injury and increase smooth muscle contraction(8). Thus, in our experiments, these complexes could exert an adverse effect on the uterine tissue affecting its contractions during parturition indirectly causing maternal-fetal mortality.

The 7S γ-globulin allotypes (Asa1 and Asa2) are not necessarily the only paternal allotypes present in the washed agglutinates used for immunization of the female mice. Antibodies to protein allotypes other than 7S γ-globulin have been produced in our laboratory by this method(9). Although all the immunized female mice (Exp. Ia, IIa) were shown to have precipitating antibodies specific only for the Asa1 or Asa2, it is possible that other allotype-isoantibody systems may be involved which are not detectable by the precipitin tests used.

In a recent study, we identified 3 additional γ-globulin allotypes Asa3, Asa4, and Asa5. The F_2 segregation tests indicated that these 3 allotypes are controlled at the same locus as the Asa1 and Asa2(10,11). Thirty-eight inbred strains were tested and each strain exhibited only one of these allotypes. With this information, the source of antigen and the specificity of the isoantibody in our current experiments can be further elucidated by mating BALB/c female mice with anti-Asa2 antibodies to male mice of inbred strains with Asa1, Asa2, Asa3, Asa4, and Asa5 allotypes, respectively. If our hypothesis is valid, maternal-fetal incompatibility will occur only in the experiment with Asa2 fathers.

Since isoantibodies are known freely to cross maternal-fetal barriers(12,13) while lymph node cells do not(14-16), it appears likely that the observed maternal-fetal death is mediated through circulating antibodies. However, such sensitized cells may leak through the barrier(17). This possibility is being investigated.

In contrast to our results with isoantibodies to γ-globulin it is of interest that Kaliss, Dagg and Stimpfling(18) reported no maternal or fetal deaths in pregnant mice with isoantibodies to histocompatibility antigens.

The Gm groups in man are analogous to the γ-globulin allotypes in mice(19); the possibility of deleterious biologic effects due to maternal-fetal incompatibility in γ-globulins and consequent formation of maternal isoantibodies to the paternal Gm group lacking in the mothers should be investigated. Maternal isoantibodies may develop as a result of immunization of the mother by γ-globulin of the paternal Gm type present in the heterozygous fetus(20) or by transfusion of blood with the paternal Gm type(21).

Summary. A high incidence of maternal and fetal deaths has been observed in pregnant mice with isoantibodies to paternal γ-globulin allotypes. BALB/c females were immunized to produce isoantibodies to C57BL/6 γ-globulin and mated to C57BL/6 males; similarly, C57BL/6 females were immunized to produce isoantibodies to BALB/c γ-globulin and mated to BALB/c males. Fifty per cent of the BALB/c pregnant mice succumbed at term; the number of progeny surviving per pregnant mouse was 30% of the control groups. No maternal deaths occurred in the C57BL/6 pregnant mice; no live progeny were delivered. Retention of fetuses *in utero* as well as grossly misshapen and severely bruised fetuses were observed in both strains.

We wish to thank Mr. William Humphrey, Jr., and Mr. Cornelius Alexander for valuable technical assistance.

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Received May 11, 1964. P.S.E.B.M., 1964, v116.

Plasma Angiotensinase Activity in Cirrhosis.* (29455)

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Plasma angiotensinase activity is of theoretic interest in cirrhosis for several reasons. If such enzymatic activity reflects production of angiotensin(1) and thus aldosterone(2), increased activity would be expected in association with secondary hyperaldosteronism, as manifested by ascites and edema. Moreover, as activation of the renin-angiotensin-aldosterone system may result from decreased renal perfusion(2,3), plasma angiotensinase activity could be augmented by renal circulatory failure(4) in hepatic disease. An alternative suggestion is that, like other enzymes, angiotensinases might be released from the liver in abnormally great amounts as a result of hepatic injury(5), regardless of changes in production of angiotensin. Under such circumstances, the resulting acceleration of angiotensin degradation could contribute to the infrequency with which hypertension is said to occur in patients with cirrhosis(6,7) and might explain the reduced pressor responsiveness to angiotensin described in this disease(8).

The degradation of angiotensin has been studied in hypertension(1,9,10), edema(1), pregnancy(1,11), and toxemia(1,12). The conflicting results could come in part from use of assay methods based on different principles, namely, removal of radioactive iodine from the angiotensin molecule(10), destruction of its pressor activity(1), and release of valine measured by colorimetry(12). When the last method was applied to patients with various diseases(11), particularly high values were found in those with hepatobiliary disorders. Since the authors did not equate the rate of liberation of valine with the rate of pressor degradation, a biologic assay of angiotensinase activity in patients with cirrhosis seemed more desirable from the physiologic point of view.

Angiotensinase activity appears to have some substrate specificity, since plasma does not destroy the pressor activity of β-aspartyl angiotensin(13) nor does it inactivate arginyl[1] angiotensin, deaminoangiotensin or poly-O-acetylseryl angiotensin(14). The optimal pH and some ionic requirements are known. Klaus and associates(12) considered an aspartic acidaminopeptidase system responsible but conceded the participation of other aminopeptidases. However, a specific enzyme has not yet been identified. It is necessary, therefore, to refer to "plasma angiotensinase activity" until further purification reveals the peptidase system(s) responsible.

* This investigation was supported in part by research grants from Nat. Inst. of Health, P.H.S.