

THE INFLUENCE OF Rh HAPTEN THERAPY ON THE COURSE OF Rh ISOSENSITIZATION IN PREGNANCY

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WITH clarification of the pathogenesis of erythroblastosis fetalis it became evident that ideal therapy should be directed either at preventing the formation of maternal Rh antibodies, or, if already present, their passage into the fetal circulation in harmful amounts. Early therapeutic attempts with these goals in mind, using vitamin C,¹ serum containing blocking antibodies,² injections of Rh-positive blood,³ typhoid vaccine,⁴ and ethylene disulfonate⁵ were uniformly unsuccessful.

The possibility that a specific Rh hapten derived from Rh-positive red blood cells might be effective in neutralizing Rh antibodies was first suggested by Belkin and Wiener in 1944.⁶ Interest in the subject was renewed two years later by Calvin, Evans, Behrendt, and Calvin,⁷ who isolated by physical methods a lipoprotein substance from Rh-positive red cells which was shown specifically to inhibit Rh antisera in vitro. In 1947, Carter⁸ prepared an ethereal extract from laked alcohol-precipitated Rh-positive cells, lipid in nature and nonantigenic, which was also said to inhibit hemagglutination by Rh antisera and to fix complement. The material was thought to represent Rh hapten in an impure form and to be essentially the same as that described by Calvin et al. in protein combination. Preliminary reports of clinical trials with the latter material by Loughrey and Carter,⁹ Carter,¹⁰ and more recently by Goldsmith¹¹ seemed to indicate that it was effective in reducing maternal Rh antibody titers and in the treatment of erythroblastotic infants. On the other hand, 11 sensitized women treated with Rh hapten by Unger¹² failed to show any immunologic response to the material.

The purpose of the present communication is to report in detail the results obtained in the treatment of 10 Rh-negative sensitized pregnant women with Rh hapten.

MATERIALS AND METHODS

The 10 patients included in this study were selected from the active files of the Baltimore Rh Typing Laboratory. All, with one exception, had evidence of isosensitization to the Rh factor during one or more previous pregnancies as indicated either by serologic study or history. Serologic studies during the current pregnancy were performed at weekly intervals by methods previously described.¹³ One or more control observations were recorded in all patients, with one exception, prior to the starting of hapten therapy. The titers of antibodies present in each case are reported as albumin, serum, and saline titers, respectively, these terms referring to the type of medium in which the Rh-positive erythrocytes are suspended. In all cases the indirect blocking test of Wiener was also employed. Titers are recorded in units, i.e., the reciprocal of the highest serum dilution giving a one-plus microscopic reaction.

Nine of the patients received Rh hapten* prepared by a method similar to that of Carter.¹⁰ The method

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* We are indebted to Dr. J. M. Maas of the Lilly Research Laboratories for the supplies of Rh Hapten used in this study. The hapten was prepared in the following manner:

Human blood, Rh positive of any type, is pooled and stored at 3 to 6 degrees C. The blood is centri-

of preparation differed only in that for purposes of greater purification, a crude acetone extraction was made after preliminary treatment of the laked red cell mass with alcohol. The final product was suspended in sesame oil (100 mg. per cc.). The dose employed ranged from 100-600 mg., injected intramuscularly, per week. Case 7 received Rh hapten prepared by Mrs. Carter and given according to her instructions. Further details of the therapy are included in the case studies below.

CASE REPORTS

Case 1. Mrs. S.N., white female 31 years of age, had her first pregnancy in 1939, which terminated in the birth of a normal full term infant (type A₁ M Rh₁rh). The second pregnancy, in 1942, resulted in the birth of a stillborn infant at 40 weeks; and the third, in 1944, of a 26 week old premature infant which lived only one-half hour. There was no history of previous transfusions.

When studied by us during her fourth pregnancy in 1946, the patient was found to be type A₁ MN rh and her husband type A₁ M Rh₁Rh₁. Rh isosensitization was demonstrated. She delivered a stillborn hydropic fetus after 32 weeks of gestation. In 1948 she was studied again by us during her fifth pregnancy. The peak antibody titers during this pregnancy were 3072 units by the albumin method, 12 units by the serum method. No antibodies were demonstrable in saline media. At 30 weeks, premature separation of the placenta occurred and a hydropic stillborn fetus was delivered (type A₁ MN Rh₁rh). The cord serum showed a circulating antibody titer of 384 units of albumin agglutinin and the erythrocytes gave a strongly positive Coombs test.

She became pregnant for the sixth time in January, 1949. The expected date of confinement was September 13, 1949. At 9 weeks of pregnancy she demonstrated the following immunologic pattern: 384 units in albumin, 12 units in serum, and 4 units of indirect blocking antibody. The antibody was of Rh₀ specificity. By 20 weeks the albumin titer had risen to 3072 units, and treatment with Rh hapten, 100 mg. weekly, was begun (fig. 1). At 27 weeks the dose was increased to 300 mg. weekly and six weeks later to 300 mg. twice weekly. Fetal death in utero occurred at approximately 34 weeks, and two weeks later she delivered a 2000 gram macerated hydropic fetus. The fetal blood was not suitable for study. Peritoneal fluid, however, showed a titer of 48 units of Rh antibody in albumin.

Case 2. Mrs. H.L., white female, age 26, gave birth to a normal Rh-positive infant in 1945. The second pregnancy a year later terminated at 40 weeks with delivery of an infant, jaundiced and anemic at birth, which died twenty-four hours later. There was no history of transfusions.

When studied here during her third pregnancy in 1947 she was found to be type A₁ MN rh, and her husband type O M Rh₀. The one living child was type A₁ M Rh₀. Antibody titers during this pregnancy reached a maximum of 2048 units in albumin, 96 units in serum, 3 units in saline. At 38 weeks she delivered a stillborn hydropic infant.

The patient again became pregnant in February, 1949. The expected date of confinement was November 12, 1949. When studied on July 1, in the fifth month of pregnancy, she was again found to be rather strongly sensitized, with titers of 2048 units in albumin, 24 units in serum, and 2 units by the indirect blocking method (Rh₀ specificity). Because of the poor obstetric history she was started on Rh

fused and the cells are laked with $\frac{1}{2}$ volume of distilled water, stirring gently for fifteen minutes. Five volumes of chilled 95 per cent alcohol, e.g., 7.5 liters of alcohol for each liter of original cells is added with agitation for fifteen minutes. The whole is allowed to stand from twelve to fifteen hours at 3 to 6 degrees C. and is then filtered by vacuum. The cake is pulled dry to such a point that it is friable to the touch. The alcohol filtrate is discarded. To the cake is added again 5 volumes of chilled acetone (calculated as above for alcohol) and the whole is stirred for fifteen minutes at 3 to 6 degrees C. The acetone mixture is allowed to stand for five days at 3 to 6 degrees C. with fifteen minutes of agitation each day. The mixture is then filtered by vacuum and the cake is washed with 2 volumes of chilled acetone. The filtrates are combined and the filter cake discarded. The combined filtrates are dried in vacuo, care being taken not to overheat the mixture, and when dry the residue is extracted in the cold with ether in sufficient volume to make an efficient extraction. The insoluble material is removed from the ether and the ether filtrate is concentrated to dryness. The dried powdered material is suspended in sesame oil.

haptens, 300 mg. weekly, at approximately 22 weeks of gestation. Antibody studies and treatment schedule are given in table 1. The titers remained unchanged until September 8, when a moderate rise was observed. On September 13 the patient reported that she had felt no fetal movements for several days, and when examined three days later no fetal heart tones could be heard. Treatment was discontinued. On October 12 she delivered a 1700 gram macerated fetus. No blood or body fluid could be obtained for study.

Case 3. Mrs. O.S., white female, age 34, gave birth to a normal full term Rh-positive infant in 1942. The second pregnancy in 1944 terminated at 40 weeks with the delivery of a stillborn infant, and the third in 1946 with the birth of a hydropic erythroblastic infant which lived for only a few minutes. There was no history of transfusions.

When studied in 1947, the patient was found to be type O MN rh, and her husband type B N Rh₂. The one living child was type O N Rh₂rh. Rh antibody studies showed 1024 units in albumin, 48 units in serum, and 1 unit in saline. The qualitative blocking test was positive.

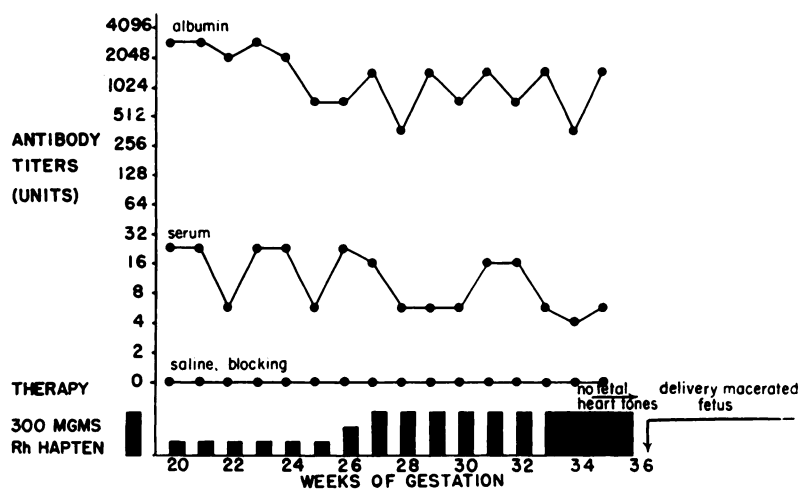


FIG. 1.—Case 1 (S.N.)

The patient became pregnant for the fourth time in December, 1948. Her expected date of confinement was September 30, 1949. She was studied on February 2, 1949, during the second month of pregnancy, and titers of 2048 units in albumin, 8 units in serum, and 2 units in saline were obtained. The antibody was of Rh₀ specificity. The indirect blocking test was negative. Rh haptens, 100 mg. weekly, was started at approximately 19 weeks of gestation. The dose was increased to 300 mg. weekly at 26 weeks and 6 weeks later to 300 mg. twice weekly (fig. 2). No significant change in titers was observed at any time. On October 22, 1949, she was delivered uneventfully of a normal full term Rh-negative infant (type B N rh). Study of the cord serum showed an albumin titer essentially the same as that of the mother at delivery. The erythrocytes gave a negative antiglobulin test.

Case 4. Mrs. B.F., white female 26 years of age, had had two previous pregnancies, both resulting in spontaneous abortions at 9 and 12 weeks respectively. At the time of the first abortion in 1948 she received one blood transfusion, the Rh type of which was not known. When first seen by us six weeks after the second abortion in February, 1949, she was found to be type A₁ MN rh, and her husband type O MN Rh₁rh. Rh antibodies were present in titers of 96 units in albumin, 6 units in serum, and 1 unit by the blocking test (Rh₀ specificity).

She was seen again on August 17, 1949, during the fourth month of her third pregnancy, when antibody titers of 256 units in albumin, 6 units in serum, and 1 unit by the blocking test were found. At 20 weeks she was started on Rh haptens therapy, receiving 300 mg. weekly for nine weeks and the same

TABLE 1.—*Immunologic Data in Ten Rh-negative Isosensitized Pregnant Women Treated with Rh Hapten*

Case No.	Therapy Begun (wk. of pregnancy)	Antibody titer at beginning of therapy*			Antibody titer at delivery			Rh Hapten Therapy Amount. Frequency	Remarks and Outcome
		Sa-line†	Ser-um†	Albu-min†	Sa-line	Ser-um	Abumin		
1. S.N.	20	0	24	3072	0	6	1536	100 mg. wkly. 20-27th wk. 300 mg. wkly. 27-33rd wk. 600 mg. wkly. 33-36th wk.	Fetal death in utero—34 wks; delivery—36 wks.
2. H.L.	22	0	24	1024	6	384	6144	300 mg. wkly. 22-28th wk.	Fetal death in utero—28th wk.
3. O.S.	19	1	4	1536	1	4	1536	100 mg. wkly. 19-26th wk. 300 mg. wkly. 26-32nd wk. 600 mg. wkly. 32-40th wk.	Delivered normal Rh negative infant. Husband heterozygous positive.
4. B.F.	20	0	6	256	0	384	12,288	300 mg. wkly. 20-29th wk. 600 mg. wkly. 29-38th wk.	Sharp rise in antibody titer at 37 wks. Delivered by caesarean section. Infant showed severe E.F. Exchange transfusion—survived.
5. C.A.	12	0	24	1536	0	32	384	300 mg. wkly. 12-40th wk.	Delivered Rh positive infant with severe E.F. Survived following multiple transfusions.
6. H.A.	22	0	6	384	0	6	256	300 mg. wkly. 22-40th wk.	Delivered normal Rh negative infant. Husband heterozygous positive.
7. S.G.	17	0	12	3072	0	24	3072	1400 mg. from 17-19th wk. 400 mg. wkly. 19-27th wk. 200 mg. wkly. 29-35th wk. 800 mg. wkly. 35-40th wk.	Delivered normal Rh negative infant. Cord Serum antibody studies identical with maternal antibody titers.
8. M.N.	29	6	48	1536	6	24	3072	300 mg. wkly. 29-36th wk.	Fetal death in utero at 36 weeks.
9. D.B.	23	0	1	24	0	1	48	300 mg. wkly. 23-40th wk.	Delivered "clinically normal" Rh positive infant. No therapy required. Maternal immunization minimal. See text.
10. A.D.	20	0	0	384	0	0	384	300 mg. wkly. 20-35th wk.	Fetal death in utero at 35 weeks.

* Titers are given in units, i.e., reciprocal of highest serum dilution giving 1+ microscopic reaction. † Refers to media used for cell suspension.

dose twice weekly during the remainder of pregnancy (fig. 3). At approximately 38 weeks, in spite of continued therapy, a sharp rise in antibody titer was observed. This finding was checked by further studies two days later. Pregnancy was promptly terminated by cesarean section.

The infant was found to be rather pale, and both liver and spleen were enlarged. Jaundice appeared shortly after birth. Cord blood study revealed 10.5 grams hemoglobin, 3.07 million red cells, and 15 nucleated red cells per 100 white cells. The infant's type was A₁ M Rh₀rh. Circulating antibodies were

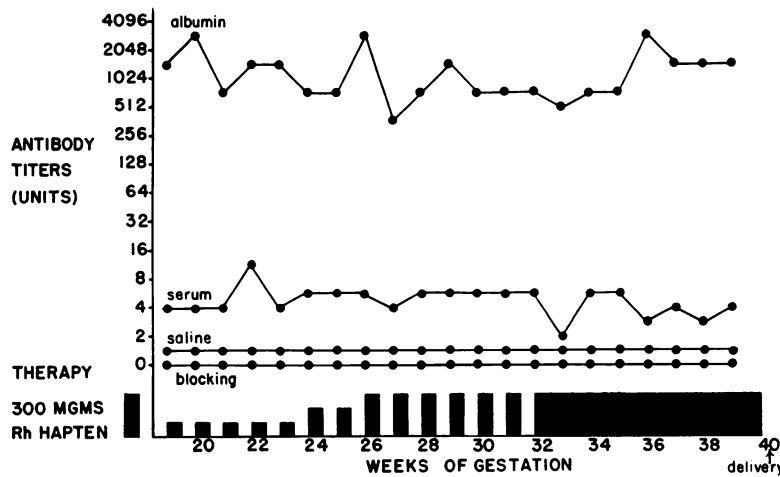


Fig. 2.—Case 3 (O.S.)

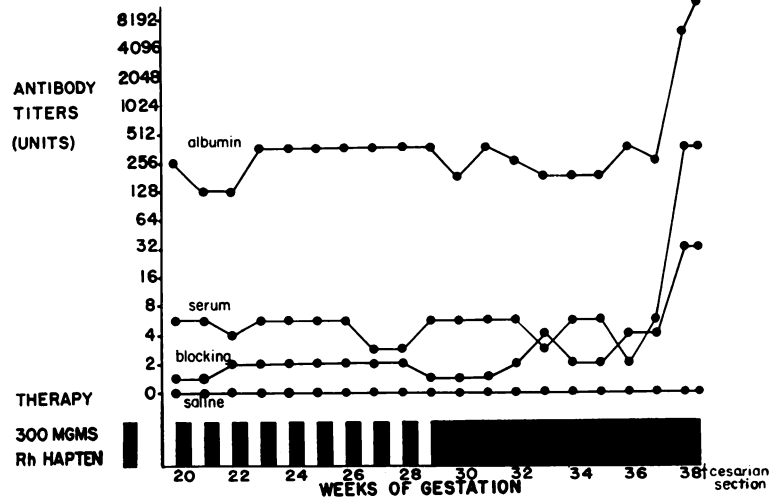


Fig. 3.—Case 4 (B.F.)

present in titers of 512 units in albumin, 24 units in serum, and 2 units by the blocking test. The anti-globulin test for coating of cells was strongly positive. At 4 hours of age an exchange transfusion with 630 cc. type rh whole blood was done without difficulty. Immediately following the procedure the circulating albumin titer dropped to 48 units, and the antiglobulin test gave only a doubtful reaction. Results in other media were negative. Three subsequent transfusions were necessary during the first week to maintain a hemoglobin of 12 to 13 grams. The baby was discharged at 2 weeks of age in good condition.

Case 5. Mrs. C. A., white female 22 years of age, received two transfusions of blood (type unknown) in 1943 when she was injured in an automobile accident. When studied during her first pregnancy in 1946 she was found to be type O MN rh, and her husband O MN Rh₁Rh₂. Rh sensitization was demonstrated and as pregnancy progressed there was a moderate increase in antibody titer, the titer in serum reaching a peak of 64 units. At term she delivered an infant (type O N Rh₁rh) which became jaundiced and anemic shortly after birth, requiring several small transfusions. Recovery was uneventful. Maternal antibody determinations two weeks after delivery showed 8,192 units in serum.

She became pregnant for the second time in July, 1949. The expected date of confinement was April 14, 1950. During the third month of pregnancy she returned for study and was found to have antibody titers of 768 units in albumin, 24 units in serum, and 4 units by the blocking test. Rh hapten, 300 mg. weekly, was started during the twelfth week and continued throughout the remainder of the pregnancy. The titers remained essentially unchanged (table 1).

On April 7, 1950, she delivered at term a rather severely affected infant, showing moderate edema, pallor, and hepatosplenomegaly at birth. Jaundice appeared within several hours. Initial hematologic studies showed 6 grams hemoglobin, 1.30 million red cells, and 480 nucleated red cells per 100 white cells. The cord blood was typed O MN Rh₁rh and gave a 4-plus positive antiglobulin test for coating. No circulating antibodies were demonstrated. An attempt to exchange-transfuse the infant was unsuccessful for technical reasons and instead a single transfusion of 75 cc. type rh whole blood was given. The next day the hemoglobin was 9.7 grams, and the baby appeared greatly improved. On each of the following two days transfusions of 80 cc. each of blood were given, raising the hemoglobin to 15.5 grams. The baby's subsequent course was uneventful.

Case 6. Mrs. H.A., white female, age 26, had had three previous pregnancies. The first, in 1942, resulted in the delivery of a normal full term infant (type O M rh). Studies during the second pregnancy, in 1946, showed her to be type A₁ M rh, and her husband A₁ MN Rh₁rh. Maximum Rh antibody titers of more than 16,000 units in albumin, 1536 units in serum, and 6 units in saline were found. She delivered a full term hydropic, stillborn infant. Her third pregnancy, in 1948, ended at 38 weeks with delivery of an infant, jaundiced and anemic at birth, which died at 36 hours of age despite an exchange transfusion. She was not studied during this pregnancy.

The patient became pregnant for the fourth time in May, 1949. The expected date of confinement was February 15, 1950. When seen on September 6, during the fifth month of pregnancy, she was found to have antibody titers of 256 units in albumin, 4 units in serum, and 1 unit by the blocking test (Rh₀ specificity). At 22 weeks, Rh hapten therapy was begun, 300 mg. weekly, and continued throughout the remainder of the pregnancy (table 1). On February 18, 1950, she delivered a normal full term type rh infant (type A M rh). The cord blood showed a circulating albumin antibody titer of 48 units, but the antiglobulin test for coating of the cells was negative.

Case 7. Mrs. S.G., white female 24 years of age, was studied by us during her first pregnancy in 1947. She had not received any blood transfusions. Blood group determinations showed her to be type A₁ M rh, and her husband O MN Rh₁rh. No antibodies were detected at any time and she delivered a normal baby at term (type A₁ MN Rh₁rh). Only one immunologic study was done during her second pregnancy. This was done at the time of delivery and showed 4096 units in albumin, 384 units in serum, and 256 units by the blocking test. The fetus was stillborn and macerated. Although an autopsy was not done, microscopic sections of the placenta revealed changes characteristic of erythroblastosis fetalis.

In June, 1949, the patient became pregnant for the third time. Her expected date of confinement was March 21, 1950. She was living in Pittsburgh at this time and was referred to Mrs. Bettina Carter at approximately the seventeenth week of pregnancy for Rh hapten therapy. She received a total of 1400 mg. during the two-week period immediately prior to her first visit here. Her husband was transferred to Baltimore and the patient was referred to us for further Rh antibody studies. Rh hapten, supplied by Mrs. Carter, was administered by her obstetrician in Baltimore. From the nineteenth to the twenty-seventh weeks she received 200 mg. twice weekly. A two-week interval without therapy followed. Thereafter she received 200 mg. twice weekly until the thirty-fifth week. From then until delivery she received 400 mg. twice weekly. Antibody studies are shown in figure 4.

On March 23, 1950, the patient delivered a normal full term type rh infant (type A MN rh). Antibody

titers on the cord blood were identical with the maternal titers at delivery and the antiglobulin test for coating of the cells was negative.

Case 8. Mrs. M.N., white female, age 21, had routine studies during her first pregnancy in 1948. She was found to be type A₂ MN rh, and her husband type A₁ M Rh₁rh. There was no history of transfusions. No Rh sensitization was demonstrated at any time during the pregnancy and an uneventful delivery of a normal baby (type A₁ MN Rh₁rh) occurred at term.

She again became pregnant in April, 1949. Her expected date of confinement was January 29, 1950. When seen on August 9, during the fourth month of pregnancy, the only evidence of sensitization was a doubtful reaction in albumin. At the end of the sixth month, however, titers of 2048 units in albumin, 6 units in serum, and 6 units in saline were found (Rh_s specificity). The blocking test was negative. On November 15, at 29 weeks of pregnancy, she was started on Rh haptens, 300 mg. weekly. On January 3, 1950, she delivered a macerated hydropic fetus considered to be at about 36 weeks of gestation. Fetal heart tones had not been heard for three weeks prior to delivery.

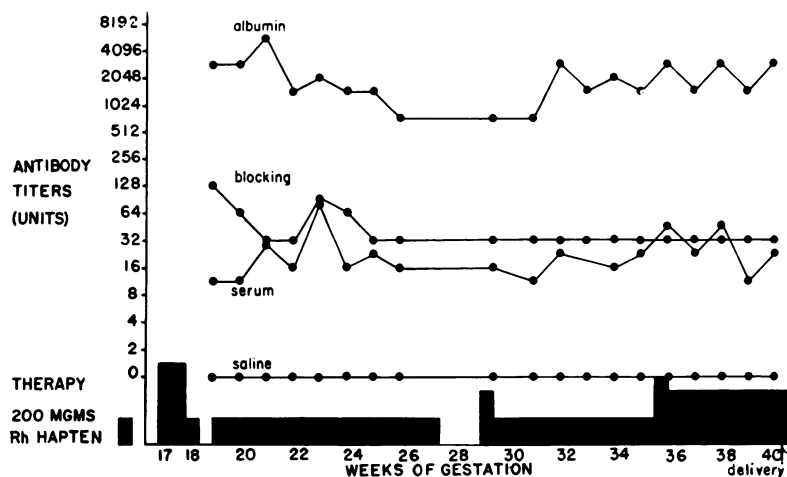


FIG. 4.—Case 7 (S.G.)

Case 9. Mrs. D.B., white female 27 years old, had had two previous pregnancies, the first in 1943 resulting in the delivery of a normal full term baby and the second in 1947, which ended with a spontaneous abortion at 12 weeks. The patient has pulmonary tuberculosis for which a thoracoplasty was done in 1948. At that time she received six blood transfusions of unknown Rh types.

She became pregnant for the third time in June, 1949. The expected date of confinement was March 11, 1950. When first studied here on August 16, during the third month of pregnancy, she was found to be type O M rh, and her husband A₁ MN Rh₂. The only evidence of Rh sensitization at that time was a titer of 8 units in albumin (Rh_s specificity). Subsequent studies showed a slight rise in titer. Rh haptens, 300 mg. weekly, was started at 23 weeks of pregnancy (table 1). The patient's degree of immunization was considered minimal. She had been advised against pregnancy because of pulmonary tuberculosis. Because future pregnancies would probably not be permitted to continue and because of the patient's overwhelming desire to bring this pregnancy to a successful conclusion, haptens therapy was employed despite the minimal sensitization.

On March 16, 1950, she delivered a term female infant appearing normal clinically, with a hemoglobin of 21 grams and no nucleated red cells. The antiglobulin test for coating of cells was found to be positive, but no circulating antibodies could be demonstrated in the cord blood. The hemoglobin reached a low level of 17 grams on the fifth day of life. Jaundice and hepatosplenomegaly did not appear. The infant was discharged from the hospital after an uneventful neonatal course.

Case 10. Mrs. A.D., white female, age 30, had her first pregnancy in 1948. Although pregnancy and labor were uncomplicated, her baby died after three days from what was said to be a congenital intestinal obstruction. There was no history of blood transfusions. When studied two months postpartum, blood group determinations showed her to be type O MN rh, and her husband O N Rh₂Rh₂. Rh antibodies were present in a titer of 1024 units in albumin.

She again became pregnant in July 1949. The expected date of confinement was April 9, 1950. On November 15, during the fifth month of pregnancy, she was found to have an albumin titer of 384 units with Rh₂ specificity. Results in other media were negative. Rh hapten, 300 mg. weekly, was started immediately. There was no significant change in antibodies at any time (fig. 5). Fetal death in utero occurred at about 35 weeks, followed by delivery of a macerated fetus one week later. The cord blood was not entirely suitable for study but was typed as O Rh₂, and the erythrocytes gave a positive anti-globulin test for coating. Autopsy showed no obvious gross cause for death. Extreme autolysis prevented adequate histologic examination.

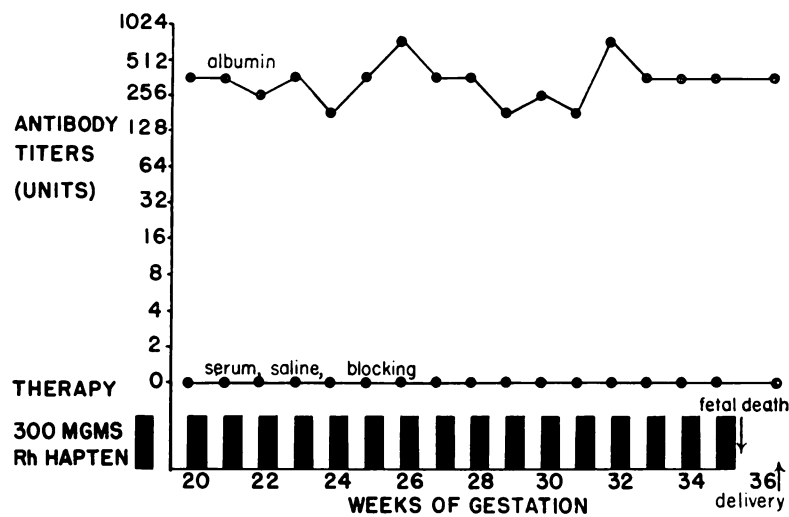


FIG. 5.—Case 10 (A.D.)

DISCUSSION

Careful analysis of the 10 cases presented here leads us to the conclusion that Rh hapten given in doses of 100 to 600 mg. weekly had no effect upon the degree of Rh isosensitization displayed by these patients. The pregnancies of 4 patients (Cases 1, 2, 8 and 10), whose previous obstetric history was poor and who displayed marked isosensitization in the current pregnancy, terminated in fetal death in utero despite continued therapy with Rh hapten. Therapy was begun at 20, 22, 29 and 20 weeks of gestation respectively. A question might be raised as to whether a greater influence upon the degree of immunization and the result of pregnancy might have occurred if treatment had been begun earlier. This does not appear likely, however. The latter statement is based on other observations in our series.

Three patients (Cases 3, 6, and 7), all of whom had heterozygous positive husbands and who had been immunized either by previous pregnancies or transfusions, failed to show any significant alteration in antibody titer while under hapten therapy, despite the fact that they were carrying Rh negative fetuses and were not

receiving further antigenic stimulation. Neutralization of antibodies would assuredly have been expected in these cases if Rh haptens were immunologically active *in vivo*.

Further evidence is afforded by analysis of Cases 4 and 5. Rh hapten therapy was begun in the former in the twentieth week of pregnancy. Despite the fact that from the twenty-ninth to the thirty-sixth week of pregnancy this patient received 600 mg. of Rh hapten weekly, a sharp rise in antibodies occurred. The infant, delivered by cesarean section, displayed evidence of severe erythroblastosis. It survived following exsanguination transfusion and subsequent smaller transfusions. The pregnancy in Case 5 likewise terminated in the birth of a severely affected infant. No significant rise in antibody titer occurred in this patient, who was treated from the twelfth week of pregnancy onward. There was, however, no significant drop in antibody titer during pregnancy. This infant also survived after multiple small transfusions. The pregnancy of Patient 9 terminated in the birth of an Rh-positive "clinically normal" infant. This patient displayed only minimal sensitization (average albumin agglutinin titer of 48 units) and was included in this series only because of other circumstances mentioned above.

The patients included in this report were selected because in most instances they displayed marked evidence of isosensitization. Of the 7 cases in which Rh-positive infants were delivered, 4 fetal deaths *in utero* occurred, a fetal mortality rate of 57.1 per cent.

The over-all mortality rate from erythroblastosis fetalis in infants born alive has shown a marked decline in the past decade. A recent study by the authors¹⁴ of 74 erythroblastotic infants treated by exchange transfusion showed an over-all mortality rate of 17.6 per cent. It is obvious that the series of cases included in the present paper is heavily weighted by cases showing a more severe degree of isoimmunization. However, it is in the latter type of case that a useful antepartum therapeutic regimen is most needed.

SUMMARY

1. Ten Rh-negative sensitized pregnant women were treated with Rh haptens in doses of 100 to 600 mg. weekly for periods varying from seven to twenty-eight weeks antepartum.
2. Three patients with heterozygous husbands delivered Rh-negative infants. Maternal antibody titers were unaffected by hapten therapy.
3. Three patients delivered Rh-positive infants displaying varying degrees of erythroblastosis fetalis.
4. The pregnancies of 4 patients terminated with fetal deaths *in utero* despite hapten therapy.
5. There does not seem to be any evidence that Rh haptens in the doses and manner employed altered the course or outcome of any of the 10 pregnancies.

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