

THE A AND B FACTORS AS A POSSIBLE CAUSE OF ERYTHROBLASTOSIS

By ALFONSO C. VÉLEZ OROZCO, M.D.

INTRODUCTION

RECENTLY, isoimmunization of the mother by the Rh factor of the fetus and its mechanism have been clearly demonstrated.^{1, 2} On the other hand, only vague references exist in medical literature regarding sensitization by other blood factors. Considering the fact that when red cells pass into the maternal circulation they carry not only the Rh factor but also the blood factors A and B, the M, N, P, Hr and other as yet unknown factors, it is not surprising that immunization by other than the Rh factor may occur.

Pregnancy offers a particularly favourable circumstance to produce isoimmunization, because of the slow administration of antigen during a lengthy period. Therefore, this subject must be carefully studied, because our lack of knowledge of the causes of fetal death is striking.

HISTORICAL DEVELOPMENTS

Dienst,³ in 1905, was apparently the first to think about blood incompatibility as a cause of pathological alterations, believing that eclampsia could be produced by the conflicting antigen-antibody between the mother and fetus. In 1923, Mc-Quarrie⁴ said that such a mechanism produced not only eclampsia but also the gravidic toxemias, and Ottenberg,⁵ in the same year, explained that the passage of blood through the placenta was the mechanism by means of which these phenomena are produced.

It was Hirszfeld⁶ who, in 1928, clarified these ideas, creating the term "hetero-specific pregnancy" and applying it to the cases in which the mother and the child had different blood groups. In such cases, the fetal injury was thought to be caused by an infiltration of agglutinins through the placenta. He also thought that the mechanism of protection for the fetus would be the poor sensibility of the erythrocytes to the agglutinins, due to the incomplete development of the fetal agglutinogens, and the presence of specific group substances in the latter's tissues and secretions. Together with Zborowski, he presented cases in which the babies weighed less than normally, as well as cases in which, contrary to what was to be expected according to the laws of heredity, the blood type of the mother was more frequent in the babies than that of the father. This was thought to be due to the fact that, in the already mentioned incompatibilities, sterility or at least a diminution in fertility was present.

Since then, much research has been directed to determine the antigenic capacity of the A and B factors. Biancalana and Teneff⁷ in 1930 were the first to prove the antigenicity of the A and B agglutinogens in human species. Jonsson⁸ in 1936 found that mothers belonging to the group O who had group A children developed

From the Institute of Medical and Biological Research, Mexico City, Mexico.

Read at the International Hematology and Rh Conference, Dallas, Texas, Nov. 15, 1946.

higher degrees of anti-A agglutinins than anti-B and that in group O mothers with B children the situation was the opposite. He suggested the possibility of isoimmunization through the placenta.

Dr. Levine⁹ in 1943 reviewed the subject, establishing that "the very suggestive data offered are extensive enough to warrant further investigations."

Let us now deal with the evidence which can be obtained on the possibility of isoimmunization against the A and B factors.

One of the evidences of the antigenic power of the A and B agglutinogens is the rising titre of agglutinins which is observed following incompatible blood transfusion, for instance A or B type in a type O patient. Immediately after blood transfusion, the titre is low due to the specific absorption of agglutinins by the incompatible cells. This negative phase lasts 2 or 3 days and afterwards the titre is increased several times its original level, reaching its maximum between 7 and 10 days. A second incompatible blood transfusion will produce, in such conditions, a stronger reaction, as has been pointed out by Thalheimer¹⁰, and Astrowe.¹¹

The increase of agglutinin titre following an abortion or the delivery of a stillbirth is another observable fact. In these conditions the incidence of abortions or stillbirths in later pregnancies increases. This is apparently the cause of the fact observed by Hirszfeld, that the frequency of type A children is less in a mating with A father and O mother, in comparison with the cases of O fathers and A mothers, the difference being considered due to the loss of type A children by abortions or stillbirths. These observations have been confirmed by the statistics of Gardiner and Yerushalmy.¹² The antibodies produced by isoimmunization are far more dangerous than the normal antibodies because of their higher titre.

Furthermore, we have been able to observe that in certain patients who receive repeated blood transfusions there can be abnormal agglutinins as a result of isoimmunization; in such patients the use of the same donor, or of another whose blood contains the same agglutigen, produces a severe hemolytic reaction. The same phenomenon can be seen in cases of intramuscular injections of incompatible blood. In cases of pregnancy atypical antibodies can be found as a result of isoimmunization even in patients who had never received a blood transfusion. In such cases the fetus in the uterus is the source of the antigen inherited from the father and, when a blood transfusion is needed, the cross-matching must be done using different bloods and being very careful with the weaker reactions, checking them until a completely compatible blood is found.

Different observations have been published about incompatibility cases against different blood factors, as A₁, O, M and P following blood transfusions, and Hill and Haberman¹³ have reported a case of sensitization produced by the N factor. In some cases, the agglutinins found do not correspond to any of the well-known blood properties; in such cases we can guess that they are due to not very well determined agglutinogens or to multiple sensitizations against several antigens. In all cases of isoimmunization one must identify the agglutinin by doing the tests under different temperatures because there are some agglutinins which react better at cold temperatures (anti-M, A and B) and others react better at 37°C (Rh.).

In some cases even if isoimmunization exists, it cannot be demonstrated by the

usual tests due to the fact that the technics are not very sensitive or because the patient is in the negative phase. Sometimes we must admit that we are dealing with antibodies present in the tissue cells, having in such cases, the biologic test as the only resource.

In other instances incompatibility can produce hemolysis instead of agglutination in the tests made in vitro. This must always be taken into account to avoid misinterpreting the results.

About the isoimmunization in pregnancy we can state the following: the agglutinogens have been demonstrated by Kempt¹⁴ in the blood of a fetus of 37 days. This can be explained if we remember that the first red blood cells are formed in the yolk sac in the fourth week of pregnancy having been found constantly after the second or third month. The agglutinins appear later, and when a child presents them at birth, it can be considered, according to Hirsfeld and to our personal observations, that they are maternal, having passed through the placenta. These

TABLE I.—*Pregnancies or Marriages*

Compatible			Incompatible		
Husbands or Fetus		Wife or Mother	Husband or Fetus		Wife or Mother
O	X	O	A	X	O
O	X	A	B	X	O
O	X	B	A	X	B
A	X	A	B	X	A
B	X	B	AB	X	O
O	X	AB	AB	X	A
A	X	AB	AB	X	B
B	X	AB			
AB	X	AB			

agglutinins disappear in approximately 10 days and the proper agglutinins are then originated.

In Rh cases, the first pregnancy almost always gives normal children even though they be Rh positive due to the fact that a sufficient degree of isoimmunization has not been developed. We have seen in several cases that when a blood type incompatibility is added the disease is presented in the first pregnancies. Many examples have been observed of Rh negative, type O mothers with Rh positive, type A husbands, who, in spite of never having had a blood transfusion, had a stillbirth in the first delivery.

When the frequencies of the blood types of a certain population are known, the frequency of the mother-fetus combination can be mathematically established. We see that this kind of sensitization is not so common as could be expected, if we consider the percentage of incompatible marriages. Attempts to explain such a difference by several mechanisms have been tried.

In the first place, we must consider the incapacity of some mothers to produce antibodies as can also be seen in Rh cases. There are some people who, because of certain genetical or constitutional characteristics, are anergic to certain allergens.

We must also take into consideration that a placental defect which permits the passing of the fetal blood is necessary although it should not be expected that a considerable lesion of the placenta must exist to produce isoimmunization. Dr. Levine¹⁵ has demonstrated that a small amount of fetal blood acting during a long period of time is sufficient to produce an effective degree of isoimmunization, and Dr. González Guzmán¹⁶ insists upon the fact that we should take into account the destruction of fetal cells during its normal processes of development, because the stroma of such cells contain the antigens and in such conditions the passage of cells through the placenta is not necessary, but the products of the lysis of the cells can yield a sufficient antigenic stimulus.

On the other hand, there are constitutional fetal factors which must be taken into account, principally their characteristic of being secretors or nonsecretors. These substances, widely studied by Witebsky,¹⁷ can be demonstrated as present in fetal tissues after the sixth month. As an important detail, we will only point out that there are haptens consisting of 2 factors, one soluble in alcohol and of lipoid origin and the other one soluble in water. These substances may be sought for in saliva, because if they do not exist there they cannot be found in any other secretion.

According to our unpublished data, 82 per cent of our population are secretors. In the remaining 18 per cent, nonsecretors, the A and B factors are limited to the erythrocytes.

The small number of cases we have studied, seems to confirm our previous supposition that when the fetus belongs to the nonsecretor group it presents a more or less typical picture of erythroblastosis and when it is secretor, the blood group substances in the tissues cause a more generalized deleterious effect of the agglutinins, producing early abortions or stillbirths. We have not been able to determine the secretor or nonsecretor character of feti or stillbirths, having deduced such characteristics through genetic studies of its possible heredity. These statistical data will be published later.

Concerning this isoimmunization we have seen in addition to typical erythroblastosis, subclinical anemia of the newborn, repetitive abortions or death of the fetus in the uterus. Therefore, it can be said as a general rule, that these troubles are not a perfectly defined unity, such as fetal erythroblastosis or hemolytic disease of the newborn, because we consider that 2 conditions must be fulfilled in order to speak about typical erythroblastosis: first, the presence in peripheral blood of erythroblasts with the typical alterations pointed out by Dr. I. González Guzmán; and second, the confirmation of sensitization by any test, preferably the "developing test," using the Race, Mourant and Coombs serum.

The presence of erythroblasts in the peripheral blood means a response to destruction of red blood cells from the bone marrow. But it is important to distinguish erythroblastemia from erythroblastosis. In the first case we only have the presence of normal erythroblasts in the blood stream and it can be taken as a slight alteration of the haematopoiesis produced by anoxemia; while in the second case, the structural changes signify a profound attack on the reticulo-endothelial system which produces erythrocytes.

In most of the cases of A and B sensitization, hemolysis is not found in spite of the increased titre of agglutinins normally present. It has been explained by some investigators as due to the existence of blood substances in the tissue cells and in the parenteral liquids and secretions of the fetus. We shall now study a very demonstrative clinical case which we have chosen among 13 cases seen by us.

TABLE 2

	Blood Type	Secretor	Other Factors
Mother.....	B	S	MN Rh ⁰ ₀
Father.....	A ₁	s	N Rh ₀
First twin (sick)	A ₁	S	MN Rh ₀
Second twin (normal).....	B	S	MN Rh ⁰ ₀ *

* The Rh subtypes were only determined recently because before we had only the 85% serum.

REPORT OF CASE

E. R., a 42 year old woman, had had 3 normal children with her first husband; with the second she had female twins. The serological characteristics are studied in table 2.

The 3 children of the first marriage were B, Rh positive.

When the first twin, a week old, was seen by us, she presented anemia of 3,200,000 R.B.C. and had been jaundiced since the fourth day of life. In her peripheral blood, 27 erythroblasts for 100 leucocytes were found, presenting the same characteristics as in erythroblastotic cases. She received four 50 cc. blood transfusions of A, Rh positive blood, perfectly tolerated and recovered completely.

The mother had never had blood transfusions or abortions. A week after delivery she presented a titre of 1:8192 against A₁ blood, the titre being actually 1:256.

Even though she was Rh⁰₀ the existence of atypical agglutinins mainly anti-Hr, was carefully investigated. They, as well as tests for syphilis were negative.

DISCUSSION

This is a case of isoimmunization due to the A factor. The A agglutinin is not a simple substance but comprises 2 properties called A₁ and A₂ by Landsteiner and Levine. Each one presents its own agglutinins. The proportion of A₂ is 25 per cent in reference to A₁. Some investigators think that they are the same antigen varying quantitatively. If this is true, it will explain why we have only seen sensitization against A₁ fraction, which seems to be the most antigenic.

This case is of great interest to us because it has its own control; the sick twin is the one who has the opposite blood type to the mother. Also, we can see the clear increase of titre, because the normal ones for Mexico, according to our small number of determinations are $\frac{1}{32}$ to $\frac{1}{128}$ with extreme values from $\frac{1}{4}$ to $\frac{1}{512}$.

We think, according to the above mentioned case, that we have demonstrated that the A and B blood factors can in certain occasions and conditioned by circumstances which we do not as yet know, be the cause of isoimmunization, which can manifest itself by erythroblastosis, repetitive abortions or fetal death.

SUMMARY

The author establishes that there is no reason to think that the isoimmunization must be limited to the Rh factor, especially in pregnancy cases, because such a condition brings the ideal means for its presence.

A brief historical review is presented, establishing some evidence for the antigenic capacity of the A and B blood factors, such as the experimental production of serum in animals, the increase of agglutinin titre following an incompatible transfusion or intramuscular blood injection and heterospecific pregnancies. Some fetal antigens are analyzed, as well as the possible pathogenic process of sensitization. Some factors of the antigen-antibody conflict in the fetal organism are studied. The pregnancies are also analyzed from the viewpoint of the fetal maternal incompatibility explaining the low frequency of the observed cases, due to nonpermeable placentas, the anergy of the mother, and the fetal characteristic of being secretor or nonsecretor, considering that the clinical form of the fetal alteration depends upon the presence or absence of A and B substances in fetal tissues. The writer gives as a basis to consider a true isoimmunization the presence in the blood stream of erythroblasts with nuclear alterations, and a positive "developing test."

A clinical case of twins in which the sick twin was of incompatible blood type in respect to the mother is presented.

ACKNOWLEDGMENT

I am in debt to Miss Graciela Romero for her assistance.

REFERENCES

- ¹ HILL, J. M., HABERMAN, S., AND VÉLEZ OROZCO, A. C.: *Sugestiones 119*: 1-14, 1945.
- ² VÉLEZ OROZCO, A. C.: *Sugestiones 121*: 1-12, 1945.
- ³ DIENST, A.: Eclampsia: further studies. *Zentralbl F. Gynäk.* 29: 353, 1905.
- ⁴ MCQUARRIE: *Bull. Johns Hopkins Hosp.* 39: 51, 1923.
- ⁵ OTTENBERG: *J. A. M. A.* 81: 295, 1923.
- ⁶ HIRZFELD, L.: *Serologic and Blood Group Investigation*. Berlin, Julius Springer, 1928.
- ⁷ BIANCALANA AND TENEFF: *Soc. internaz. di Microbiol., Boll. d. sez. ital.* 2: 397, 1930.
- ⁸ JONSSON, B.: On the question of heterospecific pregnancy. *Acta. path. et. microbiol. Scandinav.* 13: 424, 1936.
- ⁹ LEVINE, P.: *J. Hered.* 3: 71, 1943.
- ¹⁰ THALHEIMER: *J. A. M. A.* 76: 1345, 1921.
- ¹¹ ASTROWE: *J. A. M. A.* 79: 1511, 1922.
- ¹² GARDINER, E. M., AND J. YERUSHALMY: *Am. J. Hyg.* 30: 11, 1939.
- ¹³ HILL, J. M., AND HABERMAN, S.: *Texas State Jour. of Med.* 40: 177-187.
- ¹⁴ KEMPT, T.: *Acta path. et microbiol. Scandinav. Supp.* 7: 62, 1930.
- ¹⁵ LEVINE, P.: Mechanism of the isoimmunization by the Rh factor of red blood cells. *Arch. Path.* 37: 82-90, 1944.
- ¹⁶ GONZÁLEZ GUZMÁN, I.: Personal communication.
- ¹⁷ WITEBSKY, E., KLENSHOJ, N., AND SEVANSON, P.: *Blood Substitutes and Blood Transfusion* 309-326, 1942.