The limitations of the apparatus are determined by its mechanical characteristics. They come near to those of the mercury manometer itself. For a close analysis of the pulsewave, however, an inertia-free manometer and photographic recording are still necessary. The advantages of the photoelectrical recorder are as follows: 1. Any kind of recording can be used, as the power is supplied by the motor. An ink writer on a horizontal kymograph using a paper roll can be connected to the slide. 2. A very thin mercury column can be used; thus the amount of blood entering the manometer system upon a rise of blood pressure is substantially reduced. 3. The deflection of the manometer can be amplified by mechanical means (in the mechanical connection of the slide with the writer) and by inclining the manometer tube; the amplification factor is $1/\cos \alpha$, where α is the angle between the tube and the vertical. 4. Colored water can be substituted for mercury, *e. g.,* for recording venous pressure, intestinal pressure, etc.

The use of this apparatus is not limited to manometric recordings. Any lever with a broad pointer can be substitued for the manometer, and the light beam will then oscillate at the level of the upper margin of the pointer. All difficulties due to the contact of the pointer with the recording paper are thus eliminated. By removing the lamp from the slide and setting the apparatus in a horizontal position, it can be used for recording deflections of a reflecting galvanometer, eliminating the inconvenient photographic methods.

13506

Demonstration of Rh Antibody in Breast Milk.

ERNEST WITEBSKY, GEORGE W. ANDERSON AND ANNE HEIDE.

From the Buffalo General and the Children's Hospitals, the Departments of Pathology and Bacteriology, Gynecology and Obstetrics, Medical School, University of Buffalo.

Landsteiner and Wiener[1, 2, 3] recently described a new blood factor referred to by them as the Rh factor. Rabbits and guinea pigs when treated with rhesus monkey blood, produced antibodies that

[1] Landsteiner, K., and Wiener, A. S., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 223.

[2] Landsteiner, K., and Wiener, A. S., *J. Exp. Med.*, 1941, **74**, 309.

[3] Wiener, A. S., and Peters, H. R., *Ann. Int. Med.*, 1940, **13**, 2306.

agglutinated about 85% of human blood specimens, regardless of their blood group, while 15% of them were not influenced.

Levine and his coworkers, Stetson, Katzin, Burnham, and Vogel,[4-12] observed that in women who gave birth to a child exhibiting erythroblastosis fœtalis, severe transfusion-reactions would occur rather frequently. Over 90% of these women belonged to the Rh negative group, while their children were Rh positive. The Rh factor present in the child and inherited from the father should, according to the conception of Levine, act as an antigen and stimulate antibody-production in the mother. Conversely, such an antibody would then act upon the blood cells of the baby and be the cause of erythroblastosis. The number of cases studied by Levine and his coworkers seems to be large enough to justify the conclusion that isoimmunization in pregnancy might occur, and that the Rh factor plays an important part in the pathogenesis of erythroblastosis.

There are, however, quite a few problems that require clarification. Among them are: First, it is difficult to explain why only a small percentage of Rh^+ babies who have Rh^- mothers develop the disease; second, it is difficult to understand how antigenic substances originating from the child can enter the circulation of the mother and act as an antigen; third, the majority of Rh^- mothers with erythroblastotic children do not reveal any antibody on serological examination. Why should an antibody causing such a severe pathological change in the child not be demonstrable *in vitro?* Fourth, the Rh antibody usually does not produce hemolysis, but only agglutination. Fifth, because of the anticipated absence of the Rh factor in tissue cells, Rh antibodies are supposed to be absorbed exclusively by the blood cells, while antibodies against the A and B substances would be absorbed more or less by all tissue cells. However, the blood-type specific factors, M and N, have, to our knowledge, not been demonstrated as yet in normal tissue cells either. Why do not the blood factors M and N produce isoimmunization in pregnancy

4 Levine, P., and Stetson, R., *J. Am. Med. Assn.*, 1939, **113**, 126.

5 Levine, P., and Katzin, E. M., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 343.

6 Levine, P., Katzin, E. M., and Burnham, L., *J. Am. Med. Assn.*, 1941, **116**, 825.

7 Levine, P., *Am. J. Obst. and Gynec.*, 1941, **42**, 165.

8 Levine, P., Vogel, P., Katzin, E. M., and Burnham, L., *Science*, 1941, **94**, 371.

9 Levine, P., Katzin, E. M., and Burnham, L., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 346.

10 Levine, P., and Polayes, S. H., *Ann. Int. Med.*, 1941, **14**, 1903.

11 Levine, P., and Katzin, E. M., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**.

12 Levine, P., Burnham, L., Katzin, E. M., and Vogel, P., *Am. J. Obst. and Gynec.*, 1941, **42**, 925.

more frequently? Sixth, many children with erythroblastosis are born alive, and severe anemia develops shortly after birth. Provided that antibodies present in the circulation of the mother are responsible for the damage in the child, it is difficult to see why clinical symptoms in those cases become manifest after the child's birth.

We were given an opportunity to study the sixth point mentioned in a typical case of erythroblastosis fœtalis.*

Mrs. J. P.'s first pregnancy resulted in a late abortion, followed by a second pregnancy with a full-term living infant who died during the third day of its life from undiagnosed causes. On October 18, 1941, the patient was delivered of a 6 lb 8 oz living female infant. On the night of admission to the nursery, the child's temperature was elevated, and slight evidence of jaundice was present. Examination of umbilical-cord blood revealed 58% polymorphonuclear cells and 42% lymphocytes with 58 nucleated red blood cells per 100 white blood cells. The infant's jaundice increased in intensity and on the seventh day of its life, the hemoglobin was 9.6 g per 100 cc of blood.

Examination† for the Rh factor revealed Mrs. P. to be Rh^-, and the child and father Rh^+. The mother's serum, in dilutions up to 1:320, agglutinated Rh^+ blood cells, and behaved very similarly to an anti-Rh guinea pig immune serum obtained from Dr. Landsteiner. However, a few human blood specimens appeared to be Rh^+ with the guinea pig immune serum and Rh^- with the patient's serum. This discrepancy points to the fact already described, that the Rh antibodies present in serum of patients with erythroblastotic children might vary in their potency toward different blood specimens. Therefore, the Rh factor is not a sharply identifiable blood factor when examined by different patients' sera.

The child's anemia and icterus increased constantly in severity during the first week of her life. The question arose as to what factor could bring about such a change in the baby's blood picture after the contact between the mother's circulation and that of the baby's had been disrupted at the time of birth. It was considered worthwhile to examine the breast milk for its content in Rh antibodies. The possible importance of breast milk in the pathogenesis of erythroblastosis had been mentioned in older clinical observations—that of colostrum by Hooker,[13] and by Levine and his coworkers.[12] The

* We are very much indebted to Dr. Robert McDowell and Dr. Richard Downey for having given us the opportunity to study this case.

† Through the courtesy of Drs. Landsteiner, Levine, and Wiener, sera containing anti-Rh antibodies were at our disposal.

[13] Hooker, S. B., *N. Eng. J. Med.*, 1941, **225**, 871.

breast-milk specimen from patient P was centrifuged, and the layer of fat removed with a swab.

Several different methods are used for the demonstration of the Rh factor. Landsteiner and Wiener[2] mixed decreasing dilutions of their guinea pig immune serum with the blood cell suspensions. They obtained the best results by examining the sediment formed. Typical differences in positive and negative sediments are illustrated in Landsteiner and Wiener's paper. Levine keeps the mixtures of patient's serum and blood cells under investigation at 37°C for from 30 to 60 minutes and then spins them down in a centrifuge. The observations of the sediments formed gave the most satisfactory results in our experiments when breast milk was examined for anti-Rh antibodies.

The experiment to be recorded was carried out in the following way: About 1% blood-cell suspensions were prepared from nine blood clots all belonging to Group O. One tenth cc of each suspension was then added to: (1) 0.1 cc of a 1:30 dilution of anti-Rh guinea pig serum; (2) 0.1 cc of a 1:20 diluted serum of patient P; (3) 0.15 cc of breast milk of patient P; (4) 0.1 cc of saline solution. The tubes were then kept for about 2 hours in the incubator at 37°C. Rows 1, 3, and 4 in Table I were examined as to the sediment formed. Row 2 was spun down and observed for agglutination. The results obtained are shown in Table I.

As can be seen from Table I, blood specimens found to be Rh^+ with both the guinea pig immune serum and the patient's serum, gave a positive reaction with the milk also, while the Rh^- specimens were not influenced by the milk either. Forty blood specimens all belonging to Group O revealed a perfect parallelism when examined with the patient's serum and with the milk. Blood specimens that reacted positive with the guinea pig serum and negative with the patient's serum were negative with the milk. The specificity of the reaction was ascertained by examining 10 specimens of milk from different

TABLE I.
Determination of the Rh Factor in 9 Blood Specimens Belonging to Group O.

Serum	Blood Specimens								
	1	2	3	4	5	6	7	8	9
1. Guinea-pig serum	—	—	+++	+++	+++	—	+++	+++	++
2. Patient's serum	—	—	++	+++	+++	—	+++	+++	+++
3. Breast milk	—	—	+++	+++	+++	—	+++	+++	+++
4. Saline control	—	—	—	—	—	—	—	—	—

+++ Very strong agglutination or characteristic sediment-formation.
++ Strong but less marked reaction.
— No agglutination or characteristic sedimentation.

mothers. Occasionally, a positive reaction was observed with the control milk specimens. However, these reactions were not common and did not show any correlation with the distribution of the Rh factor. The milk, therefore, seems to contain the same Rh antibody as does the blood serum. Whether Rh antibodies present in the milk are absorbed unaltered from the intestinal canal, and thus, constitute a contributing factor in bringing about the infant's anemia in certain instances, cannot be decided.

It is to be noted that the anti-Rh antibody titer in the milk was very low. Upon dilution, the milk lost its capacity to react with Rh^+ cells. The demonstration of Rh antibodies in the serum of patients with erythroblastotic children succeeds only in a minority of cases. Because of the marked difference in titer between milk and blood serum, it might be even more difficult to find cases of erythroblastosis where it is possible to demonstrate the Rh antibody in the breast milk. No statement can be made regarding the titer of Rh antibodies in colostrum, because we obtained the first milk specimen about a week following delivery.

On the fourteenth day of life, the baby's hemoglobin was down to 7 g per 100 cc of blood, in spite of the fact that 2 blood transfusions with the father's blood had been given. About this time the breast feedings were discontinued. Rh^- blood (but, of course, not the mother's blood) was transfused to the child in consideration of the possible presence of potent Rh antibodies in the baby's circulation. Following the discontinuation of breast feedings and the transfusion of Rh^- blood, the child made a gradual improvement and was discharged from the hospital in an improved condition.‡

Conclusion. The occurrence of an Rh antibody in the breast milk of a woman with an erythroblastotic child is described. The patient's serum, too, contained a very potent antibody against Rh^+ blood cells.

‡ The child's mother belonged to group O, the child and father to Group A. The iso-agglutinin titer of the mother's serum was extremely high; anti-A reached a titer of 1:9640, anti-B, 1:1280. The iso-antibodies, anti-A and anti-B, were present in the breast milk too. The titer of the iso-antibody, anti-A, was 1:100.