14120

Further Investigations on Presence of Rh Antibodies in Breast Milk.

ERNEST WITEBSKY AND ANNE HEIDE.

From the Buffalo General Hospital and the Department of Bacteriology and Immunology, Medical School, University of Buffalo.

The occurrence of Rh antibodies in the breast milk of an Rh negative mother who had given birth to a child suffering from erythroblastosis was reported in a previous communication.[1] However, the first specimen of breast milk examined was taken at the beginning of the second week following delivery, which might have accounted for the low antibody titer. The possibility that Rh antibodies in breast milk could constitute a contributing factor in bringing about destruction of the child's blood cells was entertained. This warranted further studies on the occurrence of Rh antibodies in breast milk. Since then, we have had an opportunity to examine 2 more cases in which the Rh antibody was of a much higher titer than in the one previously described.

Case No. 1: A colored patient, aged 20, gave birth to a child who died 3 days after delivery with the clinical diagnosis of erythroblastosis confirmed at autopsy. The patient has 2 living children, one child who died at the age of 4 months, and one miscarriage. There was some doubt regarding the paternity of the different children. The mother, baby and putative father belonged to the blood group A. The mother was found to be Rh negative, the baby and father Rh positive. The mother's serum contained a potent Rh antibody and agglutinated the baby's cells in dilutions up to 1:256. A specimen of breast milk was obtained on the fifth day following delivery (two days after the baby's death). The technic used for the determination of the Rh antibodies was the same as that used for the determination of the Rh antibodies in blood serum; the milk specimen was centrifuged and the layer of fat removed with a swab. The turbid supernatant fluid was used for examination. Decreasing amounts of breast milk, volume .1 cc, were mixed with .1 cc each of a 1% suspension of Rh negative blood cells belonging to group O. The mixtures were incubated for 1 hr at 37°C and then centrifuged. The degree of agglutination observed was recorded following centrifugation. In dilutions up to 1:4 the resulting agglutination was very strong (4+ in character) but definite agglutination (1+) of Rh positive cells was obtained in dilutions of the breast milk up to 1:16. When tested against a number of Rh positive and Rh negative cells it was found that Rh positive cells were strongly agglutinated while Rh negative cells failed to show any agglutination.

Case No. 2: Blood specimens of parents and baby were sent to this laboratory from another hospital a few hours following delivery of the child. The child exhibited clinical symptoms of erythroblastosis. All 3 blood specimens belonged to the blood group O. The mother was Rh negative and the father and baby were Rh positive. The mother's serum agglutinated Rh positive cells up to a dilution of 1:8 though the degree of this agglutination was not too marked (2+). Colostrum was obtained about 24 hours following delivery. In spite of the fact that the Rh antibody content of the serum was not very high, examination of the colostrum revealed the presence of an Rh antibody of about the same strength as that observed in the blood serum. The colostrum specimen agglutinated Rh positive cells in a dilution up to 1:8.

Summary and Conclusions. Rh antibodies were found in the breast milk of 2 mothers with erythroblastotic children. The Rh antibody titer was much higher in both instances than in the case reported previously. In the first case, the Rh antibody titer of the serum was 1:256 while the milk specimen obtained on the fifth day following delivery showed an antibody titer of 1:16. In the second case, the Rh antibody titer of the serum was not

[1] Witebsky, E., Anderson, Geo., and Heide, A., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 179.

higher than 1:8 and the colostrum, upon examination, revealed the same titer. Those findings suggest that the content of Rh antibodies in breast milk is relatively high in the first days following delivery, especially in the colostrum. In the few cases we examined, we have not been able to demonstrate antibodies in the breast milk unless they were present in the serum. Assuming that Rh antibodies in the breast milk pass through the intestinal canal and enter the circulation, breast milk might be the cause of further damage to blood cells of erythroblastotic children.

14121 P

Inherited Differences in the Choline Requirement of Rats.*

R. W. ENGEL. (Introduced by P. H. Phillips.)
From the Laboratory of Animal Nutrition, Alabama Polytechnic Institute, Auburn.

In a previous report[1] it was emphasized that there was considerable variation in the minimum choline requirement of rats from different litters. An experiment was conducted to determine whether or not this variation was hereditary. Two female and 2 male rats which had produced offspring having a relatively high choline requirement were mated, and their progeny were designated the F_1 of strain HCR. Likewise a similar number of rats which had produced offspring having a relatively low choline requirement were mated and their offspring were designated the F_1 of strain LCR. The F_1 rats of both strains were brother-sister mated simultaneously so that seasonal and other environmental influences would be uniform in the 2 strains.

At 23 days of age the F_2 rats were placed on the choline-deficient diet 31PMC[2] supplemented with adequate amounts of thiamine, riboflavin, pyridoxin, Ca pantothenate, carotene and calciferol. A suboptimum level of 4 mg of choline chloride per rat daily was also fed. Seventy-six progeny from 4 F_1 females of the strain HCR and 126 progeny from 6 F_1 females of strain LCR were tested over a period of 6 months. Weight gains of the progeny were equal in the 2 strains and ranged from 15 to 20 g per rat during the first week. Kidney hemorrhage, characteristic of choline-deficiency, developed in 6 to 10 days, and the severe cases culminated in death. The kidneys of survivors were examined at the end of a 2-week feeding period. The presence and severity of kidney hemorrhage were used as an index to the adequacy or inadequacy of choline nutrition.

The results are presented in Table I. With a daily intake of 4 mg of choline chloride, 100% of the F_2 males of strain HCR developed kidney hemorrhage, and 58% of them died. The incidence of hemorrhage among the F_2 males of strain LCR receiving the same level of choline was only 48%, with a mortality of only 10%. As previously reported, the females required less choline than the males, but again the incidence of hemorrhage was markedly higher in the F_2 females of strain HCR. All the F_1 females of strain HCR produced young about equal in their choline requirement, and similarly the offspring of the F_1 females in strain LCR, regardless of litter were uniform in their requirement of choline.

Preliminary results indicate that the daily requirement for choline is nearly twice as great in strain HCR as in strain LCR. That such unusual differences in the requirement for a specific nutrient can be demonstrated in the second generation seems highly significant, and illustrates clearly how genetic variation in the stock used may affect the results of nutrition experiments. The physiological basis for this genetic difference in the requirement of choline is yet unknown.

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1 Engel, R. W., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 193.

2 Engel, R. W., and Salmon, W. D., *J. Nutrition*, 1941, **22**, 109.