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Hematology in Vit. B_{12} Deficient Newborn Rat.* (24618)

P. M. NEWBERNE† AND B. L. O'DELL

With isolation of Vit. B_{12}(1,2), it was shown that this vitamin fulfilled the role of Castle's extrinsic factor as well as that of the hemopoietic factor in liver(3). A number of investigators(4-7) reported the effect of Vit. B_{12} deficiency on blood of animals with rather wide variations in effects produced among different species. This paper includes results of blood and related tissue studies of newborn rats littered to Vit. B_{12}-depleted dams.

Methods. Newborn offspring of Vit. B_{12}-deficient and Vit. B_{12}-supplemented dams were used. Dams were depleted of Vit. B_{12} by maintaining them on soybean meal-glucose type diet from time of weaning. Methods employed for production of the deficiency and for histological studies were described previously(8). Blood was obtained by decapitation and hemoglobin was determined by direct photometric method(9). A Hellige-Diller colorimeter, calibrated with a sample of blood whose hemoglobin content was calculated from its iron analysis, was used for measurement. Red cell, leucocyte and differential counts on blood obtained as described above, were made by standard procedures. Erythrocytes were examined for sickling by mounting a small drop of blood with a drop of fresh, 1% aqueous solution of sodium bisulfite under coverslip and examining microscopically after 5 minutes. Hematocrits were determined in Van Allen hematocrit tubes using 1.3% sodium oxalate as diluent.

Results. Table I shows that a significant difference in blood picture existed between controls and Vit. B_{12} deficient newborn rats. Hemoglobin, hematocrit values and erythrocyte counts were considerably decreased in the deficient rats. Leukocyte counts were reduced to about one-half that of controls and the differential counts showed this difference to be manifest mainly in percentage of mature neutrophils. Neutrophilic granulocytes appeared unable to mature properly leaving more cells in the myelocytic and metamyelocytic stages.

Erythrocyte concentration was reduced significantly in peripheral blood and large numbers of nucleated red cells were present in the deficient animals. Peripheral blood films showed polychromatophilia, anisocytosis, poikilocytosis, smudge cells, and objects resembling degenerate erythrocytes (Fig. 1). Sickling mounts indicated that these were not true sickle cells.

Bone sections showed that the bone was poorly developed. There was a lack of normal calcification and a failure of normal endochondral bone growth and remodeling sequences. Osteoblasts were reduced in number and those present appeared less active than controls. Both cartilage cells and osteoblasts contained very little glycogen compared to controls. Clumps of cartilage cells were observed in the bony collar, and in many cases tongues of nucleated cartilaginous cells extended down into the marrow cavity from the epiphyseal plate (Figs. 2 and 3). Bone marrow from deficient animals showed active hemopoiesis and a 2- to 3-fold higher concentration of pro-erythrocytic elements, a large portion of which were pronormoblasts (Figs. 4

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† P.H.S. Research Fellow, Nat. Inst. of Neurological Diseases.

TABLE I. Hematology of Newborn Rats.

Vit. B_{12} status of dam	Hemoglobin, g/100 ml	Hematocrit, %	RBC $\times 10^6/mm^3$	WBC $\times 10^3/mm^3$	Lympho-cytes, %	Myelo-cytes, %	Meta-myelo-cytes, %	Neutro-phils, %
Supplemented	10.32 ± .63*(56)†	37.51 ± 3.5 (56)	2.408 ± .19 (30)	4.133 ± .83 (36)	26.1	25.9	25.6	22.4
Deficient	8.34 ±1.26 (94)	28.43 ± 3.7 (94)	1.755 ± .24 (67)	2.689 ± .97 (57)	37.2	23.2	24.9	14.7
Significance of diff. (P)‡	<0.01	<0.01	<0.01	<0.01				

Vit. B_{12} status of dam	Mean corpuscular vol (μ^3)	Mean corpuscular hemoglobin ($\mu\mu g$)	Mean corpuscular hemoglobin conc. (%)
Supplemented	148.6	42.5	28.6
Deficient	160.1	45.0	30.9

* ± stand. dev.
† Numbers in parentheses indicate number of observations.
‡ P determined by Fisher "t" test.

FIG. 1. Red blood cell smear from newborn B_{12} deficient rat showing objects resembling sickle cells. Some were irregularly rounded with pronounced vacuolations. Wright's stain, × 215.

FIG. 2. Epiphyseal area from femur of a control newborn rat. Note orderly arrangement of cartilage cells and endochondral bone growth. H. & E. stain, × 30.

FIG. 3. Epiphyseal area from femur of newborn vit B_{12} deficient rat showing irregular shaft of cartilage extending into marrow cavity. Many of these cartilage cells contain nuclei. H. & E. stain, × 30.

FIG. 4. Bone marrow from newborn control rat showing normal cellularity. H. and E. stain, × 30.

FIG. 5. Bone marrow from newborn vit B_{12} deficient rat showing increased cellularity. Much of the increase was due to immature erythroid and myeloid elements. H. & E. stain, × 30.

FIG. 6. Liver section from control newborn rat showing extramedullary hemopoiesis within sinusoids. H. & E. stain, × 267.

FIG. 7. Liver section from vit B_{12} deficient newborn rat showing lack of erythropoiesis, presence of cytoplasmic lipide accumulations, and large, irregular clumps of nuclear chromatin. H. & E. stain, × 267.

and 5). The granulocytic elements contained pale, vacuolated nuclei.

The liver is a part of the erythropoietic system in the infant rat, and for this reason the organ was studied histologically along with blood. Liver sinusoids showed vascular blocks and were plugged with fatty material and cellular debris. The nuclei showed margination and clumping of deeply staining basophilic chromatin material. The cytoplasm of cells was distended with lipid material. There was a marked reduction in hemopoietic activity with only a few erythroblastic elements scattered about in the tissue (Figs. 6 and 7). These elements were densely chromatic and many were pyknotic.

Discussion. Anemia, leukopenia and granulocytopenia observed in Vit. B_{12} deficient animals was similar to that reported by Borson *et al.*(7). The fact that the greatest change in leukocytes was in mature neutrophils, suggests that neutrophilic granulocytes were unable to mature properly in absence of Vit. B_{12}. The observation on peripheral blood films was supported by bone marrow studies. Granulocytic myelocytes contained pale, vacuolated nuclei deficient in nuclear chromatin material.

The presence of large numbers of nucleated erythrocytes in peripheral blood film of deficient animals indicated release of these cells from marrow before they were matured. This finding and the anemia which was present in deficient animals suggests that deficiency of Vit. B_{12} has an effect on the newborn rat similar to blood dyscrasia in pernicious anemia of man. Sickle-shaped objects were not true sickle cells; they may have been fragments of red blood cells which had undergone degeneration as a result of increased fragility.

The changes observed in liver agree with those reported by Jones *et al.*(10). The reduction in hemopoietic elements in liver of deficient rats no doubt contributed to the anemia, which was primarily the result of malfunctioning bone marrow. Accumulation of fatty substances in the hepatic cells suggests that lipid metabolism is deranged as a result of Vit. B_{12} deficiency.

Reduced calcification of bone as well as decreased osteoblastic activity may be related to reduced phosphatase activity in Vit. B_{12} deficient bone(11). Immaturity of bone as well as of other tissues is related to a more generalized failure of cells to undergo normal cell division and growth.

Summary. Vit. B_{12} deficiency in the dam resulted in newborn rats with anemia, leukopenia and granulocytopenia. The liver showed a marked reduction in hemopoietic elements, vascular blocks, and an accumulation of lipide in the hepatic cells. The femur of Vit. B_{12} deficient rat showed abnormal patterns of growth and bone marrow contained increased concentration of hemopoietic elements. However, most cells appeared unable to complete maturation.

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