the blood of rats receiving pyrimethamine contains a factor inhibiting development of mouse embryos, and there is every reason to suppose that this factor is pyrimethamine itself or its metabolites. Characteristically mouse embryos reacted by inhibition of cleavage to blood serum from donor rats receiving 6 mg/kg pyrimethamine. This is the threshold dose which disturbs the development of rat embryos in utero [1]. The sensitivity of of the embryonic cells themselves to this antifolic compound was thus just as high in the mouse embryos as in rat embryos.

Since the mechanism of action of pyrimethamine consists of inhibition of dihydrofolate reductase [6, 10], there is reason to suppose that the very earliest stages of development of rat and mouse embryos are equally dependent on the normal function of this key enzyme of the folate cycle in the blastomeres; moreover, the zygotes of these animals evidently have no reserves of folates which could maintain the processes taking place during cleavage.

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# EFFECT OF ANTENATAL ADMINISTRATION OF DIETHYLSTILBESTROL AND PROGESTERONE ON THE BLOOD SYSTEM OF THE NEWBORN PROGENY


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The effect of sex hormones injected into pregnant animals on the blood system of their progeny was determined. Changes were observed in various sections of the system: peripheral blood, bone marrow, spleen, and liver. Sex hormones stimulate hematopoiesis in the bone marrow and spleen of the fetus but inhibit it in the liver. The changes observed are interpreted as a process of acceleration of functional maturation of the fetal blood system.


Sex hormones have a considerable effect on the hematopoietic system for they cause changes in erythro-, leuko-, and thrombopoiesis [2, 3, 5-8]. Inhibition of hematopoiesis as a result of the action of sex hormones is manifested as a decrease in the number of cells of the erythroid and myeloid series and of karyocytes, delayed maturation of cells, and an increase in the number of reticular and endothelial cells in the bone marrow, spleen, and lymph nodes.

This paper describes a study of the effect of a combination of sex hormones (diethylstilbestrol and progesterone), injected into female rats during pregnancy, on the state of the blood system of their progeny.


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## EXPERIMENTAL METHOD

Experiments were carried out on 25 pregnant noninbred rats and on 72 of their newborn progeny. Control investigations were carried out on 60 young rats born from 12 animals not receiving sex hormones during pregnancy.

Diethylstilbestrol and progesterone were injected into the animals once daily for three successive days. One group of rats received the injections in the first half of pregnancy (9th-11th day), the second group in the second half of pregnancy (16th-19th day). The dose of the hormone injected into the animal was calculated per kilogram body weight and was equivalent to the daily therapeutic human dose, i.e., the animals each received 0.003 mg diethylstilbestrol and 0.03 mg progesterone per injection. A series of experiments in which doses of the hormones 10 times smaller were given also was set up: These animals received 0.0003 mg diethylstilbestrol and 0.003 mg progesterone.

The newborn rats were examined on the fifth day after birth. The cell composition of their peripheral blood, bone marrow, spleen, and liver was studied. At the same time the number of nucleated cells was determined in the femoral marrow, spleen, and liver by the method suggested by Belousova and Fedotova [1].

## EXPERIMENTAL RESULTS

Injection of sex hormones into rats in the second half of pregnancy caused a decrease in the number of erythrocytes in their peripheral blood (1.3 ± 0.09 million compared with 2.0 ± 0.06 million/$\mu l$; $P < 0.05$) although the hemoglobin level remained unchanged. The color index in rats exposed to the hormones was higher than in the control progeny (2.0 ± 0.09 compared with 1.4 ± 0.07; $P < 0.001$). The reticulocyte count was almost doubled (from 304 ± 17.8 to 503 ± 19.6 and 561 ± 4.3%; $P < 0.001$). The leukocyte count was lower in the experimental than in the control rats (5.0 ± 0.97 thousand compared with 6.9 ± 0.58 thousand/ $\mu l$). The decrease in the number of leukocytes took place in some cases mainly on account of neutrophils, in others on account of lymphocytes.

Under the influence of the hormones changes also took place in the bone marrow, spleen, and liver. Corresponding to the decrease in the erythrocyte count, there was an increase in the number of erythroid cells (polychromatophilic and oxyphilic normoblasts, reticulocytes) entering the blood stream from the marrow. The intrauterine action of the hormones in both doses led to an increase in the number of nucleated cells in the femoral marrow of the newborn rats (4.1 ± 0.28 million and 6.1 ± 0.35 million compared with 2.35 ± 0.14 million in the control progeny; $P < 0.001$).

The ratio between the total numbers of erythroid and myeloid cells in the bone marrow was unaffected by the action of the hormones. However, a decrease in the relative percentage of young erythroid cells was found in the experimental groups. The process of maturation of cells of the erythroid series was accelerated (maturation index increased from 0.50 to 0.70), and this was evidently the reason for the decrease in the relative percentage of young cell foms. Considerable individual variations were found in the percentage of young myeloid cells. However, statistical analys is of these data revealed no significant differences from the control group.

Changes also were found in the spleen. The total number of nucleated cells in that organ rose under the influence of the hormones from 52 ± 3.6 million to 83 ± 6.9 and 93 ± 4.6 million ($P < 0.001$). At the same time, the percentage of all erythroid cells, including young, increased ($P < 0.01$). This was evidently connected with a delay in their maturation (maturation index decreased from 0.9 to 0.6). Under the influence of the hormones the relative percentage of myeloid cells in the spleen of the young rats fell by half (from 10.8 ± 1.47% in the control to 5.6 ± 0.56% in the experimental group; $P < 0.01$).

Blood cells were found much less frequently in squash preparations from the liver of the young rats than in the bone marrow and spleen. However, all generations of cells of the erythroid and myeloid series, undifferentiated cells, and hemocytoblasts were found. The composition of the blood cells in the liver of the newborn rats was similar to that in the bone marrow.

Injection of sex hormones in the first half of pregnancy led to changes uniform with those described above in the blood system of the newborn progeny. However, they were less severe.

These experiments showed that diethylstilbestrol and progesterone, if injected into pregnant animals at different stages of pregnancy, influenced the blood system of their future progeny. In small doses these hormones stimulated the fetal hematopoietic tissue: The total number of nucleated cells in the bone marrow and spleen was increased. Meanwhile the percentage of erythroid cells in the spleen also was increased.

Not only the bone marrow and spleen, but also the liver, an organ with a cell composition in newborn rats similar to that of the bone marrow [4], also reacted to hormone administration. Under the influence of the hormones the number of nucleated cells in the liver was reduced, possibly reflecting the decline of hematopoiesis in that organ.

An external factor such as hormones thus caused acceleration of functional maturation of the blood system, in the form of activation of bone marrow function and inhibition of hematopoiesis in the liver.

The responses observed point to the ability of the fetal hematopoietic apparatus to react to an external environmental factor such as sex hormones.

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# SYNTHESIS AND LOCALIZATION OF α-FETOPROTEIN DURING REGENERATION OF THE LIVER IN MICE


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The main site of localization of α-fetoprotein (αFP) in mouse liver regenerating after $CCl_4$ poisoning or partial hepatectomy was in the typical mature hepatocytes that account for not more than a few per cent of the total number of residual hepatocytes. Morphologically they were indistinguishable from the main mass of hepatocytes and they retained on their surface bile capillary antigen. The change in their number and in the brightness of their fluorescence in liver sections corresponded to the dynamics of the αFP level in the animals' serum. During regeneration of the liver in mice αFP is evidently produced mainly by mature hepatocytes.


KEY WORDS: α-fetoprotein; localization in sections; immunofluorescence; hepatocytes; regeneration of the liver.


The appearance of the embryo-specific protein known as α-fetoprotein (αFP) in adult mice after partial hepatectomy was described by Abelev et al. [1]. In mice and rats after hepatectomy or $CCl_4$ poisoning the blood αFP level as a rule rises sharply on the second day, reaches a maximum on the third to fourth day, and then falls by the seventh to tenth day to its original values [1, 2, 4, 8]. The nature of the cells which synthesize αFP


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