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Cell Proliferation Accelerating and Inhibiting Substances in Blood Serum During Pregnancy.*

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In blood serum there are two types of factors which affect cell proliferation in bone marrow cell and tissue cell cultures *in vitro*.[1] Normal blood serum will accelerate the rate of cell proliferation in bone marrow cultures because of a predominance of accelerating over inhibiting factors. Serum from cases of neoplastic disease, pernicious anemia, aplastic anemia and leukemia have a ratio of factors such that a supplement of the serum will inhibit normal cell proliferation. In a series of conditions previously reported,[2] the sera, from 7 cases of pregnancy, inhibited the rate of cell proliferation.

A further study was made of the effect of blood serum from cases of pregnancy on cell proliferation in bone marrow cultures *in vitro*. The technique used for the culturing of bone marrow cells was the same as that previously described.[2] Rabbit bone marrow was used in the experiments reported in this paper.

Fig. 1 gives the response of the cells of bone marrow cultures *in vitro* to the addition of human pregnancy blood serum at various stages of pregnancy. Circles connected by a line represent cases in which three or more specimens of blood serum were obtained at the intervals indicated. Determinations have been made on thirty cases in addition to those shown in Fig. 1, especially in the latter stages of pregnancy. The results all fall close to the order of magnitude of values given in the curves shown and are omitted to prevent overcrowding of the graph. During pregnancy there was an increase in factors which inhibit normal cell proliferation, with a tendency toward an increasing inhibition with the progress of pregnancy. In the later stages of pregnancy, the blood sera were strongly inhibitory of normal cell proliferation similar to the blood sera from cases of neoplastic disease, pernicious and aplastic anemia, and leukemia. The anemia associated with pregnancy is consistent with the change in normal cell accelerating and inhibiting factors as is also the anemia associated with neoplastic disease.

Fig. 2 gives the response of bone marrow cultures *in vitro* to the blood serum of pregnant rats at various stages of pregnancy. The blood serum showed a progressive increase in inhibiting factors up to the time of parturition. The amniotic fluid was obtained from the uterus of the pregnant rats on approximately the eleventh and fifteenth day of pregnancy and at the time of birth. The amniotic fluid becomes strongly inhibitory during the pregnancy, but at the time of birth there was a considerable excess of normal cell, proliferation accelerating factors in the fluid.

At various stages of pregnancy, the rats were sacrificed and the cells of the embryos or fetuses cultured by a technique similar to that used for culturing normal tissue cells and cancer cells *in vitro*.[3,4] The entire embryos were disintegrated in a modified Waring blender as previously described for the preparation of tissue cell suspensions with the addition of Tyrode's solution without glucose. The cell suspension was incubated in 5 ml rubber capped vials at 37°C. Ten mg of acid hydrolyzed casein per ml of cell suspension was added as a neutralized solution of commercial casein hydrolysate. One half mg of

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1 Norris, E. R., and Majnarich, J. J., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 229.

2 Norris, E. R., and Majnarich, J. J., *Am. J. Physiol.*, 1948, **153**, 483.

3 Norris, E. R., and Majnarich, J. J., *Am. J. Physiol.*, 1948, **153**, 488.

4 Norris, E. R., and Majnarich, J. J., *Am. J. Physiol.*, 1948, **153**, 492.

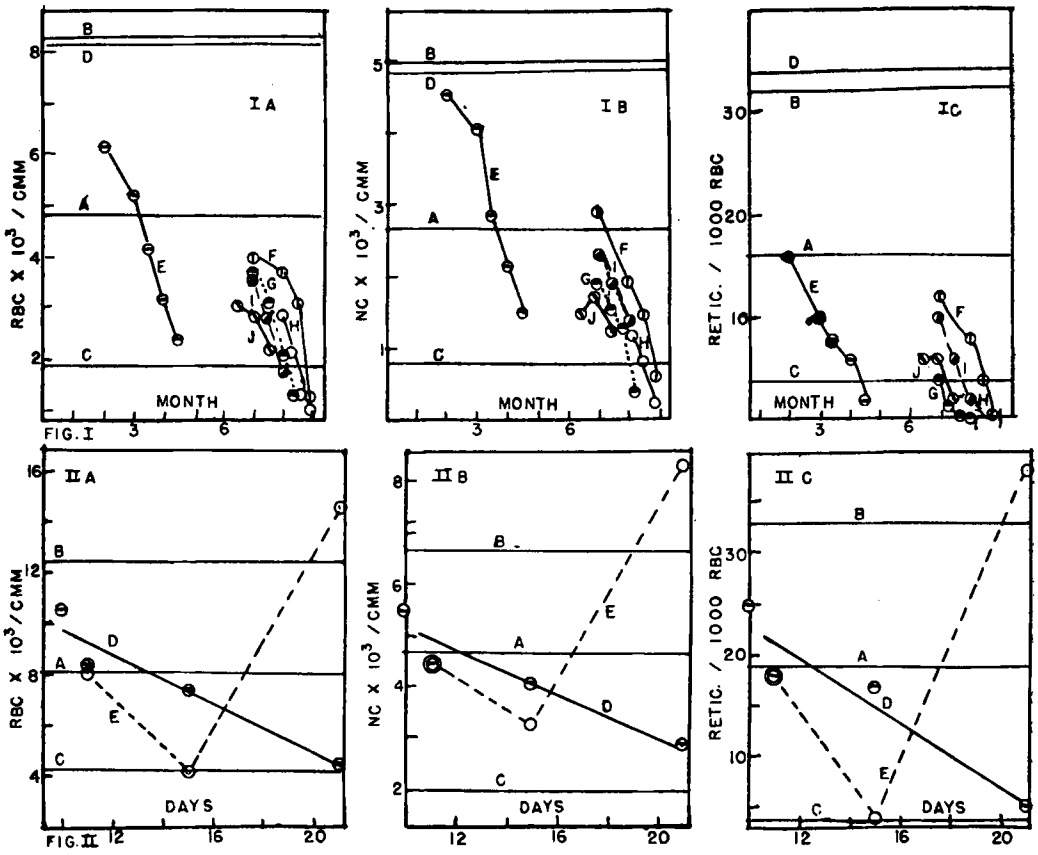

FIG. 1.

The effect of blood serum from cases of human pregnancy at different periods of pregnancy upon the rate of cell proliferation in bone marrow cell cultures *in vitro*. The initial concentration of cells in the bone marrow cell suspension used was red blood cells (RBC) 4840/emm; nucleated cells (NC) 3240/cmm; reticulocytes 10/1000 RBC. Time of incubation of bone marrow cultures is 8 hours. A, indicates the level of response obtained without supplement; B, indicates the level of response obtained with 5 γ of xanthopterin per ml of suspension; C, indicates the level of response with 5 γ of 2-amino-4-hydroxy-7-methyl pteridine per ml of suspension; D, the level of response with normal human serum; E, F, G, H, I, J, the response obtained with serum from cases of pregnancy at the period of pregnancy indicated. One tenth ml of serum was added to 2 ml of bone marrow suspension.

FIG. 2.

The effect of the blood serum and amniotic fluid of pregnant rats upon cell proliferation in bone marrow cultures *in vitro*, at various stages of pregnancy. A, is the level of response obtained with no supplement; B, is the level of response obtained with a supplement of 5 γ of xanthopterin per ml of bone marrow suspension; C, is the level of response obtained with 5 γ of 2-amino-4-hydroxy-7-methyl pteridine per ml of bone marrow suspension; D, is the response obtained with 0.1 ml of blood serum from rats, at different stages of pregnancy, added to 2 ml of bone marrow suspension; E, is the response obtained with 0.1 ml of amniotic fluid from the uterus of pregnant rats, at different stages of pregnancy, added to 2 ml of bone marrow suspension.

tryptophan was added per ml of cell suspension, and supplements as indicated below.

In the earliest stages of pregnancy, it was not possible to separate the implanted embryos from the uterus so that the entire uterus containing the embryos was disintegrated and a cell suspension obtained. Fig. 3 gives response obtained with cells of the uterus from a rat in the early stages of pregnancy compared with that of a suspension of

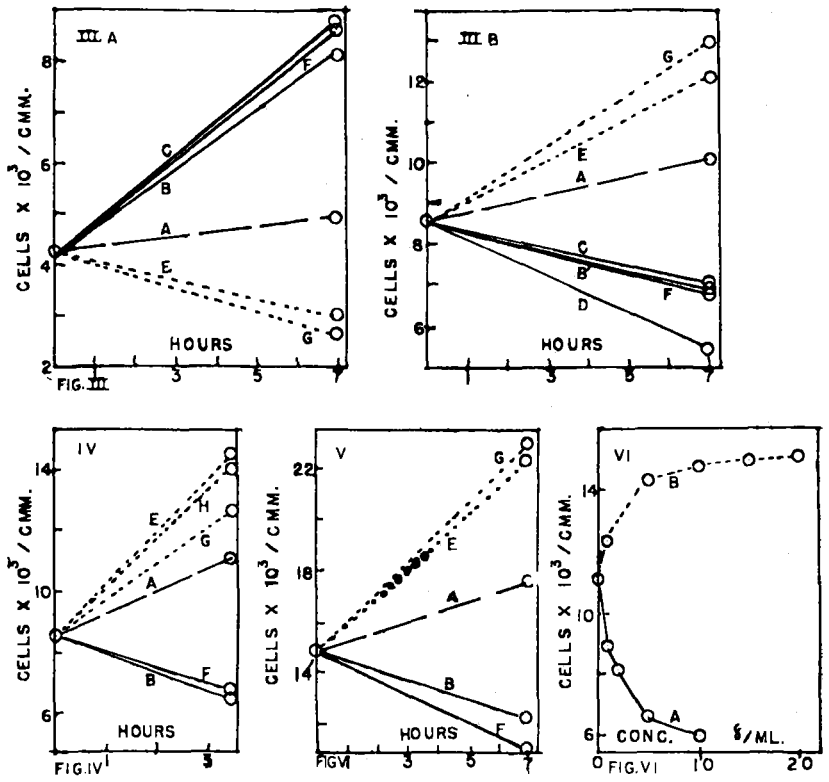

FIG. 3.

The effect of various supplements upon the rate of cell proliferation *in vitro* in a suspension of cells from the uterus of a non-pregnant rat (III A) and from the uterus and embryos of a obtained with 0.1 ml of blood serum from rats, at different stages of pregnancy, added to 2 ml of cell suspension: A, no supplement; B, 5 γ of xanthopterin per ml; C, 1×10^{-6} γ of Vitamin B_{14} per ml; D, 1×10^{-2} γ of Vitamin B_{14} per ml; E, 5 γ of 2-amino-4-hydroxy-7-methyl pteridine per ml; F, 0.1 ml of normal human blood serum; G, 0.1 ml of blood serum from a person with cancer.

FIG. 4.

The effect of various supplements upon the rate of cell proliferation *in vitro* in a suspension of cells, from rat fetuses of about 10 days. The following supplements were added to 2 ml of cell suspension: A, no supplement; B, 5 γ of xanthopterin per ml; E, 5 γ of 2-amino-4-hydroxy-7-methyl pteridine per ml; F, 0.1 ml of normal human blood serum; G, 0.1 ml of human cancer blood serum; H, 0.1 ml of human leukemia blood serum.

FIG. 5.

The effect of various supplements upon the rate of cell proliferation *in vitro* in a suspension of cells from rat fetuses of about 15 days. The following supplements were added to 2 ml of cell suspension: A, no supplement; B, 5 γ per ml of xanthopterin; E, 5 γ per ml of 2-amino-4-hydroxy-7-methyl pteridine; F, 0.1 ml of normal human blood serum; G, 0.1 ml of human cancer blood serum.

FIG. 6.

The effect of various concentrations of pteridines upon the rate of cell proliferation *in vitro* in a suspension of cells from rat fetuses of about 10 days. A, xanthopterin; B, 2-amino-4-hydroxy-7-methyl pteridine.

cells produced from the uterus of a non-pregnant rat. The non-pregnant uterus gave the response obtained with normal tissue cells *in vitro*. The response of the uterus containing the implanted embryos at an early stage gave the opposite response to that of normal cell proliferation. Xanthopterin, Vitamin B_{14}[5]

[5] Norris, E. R., and Majnarich, J. J., *Science*, in press.

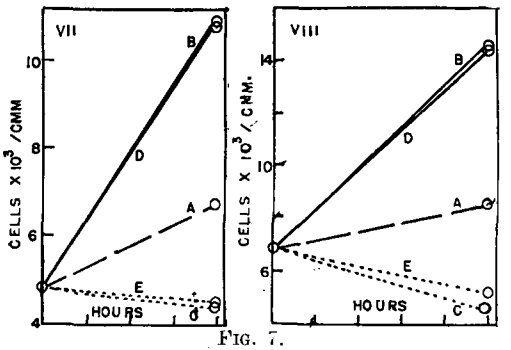

FIG. 7.

The effect of various supplements upon the rate of cell proliferation *in vitro* in a suspension of cells from rat fetuses taken at the time of birth. The following supplements were added to 2 ml of cell suspension: A, no supplement; B, 5 γ of xanthopterin per ml; C, 5 γ of 2-amino-4-hydroxy-7-methyl pteridine per ml; D, 0.1 ml of normal human blood serum; E, 0.1 ml of human cancer blood serum.

FIG. 8.

The effect of various supplements upon the rate of cell proliferation *in vitro* in a suspension of cells from rat young about 12 hours after birth. The following supplements were added to 2 ml of cell suspension: A, no supplement; B, 5 γ of xanthopterin per ml; C, 5 γ of 2-amino-4-hydroxy-7-methyl pteridine per ml; D, 0.1 ml of normal human blood serum; E, 0.1 ml of human cancer blood serum.

and normal blood serum accelerated the rate of cell proliferation of the non-pregnant uterus and inhibited proliferation of the cells of the pregnant uterus. Two-amino-4-hydroxy-7-methyl pteridine and cancer serum inhibited cell proliferation in the non-pregnant uterus cell suspension and accelerated the rate of cell proliferation in the pregnant uterus cell suspension.

Fig. 4, 5 and 6 give the response of cell suspensions obtained from fetuses on approximately the 10th and 15th day of pregnancy. The response was the same as that obtained with the uterus in the early stages of pregnancy described above and also the same as that obtained with neoplastic tissue cell suspensions.[4]

Fig. 7 and 8 give the response of cell suspensions obtained from the fetuses or young at the time of, and soon after birth. The response is that of normal cell suspensions. At some time between approximately the 15th day of pregnancy and the 21st day or the time of parturition, there was a complete change in the response of the cell proliferation to pteridines. In the early embryos and the fetuses up to at least the 15th day, the proliferation of the cells obtained as a suspension was inhibited by xanthopterin and accelerated by 2-amino-4-hydroxy-7-methyl pteridine. At the time of birth the rate of cell proliferation of the cells obtained from the young was accelerated by xanthopterin and inhibited by 2-amino-4-hydroxy-7-methyl pteridine.

Summary. 1. Two types of factors which affect cell proliferation have been observed in blood serum, one of which accelerates the rate of normal cell proliferation, and the other inhibits the proliferation of normal cells. Normal blood serum contains a predominance of factors which accelerate the rate of normal cell proliferation. During pregnancy there is a progressive change in the balance of factors which affect cell proliferation such that the factors which inhibit proliferation of normal cells become predominant.

2. The rate of cell proliferation of a cell suspension *in vitro* of cells obtained from rat embryos and fetuses, at least up to the 15th day of pregnancy, is accelerated by 2-amino-4-hydroxy-7-methyl pteridine and cancer blood serum and inhibited by xanthopterin and normal human blood serum.

3. The rate of cell proliferation of a cell suspension *in vitro,* of cells obtained from rat young at the time of birth, is accelerated by xanthopterin and normal human blood serum and inhibited by 2-amino-4-hydroxy-7-methyl pteridine and cancer blood serum.