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Cytological Studies of Mammalian Embryonic Blood Cells.

ARTHUR KIRSCHBAUM. (Introduced by R. F. Blount.)

From the Hematological Laboratory, Institute of Anatomy, University of Minnesota.

Blood cells are most readily classified when seen in blood smear preparations or dry imprints (smears) of tissues stained with Romanowsky dyes. In most laboratories, however, only paraffin sections are studied when the hematologist or pathologist is interested in the hemopoietic activity of spleen, liver, lymph nodes, etc.

American investigators have studied mammalian embryonic hemopoiesis from paraffin or celloidin sections only. It was deemed worthwhile to study embryonic hemopoietic organs using the dry imprint method, checking our observations with paraffin sections. The embryonic tissue (yolk-sac, liver, spleen, bone marrow) was touched gently to a chemically clean cover glass and waved vigorously until the imprinted material was dry. May-Grünwald-Giensa staining was used immediately as in the staining of blood smears. Preparations made in this way make possible a comparison between embryonic blood cells and elements seen in blood smears and organ imprints of the adult under normal or various physiologic and pathologic states. It is important to know which cells are found normally only in the embryo, and the elements of the embryonic hemopoietic organs which are identical with normal cells of the adult.

The material used for this study consisted of rat embryos from 9-22 days' gestation, plus various stages of rabbit, human, mouse and pig embryos and foetuses. The following were conclusions drawn:

1. The first circulating blood cells of the rat are not lymphocytes (Maximow, Jolly, Jordan), but are immature red cells of the embryonic megaloblastic series.

2. Two generations of red cells are produced in all mammalian embryos, the megaloblastic, formed primarily in the yolk-sac, and the normoblastic which appears in the liver in great numbers. The normoblasts of the liver are cytologically identical with the nucleated red cells of the fetal spleen and fetal and adult bone marrow. Megaloblasts are not the precursors of normoblasts. The normoblastic and megaloblastic series are distinct, with old and young stages in each line. Nuclear structure is the primary basis for distinguishing the megaloblast from the normoblast. In the immature stages dry

imprints are essential for classifying cells as either megaloblasts or normoblasts. In such preparations differences in the 2 series are constant.

3. The megaloblastic series of the embryo closely resembles the hemoglobiniferous series of pernicious anemia bone marrow during relapse. The cells of the rat yolk-sac are not, however, morphologically identical with the pathologic cells of pernicious anemia. The nuclear pattern of the embryonic megaloblast is coarser in the polychromatic stage. Since normoblasts, leukocytes and lymphocytes have the same nuclear patterns in all mammalian species, it is likely that this is true for the megaloblasts also. Thus, it might be assumed that in pernicious anemia red cell regeneration is similar to, but not identical with that of the embryonic yolk-sac.

4. The occasional presence of azurophilic granules in the cytoplasm of basophilic megaloblasts of the rat yolk-sac, an organ which produces only erythrocytes, proves that myeloid azure granulation in the cytoplasm of an immature blood cell does not necessarily mean the cell is a granulocyte precursor. Azurophilic granules have been observed by Jones[1] in pernicious anemia megaloblasts.

5. In the case of the rabbit the definitive (normoblastic) generation of red cells appears in the yolk-sac before hemopoiesis has begun in the liver. Only megaloblastic red cells are formed in the rat yolk-sac. Embryonic mammalian hepatic erythropoiesis is normoblastic only and primarily extravascular in location. The embryonic liver forms practically the same cellular elements as adult bone marrow, but the ratio of red cells to leukocytes is much higher in the liver.

6. Megakaryocytes of the embryonic liver are identical with those of the adult bone marrow, but it is questionable whether the yolk-sac forms megakaryocytes. As claimed by Storti[2, 3] hypertrophied megaloblasts with polymorphous nuclei and no azurophilic granules in the cytoplasm may represent the cells which have been interpreted as megakaryocytes by many authors. This point needs further study.

7. Megaloblasts circulate up to birth in animals with a short gestation period (rat, mouse). In the human and pig, where the gestation period is long, nucleated megaloblasts disappear from the circulation long before birth (in the human there are none after 12 weeks). Similar cells, according to many investigators, appear

1 Jones, O. P., *Folia Hematol.*, 1936, **55**, 195.

2 Storti, C., *Boll. d. Soc. Med.-Chir. Pavia*, 1932, **46**, 893.

3 Storti, C., *Arch. Zool. Ital.*, 1935, **21**, 241.

again in the human adult only pathologically in the liver-principle deficiency anemias (pernicious anemia, tropical sprue, bothriocephalus anemia, pernicious anemia of pregnancy), the leukemias and agranulocytosis (one case in Dr. Hal Downey's collection).

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A Micro-Bioassay Method for Acetylcholine.*

GERHARD KATZ. (Introduced by J. T. Halsey.)

From the Department of Pharmacology, Tulane University School of Medicine, New Orleans.

One of the most specific and sensitive tests for acetylcholine in body fluids is the leech muscle suspended in a saline bath containing eserine (Fuehner, Minz[1,2]). When only small quantities of blood are available they must be diluted with sufficient saline to fill the bath. This procedure naturally decreases the sensitiveness of the test. Moreover, in smaller animals, the repeated drawing of several cc. of blood is often detrimental to the carrying out of a series of tests. The method presented here eliminates these disadvantages by suspending the leech in foam from small quantities of blood.

The leech is attached to a hook at the bottom of a glass bath (2x6 cm.), the other end leading to a writing lever. A narrow opening at the funnel-shaped bottom of the bath is connected with an air tank by means of rubber tubing, so that air is allowed to enter at the desired rate. A wire loop serves as a guide to prevent the blood soaked thread from adhering to the wall of the tube. The leech is prepared in the usual manner and is suspended in eserinized saline until it is relaxed. The saline is then drained completely and the blood which is to be tested is placed in the bottom of the tube. The air passing through the blood creates a foam, which passes over the muscle as it is carried upward. If there is acetylcholine present in the blood, the contraction of the muscle then starts immediately. (See Fig. 1.) In order to obtain uniform results, it is necessary to keep the amount of air entering the bath constant. After the foam has exerted its effect on the leech, the bath is washed with saline,

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1 Fuehner, H., *Biochem. Z.*, 1918, **92**, 347.

2 Minz, B., *Arch. f. exp. Path., u. Pharmakol.*, 1932, **167**, 85; **168**, 292.