The morphometric investigation of prenatal erythropoiesis in the rat spleen

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In this study, a total of 36 white rats (Ratlus Norvegicus), 6 of each embryo being in the 14-19th days of pregnancy were used in order to determine the blood formation (erythropoiesis) in the embryonic rat spleen morphometrically. The erythropoiesis rate in the embryonic spleen was determined morphometrically through statistical methods by taking base the nucleus diameters of the erythropoietic serial cells. The spleen was observed for the first time in the 15th day of pregnancy and with the first appearance of the erythropoietic cells in the spleen. Erythropoiesis began in the 17th day and increased gradually. The nucleus diameters of the erythropoietic cells in the 15th, 16th and 17th days were 7,13 \im, 7.45 \un and 7.43 u/n *respectively, and 6,18* urn *in the 18th day and 5,60* um *in the 19th day. It is concluded that the relation between the developing spleen and the erythropoiesis was set in the 17th day and that the erythropoiesis rate gradually increased in the 18th and 19th days dependent on the development age. [Turk J Med Res 1995, 13(3): 86-89]*

Keywords: Spleen, Rat, Erythropoiesis

The blood formation (erythropoiesis) within the embryonic life In mammalians occurs in the vitellus sac, liver, spleen and the bone marrow, respectively (1-3). It is known that erythropoiesis is started and continued by hemopoietic stem cell (HSC) and that these cells branch into blood cells, save and renew the stem cell population (4,5). It has been stated by several reports that the hemopoietic cells are migrating between hemopoietic organs (6); that only definitive stem cells participate in the circulation and start blood formation in other hemopoietic tissues (7); that in the 8th and 9th days of pregnancy in rodentia (rat, mouse, etc.) the hemopoietic centers and angioblastlc cords are first seen in the vlsseral connecting tissue of the vitellus sac (8); that the HSC need an inductive exogenous support for a blood formation in the liver (9); that the HSC are transported by blood from regions which formerly were active in blood formation (10); that the stromal stem cell population undergoes important changes dependent on the pregnancy age; the number in HSC in the spleen also decreases when blood formation in the bone marrow begins (11). The different opinions about the first embryonic phase, where the spleen is observed, have caused various discussions to arise (2,12-14).

Some physiological conditions (in the cases of hypoxia, hemorrhage and erythropietin injection) related with the blood formation have also been compared parallel to the embryological development of the spleen (15-20). These reports show that discussions about the development of the spleen, the blood formation in the spleen and about the physiological conditions still continue.

In a study performed on human material (21), a decrease in the hemoglobin F (HbF) level within the intrauterine life has been interpreted as an increase of the erythropoiesis. Sasaki K and Matsumura G (14) have tried to interprete the mouse spleen erythropoiesis by measuring the nucleus diameters. From this point, we aimed to interprete the erythropoiesis rate by measuring the nuclear diameters of the erythropoietic cells for rat spleen in the prenatal period.

MATERIALS AND METHODS

A total of 36 rats (Rattus Norvegicus) including 6 of 14,15,16,17,18 and 19 days old embryos each were used in this study. The abdomens of the pregnant rats were opened under ether anesthesia. The 14,15 and 16 days old embryos were totally taken and of the

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17,18 and 19 days old embriyos, removing their heads, only their bodies were taken. Their tissue samples were fixed immediately in the Zenker-Formalln-Acetic acid solution for 6-7 hours. Following routine histological technics, the samples were put into paraffin and serial cross-sections of 6 urn were taken from the tissue blocks. Hematoxilen-Eosin, May-Griinwald-Giemsa and Maximov staining technics were applied to the cross-sections (22). An ocular scala being assembled to the Nikon optiphot microscope was used to measure the nucleus diameters of the erythropoietic cells in the embryonic spleen. The identification of the erythropoietic cells was made according to Sasaki and Matsumara (8), and the measuring of the nucleus diameter according to Sesaki and Matsumara 1987 (14). For each test group, the nucleus diameters of 10 randomly selected cells, in an area of 2500 square urn were measured for each preparation which was determined through a definite order (by intervals, like the 1st, 5th, 10th preparation) from the serial cross-sections with the spleen in it. In order to make a comment about the erythropoiesis rate according to the nucleus diameter measurements (when the nucleus diameter decreased, erythroplesls increased and when it increased, erythropiesis decreased), the nucleus diameters were compared with the following phase. For example, the nucleus diameter of the 15th day was compared with the 16th day and that of the 16th day, with the 17th day. Since no spleen was observed in the 14th day, this test group was let out of comparison. The student-t test was used for statistical evaluations.

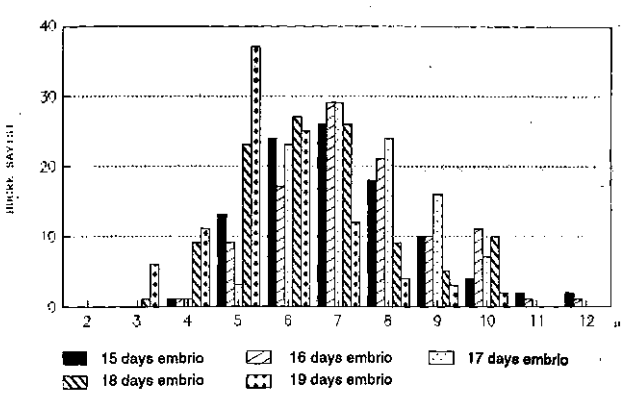
RESULTS

No spleen formation was observed in the 14th day, but in the 15th day, right under the stomach, the spleen was observed as a mesanchimal thickening in connection with the stomach and the pancreas. The cell layer which covers the organ from the outer surface was seen dark colored which lessened with the increasing age of pregnancy. Erythropoietic cells were observed in this embryonic period and the arythmetic average of their nuclear diameters was to be 7,13 urn (SD: 1,63) (range 4-12 urn) and a frequency of 7 urn, 6 urn (Table 1).

According to the measurements in the 16th day, the nuclear diameter of the erythropoietic cells was to be 7,45 urn (SD:1,65), (range: 4-12 urn) with a frequency of 7 urn (Table 1). There was no statistical difference between the 15 and the 16 days old embrios (p>0.05) (Table 2).

The connection of the spleen with the stomach and pancreas in the 17th day was more significant, the intercellular intervals observable. The nuclear diamater was 7,43 urn (SD:1,31) with a distribution of 4-10 urn and a frequency of 7 urn (Table 1). There was no statistically difference between the 16 and 17 days old embryos (p>0.05) (Table 2).

Tablo 1. The nuclear diameter distribution of the investigated erythropoietic cells



Tablo 2. The number of the erythropoietic cells being measured and the statistical comparison of the nuclear diameters

Study Days	Erythropoietic Line	
15 Days Embrio	N: 100 7.13±1.63	
		t:0.890 p>0.05
16 Day,s Embrio	N: 100 7.45±1.56	
		t:0.068 p>0.05
17 Days Embrio	N: 103 7.43±1.31	
		t:5.014 p<0.05
18 Days Embrio	N: 101 6.18±1.36	
		t:2.090 p<0.05
19 Days Embriyo	N: 100 5.60±1.44	

The connection of the spleen with the stomach and pancreas in the 17th day was more significant, the intercellular intervals observatiable. The nuclear diameter was 7,43 urn (SD:1,31) with a distribution of 4-10 urn and a frequency of 7 urn (Table 1). There was no statistical difference between the 16 and 17 days old embryos (p>0.05) (Table 2).

Besides being more developed (in terms of total size), yet, the spleen in the 18th day showed no regional differentiation (white-red pulpa). There was a significant increase in the basophyl erythroblast number and besides this, erythrocytes and normoblasts were observed in the primitive vessel tubes. No lymphocytes and megakaryocytes were appeared in this period. The nuclear diameter was 6,18 urn (SD: 1,36), the distribution to be 3-10 /am with a frequency of 6 and 7 urn (Table 1). The difference between the 17 and 18 days old embryos was significant (p<0,05) (Table 2).

In the 19th day of pregnancy, a capsule formation in the spleen and the presence of vessels in the trabeculae were observed for the first time. Some morphological changes dependent on the increase in the number of venos blood sinuses were marked. The number of erythrocytes had increased. The nucleus diameter was to be 5,6 urn (SD:1,44), the distribution to be 3-10 urn with a frequency of 5 urn (Table 1). The difference between the 18 and 19 days old embryos was significant ($p<0.05$) (Table 2).

DISCUSSION

It is a common point of view that there are different results and commentments about the development of the spleen. That the spleen formation in mice begins In the 12th (2), 14th (14) and 15th (13) days of pregnancy and in rats in the 16th (12) day shows that this discussion is continuous. The organogenesis is expected to be different for each test animal. But the occurence of different findings for the same animal may show that the subject is worth being investigated and discussed. Even as, despite other studies (2,12-14) our results showed that the spleen developed in the 15th day instead of the 16th. The Indication of the day when a spleen is observed for the first time is also important in terms of the beginning of erythropoiesis. In one of his studies, Marecki (23) stated that the change in the size of the spleen (width, height, thickness) in the embryonic period showed more increase than that of the postnatal period. The dark stained outer cell layer showed less staining character with progressing pregnancy. Excess staining is inevitable due to the numerical abundance of the cells being in the embryonic period. When in the course of time fibroid elements enclose the protecting capsule around the organ, then it takes less stain which in turn indicates that the organ in the progressed phase of pregnancy has reached the necessary size and maturity. Sasaki and Matsumura (13) have observed that the mouse spleen develops in the 15th day including the mezenchimal cell net in which free mononuclear cells are present in a small number. Our results agree with the studies of Sasaki and Matsumura (13). According to another study (14) of the same authors performed on mice, the frequency to be observed by measuring the nucleus diameters of the erythrocyte serial cells was 6-7 mm in the 16th day. In the results of this study, it was found that the nucleus diameter of the erythrocyte serial cell was to be 7,45 mm in the 16 days old rat embryo. It should not be found strange that mice and rats which are members of the same family may have a similar nucleus diameter in this period even if they are of different species. Our findings were found close to the stated results (14).

Holyoko and friends (12) have stated that the general outlook of the spleen in the 17th day does not change much in rats and that erythropoiesis is marked after that day. Sasaki and Matsumura (14) have informed that the frequency in the nucleus diameter measurements of the erythrocyte serial cells was about 5 mm in mice. When these results were compared with our findings concerning the 17 days old embryo, it was determined that the other results showed no confirmity except for the pregnancy age about the phase of erythropoiesis formation.

It is informed that in the 18 days old mouse embryo eozinofibroid granules were seen (2), that the erythropoietic activity increased and the arterioles differentiated (12); and furthermore, it is stated that mitotic figures were frequently observed in the spleen cordons and that the number of haemopoietic cells increased significantly (13). Both the histological and morphological findings of our study agree with the literature.

A study performed on the rat spleen states that the intercellular intervals gained a typical sinus sight towards the 20th day (12). Our findings agreed with those of Holyoke and friends (12).

According to the in vitro study results of Nagel and frients (24) performed on the rat spleen in the last 4 days of the embryonic life, it was stated that the erythroid colony forming cells showed a gradual decrease in number. Alter et al (21) on the other hand, have informed that the hemoglobin F (HbF) level in the human material was decreasing within the intrauterin life. The results of the investigators have been interpreted for both species as an increase in the intrauterin erythrocyte production.

Our mathematical results showing that erythrocyte formation in the last periods of pregnancy increases, agree with the results of Nagel (24) and Alter (21), Sasaki and Matsumura (13) have stated that erythropoiesis is highly active by approaching birth.

With the determination (through nucleus diameter measurements of the erythropoietic serial cells) of a gradual increase in erythropoiesis in phases close to birth, we concluded that our results agree with the studies Sasaki and Matsumura (13,14) performed on mice.

Prenatal döneme ait sıçan dalağında kan yapımının morfometrik olarak incelenmesi

Bu çalışmada, embriyona! sıçan dalağındaki kan yapımını morfometrik olarak belirlemek amacıyla gebeliğin 14-19. günleri arasındaki embriyolardan 6'şar adet olmak üzere toplam 36 adet beyaz sıçan (Rattus norvegicus) kullanıldı. Embriyonik dalaktaki kan yapımı (eritropoez) hızı, eritropoetik seri hücrelerin nukleus çaplan esas alınarak istatistiksel metodlarla morfometrik olarak belirlendi. Yapılan gözlemler sonunda: dalağın ilk olarak gebeliğin 15.gününde gözlendiği, dalakta eritropoetik seri hücrelerinin ilk olarak görülmesiyle gebeliğin 17.gününde, kan yapımının (eritropoez) başladığı

ve *bu aktivitenin giderek arttığı belirlendi. Eritropoetik hücrelerin nukleus çapları, gebeliğin 15., 16. ve 17. günlerinde sırasıyla 7,13* um, *7,45* um *ve 7,43* um, *eritropoez'in artmasıyla 18.günde 6,18 mm, 19.günde 5,60* um *olduğu hesaplandı. Sonuç olarak gelişen dalağın eritropoez ile ilişkisinin gebeliğin 17.gününden itibaren başladığı ve gelişme yaşına bağlı olarak gebeliğin 18. ve 19.günlerinde eritropoez hızının giderek arttığı belirlendi. [Türk J MedRes 1995, 13(3): 86-89)*

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