Porcine Research

Comparative characteristics of the pigs' and rats' early erythropoiesis

[1]Marina Tatoyan, [2]Liana Abroyan, [2]Aida Avetisyan, [2]Zara Semergyan, [2]Lina Hakobyan, [2] Elena Karalova, [1,2]Zaven Karalyan

[1] Department of Cytology, Histology and Embryology, The YSMU after M. Heratsy, Yerevan, Armenia; [2] Laboratory of the Cell Biology and Virology of the Institute of Molecular Biology of NAS of RA, Yerevan, Armenia. Corresponding author: M. Tatoyan, marina.tatoyan@mail.ru

Abstract. Investigation of the pig's and rat's embryos erythropoiesis showed that in rats, in distinct with pigs, there was not a significant intra-embryonic mesenchymal erythropoiesis. First time it was revealed, that in pigs during the mesenchymal megaloblastic erythropoiesis in a part of the cells an extrusion of nuclei and a formation of the primitive erythrocytes are possible to appear. According to our data got, their amount in pigs made up not more, than 2-2.5% of the whole erythroid cells population. The given type of the cells is being determined on the 15-25th days of the pig embryo's development and almost completely disappeared by the 35th days of gestation. Nevertheless, the nucleated and non-nucleated primary erythrocytes that formed generations vary on their size, most often big cells, the megaloblasts and megalocytes, could be observed. The megaloblastic erythropoiesis in rats was revealed on the 12th stage of gestation, when only megaloblasts were appeared in the yolk sac vessels, whereas erythrocytes were being revealed already on the 13th stage of that. On the 13-14 stages, when the hepatic erythropoiesis is already started, the erythrocytes number was being dramatically increased, making up more than 70% of the rats erythroid cells population. Herein, the megalocytes number in rats was a rather high, attaining up to 43% of the erythrocytes population, while the megalocytes number in the pigs' hepatic erythropoiesis did not exceed 10% of the whole erythrocytes population. At the early stages of the rats' ontogenetic development almost a complete absence of DNA synthesis was observed. The latter, in our opinion, is a consequence of the more intensive erythropoieses during the pig's physiological development process. At the same time, absence of the hemopoietic cells' hypertetraploid population in rats confirms our suggestion. On the benefit of our suggestion the lesser values of hemoglobin content in the erythroid cells of the early erythropoiesis in rats vs. the analogical indices in pigs indicate as well.
Key Words: embryonic development, hematopoiesis, erythrocytes, mammalians, blood.

Introduction. It is well known, that erythropoiesis in mammalians ontogenetically and morphologically is strictly divided into two types: a primitive embryonic and the ultimate adult erythropoiesis, which have been described for the mammalians' embryos more than a century ago (Jolly 1909; Zeidberg 1929). During embryonic development the hemopoiesis carries out a function of a rapid production the erythroid cells big number, maintaining the embryo's growth and survival, afterwards the hemopoietic stem cells (HSC) generation is being appeared, which then are preserved over the adult animals' whole lifespan. Hemopoiesis in the developing embryos of the chordates is taking place in several stages and at several different localizations. For a long time it was considered, that the first stage of that occurred in the yolk sac, the wall of which is the first hemopoietic organ in all the mammalians (Yoder & Hiatt 1999; Ghatpane et al 2002), as well as a site of the primary erythroblasts formation. Herein one-sided tendency of the stem cells differentiation towards erythropoiesis was detected (Kyunghee 2002; Zambidis et al 2005; Tada et al 2006; Isern et al 2008). The second stage occurred in the yolk sac as well, forming the erythroid, megakaryocytic and several myeloid lines. In the latter the polypotent stem cells were contained, giving birth to all the hemopoiesis blastemas (Sheng 2010). The third stage came out from the GSC, obtained in the frame of the

embryo's yolk sac major arteries, intensified in the embryo's liver and at last in the bone marrow. The definitive erythroid cells continuously were obtained from the bone marrow hemopoietic cells over the whole postnatal life of animals.

Thus, it might be considered, that the primitive erythroid cells supply the life of intensively growing embryo, meanwhile the definitive myeloid erythrocytes have a leading significance when transferring from the intrauterine life to the birth. For the recent years it became apparent, that the ontogenesis and these lines' maturation processes were more complex, than it was supposed earlier. By several researches carried out on mice (Ferkowicz et al 2003; Ferkowicz & Yoder 2005; Huber et al 2004; Baron et al 2012) it had been shown, that the first cells of blood were coming appear from the gastrulation beginning moment, much before the morphologically determined development of the blood islets in the yolk sac and that they had an intra-embryonic localization. They suggested that at first the blood cells formed the primitive erythroid lines shortly after their exit from the primary embryonic strip, having an important role in the early embryonic hemopoiesis. They differed by big sizes and had nuclei in the mammalians (Godin & Camano 2005). That is the first so-called angioblastic period of hemopoiesis (Jaffredo et al 2005; Lu et al 2008).

We have been driven by a question, whether there is an intra-embryonic hemopoiesis in the other mammalians, in connection with what an investigation of the embryonic erythropoiesis in rats and pigs was provided. It also referred to some common and the differential features in the embryonic erythroid cells development and to our comprehension of how these cells are coming to development and differentiation over the mammalians' ontogenesis period.

Material and Method. The given research was performed on the rats' and pigs' embryos at different stages of their embryonic development, starting with the yolk hemopoiesis (the 11.5-days old embryos of rat and the 10-days old embryos of pig) till the definitive bone marrow hemopoiesis (17-days old embryos of rat and the 3-months old embryos of pig). In each case sows were slaughtered in threes of the Large White breed in 11-12 months of ages upon attaining 130-140 kg, and in threes of the white laboratory rats. Each time 5 embryos (fetuses) were explored. Euthanasia was performed according to the protocol Guide for the Care and Use of Laboratory Animals, AVMA Guidelines (International Review Board/Independent Ethics Committee of Institute of Molecular Biology of NAS, IRB00004079). For the morphological investigations the material was fixed in the 96% ethanol, placed in paraffin and afterwards the slides of 5-8 mkm in width were prepared. The preparations were stained by Hematoxylin solution B according to Weigert (1904) with additional staining by eosin (Lillie 1965), azan by Mallory (Gray 1954). For cell analysis, the slides were fixed in pure methanol and stained by Giemsa modified solution (azure B/azure II, eosin and methylene blue) according to the manufacturer's protocol (Sigma-Aldrich). The cells were examined under the light microscope at 1250x magnification in a random sequence. At least 200 cells in each sample were classified. The morphological determination of stages of the primitive erythropoietic development was performed according to Baron et al (2012). Classification of the erythroid cells was encountered as per Weiss & Wardrop (2010).

Hemoglobin quantification. Hemoglobin amount in cells was determined spectrophotometrically on unstained slides. Wavelength scans of the diluted rat blood consistently showed the greatest absorbance at 414 nm, which is the Soret peak from hemoglobin.

Protein staining with nafthole yellow and the protein cytophotometry. For the total protein counting the preparations were stained with nafthole yellow by routine method (Gaub al 1975). The protein optical density measurement was performed in the wave length of 434 nm on the cytospectrophotometer SMP 05 Opton. For the cells sizes cytometric analysis the programming support ImageG was used.

DNA protein staining. All preparations were treated with the combined Feulgen-Naphthol Yellow staining (FNYS) procedure (Lillie 1965; Gaub et al 1975). This method permits simultaneous microspectrophotometric analyses of DNA and protein in single cells and the protein value is closely correlated to the amount of dry mass of the cell.

Image scanning cytometry. For image scanning cytometry and DNA measurement, blood slides were fixed in 96% ethanol for 30 minutes and stained in fresh Schiff reagent (DNA hydrolysis in 5 N hydrochloric acid for 60 minute at 22°C) by the method of Deich (1966). In order to measure DNA content (in conventional units) by image scanning cytometry, computer-equipped microscope-cytometer SMP 05 (OPTON) was used at 575 nm wave length and at 1250x magnification. Before the scanning process, each nucleus was contoured, and cytometry of nuclear DNA content of all studied types of cells were carried out at 1 to 7 dpi.

Ploidy of cells. DNA content was expressed on a "c" scale, in which 1 c is the haploid amount of nuclear DNA occurred in normal (non-pathologic) diploid populations in G0/G1. The DNA content of unstimulated swine lymphocytes was used as a diploid standard for measurements. DNA measurements identify nuclei as aneuploid if they deviate more than 10% from 2 c, 4 c, 8 c, or 16 c; i.e. if they are outside of 2c±0.2, 4c±0.4, 8c±0.8, or 16c±1.6 values. The total number of cells in euploid areas of the DNA histogram rescaled by the mean corrective factor (1.8c-2.2c, 3.6c-4.4c, 7.2c-8.8c, and 14.4c-17.6c) was also calculated. The variability of DNA content in unstimulated lymphocytes did not exceed 10%.

Statistical analysis. The significance was evaluated by two-tailed Student's t-test. P values <0.05 were considered significant. SPSS version 17.0 software package (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

Results and Discussion. First time an intra-embryonic hemopoiesis in pigs was detected by us. It was found, the whole period of the mesenchymal erythropoiesis took about three weeks: it came appear on the 13-15 days of gestation and disappeared by the 35-37th days of that. A proliferative activity of the hemopoietic cells decreased by the 25th days and practically disappeared by the 35th days of the pig embryo's development. The hemoglobin content in all the explored cells did not differ significantly from analogical cells of the yolk sac. Their number was not big, and a significant overgrowth of blood cells alike the yolk sac hemopoietic islets, in the pig embryo's body cavity mesenchyme was not formed. On the 15th stage of gestation (the 25th day of embryo's intra-uterine development), when in pigs the yolk sac erythropoiesis started, but the mesenchymal erythropoiesis was still the basic one, we can observe (Table 1) that the main erythroid cells of the pig embryos' intra-embryonic mesenchymal islets were angioblasts, among which 63% were the blasts and 37% were the nucleated forms of erythrocytes. However, although in fewer amounts there already the minor forms were being appeared, totally not exceeding 14% of the erythroid cells population (the 10th stage of embryogenesis). Most likely, that was a result of the starting yolk sac hemopoiesis, which became prevailing by the 25th day of a pig embryogenesis and practically ceased by the 35th days of the intra-uterine development, being accompanied with a complete disappearing of the mesenchymal erythroid population. It was shown by us, that at early stage of the yolk sac erythropoiesis on the 15-25th and the 35th days some part of megaloblasts (according to our data - about 2.2%) turned to the non-nucleated primary erythrocytes, megalocytes. These erythrocytes sizes, which were forming in the yolk sac, significantly exceeded the sizes of mature erythrocytes of the bone marrow origin (Table 2). In the yolk sac vessels the blast forms number was gradually decreasing, making up 58% of the erythroid cells population, whereas erythrocytes made up 42% (Table 1). Gradually the normal forms number among the blasts were increased, as well as the erythrocytes number, what was much likely connected with quenching of the hemopoietic angioblastic period along with the embryo's development process.

Table 1

Changes in the erythroid cells' population content at different stages of the pig embryogenic development (%)

Specification	*15 day*	*25 day*	*35 day*	*55 day*	*65 day*	*75 day*	*90 day*
				Blast			
Small	5.0±0.6	32.0±2.5	22.0±1.5	10.5±1.0	11.5±2.0	5.1±0.8	0.8±0.003
Large	58.0±6.1	26.0±1.9	16.0±1.5	5.9±0.05	0.6±007.	-	-
Total	63.0±3.3	58.0±2.3	38.0±1.5	16.4±1.0	12.1±1.6	5.1±0.8	0.8±0.003
				Erythrocyte			
Small	9.0±0.5	32.0±1.8	54.0±1.5	76.6±10.1	83.3±8.5	92.1±8.8	95.6±5.5
Large	28.0±3.2	10.0±0.8	8.0±0.9	7.0±0.8	4.6±0.5	2.8±1.1	1.6±0.7
Total	37.0±4.1	42.0±1.3	62.0±2.8	83.6±10.1	87.9±7.9	94.9±9.3	99.2±4.6

Table 2

Measurements of the erythroid cells' dimensional indices at different stages of pig embryogenesis

	Days								
	15			*25*			*35*		
	cell	*nucleus*	*cytoplasm*	*cell*	*nucleus*	*cytoplasm*	*Cell*	*nucleus*	*cytoplasm*
					Blast				
Small	54.6±0.9	18.0±1.6	36.6±1.2	36.5±2.0	12.3±0.6	24.2±1.8	36.1±2.0	10.9±0.5	25.2±2.0
Large	83.3±1.7	22.9±0.5	60.4±1.5	59.8±1.4	16.8±0.9	43.1±1.5	62.1±7.5	14.3±0.6	47.8±7.3
Total	80.8±1.7	22.9±0.4	58.3±1.5	48.4±2.2	14.6±0.7	32.8±1.9	49.1±4.6	12.6±0.6	36.5±4.0
					Erythrocyte				
Small	54.1±1.2	15.2±0.7	38.9±0.8	35.8±1.7	11.3±0.6	24.5±1.5	30.0±4.6	8.2±0.8	21.8±3.8
Large	86.1±2.3	23.2±0.6	62.9±2.1	60.9±2.5	17.8±1.9	41.1±4.1	61.7±5.3	11.7±0.7	50.0±5.8
Total	82.6±2.3	22.4±0.6	60.2±2.0	41.8±2.2	13.3±0.9	28.5±1.8	45.8±4.6	10.0±0.6	35.9±4.2

In the morphological study of early embryonic erythroid cells of pig it has been revealed, that for the pig's 25-days embryo a simultaneous presence of both primitive and early hepatic erythroid cells in blood was characteristic. On the 25th and 35th days a presence of non-nucleated blood cells in small amount was revealed, which were not considered by us before. The performed study of the erythroid cells' population content over the whole period of gestation, represented in the Table 1, had revealed a significant reduction of both big and minor blast forms number, almost till their complete disappearing (0.8% from the total number of the erythroid cells popultion) at the stage of the hemopoiesis embryonic bone marrow (the 90th day of gestation).

Herein, along the whole embryonic erythropoiesis duration a number of megalocytes reduced significantly, more than 17 times, decreasing from 28% till 1.6% (Table 1). That was an evidence of the embryonic erythropoiesis early forms quenching, when the two third of erythroid cells were already matured by the 55th days, making up the overwhelming majority by the 90th days of gestation (more than 95% of the erythroid cells population in the pigs' embryogenesis). A study of dimensional indices of the pig embryonic erythroid cells on the 15th, 25th and 35th days of development have revealed, that both the erythroblasts areas mean values and the minor and big erythroblasts size on the 15th and the 25th days differed from each other veraciously, but these differences between their sizes on the 25th and 35th days were not authentic. We got the analogical data for erythrocytes as well (Table 2). It is important to mention, that the nuclear-plasmatic relationship in all the types of erythroid cells were being reduced with age, but in minor erythroblasts and erythrocytes on the 15th and 25th days they reached 0.5 and in their big analogs it did not exceed 0.4. Finally, on the 35th days the nuclear-plasmatic relationships of erythroblasts and erythrocytes dropped till 0.3. Thereafter, on the 55th, 65th, 76th and 90th days of a pig embryo's development, as well as in newborn piglets erythrocytes sizes varied from 44.8±4.0 mkm^2 till 46.2±4.4 mkm^2, meanwhile in the 3-months piglets, when erythropoiesis occurred only in the bone marrow, the erythrocytes sizes reduced by 25%, reaching up to 35.4±1.6 mkm^2. The data on hemoglobin content in the erythroid cells during a pig embryogenesis testified about significant reduction of its content in a distinct cell with the gestation period increase. It should be noted, that although the primitive hemopoietic cells contained about two times more hemoglobin vs. the matured erythrocytes, their square area exceeded that of the matured cells also more than two times, so their volume exceeded the matured erythrocytes volume more than three times (without consideration of the present nucleus). The primitive non-nucleated erythrocytes contained hemoglobin also above two times more vs. the matured erythrocytes, having a volume approximately two times more. Consequently, a grade of hemoglobin saturation of the primitive hemopoietic cells, primitive erythrocytes and the matured (postnatal) erythrocytes were approximately the similar.

By us performed determination of hemoglobin content in the main erythroid cells in pig ontogenesis are represented on the Figure 1. As it is obvious from the Figure 1 (A) the highest content of hemoglobin corresponds to the early, primitive erythroid cells synthesized in intra-embryonic way in the mesenchyme of embryo and in extra-embryonic way in the yolk sac on the 15th day of gestation. It is apparent, that this population is rather heterogenic, as there are cells with a significantly great content of hemoglobin. At the later stages of ontogenesis such heterogenic distribution of hemoglobin disappears and corresponds to the normal variation line. It is necessary to add, that the increased content of hemoglobin does not bring to the oxygen more effective transportation due-to the lesser number of cells (comparably with the later terms). Because of the total surface area reduction an oxygen exchange in the tissues is slowed down as well. That is why this type of erythropoiesis is possible just in small-volume embryo.

Investigation of the mesenchymal erythroid cells' nuclei distribution as per the ploidity classes found out a presence of the hyperploids and even tetraploid cells on the 15th day and of the hyperploids on the 25th day of embryo development. At the later terms of that the main bulk of the cells were represented by the diploid population. Most likely, our got results testifying about a decline in the nuclear-plasmatic relationships on the 35th day till 0.3 are connected with the nuclei sizes reduction and indirectly testify

about quenching of these erythroid cells proliferative activity, what is highly correlated with the results of investigations of DNA content in nuclei of the erythroid population of mesenchymal islets on the 35th day, when the whole population of erythroid cells are becoming already diploid, what could be accounted for almost complete disappearing of the given hemopoiesis locus by that time.

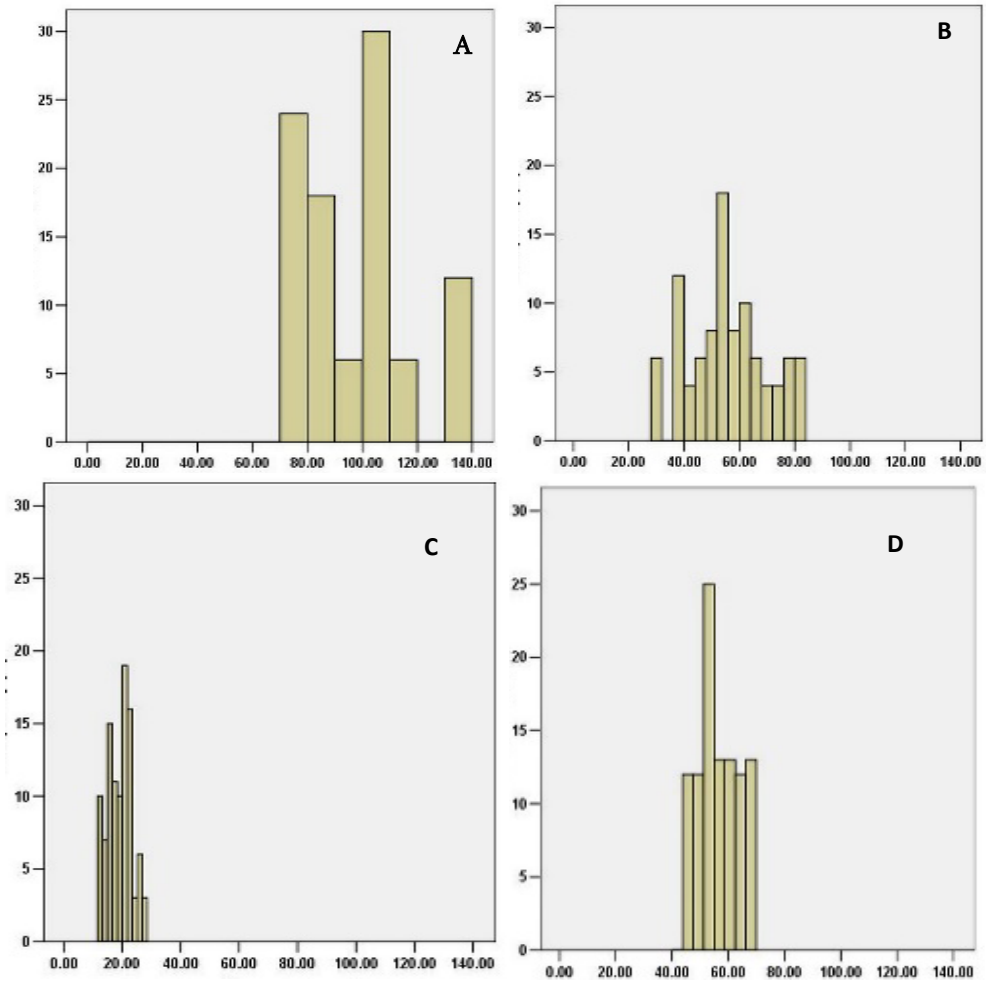

Figure 1. Distribution of the main erythroid cells' of a pig embryo and the pig mature erythrocytes as per the hemoglobin content (*pg*).
A. Primitive erythroid cell; B. Early nucleated embryonic erythrocyte;
C. Late nucleated embryonic erythrocyte; D. Matured erythrocytes.

In order to explore the common and distinctive features of the early development of erythroid cells in the mammalians' embryogenesis and also our comprehensions of how these cells develop and differentiate during the whole ontogenesis process we carried out a study of the early erythropoiesis in rats and provided its comparison with the pigs erythropoiesis. Analogical study upon the early erythropoiesis in rats did not reveal an intra-embryonic hemopoiesis in them. On the 12th stage of gestation (11th days of embryogenesis) there was a yolk sac hemopoiesis and the whole population of the primitive erythriod cells was represented by the macroblasts (Table 3), which was distinct from the early erythropoiesis in pigs, when the yolk hemopoiesis was characterized by more number of mature forms. However, starting from the 13th stage (12th day) of the rats' embryogenesis in the yolk sac all the erythroblastic forms were represented by 66% of macroerythroid cells and just by 4% of microerythroid forms of them, while a content of the appeared normoblastic forms reached up to 30%.

Their number was being gradually increased and by the 13-14th stage, when the hepatic hemopoiesis starts, a number of normocytes reached to 40%, while a number of megaloblasts decreased by 22%. This tendency continued also at the 14th stage (13th

days of embryogenesis), when a number of normoblastic forms increased up to 52%, while a number of megaloblasts dropped till 43%. Thereafter the embryonic bone marrow hemopoiesis also joined up to the hepatic one, and already at the 17th stage (15th days of embryogenesis) along with the hepatic hemopoiesis quenching a number of all types of microcytes attenuated till 1.5% from the whole erythroid population and a number of normocytes reached to 72%, wereas a number of megaloblasts decreased by over 35% in mean. Herein, a number of the mature erythroid forms (normal reticulocytes and erythrocytes) getting increased dramatically, making up more than a half of all the erythroid cells' population.

Table 3

Changes in the erythroid cells' population content during the primary erythropoiesis in rats (%)

Specification	*12 stage*	*13 stage*	*13-14stages*	*17 stage*
Proerythroblasts	5.6±1.2	4.1±0.6	2.8±0.3	0.9±0.8
Macroblast	94.4±5.6	37.0±2.2	7.2±0.5	3.1±0.3
Normoblast	-	17.8±15	12.7±1.1	17.6±2.1
Microblast	-	3.4±0.7	3.6±0.02	0.8±0.05
Macrocytes	-	26.1±3.1	43.6±4.1	22.9±2.2
Normocytes	-	11.6±1.6	26.9±2.2	53.9±4.5
Microcytes	-	-	3.2±1.1	0.8±0.03

According to our data got, although microcytosis proceeded during the rats' embryonic erythrocytosis, anyway it had not have a significant role in the rats' embryonic erythropoiesis, whereas macrocytosis was crucially important in the rats' primary erythropoiesis. Sizes of the forming in the yolk sac erythrocytes significantly prevailed that of not only mature erythrocytes of the bone marrow origin, but also of those, which were being formed in the early embryonic erythroblastic islets with a central macrophage inside.

Thus, along with the rats' embryos development there was the same tendency towards increasing of the minor erythrocytes, as it took place in the pigs' embryogenesis, which was most likely connected with a quenching as far as the angioblastic hemopoietic period of embryo developed. Thereafter the embryonic bone marrow hemopoiesis also joined up to the hepatic one, and already on the 17th stage (15th days of embryogenesis) along with the hepatic hemopoiesis quenching a number of all types of microcytes dropped till 1.6% from the whole erythroid population and a number of normocytes reached to 67%. Correspondingly, a number of megaloblasts decreased by over 35% in mean (Table 4), attenuating by approximately 25%, although at the primary erythropoiesis beginning moment they made up the overwhelming majority of blood cells, composing 95% of the population. At the same time, a number of the mature erythroid forms (normal reticulocytes and erythrocytes) getting enhanced significantly, making about 65% of all erythroid forms population.

Table 4

Changes in the erythroid cells' sizes (mkm^2) in the primary erythropoiesis process in rats

Specification	*12 stage*	*13 stage*	*13-14 stages*	*17 stage*
Proerythroblasts	70.0±6.5	56.3±18.2	52.6±19.6	52.4±17.5
Macroblasts	86.2±7.9	67.1±13.3	48.7±4.2	44.3±4.2
Normoblasts	-	38.0±3.2	38.0±3.5	35.7±4.6
Microblasts	-	26.5±2.2	26.2±2.3	21.9±1.9
Macrocytes	-	54.5±4.6	54.5±4.6	41.6±4.8
Normocytes	-	37.7±3.5	37.7±3.4	34.7±3.4
Microcytes	-	25.6±1.8	22.7±1.8	18.4±1.5

From the above-mentioned one could conclude that a process of microcytosis is taking place in the rats' embryonic erythropoiesis, nevertheless its role in that is insignificant, while macrocytosis plays an important role in the rats' primary erythropoiesis. It is important to note that sizes of megaloblasts, a number of which decreases by 50% in mean on the 17th day of erythropoiesis in rats. According to the more detailed represented data upon the all erythroid cells types in Table 4 the erythroid cells sizes pointed on to the fact, that along with starting of the yolk sac hemopoiesis (the 13th stage of gestation) sizes of proerythroblasts and of the big basophilic blasts in mean decreased by 25%, but further the sizes of proerythroblasts remained unchanged over the whole duration of the study. As respects to the blastic forms sizes changes during the embryonal process of development we noted non-veracious decrease with exception of the normal blasts, the sizes of which remained without changes. The more mature erythroid cells, and namely, the big and small reticulocytes and erythrocytes were also being reduced by 25-30% during the process of gestation. Nevertheless, sizes of the normal mature non-nucleated erythrocytes were not subjected to any changes afterwards.

A study of the hemoglobin content changes' dynamics found out non-veracious reduction in its values in all the erythroid cells during the rats' embryonic development process, except for normocytes, the hemoglobin content in which in the bone marrow hemopoiesis increased by 22% (Table 5). The hemoglobin content authentically reduced in the big, normal and small reticulocytes and also in microcytes during the process of primary erythropoiesis, whereas in the big and normal erythrocytes its content authentically increased.

As it was shown before, at the early stages of the yolk sac hemopoiesis more than 80% of erythroid cells were represented by the basophilic macroerythroblasts, the hemoglobin content in which, as well as in proerythroblasts was 1.5 times lesser comparably with that of reticulocytes and erythrocytes. Starting from the 13th stage of the rats embryogenesis about 25% of embryonic cells were represented by macroerythrocytes, sizes of which in one and half times more of those of normocytes and in two times bigger comparably with microcytes. Herein, the hemoglobin content in macrocytes correspondingly was by 50% more than in normocytes and in four times more than in microcytes. It is noteworthy, that the hemoglobin content in the primary erythropoiesis process gradually and steadily attenuated, but in normocytes increased approximately by 1.5%.

Table 5

Dynamics of the hemoglobin content changes (in conventional units) in the primary erythropoiesis erythroid cells in the process of their differentiation

Specification	*12 stage*	*13 stage*	*13-14stages*	*17 stage*
Proerythroblasts	32.1±0.9	30.1±1.1	19.5±0.7	20.1±0.9
Macroblasts	45.4±4.1	43.4±6.2	41.0±4.1	34.9±4.1
Normoblasts	-	13.9±1.2	31.6±3.2	21.9±1.5
Microblasts	-	12.1±0.3	11.3±1.4	12.4±1.5
Macrocytes	-	64.9±6.7	71.4±3.9	56.8±3.5
Normocytes	-	43.8±6.0	46.7±1.9	46.7±1.7
Microcytes	-	23.3±4.0	17.2±1.9	16.8±1.7

Considering the performed analysis results of DNA content at the 13-14th stages of the primary erythropoiesis development in rats, which are represented in the Table 6, and also the DNA distribution histograms (Figure 2) in different types of cells it comes apparent that the early, the median and the late erythroblasts are represented by cells in G_1, S and G_2 phases of the mitotic cycle. Herein, the main amount of 3c and 4c cells (more than 70%) are the early erythroblasts, meanwhile in the median and the late erythroblasts the diploid cells make up over 70%, and the protein content in the median erythroblasts is about by 25% more, and in the late erythroblasts it is about two times more than in diploid analogs of the early erythroblasts.

Table 6

Content of DNA and the total protein on the 13-14-th stages of the rats' primary erythropoiesis

Stages of the development	*DNA*	*Amount of cells (%)*	*Total protein*
Early erythroblasts	2c	28	28.2±1.4
	3c	44	41.1±4.0
	4c	28	52.1±3.8
Medium erythroblasts	2c	72	35.1±3.9
	3c	15	56.1±5.8
	4c	13	75.1±8.9
Late erythroblasts	2c	77	54.2±2.8
	3c	13	68.3±9.5
	4c	10	81.2±9.3
Medium erythroblasts with additional nuclei	2c	34	37.7±4.2
	3c	66	50.0±7.1
Late erythroblasts with additional nuclei	2c	90	56.3±3.8
	3c	10	76.3±9.5

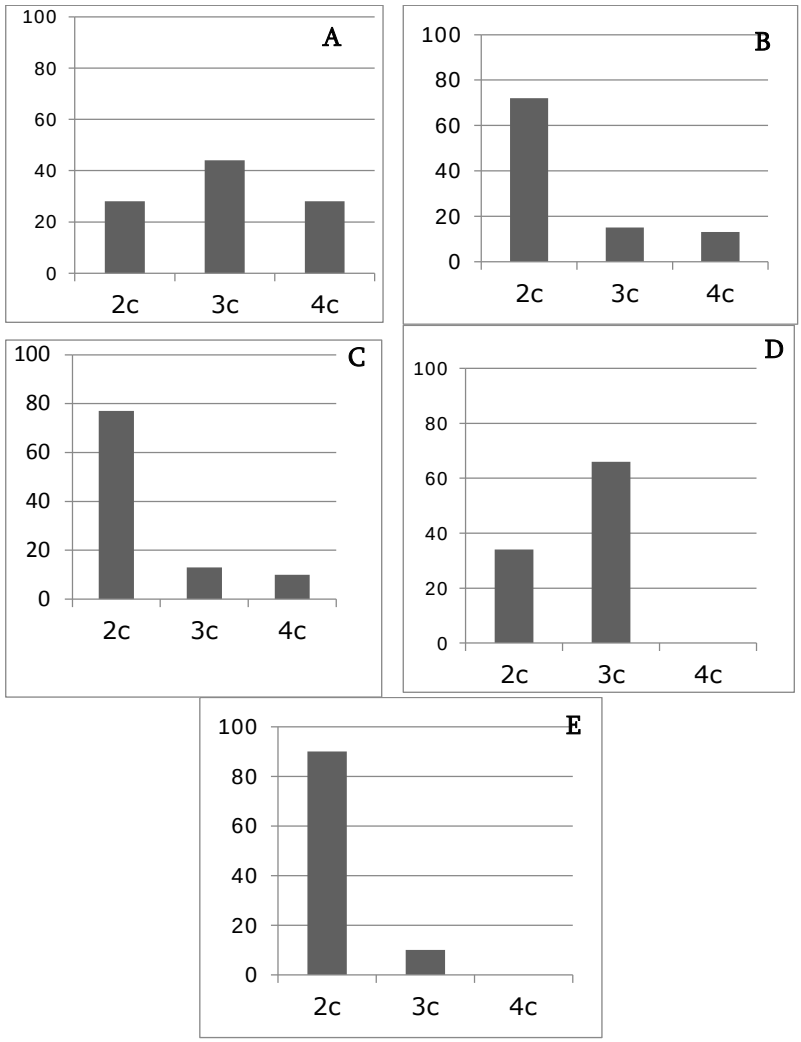

Figure 2. Distribution of the rat erythroid cells as per classes of ploidity.
A- Early erythroblasts; B- Medium erythroblasts; C- Late erythroblasts; D- Medium erythroblasts with additional nuclei; E- Late erythroblasts with additional nuclei.

Considering that "total protein" index mainly reflects the hemoglobin content in erythroblasts, one may assume that the data represented in the Table 6 reflect a dynamics of the hemoglobin content changes in erythroblasts in the process of their differentiation. As it turned out, among the medium and the late erythroblasts there was about 3% quantity of cells with additional nuclei. Their maximal amount was observed at the 13-14th stages of the embryonic development, after which it was dramatically reduced. After a performed separate photometry of the main and additional nuclei it was found, that if about 70% of median erythroblasts contained diploid amount of total DNA of the main and additional nuclei together, already at the late erythroblast stage almost all the cells with additional nuclei (about 90%) were with a diploid amount of DNA, and the protein content in them did not exceed that in the diploid analogs of late erythroblasts. It testifies about the fact that in nuclei during the primary erythropoiesis in rats mainly not an additional synthesis of DNA takes place with a corresponding increase of the total hemoglobin content, but a fragmentation of nuclei, and only in 10% of cells the total DNA content increases authentically because of additional synthesis of DNA in the erythroblasts nuclei, which is associated with an increase of total protein content. This phenomenon confirms our assumption about the absence of additional DNA synthesis in the remarkable part of erythroid cells with accessory nuclei during the rats' primary erythropoiesis. Anyway, a presence of this factor even in insignificant part of the late erythroblasts with a corresponding increase of the total protein content in them tells about an existence of this phenomenon during the evolution process.

Conclusions. Investigation of the pig's and rat's embryos erythropoiesis showed that in rats, in distinct with pigs, there was not a significant intra-embryonic mesenchymal erythropoiesis. First time it was shown that in the pigs' mesenchymal megaloblastic erythropoiesis in some part of cells an extrusion of nuclei and formation of non-nucleated primitive erythrocytes is possible. According to our data got in relation to pigs, they made up not more than 2-2.5% of erythroid cells. The given type of cells was being detected on the 15-25th days of the pig embryo development and almost fully disappeared by the 35th day of gestation. The forming generation of nucleated and non-nucleated primary erythrocytes varied on sizes, however large-sized cells, megaloblasts and megalocytes, are found most often. The megaloblastic erythropoiesis in rats was found already at the 12th stage of gestation, when in the yolk sac vessels only megaloblasts have been revealed and just at the 13th stage erythrocytes came appeared, but at the 13-14th stage, when the hepatic hemopoiesis also included, the erythrocytes number remarkably increased, composing over 70% of the rats' erythroid cells population. Herein, a number of megalocytes was rather great, reaching up to 43% of erythrocytes population, whereas a number of megalocytes in the pigs' hepatic erythropoiesis did not exceed 10% from erythrocytes population. At the early stages of ontogenetic development of rats an almost complete absence of DNA synthesis was observed, what in our opinion was a consequence of the more intensive erythropoiesis in the physiological development of pigs, but absence of the hemopoetic cells' hypertetraploid population in rats instantiates our suggestion. On the benefit of this suggestion a lesser values of the hemoglobin content in the rats' erythroid cells of early erythropoiesis testify in comparison with the analogical indices in pigs.

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Authors:
Marina Tatoyan, Yerevan State Medical University, M. Heratsi Department of Histology, Cytology and Embryology, Armenia, Yerevan, 0025, Koryun St., e-mail: marina.tatoyan@mail.ru
Liana Abroyan, Institute of Molecular Biology of NAS RA, Laboratory of Cell Biology, Armenia, Yerevan, 0014, Hasratyan St. 7, e-mail: z_karalyan@mb.sci.am
Aida Avetisyan, Institute of Molecular Biology of NAS RA, Laboratory of Cell Biology, Armenia, Yerevan, 0014, Hasratyan St. 7, e-mail: aida.avetisyan@inbox.ru
Zara Semergyan, Institute of Molecular Biology of NAS RA, Laboratory of Cell Biology, Armenia, Yerevan, 0014, Hasratyan St. 7, e-mail: hayk_aslanyan@mail.ru
Lina Hakobyan, Institute of Molecular Biology of NAS RA, Laboratory of Cell Biology, Armenia, Yerevan, 0014, Hasratyan St. 7, e-mail: naira_karalyan@yahoo.com
Elena Karalova, Institute of Molecular Biology of NAS RA, Laboratory of Cell Biology, Armenia, Yerevan, 0014, Hasratyan St. 7, e-mail: karalovae@gmail.com
Zaven Karalyan, Yerevan State Medical University, M. Heratsi Department of Histology, Cytology and Embryology, Armenia, Yerevan, 0025, Koryun St.; Institute of Molecular Biology of NAS RA, Laboratory of Cell Biology, Armenia, Yerevan, 0014, Hasratyan St. 7, e-mail: zkaralyan@yahoo.com

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