Open Access
International Journal of Physiological Sciences
RESEARCH ARTICLE

# Erythrocyte Deformability Dynamics in Early Postnatal Ontogenesis of Mature and Immature Newborn Animals

**Katiukhin LN***

*Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, (IEPhB RAS) 44 Thorez Avenue, Saint-Petersburg, Russian Federation.*

***Corresponding author:** Katiukhin LN, Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, (IEPhB RAS) 44 Thorez Avenue, Saint-Petersburg, Russian Federation. Tel: +891126731 92 E-mail: lion@iephb.ru

**Citation:** Katiukhin LN (2022) Erythrocyte Deformability Dynamics in Early Postnatal Ontogenesis of Mature and Immature New born Animals. Int J Physiol Sci 1: 1-9

**Copyright:** © 2022 Katiukhin LN This is an open-access article distributed under the terms of Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

## ABSTRACT

The formation of the rheological properties of erythrocytes in ontogenesis of mature newborn (rats) and immature newborn (guinea pigs) animals was studied in an artificially created model environment using osmotic gradient ektacytometry. A phase change in the deformation properties of erythrocytes in early postnatal ontogenesis of the animals has been shown. An increase in the water permeability capacity of red blood cell membranes occurs during the process of maturation in rats. In contrast, a decrease in this ability was observed in guinea pigs.

**Keywords:** Mature Newborn and Immature Newborn Animal; Rats; Guine Pigs; Red Blood Cells; Erythrocytes; Deformability; Ektacytometry

## Background

In early postnatal ontogenesis in mammals, the erythronium is reformatted. Therefore, the rheological properties of blood change. The formation of erythrocyte deformation abilities during ontogenesis of immature newborn animals using the method of gradient ektacytometry has not been described in the literature.

## Objective

To study the formation of the erythrocyte deformability in early postnatal ontogenesis of mature and immature newborn animals.

## Methods

The dynamics of the rheological properties of erythrocytes in Wistar rats and guinea pigs of both sexes were studied 1, 3, 10 days and 1, 2 and 3 months after birth according to the Rules of work with experimental animals, approved by the Bioethics Commission of the Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences (Protocol № 6, 2020). The experiments were conducted with the approval of the Animal Welfare Committee in accordance with the guidelines outlined NIH in the United States regarding the care and use of animals for experimental procedures. Measures to minimize the pain of non-narcotized animals were taken by guillotining them. After the decapitation, mixed blood was collected in tubes with EDTA. The deformation properties of erythrocytes were assessed by gradient ektacytometry at 37°C ex tempore [1]. The classical profile of the osmotic deformability of native erythrocytes (osmoscan), which represents the change in the deformability index (EI) of erythrocytes in the range of osmotic concentrations of suspension medium, is characterized by the following parameters: the integral deformability index ($EI_{max}$) at isotonic osmolality O($EI_{max}$), the $EI_{min}$ parameter at the osmoscan inversion point $O_{min}$, the $O_{hyper}$ index changing linearly with MCHC, which serves as an estimate of cytoplasmic viscosity, or degree of hemoglobin hydration in the red blood cell and the range of osmotic deformability of red cells ($\Delta O=O_{hyper} - O_{min}$). Statistical processing of the results was performed using Microsoft Office Exel 2007. The data are presented as arithmetic mean values with their standard deviations. Significance of the changes found in the compared groups was determined using two-pair Student's test with unequal deviation.

## Results

Figures 1 and 2 show the osmoscans of rats and guinea pigs at different stages of postnatal development.

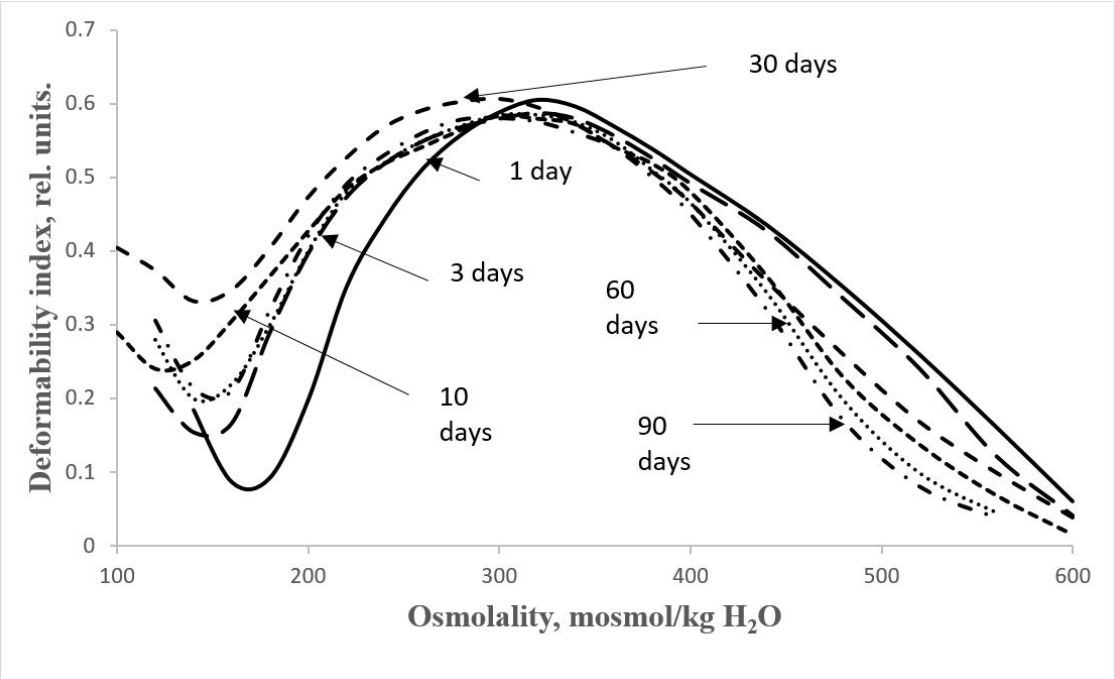


**Figure 1:** Representative osmoscan curves of rat at different stages of postnatal development

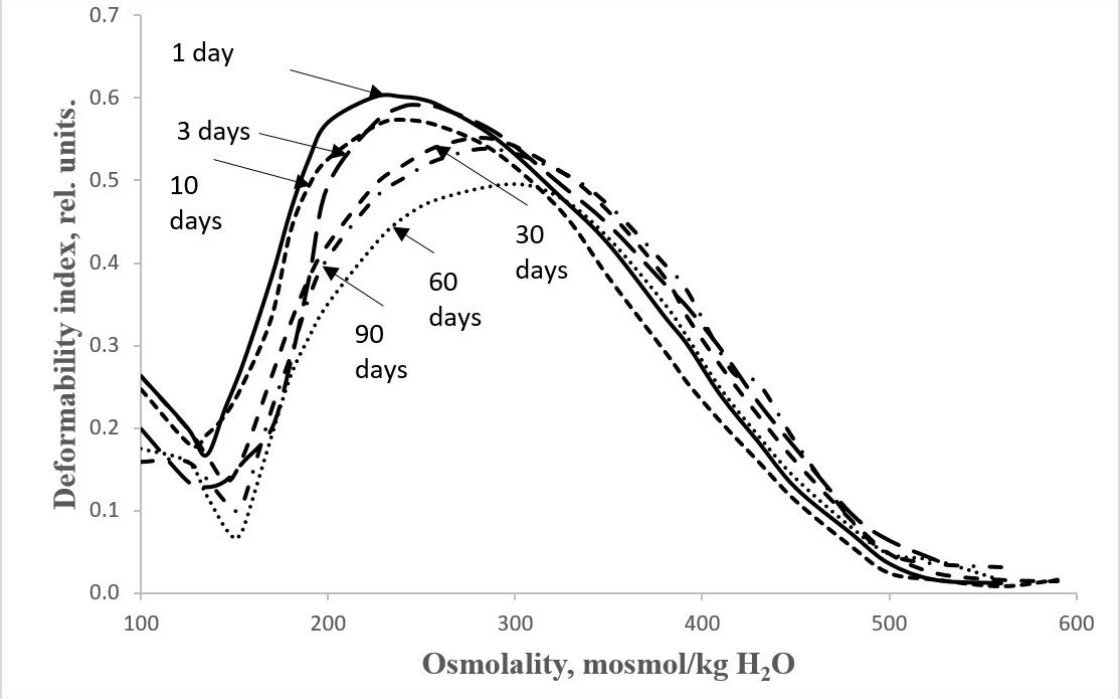


**Figure 2:** Representative osmoscan curves guinea pig erythrocytes at different stages of postnatal development

Tables 1 and 2 present the numerical data of the obtained osmoscans.

Analysis of the experimental results shows that the dynamics of the formation of the deformability properties of erythrocytes in the studied animal species has a distinct phase character. Thus, a significant decrease in the erythrocyte deformability index $EI_{max}$ was observed in rats from the first day (Table 1). However, by day 30 of postnatal ontogenesis, its value does not differ from the initial one. During this period of animal life, there is a gradual decrease in such a determinant of deformation properties as the degree of hemoglobin hydration $O_{hyper}$, which means an increase in the internal viscosity of the red cell. At the same time, the index $O_{min}$, reflecting the degree of erythrocyte toroidality (S/V), decreases. These changes are accompanied by a shift of isotonic osmoscan point $O(EI_{max})$ in hypo-osmotic direction and narrowing of red cell osmotic range ΔO, which are already significantly reduced by day 30. The $EI_{min}$ index is the lowest on the first day, and increases with high confidence up to day 30 thereafter.

**Table 1:** Ektacytometric parameters of rat erythrocytes at different stages of postnatal ontogenesis (M±σ)

| Age, days | $EI_{max}$, | $EI_{min}$, | $O_{min}$, | $O(EI_{max})$, | $O_{hyper}$, | ΔO, |
|---|---|---|---|---|---|---|
| | rel. units | | mosmol/kg $H_2O$ | | | |
| 1, n=5 | 0,608±0,007 | 0,076±0,004 | 168±3 | 320±3 | 503±5 | 335±4 |
| 3, n=5 | 0,590±0,006# | 0,150±0,010¥ | 148±3# | 325±3 | 498±4 | 350±5* |
| 10, n=5 | 0,586±0,006# | 0,238±0,015¥ | 126±4¥ | 310±3 | 460±4# | 334±4 |
| 30, n=5 | 0,607±0,008 | 0,330±0,018¥ | 145±5# | 300±3* | 462±4# | 317±4# |
| 30, n=5 | 0,607±0,008 | 0,330±0,018 | 145±5 | 300±3 | 462±4 | 317±4 |
| 60, n=5 | 0,585±0,006¥ | 0,196±0,016¥ | 144±3 | 304±4 | 455±6 | 311±3 |
| 90, n=3 | 0,580±0,006¥ | 0,200±0,020¥ | 151±6 | 291±3* | 446±7* | 293±3* |

Note: n - number of animals; * - p<0.05; # - p<0.01; ¥ - p<0.001

**Table 2:** Ektacytometric parameters of guinea pig erythrocytes at different stages of postnatal ontogenesis (M±σ)

| Age, days | $EI_{max}$, | $EI_{min}$, | $O_{min}$, | $O(EI_{max})$, | $O_{hyper}$, | ΔO, |
|---|---|---|---|---|---|---|
| | rel. units | | mosmol/kg $H_2O$ | | | |
| 1, n=7 | 0,602±0,002 | 0,207±0,022 | 118±8 | 235±26 | 394±13 | 267±10 |
| 3, n=6 | 0,592±0,025 | 0,128±0,024# | 128±6* | 245±17 | 411±4# | 283±5# |
| 10, n=11 | 0,574±0,024 | 0,179±0,026 | 127±9 | 239±23 | 382±27 | 255±22 |
| 10, n=11 | 0,574±0,024 | 0,179±0,026 | 127±9 | 239±23 | 382±27 | 255±22 |
| 30, n=8 | 0,550±0,054 | 0,135±0,041 | 146±25 | 289±21 | 410±16 | 263±28 |
| 60, n=6 | 0,496±0,026* | 0,066±0,020¥ | 151±4* | 299±10* | 410±6 | 258±2 |
| 90, n=4 | 0,539±0,032 | 0,100±0,007¥ | 151±6* | 289±7* | 416±3 | 265±4 |

Note: n - number of animals; * - p<0.05; # - p<0.01; ¥ - p<0.001

One month after birth and up to the end of observation, $EI_{max}$ begins to decrease again, as well as $O_{hyper}$. The Omin index does not change any more. The osmoscan isotonic point $O(EI_{max})$ continues to shift to the hypotonic zone and ΔO decreases, which are already significantly reduced by the end of observation. From day 30 $EI_{min}$ begins to decrease with a high degree of reliability.

Erythrocytes in guinea pigs of the first day of life are characterized by the highest index of deformability $EI_{max}$, which has a stable tendency to decrease until the 10th day of observation. The index $O_{hyper}$ after increasing by the 3rd day remains unchanged for 10 days. $O_{min}$, after a significant increase by day 3, remained elevated until day 10. The position of the $O(EI_{max})$ point during this period remains unchanged, and the ΔO range, which increased by day 3, is already equal to the initial value by day 10. The $EI_{min}$ value decreases sharply from the first day.

From the 10th day of observation, $EI_{max}$ continues to decrease, $O_{hyper}$ is unchanged, and $O_{min}$ significantly increases. The maximum osmoscan point $O(EI_{max})$ reliably shifts in the hyperosmotic direction with ΔO unchanged. $EI_{min}$ experiences a second wave of decrease, which occurred by day 10 after birth of the animals.

The changes in the rheological parameters of erythrocytes in the studied animal species depending on the observation phase are clearly demonstrated in the histograms shown in Figure 3.

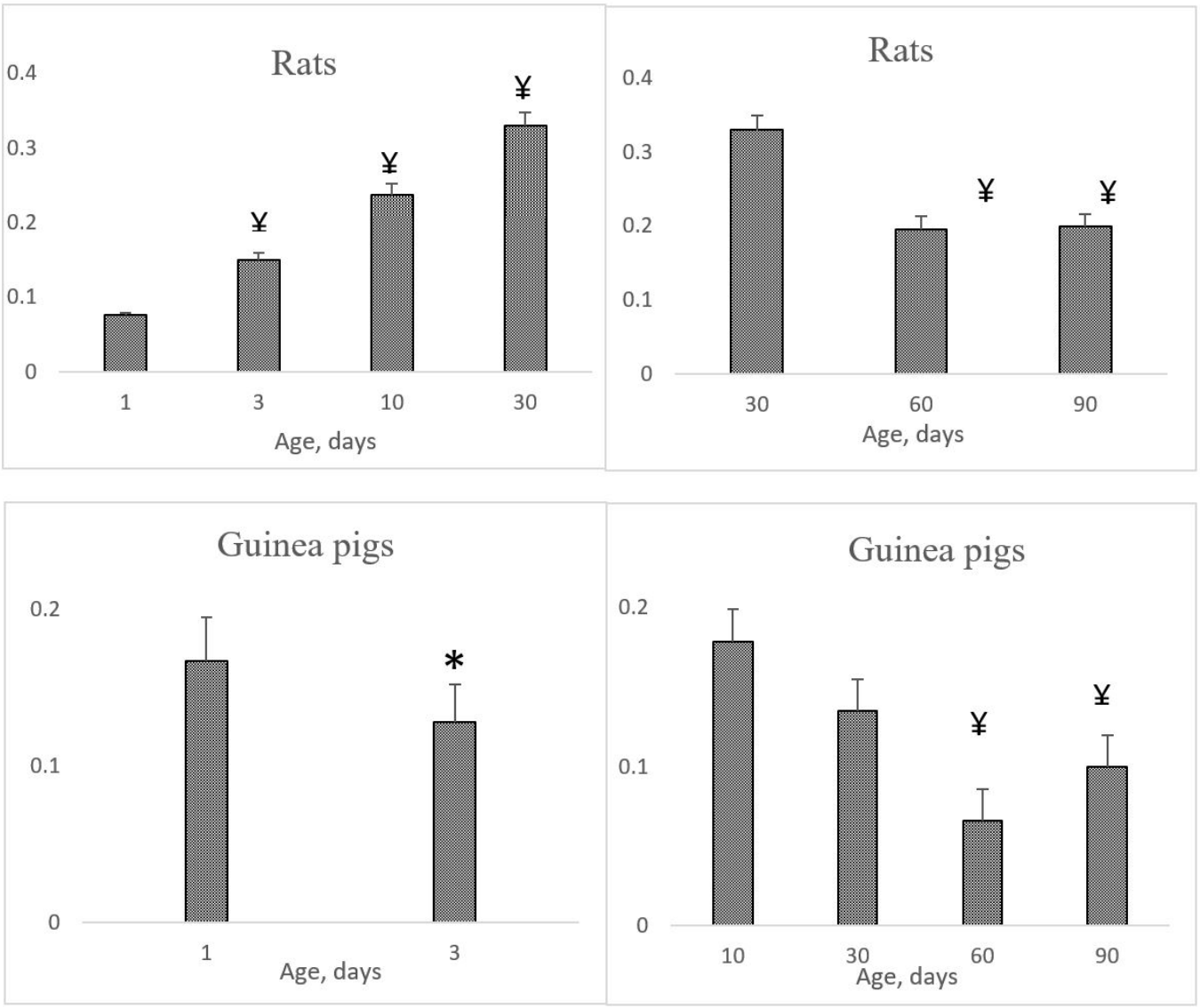


**Figure 3:** EImin values calculated from the original osmoscan raw data of rat and guinea pig blood samples

Means - SD, * $p < 0.05$, # $p < 0.01$, ¥ $p < 0.01$

## Discussion

The biological basis of ontogenesis is a transition process in functional systems characterized by certain regularities, the genetic basis of which is the programmed repression of some genes and depression of others. For a developing organism, the concept of dynamic norm is acceptable, which depends on the age of the organism and the rate of its development. One of the tasks of age physiology is to study the laws of hematological changes in mammals, starting from intrauterine development and into postnatal ontogenesis. In prenatal and early postnatal periods there are significant functional and structural transformations of the erythron and associated changes in the rheological properties of blood. Of particular interest is the formation of the deformation abilities of erythrocytes in mature and immature animals.

The rat is an interesting object for study due to the fact that they are immature animals, and the character of the development of a number of indicators in them is similar to the changes in humans since birth. The main hematological process of early postnatal ontogenesis is the replacement of the red blood cell population, formed before birth and containing fetal hemoglobin, by cells with adult hemoglobin, which provides dissociation of oxyhemoglobin at high concentrations of dissolved oxygen [2, 3]. In rats there are two critical ages: the neonatal period and the period between 9 and 17 days of development, when the number of reticulocytes in the blood drops sharply and mature red blood cells begin to prevail over the latter, i.e., the red bone marrow transitions to a new level of functioning. By the end of the 3rd week of postnatal ontogenesis, the transition to independent feeding occurs. There are few data on hematopoietic hemorheology in the ontogenesis of mature guinea pigs. It has been established only that hematological

parameters during their postnatal ontogenesis are fairly constant with respect to both the number of erythrocytes and hematocrit index and hemoglobin concentration in blood [4, 5, 6]. Individual development of rat cubs during the stages of early ontogenesis is characterized by polymodal distribution curves of functional and morphological properties of erythrocytes, which is associated with the simultaneous presence of fundamentally different cell populations in peripheral blood [7]. As the study showed, the deformability of erythrocytes, although wave-like (end of monthly development), from birth decreases, as does the degree of hemoglobin hydration, leading to an increase in the internal viscosity of the red cell. At the same time, the erythrocyte toroidality increases, which can be explained both by a decrease in the number of reticulocytes with higher viscoelastic properties than those of mature erythrocytes and by the process of their maturation [8, 9]. Up to the end of the first month after birth, the $EI_{min}$ significantly increases, and thereafter has a strong tendency to decrease.

In this regard, it is appropriate to explain the physical essence of the manifestation of the deformation ability of erythrocytes in the isotropic sphere stage at the osmolality of $O_{min}$ at the point of osmoscan inversion. The fact is that due to the hydrophobicity of phospholipid bilayer the membrane itself is impermeable to hydrophilic substances. However, the permeability of the natural biological membrane to small and electrically neutral water molecules is very high. Passive penetration of water into cells is ensured by the presence of specific "water channels" in membranes, or aquaporins, universal water channels responsible for rapid response of cell volume to changes in plasma tonicity. Aquaporins, as independent protein complexes, are designed for transmembrane transport of water along the osmotic gradient. They selectively transport water molecules across the membrane [10]. With their help, water molecules enter and leave the cell, preventing the flow of ions and other soluble substances. Each cell has its own set of aquaporins, which is determined by the evolution of the organ and its function. Thus, water permeability directly depends on the number of aquaporins in the membrane [11]. For example, each erythrocyte contains up to 120-160 thousand molecules of aquaporin-1 (AQP1) [12]. Assessing the water permeability of biological membranes is a very challenging task. With the advent of the method of gradient osmotic ektacytometry there is such a possibility [13]. The essence of the method is that under hypo-osmotic conditions water penetrates into erythrocytes along a concentration gradient. At the prehemolytic stage, the erythrocyte has the shape of an isotropic sphere. The sphere has a maximum volume for a given surface area, a change in its morphology suggests two alternatives. If the volume is preserved, this transformation must be accompanied by an increase in the surface area, and if the area is preserved, the volume must decrease. Thus, deformation of a spherical erythrocyte will lead to a decrease in its volume, since biological membranes are inextensible [14, 15]. At the isotropic sphere stage, the erythrocyte retains its structural integrity, undergoes transformation and is capable of deformation under the action of shear stress in the Couette cell. An increase in hydrostatic pressure leads to a decrease in volume compared to the maximum critical one due to the exit of the liquid phase through the hydrophilic pores into the suspension medium. The $EI_{min}$ index clearly reflects the degree of change in the deformation capacity of the membrane during the transformation of the erythrocyte at the $O_{min}$ point. However, one cannot deny that the character of osmoscan behavior in the zone of inversion (at the point $O_{min}$) is also determined by the degree of erythrocyte anisocytosis in the blood sample.

The study showed that from the first day the deformability of erythrocytes has a stable tendency to decrease with unchanged internal viscosity, but with a steady swelling of the red cell. Membrane permeability for water molecules decreases in a wave-like manner immediately after birth up to the end of the observation period of 3 months. Wave-like changes in the deformation properties of erythrocytes in postnatal ontogenesis are explained by physiological features of the animal organism in the juvenile period, when the activity of the erythropoietic system and bone marrow periodically changes.

Currently, a promising direction in molecular biology is the search for aquaporin inhibitors and activators [16]. Artificial modulators of aquaporins will find wide application in the prevention of heart diseases [17-19], treatment of blood diseases [20] and tumor processes [21, 22]. Literature analysis confirms the increase of aquaporins expression in cell membranes during mammalian ontogenesis. Thus, the level of AQP1 increases fivefold from the last gestational day to the first postnatal day and remains at a high level in adulthood [23, 24]. The content of AQP1 found in the rat myometrium increases early in early pregnancy from day 1 to day 6 from conception [25]. AQP1 levels in erythrocytes also increase after birth [26, 27]. Compared to adults, human fetuses in the second and third trimesters have been shown to contain less AQP1 and have lower osmotic permeability to water [28]. There are no data on the expression of the erythrocyte AQP1 pool in the ontogeny of mature animals.

Figure 4 shows osmoscans of all examined animals (30 rats and 42 guine pigs).

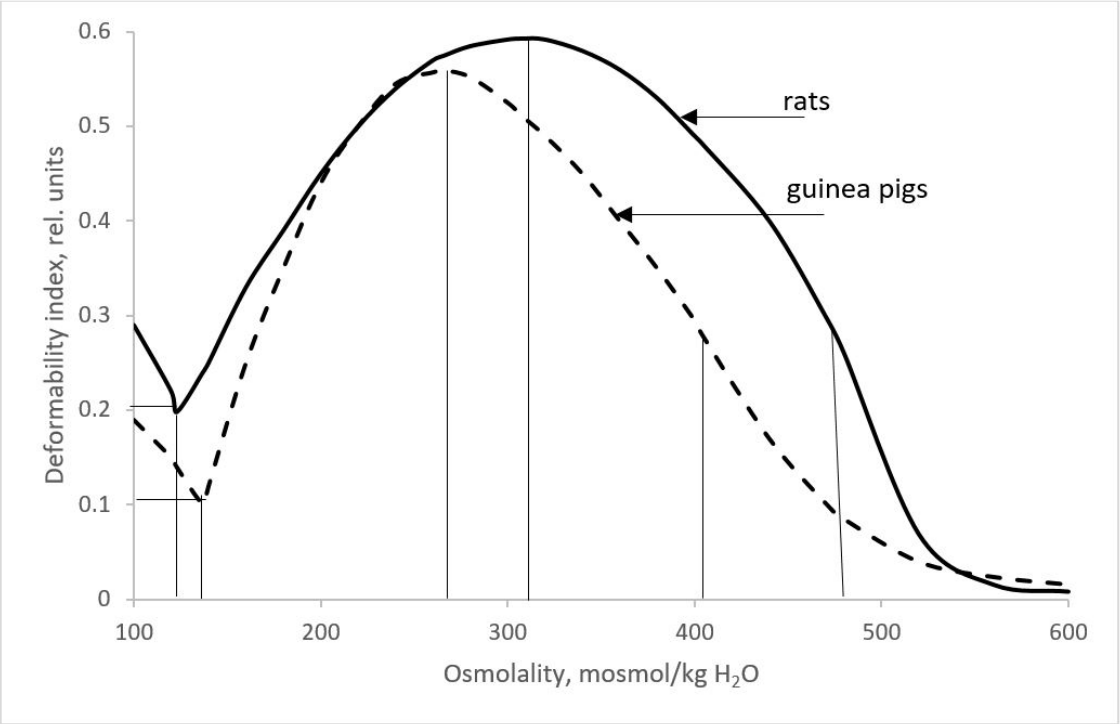


**Figure 4:** Representative averaged osmoscan curves of all examined animals

The average deformability of the erythrocyte population ($EI_{max}$) in rats is higher, they have a preferable rheological shape ($O_{min}$) and are more hydrated ($O_{hyper}$). Moreover, the osmotic range of rat erythrocyte deformability (ΔO) is (323±6) mosmol/kg $H_2O$ versus (264±21) mosmol/kg $H_2O$ in pigs. Comparison of Imin values indicates a significantly higher permeability of rat erythrocyte .membranes for water molecules

## Conclusions

The study suggests that immature rats show a gradual increase in the water permeability of erythrocyte membranes in the first month of ontogenetic development, which is reflected in the pronounced expression of the AQP1 pool. Subsequently, as the animals age, the ability of erythrocyte membranes to permeate water molecules decreases. Mature pigs are born with an established AQP1 pool, which decreases as they mature. In the compared animal species, the dynamics of membrane water permeability .formation is diametrically opposite. As immature rats mature, it increases, whereas in mature rats it decreases

## References

1. Johnson RM (1989) Ektacytometry of red blood cells. Meth Enzymol 173: 35–54. DOI: 10.1016/s0076-6879(89)73004-4.

2. Iwahara SI, Abe Y, Okazaki T (1996) Identification of five embryonic hemoglobins of rat and ontogeny of their constituent globins during fetal development. J Biochem 119: 360-6. doi: 10.1093/oxfordjournals.jbchem.a021248.

3. Nazarov SB (1993) The developmental dynamics of the erythroid stem cell in rat hematopoietic organs in ontogeny. Fiziol Zh Im I M Sechenova 79: 54-9.

4. Constable BJ (1963) Changes in blood volume and blood picture during the life of the rat and guinea-pig from birth to maturity. J Physiol 167: 229-38. doi: 10.1113/jphysiol.1963. sp 007143.

5. Lucarelli G, Porcellini A, Carnevali C, Carmena A, Stohlman F (1968) Fetal and neonatal erythropoiesis. Ann N Y Acad Sci 149: 544-59. doi: 10.1111/j.1749-6632. Tb 15194.x.

6. Kitagaki M, Yamaguchi M, Nakamura M, Sakurada K, Suwa T, et al. (2005) Age-related changes in haematology and serum chemistry of Weiser-Maples guineapigs (Cavia porcellus). Lab Anim 39: 321-30. doi: 10.1258/0023677054307042.

7. Katyukhin LN, Novozhilov AV (2021) Dynamics of Deformation Properties of Erythrocytes in Wistar Rats during Postnatal Ontogeny. Bulletin of Experimental Biology and Medicine 172: 5-8.

8. Borysiuk MV (1983) Systemic analysis of the mechanisms of blood oxygen affinity regulation. 1. Intraerythrocyte regulation of hemoglobin affinity for oxygen Uspekhi physiologicheskikh nauk. 14: 85-101.

9. Bessmeltsev S, Fedorova ZD, Abdulkadyrov KM (1990) Rheological properties of erythrocytes and hemostasis in patients with acquired autoimmune hemolytic anemia who received anti-lymphocytic globulin // Hemat. and Transfus. 35: 13-15.

10. Tsunoda SP, Wiesner B, Lorenz D, Rosenthal W, Pohl PJ (2004) Aquaporin-1, nothing but a water channel. Biol Chem 279: 11364-11367. doi: 10.1074/jbc.M310881200.

11. Zeidel M, Nielsen S, Smith B, Ambudkar S, Maunsbach A (1994) Ultrastructure, pharmacologic inhibition, and transport selectivity of aquaporin channel-forming integral protein in proteoliposomes. Biochemistry 33: 1606-15.

12. Denker BM, Smith BL, Kuhajda FP, Agre P (1988) Identification, purification, and partial characterization of a novel Mr 28,000 integral membrane protein from erythrocytes and renal tubules. J Biol Chem 263: 15634-42.

13. Katyukhin LN (2014) A Method for Evaluation of Membrane Permeability for Water by the Erythrocyte Osmotic Deformability Profiles. Bulletin of Experimental Biology and Medicine. 157: 116-18. doi: 10.1007/s10517-014-2505-1.

14. Evens I, Skailak R (1982) Mechanics and thermodynamics of biological membranes. M: 304.

15. Hennis R (1997) Biomembranes. Molecular structure and functions. M: Mir. 624.

16. Song D, Yang Y, He N, Tian X, Sang DS (2018)The involvement of AQP1 in myocardial edema induced by pressure overload in mice. Eur Rev Med Pharmacol Sci 22: 4969-74. doi: 10.26355/eurrev_201808_15637.

17. Ding F.B., Yan Y.M., Huang J.B., Mei J., Zhu J.Q., Liu H. The involvement of AQP1 in heart oedema induced by global myocardial ischemia. Cell Biochem Funct. 2013; 31(1): 60-4. doi: 10.1002/cbf.2860.

18. Li L, Weng Z, Yao C, Song Y, Ma T (2015) Aquaporin-1 Deficiency Protects Against Myocardial Infarction by Reducing Both Edema and Apoptosis in Mice Sci Rep 5: 13807. doi: 10.1038/srep13807.

19. Chow PH, Cox CD, Pei JV, Anabaraonye N, Nourmohammadi S (2022)Inhibition of the Aquaporin-1 Cation Conductance by Selected Furan Compounds Reduces Red Blood Cell Sickling. Front Pharmacol 12: 794791. doi: 10.3389/fphar.

20. Wang Y, Fan Y, Zheng C, Zhang X (2017) Knockdown of AQP1 inhibits growth and invasion of human ovarian cancer cells. Mol Med Rep 16: 5499-5504. doi: 10.3892/mmr.2017.7282.

21. Simone L, Gargano CD, Pisani F, Cibelli A, Mola MG (2018) Aquaporin-1 inhibition reduces metastatic formation in a mouse model of melanoma. J Cell Mol Med 22: 904-912. doi: 10.1111/jcmm.13378.

22. Titovets EP (2007) Human and animal aquaporins: fundamental and clinical aspects. Minsk Belorus Nauka 239.

23.Tsunoda SP, Wiesner B, Lorenz D, Rosenthal W, Pohl P (2004) Aquaporin-1, nothing but a water channel. J Biol Chem 279: 11364-67. doi: 10.1074/jbc.M310881200.

24. King LS, Nielsen S, Agre PJ (1996) Aquaporin-1 water channel protein in lung: ontogeny, steroid-induced expression, and distribution in rat. Clin Invest 97: 2183-91. doi: 10.1172/JCI118659.

25. Natochin YuV, Tkachuk VA (2009) Modern course of classical physiology M: Geotar-Media 384p.

26. Orlov RS, Nozdrachev AD (2005) Normal Physiology. Textbook. Moscow: Geotar-Media. 388p.

27. Agre P, Smith BL, Baumgarten R, Preston GM, Pressman E (1994) Human red cell Aquaporin CHIP. II. Expression during normal fetal development and in a novel form of congenital dyserythropoietic anemia. J Clin Invest. 94: 1050-58.

28. Benga G (2012) The first discovered water channel protein, later called aquaporin 1: molecular characteristics, functions and medical implications. Mol Aspects Med 33: 518-34. doi: 10.1016/j.mam.2012.06.001.