ERYTHROCYTE PRSERVING AND DYNAMICS OF CHANGES IN CONCENTRATION OF POTASSIUM AND SODIUM CATIONS IN ANIMAL BLOOD DEPENDING ON METHOD OF ITS CONSERVATION

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The article represents the results of researches of the effectiveness of blood conservation impact of the environment (containing the different forms of carbon dioxide; pCO_2, HCO^{3-}) when compared to the standard glucose-citrate additive «Glugitsyr». Estimation criteria of blood conservation effectiveness were preserveness of erythrocytes and dynamics of concentration of potassium and sodium in animal blood plasma. It was found that in terms of the combined action of carbon dioxide, bicarbonate, and low temperatures (2-4 ^{0}C), the preservation effectiveness of erythrocytes is significantly higher than in control. In the test samples of preserved blood, the sodium-potassium gradient remains stable. Preserving in bicarbonate and carbonic acid provides better preservation of blood and prolongs the term of its technological use.

Key words: *hypobiosis, conservation, preservation, blood, erythrocytes, ions, sodium, potassium, carbon dioxide, sodium bicarbonate.*

During the history of blood transfusion development there were offered many different additives for blood conservation. National science also has considerable achievements in this field. Though, the considerable drawback of national blood additives is a short term of qualitative blood conservation. Native glucose-citrate additives make it possible to store blood during twenty one day. Though, an experience of blood transfusion stations shows that the optimal term for qualitative storage of preserved donor blood ranges between seven and ten days [10]. So, in spite of the fact that the problem of blood conservation is quite an old one, the development of new, more effective environments for blood storage still remains actual.

Interesting and at the same time actual, in the problem of development and introduction into practice of new methods of biological material preservation, is the use of the state of hypobiosis with temporal decline of vital functions, when metabolism and functioning of the living systems declines due to the action of certain hypobiosis factors [2, 5, 9].

It is known that in the preserved blood while storage they take place different metabolic changes can considerably influence the preserving of formed elements, including erythrocytes. It is known that the system of electrolyte transporting through a cellular membrane, especially of sodium and potassium, is quite sensitive to the factors of storage of the preserved blood in erythrocytes [4, 6]. So, to lengthen viability of erythrocytes of the preserved donor blood it is important to provide stability of this system. To solve this task we used the state of artificial CO_2 hypobiosis. Once its use gave positive result while conservation of sperm of agricultural animals [7, 8]. The preservation influence of this type of hypobiosis is explained by the fact that under the action of strong concentrations of different forms of carbonic acid it takes place the considerable inhibition of metabolic processes [1, 9].

Research objective. Studying of the influence of strong concentrations of different forms of carbonic acid (pCO_2; HCO^{3-}) on stability of concentration gradient between plasma and blood elements of the preserved donor blood of such electrolytes, as sodium and potassium (Na, K) and analysis of erythrocyte conservation.

Materials and research methods. For researches they were taken blood samples of bullocks aged 10 – 12 months. For investigation they were chosen healthy animals by the method of analogues. In order to study the action of different forms of carbonic acid on conservation of formed animal blood elements they were formed study and control groups of blood samples.

For forming of control groups there were used the blood preserved on the additive «Glugitsyr». This is a glucose-citrate solution (glucose – 0,029 g/ml; sodium hydrocitrate – 0,019 g/ml; water for an injection – 250 ml) intended for

preservation of donor blood in correlation of 1 volume of solution to 4 volumes of blood. Maximal shelf life of preserved blood at a temperature from 2 to 6 C is 21 day [11].

For forming study group of blood samples, in some part of bottles with Glugitsyr it was added sodium bicarbonate, in a concentration of 40 mmol/l, whereupon this solution was saturated with CO_2. Thus, pH level of carbonic medium was 7,40. It is necessary to say that pH of Glugitsyr was about 5,0 though normal pH for bullock blood is 7,35 – 7,45. In our model after adding sodium bicarbonate the medium was saturated with carbon acid to approach maximum precise pH to the physiological indices. Blood was taken from jugular vein by means of special systems and put into bottles of 250 ml with control and study medium. To study the state of preserved blood during all storage period (30 days), it was poured into sterile small bottles of 20 ml, and put into a coolroom (temperature + 4 ^{0}C). Thus, they were formed control and study groups – 35 blood samples in each.

The concentration of potassium and sodium cations was determined on the atomic-absorption spectrophotometer AAS – 30. The degree of pH in an investigated blood-preserving solution was measured by ion-meter И-130. The amount of erythrocytes was counted up in the chamber of Gorjaev using standard methodology [3].

Digital data of research results were processed using the methods of variation statistics with the help of computer programs.

All researches were conducted with the observance of the general principles of the humane handling with experimental animals, accepted on the First National Congress on Bioethics (Kyiv, in 2001).

Results of researches. Researches showed that increase of concentration of potassium cations, both in investigation and control samples was observed during all period of storage. Though, the analysis of dynamics of these changes demonstarated their greater stability in the study samples of the preserved animal donor blood when compared to the control samples where they are considerably

more intensive (Figure1). For example, in the day of blood obtaining the concentration of potassium cations in plasma of study samples was 3,11 mmol/l, that actually is at the same level with control ones – 3,12 mmol/l.

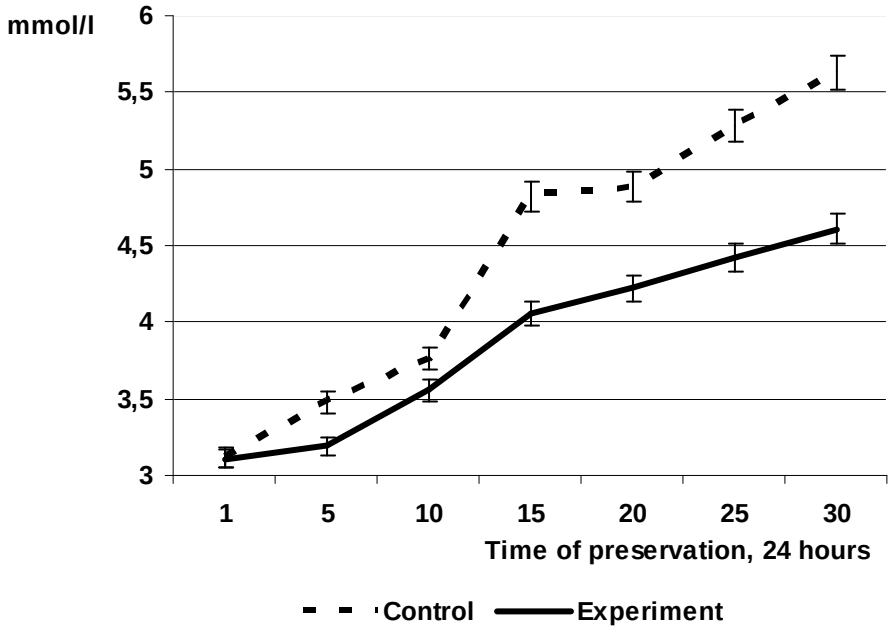

Figure 1. Change of concentration of potassium catiions of the study samples of the preserved blood of bulls

On the tenth day increase of potassium cation concentration both in study and in control groups of blood samples was almost the same. Substantial changes were noticed on the fifteenth day, in particular in the study group the concentration of potassium cations increased 1,3 times, and in control one – 1,5 times, forming 4,06 mmol/l and 4,82 mmol/l respectively. On the thirtieth day the average content of potassium cations for study group was 4,61 mmol/l, and for control – 5,63 mmol/l, that 1,4 and 1,8 times more when compared to the initial data. It is important that the concentration of potassium cations in study samples did not exceed possible physiological limits (3,05 – 4,74 mmol/l) during all period of storage if to compare with the control.

Unlike potassium, the change of sodium cation concentration had the opposite behavior and its amount in plasma declined (Figure 2) gradually. In the day of obtaining of blood its concentration in plasma of study samples was 120,2 mmol/l, and in control – 123,2 mmol/l. First five days of study group blood storage

were characterized by relative stability of sodium cation concentration which hadn't changed in study samples by the 15 th day of storage.

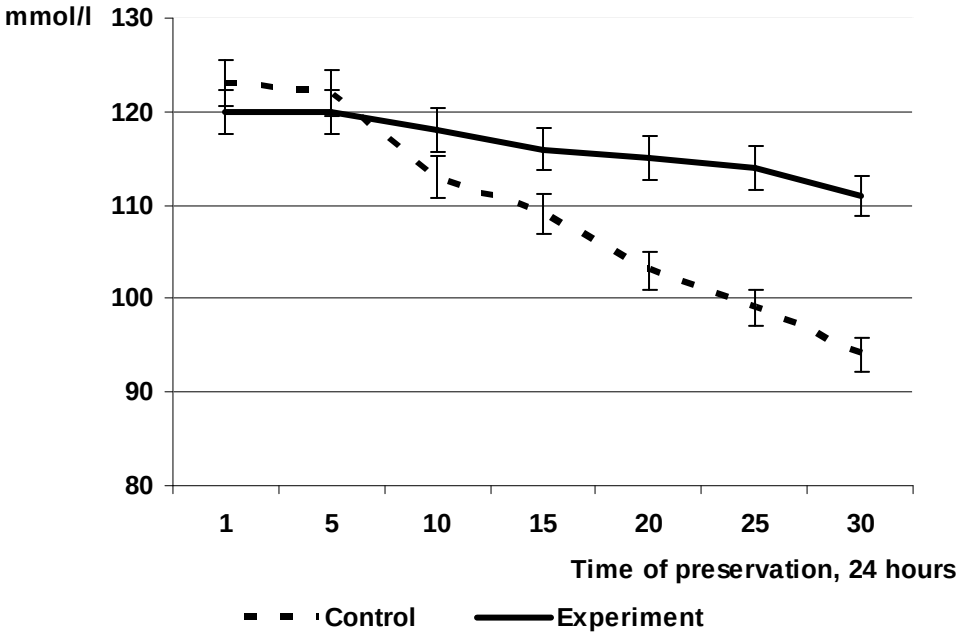

Figure. 2. Change of odium cation concentration in the study samples of the preserved blood of bulls

On the other hand, in plasma of control sample group of the preserved blood the decline of content of sodium cations was noticed on the tenth day (113,1 mmol/l) and dynamically continued during all period of researches. On the thirtieth day the concentration of sodium cations declined just 1,1 times when compared to the initial data and was 111,0 mmol/l. In a control group the level of sodium cations declined 1,3 times and was 94,8 mmol/l.

The level of undamaged erythrocytes in study and control samples of the preserved animal donor blood during the storage period (30 days) showed considerable changes (Figure 3). On the first day of storage the middle indices in study and control blood types were the same – 6,3 T/l and 6,4 T/l. During the subsequent days the amount of erythrocytes was declining, but with different intensity. Thus, on the tenth day in study and control groups the amount of erythrocytes was 6,1 T/l (3,2 %) and 5,4 (15,6 %) T/l respectively.

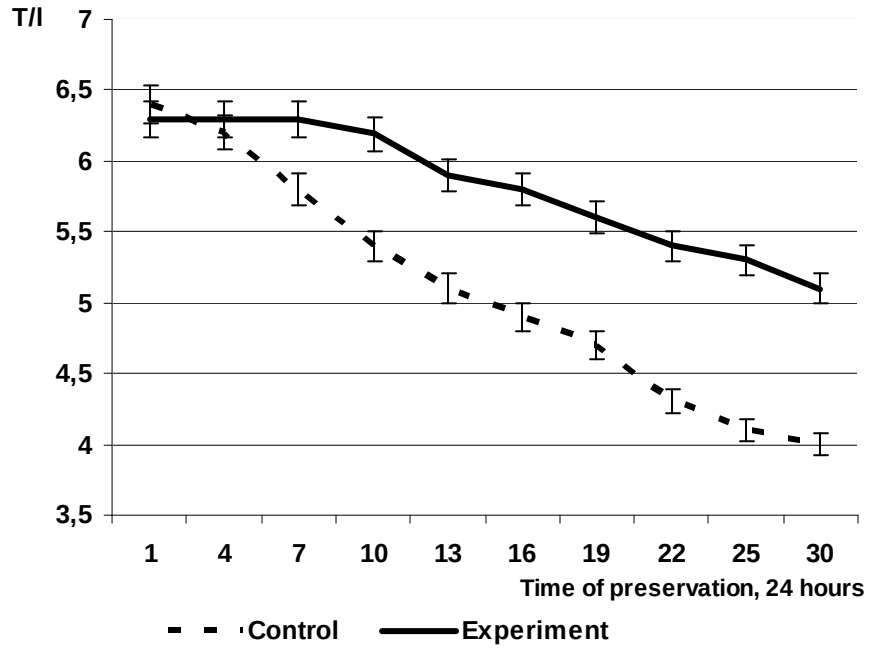

Figure. 3. Change of erythrocyte amount of the investigated samples of the preserved blood of bulls.

The minimum amount of erythrocytes, both in study and control groups was noticed on the thirtieth day. In particular, the amount of erythrocytes in the study samples of the preserved blood was 5,1 T/l (19,1 % down), and in the control group – 4,0 T/l (37,5 % down) when compare to the initial data.

Comparing the results of research of concentration changes of potassium and sodium cations to the dynamics of changes of erythrocyte amount of the preserved donor blood, it is obvious a considerable dependence of blood quality on composition of blood additive used in research. This can prove that the combined action of sodium bicarbonate and carbon dioxide in blood-preservative medium resulted in greater functional stability of cellular membranes and stipulated the best storage of erythrocytes when compared to the control.

Conclusions:

1. Use of blood additive with sodium allows prolonging stability of concentration of potassium cations as well as sodium bicarbonate and carbon dioxide in plasma of the preserved blood ranging from three to five days when compared to the control.

2. Preservation of animal blood with the use of factors of artificial CO_2 – hypobiosis during thirty days provides 27,5 % better storage of erythrocytes when compared to the standard glucose-citrate medium.

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СОХРАННОСТЬ ЭРИТРОЦИТОВ И ДИНАМИКА ИЗМИНЕНИЙ КОНЦЕНТРАЦИИ ИОНОВ КАЛИЯ И НАТРИЯ КРОВИ ЖИВОТНЫХ В ЗАВИСИМОСТИ ОТ СПОСОБА ЕЁ КОНСЕРВИРОВАНИЯ

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Представлены результаты исследований по изучению эффективности действия кровесохраняющей среды, в составе которой разные формы угольной кислоты (pCO_2; HCO_3^-), в сравнении со стандартным глюкозо-цитратным консервантом Глюгицир. Критериями оценки эффективности гемоконсервантов была сохранность эритроцитов, а также динамика концентрации ионов калия и натрия плазмы консервированной крови животных. Установлено, что в условиях комбинированного действия углекислого газа, бикарбонатов и низких температур (2-4^0 С), сохранность эритроцитов значительно выше, чем в контроле. В опытных образцах консервированной крови сохраняется стабильность натрий-калиевого градиента. Консервирование бикарбонат-углекислотной средой обеспечивает

лучшую сохранность донорской крови и продлевает срок её технологического использования.

Ключевые слова: *гипобиоз, консервирование, кровь, сохранность эритроцитов, ионы, натрий, калий, углекислый газ, натрия бикарбонат.*

ЗБЕРЕЖЕНІСТЬ ЕРИТРОЦИТІВ ТА ДИНАМІКА ЗМІН КОНЦЕНТРАЦІЇ ІОНІВ КАЛІЮ І НАТРІЮ КРОВІ ТВАРИН ЗАЛЕЖНО ВІД СПОСОБУ ЇЇ КОНСЕРВУВАННЯ

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Висвітлено результати дослідної роботи щодо вивчення ефективності кровозберігаючої дії середовища, яке містить різні форми вугільної кислоти (pCO_2; HCO_3^-), порівняно із стандартним глюкозо-цитратним консервантом Глюгіцир. Критеріями оцінки ефективності гемоконсервантів була збереженість еритроцитів і динаміка концентрації іонів калію та натрію плазми консервованої крові тварин. Встановлено, що за умов комбінованої дії вуглекислого газу, бікарбонатів і низьких температур (2-4^0 С) збереженість еритроцитів значно вища, ніж у контролі. У дослідних зразках консервованої крові зберігається стабільність натрій-калієвого градієнту. Консервування бікарбонат-вуглекислотним середовищем забезпечує кращу збереженість донорської крові і подовжує термін її технологічного використання.

Ключові слова*: гіпобіоз, консервація, кров, еритроцити, іони, натрій, калій, вуглекислий газ, натрію бікарбонат.*