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On the Preservation of Avian Blood Cells

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The functional state of erythrocytes from hen during their conservation with a preserving solution for 24 days at 4° C, has been estimated by studying some biochemical and hemorheological parameters. Results show an initial phase in the preservation period (4-5 days) in which red blood cells maintain their values at levels similar to those at the beginning of the experience, except for osmotic resistance. Furthermore a progressive erythrocyte deformability loss, linked to ATP depletion (with rise in inorganic phosphate levels) as well as a gradually higher rate of hemolysis, were detected.

Key words: Blood storage, RBC preservation, Erythrocyte microrheology, Intraerythrocytic phosphates.

The preservation and storage of human whole blood and red cells is of great clinical interest and has become a routine procedure in hospitals and blood banks. However, withdrawal of the blood cells from the circulation drastically alters its external environment, depriving the cell of necessary nutrients as well as removing certain protective factors. Attempts are made to reduce cellular metabolism, hence cell demand for energy, by using low pH and cooling the blood, and at the same time, supplying glucose and/or some other substrates as inosine in order to provide a complementary energy source.

Different solutions are used with these purposes such as Acid-Citrate-Dextrose (ACD), Citrate-Phosphate-Dextrose (CPD) and also Alsever's solution that was introduced during World War II as an anticoagulant for donor blood (1). Though long since abandoned for this application, the original formula has been modified in a variety of different ways to yield suitable suspending and preserving mediums for laboratory test cells. Inosine was shown as a good metabolic substrate in order to maintain the metabolic activity of red cells, so it would be an ideal component for a preserving solution if were not by their toxic effects after transfusion in human. This is the reason that solutions such as ACD or CPD are complemented with adenine for clinical pur-

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poses. However, Alserver's solution, containing inosine, may be considered more adequate for delayed analysis and experimental preservation of red blood cells.

The aim of this study was to determine the suitability of Alsever's solution in the conservation and storage of blood with nucleated erythrocytes for experimental and veterinary purposes. This would be beneficial in several circumstances such as the obtention of blood samples from wild animals in field work, or domestic species in farms or pisciculture installations, in order to avoid the possible alterations in blood resulting from the elapsed time between withdrawal of samples and its posterior transport and analysis in the laboratory.

Materials and Methods

The animals used in this study were three lots of nine domestic hens (Rhode Island strain). Blood samples were obtained from radial vein with heparinized syringes (5 U/ml of blood).

A blood volume of approximately 15 ml for each specimen, was immediately mixed, under sterile conditions, with Gamma modified Alsever's solution with pH = 6.8-6.9 (Gamma Biologicals, USA) in two different ratios 1:1 and 1:2 (blood: Alsever) and kept with control samples of whole blood, without any other treatment, at 4° C for 24 days. Units were stored undisturbed between intervals of sampling and mixed only prior to the sampling procedure.

During the entire experimental period, analyses were performed from these three different lots of samples. Some of the more representative biochemical and rheological parameters of the functional state of the erythrocyte were analyzed.

The intraerythrocytic ATP and inorganic phosphates (P_i) were analyzed by the methods of BUCHER (6) and AMES and DUBIN (2) respectively. The amount of free hemoglobin was measured by the spectrophotometric technique of DRABKIN and AUSTIN (7). The protein concentration in the storage medium was determined by the method of LOWRY *et al.* (12).

Red blood cell osmotic fragility was determined by the method previously described (19) modified from the classical technique by PARPART *et al.* (14). Erythrocyte deformability was estimated by the microfiltration technique (9), using polycarbonate membrane sieves with 5 μm pore diameter as described in a previous study (20).

Erythrocyte dimensions were measured from microphotographs from wet mounts of red cell suspensions at the start and after 1 and 17 days of storage.

The hematocrit was determined by the microhematocrit method by spunning the samples at 15,000 *g* during 8 min (Hemofuge, Heraeus). Results in Alsever's solution treated samples were corrected (x2 and x3 for 1:1 and 2:1 doses, respectively) in order to considering their dilution.

Analysis of variance of the data and the Student's t test for paired samples were used to statistically compare the differences between the groups and the values during the experimental period.

Results

Figure 1 shows the intraerythrocytic concentration of organic phosphates during the conservation period in the three experimental lots of samples. Upper pannel presents the ATP contents that was along the experience significatively different only at the first day of conservation; on the other hand, not differences were detected between the control and treated samples, with exception in the increase at 24 hours of conservation that was much smaller in control samples. Medium pan-

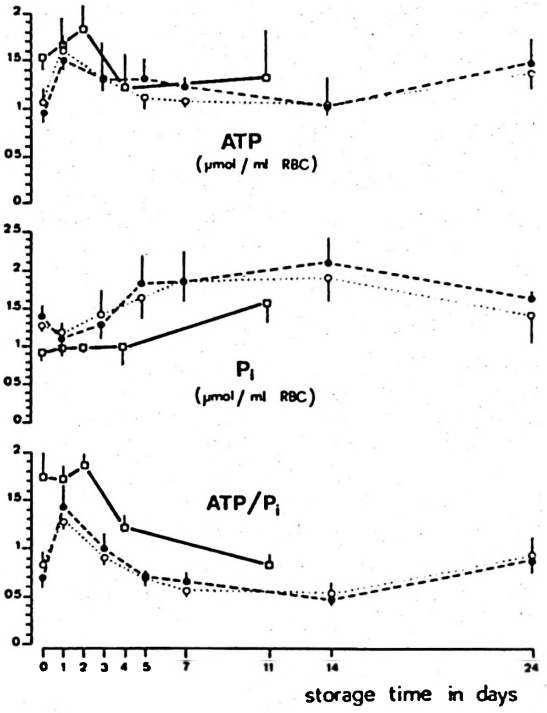

Fig. 1. *Intraerythrocytic phosphates during storage.*
Upper pannel: Adenosine triphosphate. Medium pannel: Inorganic phosphate. Lower pannel ATP/P_i ratio. Symbols represent: (□) Control samples, (●) 2:1 and (O) 1:1 Alsever/blood samples. Bars represent a half of standard deviation. If bars are not present, deviations are smaller than symbols themselves.

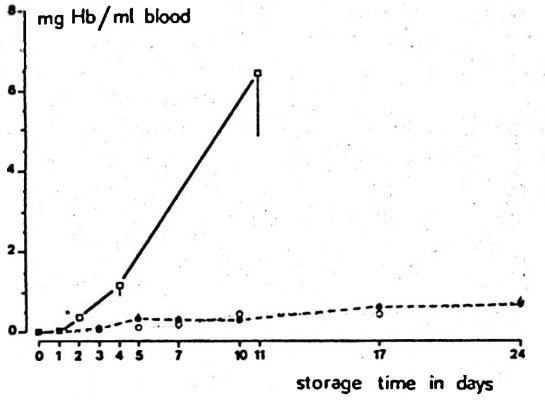

Fig. 2. *Free hemoglobin in storage medium.*
Control samples (□), and Alsever treated samples (●) 2:1 and (O) 1:1 Alsever/blood in volume. Bars represent a half of standard deviation. If they are not presented, the standard deviation are smaller than symbols themselves.

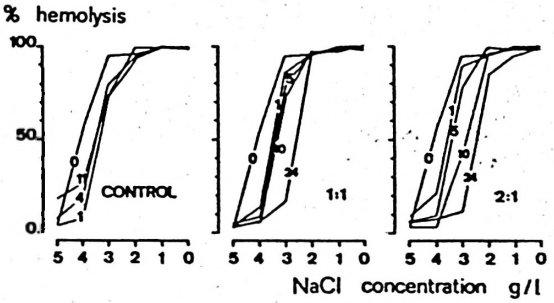

Fig. 3. *Osmotic fragility curves during storage experience.*
Left: Control samples. Center: Alsever/blood 1:1 mixed samples. Right: Alsever/blood 2:1 mixed samples. Symbols and deviations are neglected for more clarity. Lines present inserted the different times of storage in days.

nel shows the inorganic phosphate contents in red cells that follows an inverse trend than ATP levels but without statistically significant differences. Lower pannel showing the intracellular ratio ATP/P_i presents a rise at the first day of conservation in treated samples and a subsequent decreasing trend, while in control samples the continuous fall in ATP/P_i ratio after 48 h of storage was statistically significant.

The free-hemoglobin concentrations into the suspending medium in the control and treated samples are shown in figure 2. The preservating solution prevents the hemolysis until 5 days from blood sampling and later the rate of hemolysis is much smaller than in blood under control conditions. There was no statistical differences between the two doses applied.

Protein concentration in the preserving solution during the storage period does

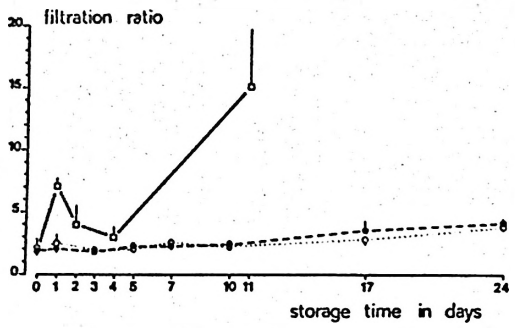

Fig. 4. *Filtration ratio (expresed as time of passage of RBC suspension/time of passage of RBC-free suspending medium) through sieves with pores of 5μm diameter.*
Symbols represent the mean values and bars a half of standard deviation for each lot of samples: Control (□), and Alsever's treated samples with 2:1 (●) 1:1 (O) ratio.

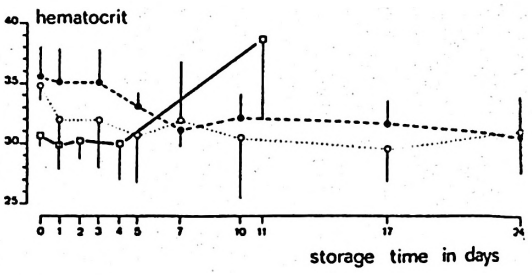

Fig. 5. *Evolution of hematocrit during storage time.*
Symbols represent mean values for each group of samples: Control (□), and Alsever's solution treated (O) 1:1 and (●) 2:1. Bars represent a half of standard deviation.

not show any significant change, indicating that bacterial growth was negligible, which is an important cause for the hemolysis of red blood cells in control conditions.

Figure 3 presents the osmotic fragility curves for the three experimental conditions during the conservation period. In the control samples, after the first day of storage, the osmotic fragility did not show significant changes but the hemolysis values at the less hypotonic levels improved during the storage period as a consequence of the spontaneous hemolysis that affects the samples. The application of preservating solution also results in increased osmotic resistance which was already manifested at 24 hours of conservation. Slight differences, which proved not to be significant, were observed between the two doses studied.

Data for the filterability of the erythrocyte suspensions through microsieves with 5 μm pore diameter are plotted in figure 4. The blood samples stored under control conditions showed an increased resistance to filtration as compared to the treated samples. Nevertheless, no statistically significant differences were detected for this parameter in the preservated samples during the first 4-5 days of storage, nor when comparing the two doses analyzed during all the time of storage.

Table I presents the values of erythrocyte dimensions at sampling and also at 1 and 17 days after storage in both control

Table I. *Erythrocyte dimensions from wet mounts.*
Length (D), width (d) and shape ratio (D/d) for control and Alsever's 1:1 treated samples. Mean values and standard deviation. A total number of 250 red blood cells were measured in each lot of samples.

	CONTROL			ALSEVER'S Sol.		
Days of storage	D	d	D/d	D	d	D/d
0	12.56 ± 0.48	7.42 ± 0.39	1.65 ± 0.15	12.65 ± 0.51	7.47 ± 0.45	1.62 ± 0.12
1	12.70 ± 0.68	7.41 ± 0.56	1.72 ± 0.12	11.99 ± 0.89	7.08 ± 0.40	1.70 ± 0.17
17	11.23 ± 1.52	6.75 ± 0.67	1.67 ± 0.21	12.98 ± 0.56	7.50 ± 0.68	1.74 ± 0.17

and treated red cells. A slight decrease in red blood cell diameters was detected at 1 day of conservation in preservated erythrocytes while there were no changes under the control conditions. At 17 days of storage the preservating solution allows the cell to maintain their normal dimensions and control samples appear with noticeably lower dimensional values.

Hematocrit values of the three groups along the experience are represented graphically in figure 5. In the samples stored with conservating solutions no significant changes were observed, in spite of a slight fall, while the control group increased significantly.

Discussion

The storage of hen blood with Alsever's solution results in a rise of intraerythrocytic ATP levels in the first days of conservation that could be justified by the metabolic utilization of inosine present in the preserving medium. However, it is necessary to note that a slight increase in the control samples also at 48 h of storage was observed, which may be related to the decrease of metabolic requirements due to the low temperature of storage. These results are in agreement with the data obtained by BARTLETT (4) who found a marked decrease of ATP and DPG in human erythrocytes stored for 4 weeks in ACD at 4° C, and after incubation with adenine+inosine a noticeable resynthesis of intraerythrocytic organic phosphates.

SHIELDS *et al.* (17) described some alterations in human erythrocytes during preservation in ACD at 4° C. They found a loss of Hb and K^+ content in red cells and an increase in osmotic fragility. These effects not appeared in the present results on preservated avian erythrocytes, since a very low rate of hemolysis was detected while a much higher loss of hemoglobin occurs in control samples and, on the other hand, storage equally affects control and conservating solution mixed red cells, increasing their osmotic resistance. The antibiotics (neomycin sulfate 0.0068 g/dl activity and chloramphenicol 0.033 g/dl) included in the Gamma Alsever's solution as preservative components might be the hemolysis preventing agents although the mechanism affecting osmotic fragility is not clear as the same effect also appears in the control samples. This might be related to cell volume changes and/or membrane stiffness alterations.

Some authors reported the relationship between intraerythrocytic ATP levels in human stored blood and other red cell parameters such as morphology (13) and mechanical or rheological properties (3, 10, 11, 18). These works proved a clear correlation between the maintenance of intraerythrocytic ATP levels, by supplying different energy sources to the storage medium, and the conservation of the morphological and rheological properties of red cells.

The chicken nucleated erythrocytes are unable to easily catabolise glucose (16) in spite the fact that bird red blood cells possess a higher oxygen consumption rate as compared to mammalian erythrocytes (5).

These metabolic aspects of chicken erythrocytes remain unclear since they are equipped with the whole set of glycolytic enzymes although their function is impaired at the level of hexose-phosphate but not at the level of triose-phosphate metabolism (15). This could be a reason for the ability of inosine to act as an important energy source for the erythrocytes of this species.

In agreement with the results obtained in human red cells stored into ACD+-adenine (11) the Alsever's solution treated red blood cells show a very low rate of deformability loss in the hen, while in control samples a high increase in resistance to filtration through microsieves with 5 μm of pore diameter gave a noticeable fall in erythrocyte deformability

which can be related to metabolic dependence as was observed in humans (21). However, FEO and MOHANDAS (8) found in human red cells that deformability appears independent of the intracellular ATP level and only dependent on the shape of the red cells.

The changes in erythrocyte dimensions observed at 24 h of storage in preserved samples are accompanied only by modification of osmotic fragility, which remains at similar values during the entire storage period while red cell dimensions recover their initial values. Nevertheless, in the control samples the diminution of erythrocyte morphological parameters after the storage period reflects the alterations also detected in other parameters.

The rise in the hematocrit of the control samples, in spite of their high hemolysis levels, could be justified by the progressively greater cell rigidity which produces an increase in the plasma trapping factor.

In conclusion, it was found that Alsever's solution, as modified by Gamma, is able to maintain the main hematological, biochemical and rheological parameters in nucleated avian erythrocytes at least during four to five days under refrigeration, with the exception of osmotic fragility which probably is affected by the ionic or oncotic changes in the red cell suspending medium as a consequence of mixing plasma with these conservating solution.

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Resumen

Se estudia el estado funcional de eritrocitos de gallina durante su conservación en una solución preservante a lo largo de 24 días, con refrigeración (4° C), por medio de la determinación de algunos parámetros bioquímicos y microrreológicos. Los resultados muestran que en una fase inicial del período de conservación (durante los primeros 4 a 5 días) los eritrocitos mantienen sus características funcionales intactas, excepto ciertas diferencias en su resistencia osmótica. Posteriormente, se detecta una progresiva pérdida de plasticidad en las células acompañada por una caída en el nivel de ATP intraeritrocitario, simultáneo a un aumento en fósforo inorgánico, apreciándose también un continuo y prolongado incremento en hemólisis.

Palabras clave: Conservación de sangre, Preservación de eritrocitos, Microrreología eritrocitaria, Fosfatos intraeritrocíticos.

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