individuals of these 3 species, completely adsorbed with sheep erythrocytes, were also tested for their ability to serve as a complement source for rabbit-anti-sheep hemolysin (GIBCO). All showed this capacity. However, when unimmunized rat serum was used as a complement source for rabbit-anti-sheep hemolysin, no lysis was obtained at 15 °C or lower. Legler et al.[28] have shown that *Bufo marinus*, *Necturus maclosus* and *Rana pipiens* complement can function with mammalian but not fish antibody.

Thus, complement and antibody of these poikilotherms seem best adapted to function at environmental temperatures in which these organisms normally live. While the laboratory temperature of 25 °C has been used in the past in an effort to provide optimal temperature conditions for these particular amphibia, our data suggest that it is not optimum for serum agglutination activity. Since our data show clearly that amphibian complement functions in vitro at the hibernation temperatures which have been found to reduce the amount of complement activity recoverable from *Rana* serum[21], it would seem that immune inhibition at cold temperatures may be partly due to a suppression of synthesis rather than activity of complement. Therefore previously activated amphibian immunocytes can continue to generate and release antibody in vitro[19,20], complement function is normal, although its synthesis in vivo is depressed[21] and antigen-antibody complexing is enhanced by the cold. If the effect of low temperature on the in vivo immune response has to do with threshold activity of a particular temperature sensitive phase, only the phenomena associated with activation of lymphocytes by antigen remain.

## Changes in rate of methemoglobin reduction and oxygen affinity of erythrocytes incubated with inosine, pyruvate and phosphate

L. Zolla, Carmela Ioppolo, G. Amiconi, A. Benaglia and E. Antonini

*Institute of Chemistry, Faculty of Medicine, University of Rome, C. N. R. Center of Molecular Biology, Rome, and Istituto Regina Elena, Divisione I di Chirurgia Generale, Rome (Italy), 23 March 1977*

*Summary.* Incubation of human erythrocytes with inosine, pyruvate and phosphate increases several fold the ferrihemoglobin reductase activity, the values of which, however, depend on the age of blood (by 6 to 2 times with respect to the normal value of fresh blood).

Blood stored in acid-citrate-dextrose (ACD) shows a progressive decrease in the activity of the ferrihemoglobin reduction systems and a concomitant increase in oxygen affinity[1,2]; these functional changes are related to the metabolism of the erythrocyte and to the exchange of materials between cells and preservation solution. Incubation at 37 °C of outdated whole blood or concentrated red cells in a medium containing inosine and adenine[3,4] or inosine, pyruvate and phosphate[5,6] restores the defective oxygen transport function and the depleted 2,3-diphosphoglycerate (DPG) concentration of erythrocytes. Moreover the incubation with rejuvenation solutions determines an increase in the 24-h post-transfusion survival[6,7]. Due to the possible practical utilization of this procedure, several studies have been carried out on biochemical modifications of erythrocyte metabolism after incubation with various substances[8–11]. The purpose of this comunication is to report the effect of inosine (10 mM), pyruvate and inorganic phosphate (5 mM) (IPP solution) on the activity of the ferrihemoglobin reduction system, the oxygen affinity and the osmotic fragility of stored erythrocytes. The techniques used have been described previously[2].

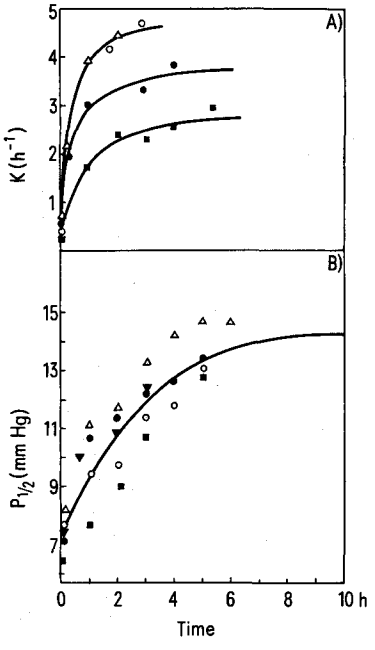


Fig. 1. Effect of incubation erythrocytes with inosine (10 mM), pyruvate (10 mM) and phosphate (5 mM) at 37 °C in isotonic Tris buffer pH 7.3. *A* Change in the rate of ferrihemoglobin reduction as a function of time of red cells stored at 4 °C for 2 h and 3, 16 and 30 days; *B* change in $p^1/_2$ (the oxygen tension at 50% oxygen saturation), measured at 20 °C in isotonic Tris buffer pH 7.3, of red cells. ▼, △, 2 days old; ○, 4 days old; ●, 16 days old, ■, 30 days old.

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From figure 1 it becomes evident that after 2–3 h of incubation with IPP at 37 °C, the activity of the ferrihemoglobin reduction system (expressed as a pseudofirst order constant, k) reaches its highest level; however, though the maximal velocity of reduction is achieved at the same time by all the samples, its absolute value depends on the age of blood (see also figure 2). Figure 1 also shows the decrease in oxygen affinity (expressed as $p^1/_2$, the partial pressure of oxygen required to produce 50% saturation of ligand binding sites) as a function of incubation time. After 7 h of incubation, the values of $p^1/_2$, determined at 20 °C and isotonic Tris buffer pH 7.3, have reached a maximum, which is independent of the age of the blood. This result is in agreement with that reported by Duhm et al.[5], even though these authors noticed a little difference in the oxygen affinity of fresh and 28 days old rejuvenated erythrocytes; this fact might be explained by the difference in the experimental conditions used.

The functional changes induced by a 6-h-incubation with IPP in freshly drawn blood as well as in blood stored in ACD at 4 °C up to 30 days are shown in figure 2. The incubation increases several-fold the rate of ferrihemoglobin reduction, the values of which however depend on the age of the blood. On the other hand, the decrease of oxygen affinity on incubation with IPP is independent of the time of storage. As is well-known, the increase in the values of $p^1/_2$ depends on the augment of DPG concentration formed during the incubation[5]; and therefore these results confirm that storage of blood in ACD up to 30 days does not impair the erythrocyte capacity of synthetizing DPG[5].

In order to investigate the stability of the effects obtained by the rejuvenation solution, the incubated suspension of erythrocytes has been kept at 4 °C and the efficiency of ferrihemoglobin reduction rate, as well as the oxygen affinity of red cells, has been determined as a function of time (figure 3). Thus, the reductase activity follows the same trend in rejuvenated erythrocytes as in fresh blood, i.e. its progressive decline starts just after the end of incubation. On the other hand, the oxygen affinity maintains its value for about 2 weeks and then begins to increase with a trend parallel to that of fresh nonincubated blood; in particular, about 24 days after the incubation with IPP, oxygen affinity reaches the highest value, which is similar to that of 'old' blood.

It is well-known that human erythrocytes lose their biconcave disc shape, concomitant with decreasing ATP level, during a normal blood bank storage period[12]. This structural and functional damage of membrane is reflected, among other things, in the increased osmotic fragility of erythrocytes. The incubation with IPP solution restores in part the resistance to hypotonic medium of outdated stored red cells, as indicated by figure 4.

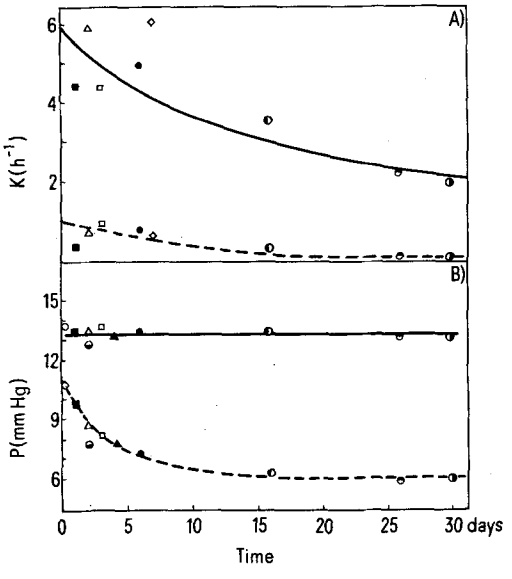


Fig. 2. Effect of incubation with IPP at 37 °C on some functional properties of human erythrocytes stored at 4 °C for different period of time. Different symbols refers to different samples of blood. *A* Reduction rate of ferrihemoglobin as a function of storage time, before (symbols around the dashed line) and after (symbols around the continuous line) incubation. *B* Oxygen affinity at 20 °C (indicated as $p^1/_2$) as a function of storage time, before (symbols around the dashed line) and after (symbols around the continuons line) incubation.

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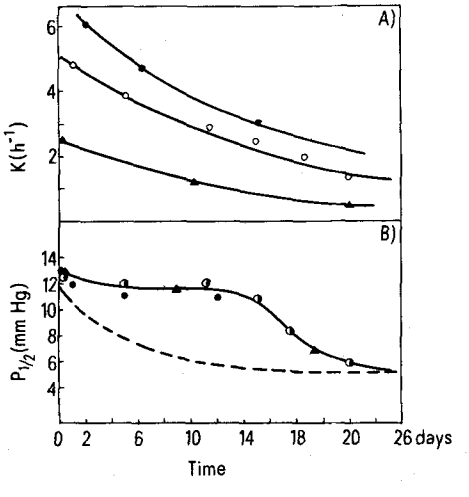


Fig. 3. Effect of storage at 4 °C of rejuvenated erythrocytes: *A* on reductase activity; *B* on oxygen affinity. Storage periods prior to incubation with IPP: ●, 3 days; ○, 8 days; ◑, 16 days; ▲, 30 days. Dashed line in *B* indicates the decrease in oxygen affinity of fresh blood as a function of time.

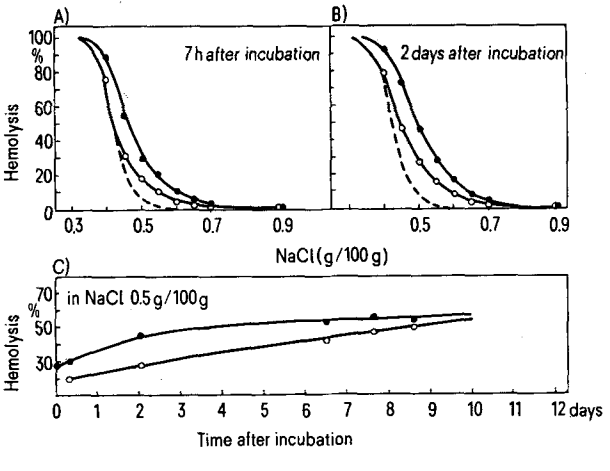


Fig. 4. Osmotic fragility measured on human red cells stored in ACD at 4 °C for 21 days and then rejuvenated with inosine, pyruvate and phosphate. Open symbols refer to incubated erythrocytes and closed symbols to the control. Dashed lines indicate the osmotic fragility of normal fresh erythrocytes.

In conclusion, the results reported here show that: a) The ferrihemoglobin reductase activity increases largely on incubation with IPP, and in particular 30 days old erythrocytes show, after incubation, a velocity of reduction which is twice that of fresh cells. However, this value is 3 times lower than that obtained by incubation of fresh erythrocytes, implying that by ageing the reductase systems are irreversibly modified. It is not yet clear where this block is localized, since the reduction as a function of time of ferrihemoglobin reductase activity of IPP incubated red cells depends not only on the intrinsic reductase system but also on the entire chain of enzymes and cofactors interposed between the substrates applied and methemoglobin. b) The oxygen affinity is lowered on incubation with IPP; the value of $p^1/_2$ obtained after rejuvenation is, however, the same for blood of different age. c) By incubation with IPP, the increased osmotic fragility of outdated blood tends towards the normal range, even though it is not fully restored.

## Antigenic correlation between rat thymus and superior cervical ganglion[1]

B. D. Janković, J. Horvat, K. Mitrović and M. Mostarica

*Immunology Research Center, Vojvode Stepe 458, 11000 Belgrade (Yugoslavia), 14 March 1977*

*Summary.* By using rabbit anti-rat thymocyte and anti-rat superior cervical ganglion sera in cytotoxicity, immunofluorescence and absorption assays, it has been shown that surface membranes of rat thymocytes and cervical ganglion cells (i.e. peripheral nervous tissue cells) contain common antigenic determinants.

The brain-thymus antigen[2] has been described in various tissues of the rat[3,4]. The subject of this paper is to demonstrate antigenic correlation between the rat thymus and the rat superior cervical ganglion.

*Materials and methods.* The sympathetic superior cervical ganglia were isolated from Wistar rats perfused with saline. The fraction which predominantly contained disrupted ganglion cell membranes and fibrillar structures was employed for the production of anti-cervical ganglion (anti-CG) serum in rabbits[5]. Rabbit anti-CG sera used in this experiment were absorbed with rat erythrocytes, liver-cell membranes, glutaraldehyde-insolubilized serum proteins, and kidney homogenate as previously described[6]. Absorbed anti-CG sera when tested against the corresponding antigen exhibited antibody titers between 1:512 and 1:2048 in complement-fixation reaction[7] using 5 50% haemolytic units of guinea-pig complement, and produced 3 precipitin lines in double diffusion in 0.8% agarose[8].

Thymocytes were separated from the thymus of saline-perfused Wistar rats and purified on Isopaque-Ficoll[9]. Anti-thymocyte serum (ATS) was prepared in rabbits[9] and absorbed in an identical manner as described above

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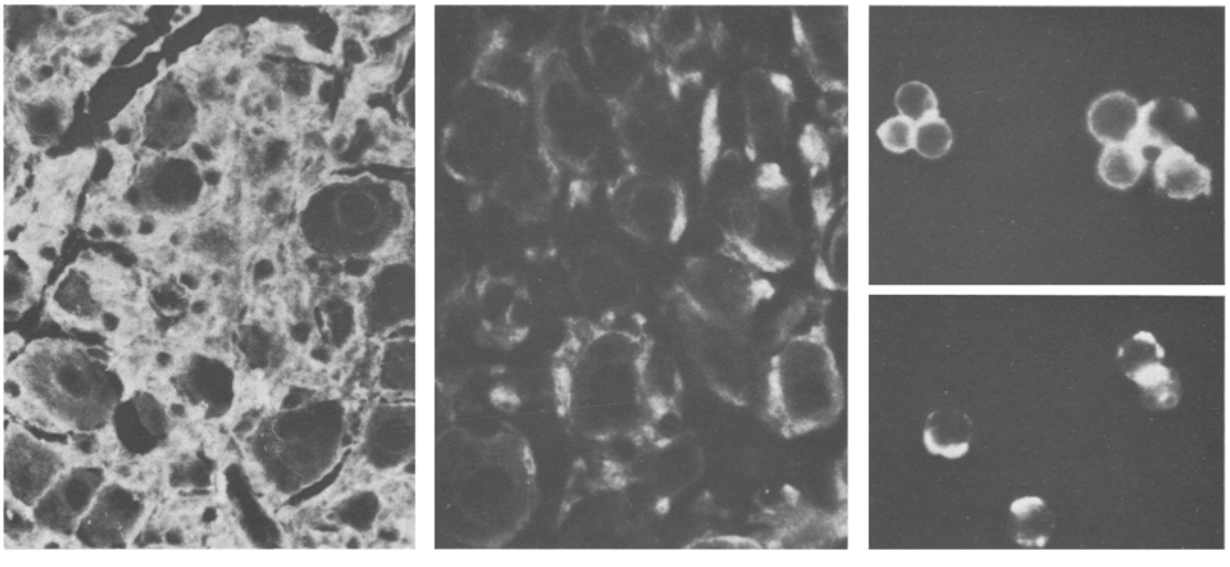

Immunofluorescence microphotographs. *a* A section of the rat superior cervical ganglion (×300) exposed to anti-rat cervical ganglion serum and fluorescein-conjugate. *b* A section of the rat cervical ganglion (×400) treated with anti-rat thymocyte serum and conjugate. *c* 2 groups of rat thymocytes (×800) treated with anti-rat thymocyte serum and conjugate. *d* 4 specifically fluorescing rat thymocytes (×800) exposed to anti-rat cervical ganglion serum and conjugate.