Current Comment

S. G. HERSHEY, M.D., *Editor*

The Osmotic Fragility of Human Red Cells Caused by Urea Administration During Hypothermia

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Intravascular hemolysis following the intravenous administration of urea during hypothermia in 2 neurosurgical patients was recently observed.[1] Hematologic studies including determination of red cell fragility and filter paper electrophoresis could not demonstrate a defect in either patient. We have recently encountered another instance of intravascular hemolysis following administration of urea during hypothermia. Because of these complications, a study was undertaken to determine the effects of the intravenous administration of urea on the osmotic fragility of red cells in anesthetized hypothermic neurosurgical patients.

METHOD

Two comparable groups of patients received intravenous urea during hypothermia to reduce brain volume. In the first group the osmotic fragility of erythrocytes in *venous blood* was studied; in the second group the red cell fragility in *arterial blood* was analyzed.

There were 10 patients in each group. The average age of the first group was 44, that of the second group 45 years. The first group contained 8 females and 2 males; two of the females were Negro, the remainder, white. The second group contained 8 females and 2 males; one female patient was Negro, the others, white. The average dose of urea was 0.57 g./kg. (0.52 to 0.69 g./kg.) in the first group and 0.60 g./kg. (0.52 to 0.63 g./kg.) in the second.

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The patients were given secobarbital 50 to 100 mg. and atropine 0.5 mg. or scopolamine 0.4 mg., intramuscularly, 45 to 60 minutes prior to the induction of anesthesia. Anesthesia was induced with intravenous sodium thiopental (150 to 400 mg.), and endotracheal intubation performed with the aid of either succinylcholine (100 mg.) or *d*-tubocurarine (1 mg./5 pounds). Anesthesia was maintained with 70 per cent nitrous oxide–30 per cent oxygen supplemented with intravenous *d*-tubocurarine (6 to 36 mg.), chlorpromazine (25 to 50 mg.) and thiopental (50–750 mg.). Ventilation was mechanically controlled at a minute volume of 125 to 150 per cent of that estimated from the uncorrected Radford nomogram.[2] Ventilation was constant throughout the experiment. Esophageal temperature was measured throughout operation.

Prior to the induction of anesthesia, a Teflon catheter was inserted percutaneously into the bracheal arteries of all patients. In the one group a polyvinyl catheter was threaded into an antecubital vein to obtain venous blood samples. Arterial and venous blood samples were collected in heparinized Luer-Lok syringes: (1) before the induction of anesthesia, to serve as a control; (2) 30 minutes after anesthesia was established; (3) immediately before, and (4) immediately after the intravenous infusion of urea.

Urea as a 40 per cent solution in 5 per cent invert sugar was administered intravenously over a 15 to 20-minute period after anesthesia and hypothermia were established. Blood samples were immediately analyzed for serum osmolarity, *p*H, P_{CO_2}, oxygen saturation, microhematocrit and osmotic fragility. Serum

osmolarity was determined with an Advanced Instrument Osmometer, oxygen saturation with an American Optical Oximeter. An Instrumentation Laboratory pH-P_{CO_2} electrode assembly or an Astrup apparatus (Radiometer AME-1B) was used for the acid-base studies. Osmotic fragility was determined by the quantitative method of Shen as described by Page and Culver [4] using a Beckman Model B spectrophotometer. Since it has been reported that washing erythrocytes in isotonic saline prior to analysis may alter their fragility,[5, 6] the cells were not washed.

An osmotic fragility curve was constructed for every sample with percentage hemolysis as the ordinate and the concentration of hypotonic sodium chloride (g./100 ml.) as the abcissa. The concentration of sodium chloride at which 50 per cent hemolysis of the red cells occurred was derived from the fragility curve. All osmotic fragility analyses were performed at room temperature (25° to 28° C).

Results

The data for venous blood are presented in table 1. The difference between the mean osmotic fragility of any of the four venous samples at which 50 per cent of the red cells hemolyzed was not statistically significant. (Student's t test $P < 0.05$). Esophageal temperature immediately before urea infusion averaged 32.7° C. (35° C. to 29.4° C.). Following infusion of urea the temperature averaged 31.3° C. (33.9° C. to 27.5° C.). The microhematocrit in both venous and arterial samples tended to increase as the patient was cooled.

Table 1 likewise lists the concentration of sodium chloride at which 50 per cent of the red cells in the arterial samples hemolyzed. There was no significant difference among any of these samples. Esophageal temperature immediately before infusion of urea averaged 32.7° C. and following the urea averaged 30.6° C. The lowest oxygen saturation in any patient was 92 per cent.

Table 1. Data from Venous and Arterial Blood Samples

1. Venous Blood

Blood Samples	50% Hemolysis Conc. NaCl (g./100 ml.) (mean ±S.D.)	Osmolarity (mOs) (mean)	pH (mean)	pCO_2 (mm. Hg) (mean)	O_2 Sat. (%) (mean)
1	0.372±0.004	289	7.41	41.5	81.0
2	0.376±0.023	290	7.45	33.5	81.5
3	0.363±0.023	291	7.48	30.0	78.0
4	0.366±0.025	328	7.45	32.7	84.8

2. Arterial Blood

1	0.361±0.022	290	7.42	39.0	95.6
2	0.359±0.018	289	7.50	33.9	96.4
3	0.357±0.028	294	7.50	26.0	96.7
4	0.361±0.025	333	7.47	26.0	96.0

Blood 1 before anesthesia; Blood 2 after 30 minutes of anesthesia before hypothermia; Blood 3 immediately before urea; Blood 4 immediately after urea.

Discussion

Ravin *et al.* described hemoglobinuria in two Negro female patients following infusion of urea during hypothermia.[1] Despite the fact that no previous hematological abnormalities could be demonstrated in these patients, the suspicion existed of either a sexual or racial predisposition to hemoglobinuria. Subsequently the appearance of hemoglobinuria in a white male following urea infusion during hypothermia led us to discount the idea of a sexual or racial predisposition. Wurster and Shapiro, using beef red cells suspended in isotonic concentrations of univalent salts such as sodium chloride and potassium chloride, demonstrated that the addition of high concentrations of urea caused hemolysis of erythrocytes.[7] Javid and Anderson infused urea in 5 per cent dextrose intravenously in man and observed occasional hemoglobinuria.[8] Substitution for 5 per cent dextrose of 10 per cent invert sugar, a substance that does not easily penetrate red cells, eliminated this problem. Mortensen reviewed many of the factors which can affect erythrocyte fragility *in vitro*.[9] A decrease in both pH and blood oxygen saturation increases osmotic fragility.[10] Combination of these two factors accounts for the slightly greater osmotic fragility of cells taken from venous blood compared with those obtained from arterial blood. An increase in P_{CO_2} will increase osmotic fragility.[10] There is some disagreement as to whether this is a direct effect of CO_2 or if mediated indirectly

through a change in *p*H.[11] The time elapsed after a sample is drawn as well as the temperature at which the analysis is carried out can also affect fragility of the erythrocyte.[10, 11, 12]

During anesthesia in neurosurgical patients, hyperventilation of the lungs was employed. There was a rise in *p*H, a decrease in P_{CO_2}, and usually an increase in O_2 saturation (table 1). All these factors tend to reduce osmotic fragility. The concentration of NaCl at which 50 per cent of the erythrocytes hemolyzed in both arterial and venous blood at 30 minutes did not differ from the controls. This finding suggests that nitrous oxide-oxygen anesthesia had little effect on red cell osmotic fragility.

Induction of hypothermia during neurosurgical anesthesia accentuates the acid-base and oxygen saturation changes described above. These would in fact tend to reduce osmotic fragility. Mortensen demonstrated *in vitro* that there is a 20 per cent increment in hemolysis when the temperature falls from 40° to 30° C.[13] In the past three years 85 patients at Neurological Institute anesthetized and maintained at normal temperatures have received intravenous urea without exhibiting hemoglobinuria. In the same period 3 of 161 hypothermic patients receiving urea developed hemoglobinuria (1.9 per cent). This complication occurred despite the fact that the hypothermic patients were given one-half the average dose of urea that would have been given to normothermic patients. Hypothermia, therefore, appeared to be a predisposing factor in causing hemoglobinuria following urea infusion. Although the occurrence of hemoglobinuria in these patients was disturbing, there were no apparent undesirable sequelae.

A significant change in erythrocyte osmotic fragility following the infusion of urea was not found in this study. It is possible, however, that during hypothermia intense peripheral vasoconstriction could give rise to stagnant areas in the circulation where there would be a decrease in *p*H, an increase in P_{CO_2} and a marked decrease in O_2 saturation. Red cells in such an area would probably have an increased osmotic fragility. Infusion of urea might cause hemolysis of such cells. Although this change was not demonstrated in this study, we believe this hypotheses is a reasonable one and the most likely explanation for the hemolysis observed in the 3 patients in whom the complication was seen.

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