Acute physiological responses during blood substitution with colloidal gelatin in conscious rats

K.C. Lowe and D.C. McNaughton

Department of Zoology, University of Nottingham, University Park, Nottingham NG7 2RD (UK), and Department of Surgery, Addenbrooke's Hospital, Cambridge CB2 2QQ (UK), 30 June 1983

Summary. The effects of exchange-transfusion with a proprietary gelatin-based plasma volume expander, Haemaccel, have been investigated in conscious, chronically-catheterized rats. No adverse changes in basic cardiovascular or respiratory functions occurred during the procedure although an increase in intravascular fluid sodium but not potassium concentration was observed.

Previous work has shown that it is possible to replace the blood of small laboratory mammals with synthetic microemulsions containing perfluorochemicals[2-7]. Recently, the development of a unique and routine method for isovolemic exchange-transfusion of conscious, chronically catheterized rats with the proprietary emulsion: Fluosol-DA 20% (Green Cross Corporation, Japan) has enabled the acute physiological responses to this procedure to be examined in the absence of anesthesia[8]. This technique provides a convenient model system for investigating the short-term homeostatic changes during varying degrees of exchange-transfusion with different blood replacement preparations.

In the present experiments, the physiological responses to blood substitution with a gelatin-based colloidal plasma volume expander: Haemaccel (Hoechst) have been examined in conscious rats. Particular emphasis has been placed on the acute cardiovascular and respiratory responses during blood replacement together with changes in the principal intravascular fluid cation concentrations. Survival time post-perfusion has been used to assess the ability of Haemaccel to maintain normal homeostatic function in animals exchange-transfused to low hematocrits while breathing air containing an increased oxygen concentration.

Materials and methods. Mature female Sprague-Dawley/OLA rats (mean ± SEM b wt. 289 ± 10 g; n = 4) were used in these experiments. Indwelling femoral arterial and venous catheters were inserted under Equithesin anesthesia as described previously[8]. Animals were allowed at least 24 h for recovery from anesthesia and surgery. Continuous isovolemic exchange-transfusion using Haemaccel (Hoechst Pharmaceuticals, Hounslow, U.K.) was performed at 1 ml min^{-1} for 40 min with animals contained within specially constructed transparent gas-tight chambers[8]. The composition of Haemaccel is given in table 1. The oxygen tension inside each animal chamber was maintained at 80–90% during the procedure and monitored by means of an oxygen-sensitive electrode connected to an Electrodyne IMI 3700 oxygen controller (Deva Medical Electronics, Runcorn, U.K.).

Mean arterial blood pressure and heart rate were measured at 10 min intervals during blood substitution using a Bell & Howell type 4-422-001 pressure transducer coupled to an amplifier and conventional chart recorder. Respiration rate was monitored by direct observation. Animals were allowed free access to food and water throughout the procedure.

The arterial effluent was collected and separated using an LKB Ultrorac II fraction collector. The hematocrit (packed cell volume %) was measured on samples of this effluent or whole blood using an automatic Adams 'Autocrit' microhematocrit centrifuge (Clay Adams, Parsippany, USA). The cellular elements in the remainder of each sample were separated by centrifugation at 2500 rpm for 30 min at 4 °C; plasma or supernatant was removed and stored at −20 °C prior to analysis. Sodium and potassium concentrations in plasma or arterial supernatant were measured using a Corning 455 Flame Photometer. Statistical analyses were made according to the methods of Snedecor and Cochran[9]; statistical significance between mean values was asessed using a conventional Student's t-test or paired t-test accordingly.

Results and discussion. Exchange-transfusion at 1 ml min^{-1} for 40 min reduced the mean hematocrit from 43% to 6% ($p < 0.001$). No significant changes in mean arterial blood pressure, heart rate and respiration rate were observed during the procedure (table 2). All animals behaved in an apparently normal manner throughout; moving freely about their cage and taking food and water.

A significant ($p < 0.01$) increase in plasma sodium concentration occurred in response to blood replacement with Haemaccel but no corresponding change in potassium concentration was observed (table 2).

The mean survival time of animals following exchange-transfusion was 33 ± 17 h. In each case the animals remained active with normal blood pressure and heart rate for some hours post-perfusion but death eventually occurred due to deterioration of hemodynamic function as reflected by progressive hypotension and bradycardia.

The results of these experiments show that rats will survive for considerable periods following exchange-transfusion to hematocrits of less than 10% with the gelatin-based plasma expander, Haemaccel. Moreover, these results demonstrate that extensive blood substitution with Haemaccel can be achieved with no immediate disruption of normal cardiovascular or respiratory functions.

The subsequent survival time of animals following exchange-transfusion with Haemaccel was similar to that observed after near total blood substitution using the oxygen-carrying per-

Table 1. Composition of Haemaccel

Constituent	Concentration
Degraded gelatin: mean mol.wt 35,000 ($g \cdot l^{-1}$)	35.0
Ions ($mmol \cdot l^{-1}$):	
Na^+	145
K^+	5.1
Ca^{2+}	6.3
Cl^-	145
$PO4^{3-}$	trace
$SO4^{2-}$	trace

pH 7.3 ± 0.3; Oncotic pressure (mm Hg): 350–390.

Table 2. Responses to exchange-transfusion with Haemaccel in conscious rats[a]

	Initial	Post-perfusion[b]	Significance
Hematocrit (%)	43 ± 2	6 ± 2	$p < 0.001$
Mean arterial blood pressure (mm Hg)	109 ± 4	90 ± 6	NS[c]
Heart rate (beats min^{-1})	413 ± 18	385 ± 15	NS
Respiration rate (breaths min^{-1})	88 ± 6	86 ± 6	NS
Sodium ($mmol \cdot l^{-1}$)	142.9 ± 1.6	148.3 ± 0.2	$p < 0.01$
Potassium ($mmol \cdot l^{-1}$)	3.6 ± 0.2	3.9 ± 0.2	NS

[a] All values are mean ± SEM (n = 4); [b] Post-perfusion, measurements taken immediately following 40 min exchange-transfusion; [c] NS, not significant ($p > 0.05$).

fluorocarbon emulsion: Fluosol-DA[8]. Thus, it is evident that short-term survival of animals following perfusion to very low hematocrits does not depend on the intrinsic capability of the replacement medium to transport oxygen other than in simple solution.

The present finding of hypernatremia in response to blood substitution with Haemaccel was unexpected since the sodium concentration in this solution is similar to that found normally in rat plasma[10]. However, since the mean plasma sodium concentration in animals immediately prior to exchange-transfusion (table 2) was slightly lower than that found in uncatheterized control animals[10], it might be expected that extensive perfusion with an essentially isonatric fluid would serve to restore plasma concentrations back to normal levels. Support for this conclusion is provided by previous observations that in the rat, a 3% reduction of plasma sodium concentration occurred in response to repeated blood sampling[11]. Thus, removal of blood for measurement of baseline parameters prior to exchange-transfusion coupled with the slight but unavoidable loss which inevitably occurs during catheterization, would help to explain the reduced initial plasma sodium concentrations seen in the present experiments.

In conclusion, the present finding that a relatively simple, gelatin-based, colloidal plasma volume expander can be used for near total blood replacement in conscious rats provides a convenient model system for assessing the acute responses to blood substitution while avoiding potentially disturbing influences from components of more complex preparations.

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On the pathway of the rectosphincteric reflex

T. Kunieda, S. Azuma and K. Sakata

2nd Department of Surgery, Gifu University School of Medicine, Gifu City (Japan), 24 June 1983

Summary. In several rat models, including those with circular and semicircular rectal aganglionosis, the rectosphincteric reflex was examined. The reflex was confirmed to be essentially an intramural one and its route is considered to run mainly in the longitudinal and partly in the oblique directions.

The presence of an intact rectosigmoid canal is known to be required for maintenance of the internal sphincter relaxation reflex, or the rectosphincteric reflex[1], which is widely used for examining Hirschsprung and other chronic constipation diseases. Since the reflex is known to be preserved in cases of lumbosacral meningomyelocele and spinal cord injury[2], it is considered to be an essentially intramural reflex. However, the details of its pathway are obscure. In the present study, we reconfirmed experimentally that the reflex was mediated by intramural ganglia and made some interesting observations on its pathway.

Materials and methods. Wistar rats of either sex, weighing 200–400 g, were used. The rat, kept fasted for 24 h and treated with a glycerol enema 2 h before the experiment, was fixed supinely under nembutal anesthesia, and a small rubber balloon with a polyethylene catheter, filled with 0.15 ml of water and connected to a pressure transducer, was inserted and fixed within the anal canal. The rat was laparotomized and a rubber balloon with a polyethylene catheter having a content of 2.0 ml, was inserted into the rectal lumen through an incision made at the descending colon and was fixed at a predetermined level. Stimulation was applied by the inflation of the latter balloon with 1.0 ml of air. Square wave electrical stimulation, 0.5 msec in duration, 10 Hz, 30 V and continuing for 3 sec, was applied on the rectal serosa with a bipolar electrode which had an inter-electrode distance of 3 mm. A total or an anterior half rectal transection was done at the level 3 cm oral to the anal orifice. A total circular or an anterior half rectal aganglionosis, at the same level and about 1.5 cm wide, was produced by serosal application of 0.1% benzalkonium chloride(BC) solution 6 weeks before experimental observation[3], and aganglionosis was later confirmed histologically (fig. 1). A total freeing of the recto-anal canal from the surrounding structures, keeping the internal sphincter muscle intact, was done under nembutal anesthesia; the symphysis pubis and the urinary bladder were removed, and experimental observations were made about 20 min later, when sphincter tonus recovered after a transient drop.

Results (fig. 2). In all 5 normal rats, balloon and electrical stimulations at the rectum up to 6 cm oral to the anal orifice induced a positive rectosphincteric reflex (a transient drop in intra-anal pressure), while balloon and electrical stimulations 8 cm oral to the anus failed to cause the reflex in 1 of 5 rats and in all 5 rats, respectively. In the rats in which the rectum had been totally transected or made circularly aganglionic, both kinds of stimulation of the anal portion always induced a posi-