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Effects of hypertonic NaCl solution on microvascular haemodynamics in normo- and hypovolaemia

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The aims of this study were to investigate possible resuscitation effects of a single, 10-min, 350-μl intravenous infusion of 7.5% NaCl in hamsters in haemorrhagic shock and to compare the effects of such infusion with an identical one of 0.9% NaCl on the hamster cheek pouch microcirculation during normovolaemia and after acute bleeding to a hypotension level of about 40 mmHg. No significant differences could be detected between the effects of either infusion given to normovolaemic normotensive hamsters. In the animals subjected to haemorrhage, upon bleeding, arterioles larger than 40 μm constricted, arterioles smaller than 40 μm dilated and venular diameter did not change, while blood flow decreased in all vessels. The main differences between the infusions after haemorrhage were a significant increase in mean arterial pressure and arteriolar blood flow, venoconstriction and a tendency for the smaller arterioles to remain more dilated and the larger ones more constricted after the hypertonic infusion. Central nervous and/or reflex excitation of the sympathetic nervous system could account for the constriction of venules and larger arterioles, while a direct effect of hyperosmolarity could explain the dilatation of the smaller arterioles. The study can therefore help to explain some of the mechanisms underlying the reported resuscitation effect of 7.5% NaCl infusion in animals during severe haemorrhagic hypovolaemia.

Key words: arterioles, blood flow, diameter measurements, hyperosmotic solution, hypovolaemic shock, microcirculation, venules.

Haemorrhagic hypotension leads to a fall in blood flow in virtually all tissues. This reduction is a consequence of both reduced perfusion pressure and increased vascular resistance. It is believed that the rise in resistance is principally the result of constriction of precapillary vessels (Lundgren *et al.* 1965), but elevated blood viscosity (Lundvall & Gustafsson 1982), obstruction of capillaries by the formed elements of the blood (Zhao *et al.* 1986), luminal narrowing of capillaries by endothelial cell swelling (Mazzoni *et al.* 1989) and venular constriction (Zhao *et al.* 1985) might also contribute modestly.

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Studies of diameter changes in various segments of the microcirculation following haemorrhage have yielded conflicting results. Arterioles under observation were reported to constrict (Gray 1972, Zhao *et al.* 1985); to constrict or dilate depending on their initial diameter (Hutchins *et al.* 1973, Flint *et al.* 1984, Colantuoni *et al.* 1985, Cryer *et al.* 1985, Torres Filho *et al.* 1989); to constrict or remain unchanged depending on their initial diameter (Baker *et al.* 1988); or to constrict and subsequently dilate (Eriksson & Lisander 1972). Complex responses have also been observed in the venules during haemorrhagic hypotension. They were reported to constrict (Eriksson & Lisander 1972, Gray 1972, Flint *et al.* 1984, Cryer *et al.* 1985), to constrict or dilate depending

on their initial diameter (Harris *et al.* 1975, Hutchins *et al.* 1975) and to not change (Colantuoni *et al.* 1985, Baker *et al.* 1988, Torres Filho *et al.* 1989).

There are very few studies, to our knowledge, which report specifically on blood flow alterations in the microcirculation during haemorrhage (Zhao *et al.* 1985, 1986, Torres Filho *et al.* 1989).

In 1980, Velasco and co-workers showed that acutely bled dogs (shed volume 42 ml kg^{-1}, equivalent to approximately 50% of total blood volume) could be permanently resuscitated, within 45 min of the start of bleeding, by a single intravenous infusion of hypertonic NaCl solution (concentration 7.5%, equivalent to 2400 mosmol l^{-1}; infused volume 10% of total blood loss). This hypertonic infusion rapidly restored blood pressure and acid–base equilibrium towards normality. After hypertonic resuscitation in this manner, blood was not retransfused, nor was any other treatment or intravenous fluid replacement necessary to sustain the response. The initial effects were sustained indefinitely: long-term survival was 100% compared with 0% for a similar group of controls treated with isotonic saline. The tonicity of the infused NaCl solution in these experiments was several hundred times higher than the tissue hyperosmolarity measured *in vivo* after heavy exercise, which is considered one of the metabolic mediators of the vasodilatation and the net transcapillary fluid flux in excercising skeletal muscle (Mellander *et al.* 1967, Lundvall 1972). The experiments with 7.5% NaCl infusion in animals suggested that resuscitation from hypovolaemic shock could be achieved with very small infused volumes. Such small-volume resuscitation might be ideal in the field treatment of injured patients. In fact, DeFelippe *et al.* (1980) reported that hyperosmotic (7.5%) NaCl promptly reversed the shock in 11 out of 12 patients in terminal hypovolaemic shock who had not responded to vigorous volume replacement or corticosteroid and dopamine infusions. The mechanisms behind this resuscitation effect are not firmly established but might involve the central nervous system and/or direct peripheral vascular actions. To our knowledge, the effects of such infusion on the microcirculation have not been studied.

The aims of the present study were (a) to investigate possible resuscitation effects of 7.5% NaCl intravenous infusion in hamsters subjected to haemorrhagic shock and (b) to compare the effects of 7.5% and 0.9% NaCl infusions, in the same conditions, on the hamster cheek pouch microcirculation. The experiments were performed during normovolaemia and normal arterial pressure and after acute bleeding to a hypotension level of 40 mmHg. Changes of arteriolar and venular diameters, red blood cell velocity, volume flow and of arterial and venous pressures were measured.

MATERIALS AND METHODS

Experiments were performed on 30 hamsters of either sex weighing 93.3 ± 3.2 g. Anaesthesia was induced by an intraperitoneal injection of 0.1–0.2 ml of sodium pentobarbital (Mebumal vet., 60 mg ml^{-1}) and maintained with α-chloralose (100 mg kg^{-1}) administered intravenously. Throughout the surgery and subsequent experiment the animal rested on a heating pad controlled by a rectal thermistor, and body temperature was maintained at 36.5 °C. The femoral arteries and veins were cannulated for pressure measurements, bleeding, anaesthetic and 0.9% or 7.5% NaCl infusion. A tracheal tube was inserted to facilitate spontaneous breathing. The hamster was placed on a plexiglass stage similar to that described by Duling (1973) and modified by Svensjö *et al.* (1978). In the middle of the stage there was was a well with a silicon rubber ring surrounding a trans-illumination window. The cheek pouch was carefully everted with the aid of a moist cotton stick, and the distal, non-muscular part of the pouch identified and pinned to the silicon ring. Dissection was performed under a stereomicroscope: a crescent-shaped incision was made in the top layer, the flap was pinned to the side and the areolar connective tissue removed to expose the bottom-layer vasculature for microscopic observations. During the preparation and throughout the experiment, the cheek pouch was constantly superfused with a bicarbonate-buffered saline solution (composition in mM: NaCl 131.9, KCl 4.7, $CaCl_2$ 2.0, $MgSO_4$ 1.2, $NaHCO_3$ 18.0) at a rate of 6 ml min^{-1}. The solution was bubbled continuously with 5% CO_2 in N_2 and the heater device was adjusted to give a temperature of 36.5 °C on the cheek pouch. The pouch was left to equilibrate for 30 min before data acquisition.

An intravital videomicroscope was used to observe the microcirculation and make microcirculatory measurements. Total magnification of the video image was 1000 ×. Red blood cell (RBC) velocity in arterioles and venules was measured continuously by the dual-slit photometric technique (Wayland & Johnson 1967). The TV monitor display was used to obtain arterial and venular internal diameter measurements by an image shearing monitor (IPM model 907). Systemic arterial and venous pressures were monitored using

Statham transducers connected to cannulas in the femoral artery and vein. All the measured variables were recorded in a six-channel stripchart record (Grass polygraph model RCS 7C8). Microvessel volume flow, Q, was calculated from the recorded diameters, D, and RBC velocity, V, using the equation:

$$Q = \frac{\pi}{4} D^2 \times 1.6\ V_{RBC}.$$

In this equation, the factor 1.6 was used to convert dual-slit velocity to whole-blood velocity according to Baker & Wayland (1974) and Lipowski & Zweifach (1978). All the vessels observed were bigger than 10 μm.

The experimental protocol consisted of sets of measurements performed every 10 min. For each set, data on vessel diameter and on RBC velocity for one arteriole and one venule as well as on arterial and venous pressures were collected during a period of at least 1 min. The first three sets were taken 10 min apart at normal arterial pressure and normovolaemia; they constituted the control period. After this, blood was withdrawn from the cannulated femoral artery so as to reduce the mean arterial pressure to 40 mmHg. A new set of measurements was taken, and subsequently a single, 10-min intravenous infusion of either hypertonic NaCl solution (concentration 7.5 %) or physiological NaCl solution (concentration 0.9%) was given. In either case, the volume infused was 350 μl, equivalent to 10% of the measured blood loss. Measurements were resumed immediately after the end of the infusion and repeated every 10 min for 1 h.

In a separate group of experiments, arterioles and venules were studied using the same protocol described above except for the haemorrhage. These experiments were performed on 18 hamsters of either sex weighing 91.5 ± 3.8 g.

All data are expressed as mean $\pm$ standard error of the mean. Statistical significances were determined by use of Student's t-test, and probabilities of less than 5% ($P < 0.05$) were considered significant.

RESULTS

Normovolaemic animals

Eighteen arterioles with internal diameter between 25 and 75 μm and 18 venules, 38–75 μm i.d., were studied in 18 hamsters during control experiments. Ten hamsters received hypertonic NaCl and the rest physiological NaCl infusion.

The results obtained are shown in Fig. 1. Figure 1(a) shows mean arterial pressure data obtained during the control period and after 10 min infusion of either 350 μl of 0.9% NaCl or 350 μl of 7.5% NaCl and Fig. 1(b) the corresponding venous pressure data. Figure 1(c) shows arteriolar internal diameter data obtained during the control period and after each infusion and Fig. 1(d) shows venular internal diameter data. Fig. 1(e) depicts volume flow data from arterioles during the control period and after each infusion, and Fig. 1(f) shows venular volume flow data. In these experiments, in which no blood was withdrawn, no consistent changes occurred in arterial or venous pressure, vessel diameter or calculated volume flow during the experimental period with either infusion. Moreover, no significant differences could be detected between the two infusions. In the group in which hypertonic NaCl was infused, mean arterial pressure varied from 90.5 ± 5.7 to 95.3 ± 4.8 mmHg and venous pressure from 4.8 ± 0.4 to 5.2 ± 0.5 mmHg. In the group in which physiological NaCl was infused, mean arterial pressure varied from 90.5 ± 5.2 to 96.4 ± 4.4 mmHg and venous pressure from 4.0 ± 0.7 to 4.8 ± 0.8 mmHg. Using the mean value of vessel diameter during the first half-hour as a reference level (as was done in animals subjected to haemorrhage), mean diameters of arterioles and venules during the subsequent period (after infusion) were within 5% of the reference levels and standard errors were less than 5% of the mean. Greater variability was seen in volume flow, where mean values for individual measurement periods were usually within 10% of the reference level and standard error was ordinarily within 10% of the mean for that period. No significant long-term trends were evident in diameter, volume flow or mean arterial and venous pressures.

Hypovolaemic animals

The results obtained are shown in Figs. 2–4. Figure 2, top, depicts mean arterial pressure data obtained during the control period, immediately after haemorrhage and after 10 min intravenous infusion of either 350 μl of 0.9% NaCl or 350 μl of 7.5% NaCl. The blood volume that needed to be withdrawn to reduce the mean arterial pressure to 40 mmHg was 3.5 ± 0.3 ml for both groups. Since the total blood volume of this size of hamster is 8.2 ± 0.6 ml (mean $\pm$ SD, E. Svensjö personal communication), the shed blood volume was approximately 40% of the total blood volume. There was a significant increase ($P < 0.001$) in mean arterial pressure *in*

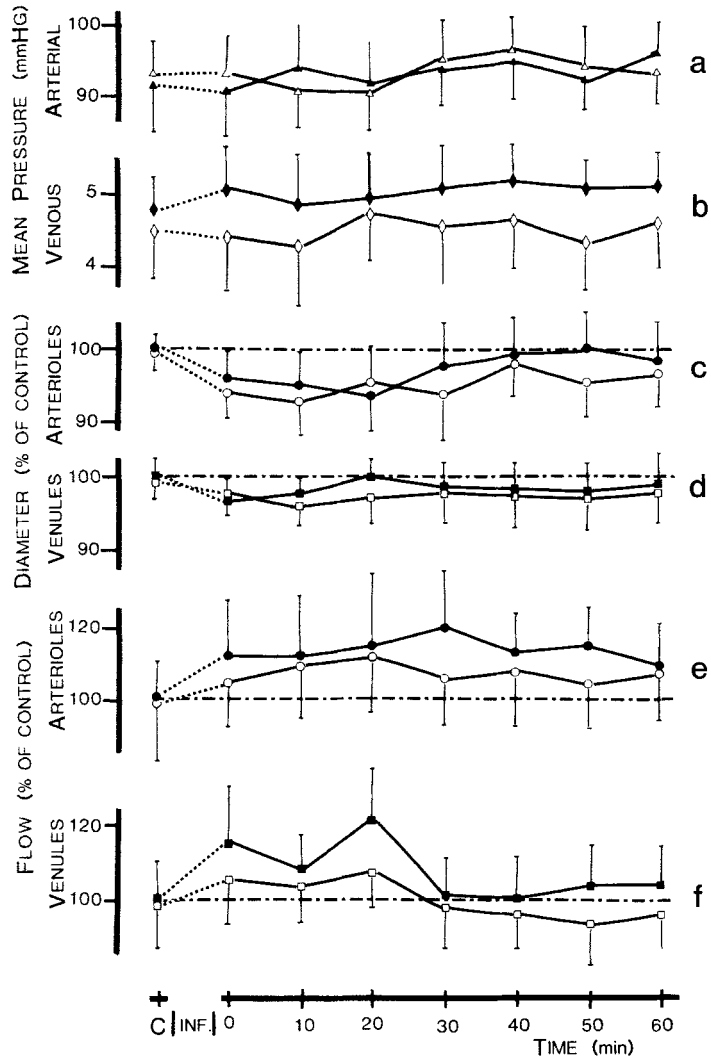

Fig. 1. Effects of hypertonic (closed symbols) or isotonic (open symbols) NaCl infusion on mean arterial pressure (a), venous pressure (b), internal arteriolar diameter (c), internal venular diameter (d), arteriolar volume flow (e) and venular volume flow (f) in normovolaemic, normotensive hamsters. Each point indicates mean ± SEM, C represents the mean (normalized in c–f) obtained during the control period (first three sets), and INF. indicates the infusion period.

the group treated with 7.5% NaCl compared with the one which received the same volume of 0.9% NaCl (comparison was between data obtained at the same time interval). There was, however, also a significant increase ($P < 0.05$) in mean arterial pressure after the infusion of 0.9% NaCl, compared with the value obtained immediately after haemorrhage. Figure 2, bottom, shows venous pressure, which did not change significantly throughout the observed period with either infusion used.

The diameter changes in arterioles with haemorrhage and thereafter were highly dependent on the control diameter. The transition point appeared to be at about 40 μm. Figure 3 shows the mean responses of arterioles grouped below (a) and above this size (b). Because large arterioles constricted and small arterioles dilated

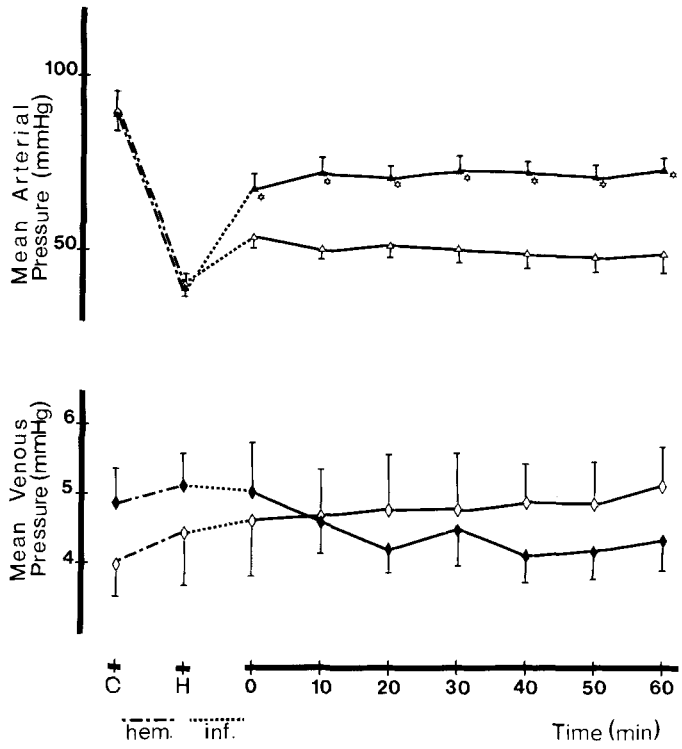

Fig. 2. Effects of haemorrhage and either hypertonic (closed symbols) or physiological (open symbols) NaCl infusion on mean arterial (top) and venous (bottom) pressure in hamsters. Each point indicates mean ± SEM; C represents the mean (normalized in Figs 4 and 5) obtained during the control period (first three sets); hem. and inf. indicate the haemorrhage and infusion periods respectively. * Statistical significance between the two infusions ($P < 0.05$).

during hypotension, the variation in diameter among all arterioles was decreased. We could not detect any temporal variations in mean arteriolar diameter during the hypotensive period with either infusion. There were no statistical differences between the data obtained after infusion of 7.5% or 0.9% NaCl, but there was a tendency for the smaller vessels to dilate more and the larger arterioles to constrict more after the hypertonic infusion. Figure 3(c) shows venular internal diameter data. The venules did not show a significant change from the control diameter following haemorrhage and infusion of 0.9% NaCl. However, the animals treated with 7.5% NaCl showed a significant venoconstriction after the hypertonic infusion. There were no significant differences in behaviour between large and small venules in either group.

After haemorrhage, the studied arterioles and venules showed similar reductions in calculated volume flow, about 60% reduction (Fig. 4 top and bottom). Figure 4, top, shows arteriolar volume flow data. In the animals which received 0.9% NaCl, the mean arteriolar flow was kept below or equal to 75% of the control level during the experimental period. In contrast, in the group treated with 7.5% NaCl, there was an increase in flow was statistically significant (compared with the other group) which was maintained throughout the experiment. This increase in flow was stastically significant compared with the group which received 0.9% NaCl 30 min after the end of the infusion and thereafter. Venular volume flow (Fig. 4, bottom) in the animals which received 0.9% NaCl returned to normal 10 min after the end of the infusion, and in the group with 7.5% NaCl flow returned to the control level 50 min after the infusion. Venular blood flow was statistically different between the two groups 10 and 20 min

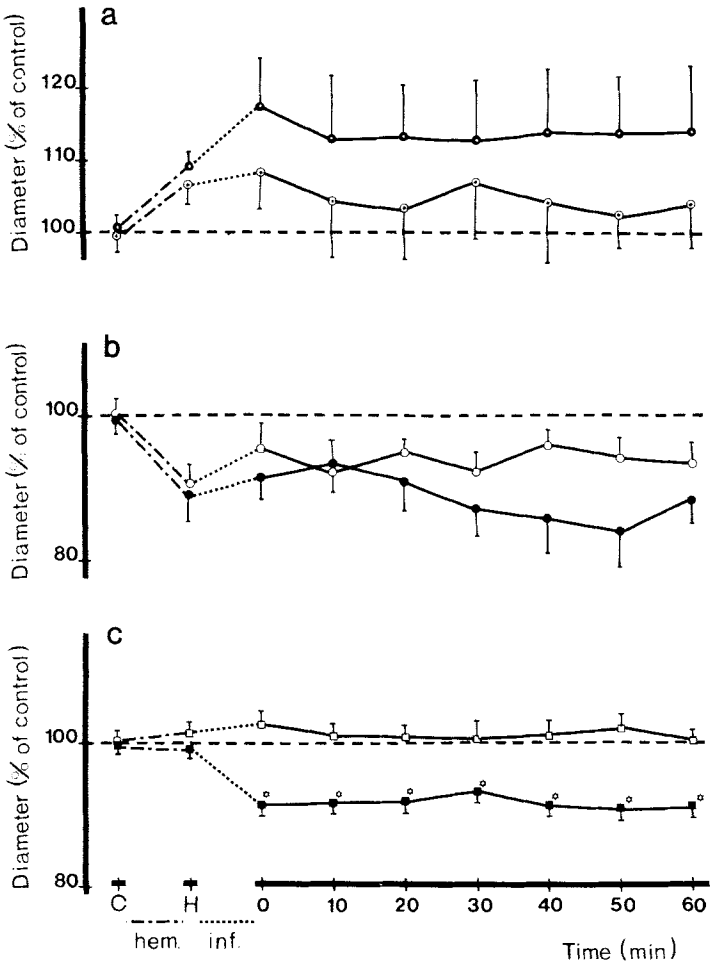

Fig. 3. Effects of haemorrhage and either hypertonic (closed symbols) or isotonic (open symbols) NaCl infusion on internal diameter of arterioles smaller than 40 μm (a), bigger than 40 μm (b) and venules (c). For details see Fig. 2.

after the end of the infusion. Complete flow stoppage was not observed in any vessels.

DISCUSSION

We could not detect any significant changes in any of the measured parameters in the animals in which no blood was withdrawn as a result of infusion of 7.5% NaCl. Similarly, Velasco *et al.* (1980) reported that an identical injection of hyperosmotic NaCl solution into normotensive normovolaemic dogs was practically without effects. It must be noted, however, that this is not an entirely valid control. Normovolaemic animals have roughly twice the plasma volume of the shocked ones, and this means that the injection or infusion of the hypertonic NaCl will dilute in a larger pool (hence at lower concentration) in the normovolaemic animal.

In hamsters subjected to haemorrhage, we found that smaller arterioles dilated while larger ones constricted during hypotension. Similar observations have been reported previously for the cat tenuissimus muscle (Eriksson & Lisander 1972), cat sartorius muscle (Torres Filho *et al.* 1989) and rat cremaster muscle (Hutchins *et al.* 1973) as well as for skin preparations of the

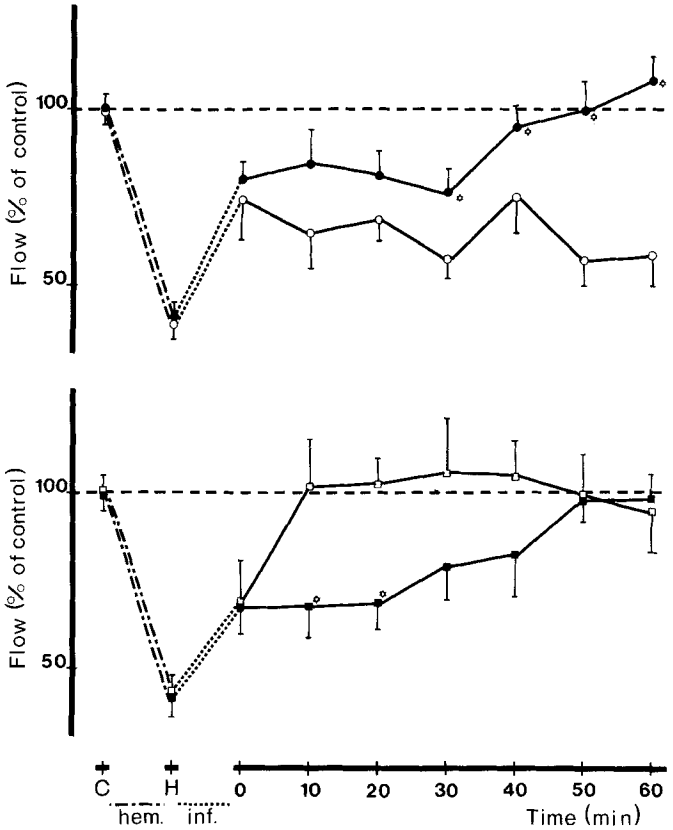

Fig. 4. Effects of haemorrhage and either hypertonic (closed symbols) or physiological (open symbols) NaCl infusion on arteriolar (top) and venular (bottom) volume flow. For details, see Fig. 2.

unanaesthetized bat (Harris *et al.* 1970) and hamster (Colantuoni *et al.* 1985). Our results do not appear to agree with those in the rat spinotrapezius muscle and other studies on the rat cremaster muscle where all arterioles were reported to constrict (Gray 1972, Zhao *et al.* 1985, 1986). However, those studies were performed in skeletal muscle preparations, and this could explain the difference.

In our study, the variation between arterioles in their response to haemorrhage could be partly attributable to differences in their initial diameter. Using 3-min periods of sympathetic stimulation, Boegehold & Johnson (1988) found, in cat sartorius muscle, a gradient of escape from sympathetic stimulation: proximal (first- and second-order arterioles by the centrifugal ordering scheme) dilated only modestly following initial constriction, while the most distal arterioles (sixth order by the same scheme) initially constricted to the same degree but dilated above the control diameter by the end of the stimulation period. The present study, however, involved a greater reduction in volume flow than Boegehold & Johnson's study (60% as compared with 40–50%) and a much longer period of reduced flow. That sympathetic activity is importantly involved in the constriction was indicated by observations on the dog gracilis muscle where denervation greatly reduces the constriction during the haemorrhage (Bond 1986). The activation of vascular β-receptors by circulating catecholoamines might also contribute to the arteriolar dilatation (Lundvall *et al.* 1982). The influence of metabolites on the vasodilatation was depicted by Lombard and coworkers (1981) by showing that dilatation of small arterioles in the hamster cheek pouch during systemic hypotension was abolished by elevated suffusate Po_2.

However, part of the resistance increase in the microcirculation during haemorrhagic shock might also be attributed to luminal narrowing and endothelial cell swelling observed in capillaries (Mazzoni *et al.* 1989). The luminal diameter of rabbit tenuissimus muscle capillaries was examined by intravital microscopy throughout a 1-h shock period (40% haemorrhage) using the red blood cells within the capillary as an intraluminal marker. By the end of the shock period, RBC flux was reported to have decreased by over 67% with a -24.3 ± 9.3% and $+22.8 \pm 6.4$% change in RBC width and length respectively.

In our study, the increased overall vascular resistance after infusion of 0.9% NaCl could not be attributed to constriction of venules, as no significant changes were observed in these vessels. These results are consistent with studies on hamster skinfold during haemorrhagic hypotension (Colantuoni *et al.* 1985).

The changes in mean arterial pressure observed after 7.5% NaCl infusion in hamsters in haemorrhagic shock showed the same resuscitation effect as reported by Velasco *et al.* (1980) in dogs. Following the infusion of hyperosmotic NaCl, plasma volume and arterial haemotocrit measurements independently showed no appreciable or lasting expansion of plasma volume (Velasco *et al.* 1980), which could account for the long-term recovery of normal circulatory function. Wolf (1971) has shown that a rapid intravenous injection of 12 ml kg^{-1} NaCl at 833 mosmol l^{-1}, which is almost exactly identical to the 4 ml kg^{-1} at 2400 mosmol l^{-1} used by Velasco *et al.* (1980), induced a maximal plasma expansion at zero time. This expansion was, however, transient. At 30 min post-injection, expansion was already down to 25%, and at 90 min it had further dropped to 10%. Mazzoni *et al.* (1988) have developed a model, based on irreversible thermodynamic transport equations, to describe blood volume restoration after haemorrhage with resuscitative fluids, particularly hyperosmotic solutions. The model shows that immediately after hyperosmotic infusion water shifts into the plasma, first from the red blood cells and endothelium and then from the interstitium and tissue cells. The endothelial cell shrinkage, if present during hyperosmotic infusion, decreases capillary hydraulic resistance.

There is evidence to indicate that hyperosmolarity increases myocardial contractility and cardiac efficiency both in normotensive and in shocked subjects (Baue *et al.* 1967, Wildenthal *et al.* 1969). Although no direct measurements of cardiac contractility were performed in the present experiments, the large increase in pulse pressure that follows the hypertonic infusion is compatible with the concept of increased myocardial contractility. Precapillary resistance vessels have been reported to dilate with hyperosmolarity (Bauer *et al.* 1967, Mellander *et al.* 1967, Gazitua *et al.* 1971, Lundvall 1972). In our study, no statistical differences between the data obtained after infusion of 7.5 or 0.9% NaCl were found; however we observed a tendency for the smaller arterioles to dilate more after the hypertonic infusion. Hypertonic perfusion of the cerebroventricular system of dogs evoked marked centrally mediated increases in arterial blood pressure, heart rate, respiratory rate and ventilation (Mellander & Hillman 1975). Intravenous infusion of hyperosmotic (7.5%) NaCl in dogs with vagal blockage produced only a transient recovery of cardiac output, with no long-term survival (Lopes *et al.* 1981). It was suggested that the first passage of hyperosmotic blood through the pulmonary circulation, at a time when vagal conduction was unimpaired, was essential for the production of the full haemodynamic–metabolic response which is needed for indefinite survival. Therefore hyperosmolarity can elicit central nervous (Mellander & Hillman 1975) and/or reflex (Lopes *et al.* 1981) excitation of the sympathetic nervous system to the heart and blood vessels. It could also induce constriction of the systemic and pulmonary capacitance vessels (Gazitua *et al.* 1971, Hauge & Bö 1971). These findings could explain the venular constriction and the tendency for the larger arterioles to constrict more after the hypertonic infusion observed in our study.

In conclusion, the results obtained after hypertonic infusion to hamsters as studied in the cheek pouch microcirculation seem to be compatible with the ones shown by Velasco *et al.* (1980). Our study has shown venular constriction and a tendency for the larger arterioles to constrict more and for the smaller ones to dilate more after the hypertonic infusion. These findings can help to explain some of the mechanisms underlying the resuscitation effect of 7.5% NaCl infusion in animals during severe haemorrhagic hypovolaemia.

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