THE INFLUENCE OF HIGH AND LOW SODIUM INTAKE ON BLOOD VOLUME IN THE DOG

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SUMMARY

Plasma volume and blood volume were measured using T-1824 in two groups of dogs which were kept on a diet differing only in its content of sodium chloride for periods exceeding six weeks. The group on low sodium intake received 0.55 mmol Na⁺ . kg⁻¹ . day⁻¹ and the group on high sodium intake received 12.4 mmol Na⁺ . kg⁻¹ . day⁻¹. Both plasma and blood volumes were distinctly greater in the group on high sodium intake than those in the group on low sodium intake, and there was no difference in the haematocrit between the two groups of dogs. It is concluded that a greater plasma volume and red cell volume resulted from high sodium intake than from low sodium intake.

INTRODUCTION

In the course of an investigation into the effect of changes in blood volume on the diuresis and natriuresis observed in response to stimulation of atrial receptors in anaesthetized dogs, it was necessary to prepare animals with differing blood volumes. It has been previously shown that there is an increase in blood volume when sodium retention is induced by a combination of subtotal resection of the kidney mass and the administration of saline (Douglas, Guyton, Langston & Bishop, 1964). Also, a decrease in the volume of extracellular fluid has been demonstrated following sodium depletion for a period of four to six weeks in unanaesthetized dogs (Reinhardt & Behrenbeck, 1967; Kramer, Boylan & Keck, 1969). Thus these effects of chronic ingestion of supplements of sodium chloride and of chronic deprivation of sodium on the volumes of plasma and blood suggested a means of altering blood volume in dogs with intact kidneys, but the evidence was inadequate to support a definite conclusion.

Thus, in the present study, dogs were randomly assigned to two groups in which the diets differed only in their content of sodium chloride; the plasma volume and blood volume were measured and compared. The study has been the subject of a preliminary communication to the Medical Research Society (Gupta, Mary, Weatherill & Linden, 1980).

METHODS

Dogs were anaesthetized with an i.v. injection of chloralose (0.1 g . kg⁻¹, Establissemegements Kuhlman, Paris) and artificially resired using methods described previously (Kappagoda, Linden & Snow, 1972). The solution of chloralose consisted of 1 g alpha chloralose dissolved in 100 cm³ saline. Anaesthesia was maintained by a continuous infusion of chloralose (0.5–1.0 mg . kg⁻¹ . min⁻¹) through a nylon cannula (Portex surgical quality No. 2, Portland Plastics Ltd, Hythe, Kent), inserted through

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the left femoral vein. The left femoral artery and the right femoral vein were cannulated and used respectively for obtaining samples of arterial blood from the abdominal aorta and injecting T-1824 (Evans' blue dye) into the inferior vena cava.

The pressure in the aorta was obtained via a cannula inserted through the right femoral artery and was recorded along with the tracheal pressure, end tidal $P_{CO_2}$, and e.c.g. The temperature and acid–base status of the animal were monitored and maintained within normal limits, as previously described (Kappagoda et al. 1972).

**Diets.** Dogs were kept in cages under supervision and were randomly fed diets providing either a low sodium intake (LSI) or a high sodium intake (HSI) for a period of 7-5 weeks (mean; range 6–10). Each dog was fed once daily and allowed free access to water.

The group of animals on LSI were fed lean beef meat (14 g. kg$^{-1}$. day$^{-1}$) containing about 0.038 mmol Na$^+$. g$^{-1}$ (O'Connor, 1977), boiled rice and suet. The rice and suet provided carbohydrate and fat, and minimal amounts of sodium of less than 0.0091 mmol. g$^{-1}$, and the amounts used were chosen to maintain the body weight of the animal by providing approximately 60 cal. kg$^{-1}$. day$^{-1}$. No additional sodium was allowed in this diet which provided 0.55 mmol Na$^+$. kg$^{-1}$. day$^{-1}$. The group of dogs on HSI received additional sodium in the form of sodium chloride (0.69 g. kg$^{-1}$ body weight) and their diet provided about 12-4 mmol Na$^+$. kg$^{-1}$. day$^{-1}$. These intakes of sodium were used in previous studies on the inulin space and urinary responses to stimulation of atrial receptors (Kaczmarczyk, Eigenheer, Gatzka, Kuhl & Reinhardt, 1978; Reinhardt & Behrenbeck, 1967), and were different from those usually given to our experimental dogs which provide a maximum intake of Na$^+$ of 3–4 mmol. kg$^{-1}$. day$^{-1}$; dogs have been maintained on these diets for up to four months, remaining in good health (O'Connor, 1977). In both groups no food was allowed during the 12 hr period immediately before the experiment.

**Plasma volume.** Measurements of plasma volume were made after a steady state had been reached with respect to heart rate, arterial blood pressure and a constant dose of chloralose. Plasma volume was measured as the dilution space of T-1824 (Evans' blue dye) after a period of 10 min to allow complete mixing. Studies in anaesthetized and conscious dogs have shown that mixing of the dye is complete at about the sixth minute after injection using the pattern of decay of dye concentration (Courtice, 1943; Lawson, 1962; Miller, 1947; Pickering & Dow, 1950; Surthshin & Rolf, 1950). The same conclusion was arrived at by Gilder, Muller & Phillips (1940) who studied anaesthetized dogs and obtained simultaneous samples from veins in the forelimbs, the hind limbs, the jugular vein and the portal vein. They showed that the concentrations of dye were identical within five minutes in all these samples.

Before the injection of T-1824 a sample of arterial blood was withdrawn for measurement of haematocrit value and to obtain plasma for use as standard (blank) specimens for subsequent analysis. The weight of dye administered was that calculated to give a plasma concentration of approximately 25 mg. litre$^{-1}$ as shown in previous experiments and amounted to a dose of about 1 mg. kg$^{-1}$. This dose was given as 0·1% solution in 20 cm$^3$ saline through the venous catheter and was immediately flushed through by 10 cm$^3$ saline. Arterial blood samples, each of 10 cm$^3$, were then taken every 10 min for 60 min and this volume was replaced with Dextran in 5% dextrose (Dextraven 150; Fisons Ltd, Loughborough, Leics.). The optical density of plasma in these samples was read at 625 nm on a Unicam Spectrophotometer (Unicam Instruments Ltd) in comparison with the specimen of plasma obtained before injection of the dye. The concentration of dye in each sample was obtained from calibration curves, using dog plasma and the same spectrophotometer. The rate of decay of T-1824 from plasma for 60 min after injection was described by a linear relationship of the concentrations on a logarithmic scale against time, using the best fit regression line (Fig. 1). The initial concentration of T-1824 at injection time (zero time) was then obtained by the intercept of the regression line with the ordinate at zero time and, in conjunction with the weight of dye injected, was used to calculate plasma volume. This method is a widely accepted technique for measuring plasma volume (Chien & Gregersen, 1962; Courtice, 1943; Gregersen & Rawson, 1959; Jones, 1970; Lawson, 1962; Mayerson, 1965). Interference in the determination of optical density from haemolysis or turbidity was encountered only very occasionally because the blood samples were centrifuged immediately after withdrawal, an optimum amount of dye was used (Allen & Gregersen, 1953), and because the animals had not been fed during the preceding 12 hr period. Errors from traces of dye in injection and
Fig. 1. An example of a time-concentration curve relating concentrations of T-1824 in plasma (on a logarithmic scale) to time (on a linear scale). The continuous line is the computed regression line of the concentration–time relationship. This line is extrapolated back (interrupted line) to give T-1824 concentration in plasma at zero time (injection time) which is used in the calculation of plasma volume. Dog No. 74/79.

Sampling catheters were minimized by flushing the catheter with saline; thus catheters were cleared reliably of the dye by the saline bolus.

Haematocrit value. Four micro-haematocrit tubes were filled with well mixed arterial blood obtained from the abdominal aorta just before the injection of T-1824, and at 30 min and 60 min afterwards. Flame-sealed tubes were then spun for 5 min at 10,000 rev. min⁻¹ in a micro-haematocrit centrifuge (Hawksley, London) and the red cell fraction was obtained by the method of similar triangles using a micro-haematocrit reader (Hawksley, London). The mean of four results was used in each measurement. In three dogs, independent comparison between haematocrit value, obtained using either heat-sealed tubes or tubes sealed by a sealer compound (24 pairs of observations) showed no significant differences in haematocrit value: the mean difference was 0.05% (range 0–1). The haematocrit value obtained before injection of T-1824 was used for the calculation of blood volume.

Comparison of haematocrit values obtained at 30 min and 60 min from the time of injection of the dye to those obtained before the injection (sixty comparisons) showed a small difference of −0.93% (mean; range −2.5 to 0.5).

Blood volume. The measurement of plasma volume was combined with the haematocrit value to provide an estimate of blood volume according to the relationships:

$$\text{blood volume} = \frac{\text{Plasma volume}}{100 - \text{haematocrit value}} \times 100$$

(e.g. Gregersen & Rawson, 1959; Lawson, 1962).

To assess the confidence limits of the difference between repeated measurements, plasma volume and blood volume were measured in duplicate. Care was taken to perform the measurements in
succession at the same steady state with respect to heart rate, arterial blood pressure and a constant dose of chloralose solution. It has been shown that the initial concentration of dye T-1824 (zero time) of a repeat injection, 60 min following the first injection, equalled the sum of the initial concentration and the residual concentrations of the dye in the first injection. The rate of disappearance of the dye remained the same (Bonncastle, 1947; Miller, 1947; Pickering & Dow, 1950; Surtshin & Rolf, 1950). In the present investigation, the residual concentration of the first injection was subtracted from those of the repeat injections, and the volumes of plasma and blood were calculated as described and compared independently to those of the first injection.

The values of blood and plasma volume in each dog were expressed in relation to body weight since it has been shown that this relationship in the dog remains constant as the animals increase in weight and size (Courtice, 1943).

RESULTS

Thirty dogs weighing 21.3 kg (mean; range 16–28 kg) were investigated. When the steady period for the measurement of blood volume was attained one hour after completion of the surgical procedure, the pH, $P_{\text{CO}_2}$ and $P_{\text{O}_2}$ were 7.377 (mean; range 7.35–7.42), 5.36 kPa (mean; range 4.79–5.72) and 21.65 kPa (mean; range 15.96–26.86). The heart rate was 149.6 beats.min$^{-1}$ (mean; range 65–220), and the mean arterial pressure was 17.89 kPa (mean; range 12–22).

During measurement of plasma volume, blood samples were obtained ten minutes after injection of the dye to allow complete mixing. In three dogs, mixing was assessed using arterial and venous blood samples, obtained simultaneously every two minutes for 15 min. In these dogs the concentration of dye in venous blood was identical with that in arterial blood after a period of 5 min (mean; range 4–6 min), and the results were similar to those obtained by Pickering & Dow (1950) in anaesthetized dogs.

Two groups of dogs were studied; the first consisted of sixteen dogs kept on HSI and the second group consisted of fourteen dogs kept on LSI. The respective data on body weight, rate of infusion of chloralose solution (1%) to maintain steady anaesthesia, heart rate, and mean femoral arterial pressure were similar in both groups of animals as shown in Table 1.

Repeated estimation of volumes. Two consecutive estimations of plasma volume and blood volume were obtained independently in each animal and were compared as shown in Figs. 2 and 3. Twelve pairs of measurements were obtained in twelve dogs. Plasma volume (Fig. 2) obtained by the first measurement was 43.1 cm$^3$. kg$^{-1}$ ± 14.1 (mean ± S.D.) and by the second measurement was 43.3 cm$^3$. kg$^{-1}$ ± 14.2. The 95% confidence limits of the difference between paired measurements of plasma volume was 0.46 cm$^3$. kg$^{-1}$ (1.1% of mean value). The respective volumes of blood shown in Fig. 3 were 75 cm$^3$. kg$^{-1}$ ± 20.2 and 74.7 cm$^3$. kg$^{-1}$ ± 20.2 and the 95% confidence limits were 0.82 cm$^3$. kg$^{-1}$ (1.1% of mean value).
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Fig. 2. Comparison between two consecutive measurements of plasma volume in twelve dogs. The first measurement on the abscissa is plotted against the second measurement on the ordinate. Each point represents two consecutive measurements in the same animal. The continuous line is the estimated regression line; the intercept and the slope of this line were not significantly different from zero ($P > 0.2$) or from unity ($P > 0.6$).

Fig. 3. Comparison between two consecutive measurements of blood volume in the same dogs as in Fig. 2. The continuous line is the estimated regression line whose intercept and slope were not significantly different from zero ($P > 0.2$) or from unity ($P > 0.8$).

value). In these animals the haematocrit during the first measurement was $42.67\% \pm 8.13$ (mean ± S.D.) and during the second measurement was $42.08\% \pm 8.23$.

Blood volume. The measured volume of plasma in the group of dogs on HSI was $53.54 \text{ cm}^3 \cdot \text{kg}^{-1}$ (mean; range 37.0–66.4) and in the group of animals on LSI was $36.36 \text{ cm}^3 \cdot \text{kg}^{-1}$ (mean; range 20.4–43.5) (Fig. 4). Most of the dogs in the HSI group (thirteen of sixteen animals) had a plasma volume which was greater than that in most of
Fig. 4. Plasma volume (cm$^3$·kg$^{-1}$) in each of sixteen dogs kept on high sodium intake (HSI) and of fourteen dogs kept on low sodium intake (LSI). The mean ± S.E.M. and $P$ value of the difference between the two groups are also shown.

Fig. 5. Blood volume (cm$^3$·kg$^{-1}$) in each of the dogs in the two groups shown in Fig. 4. The mean ± S.E.M. and $P$ value of the difference between the two groups are also shown.
Fig. 6. Plasma volume (cm$^3$) in each of the dogs in the two groups shown in Fig. 4. The mean ± S.E.M. and the $P$ value of the difference between the two groups are also shown.

Fig. 7. Blood volume (cm$^3$) in each of the dogs in the two groups shown in Fig. 4. The mean ± S.E.M. and the $P$ value of the difference between the two groups are also shown.
the dogs in the LSI group (twelve of fourteen animals); in every instance the difference exceeded the confidence of limits of repeated measurement of 0·46 cm$^3$. kg$^{-1}$. Considering plasma volume in the two groups as a whole, there is a statistically significant difference ($2P < 0·001$).

The estimated blood volume (Fig. 5) in the group on HSI averaged 92·57 cm$^3$. kg$^{-1}$ body weight (range 64–118·5) and was significantly greater ($2P < 0·001$) than that in the group on LSI of 54·16 cm$^3$. kg$^{-1}$ body weight (mean; range 33·1–69·7). This difference in blood volume exceeded the confidence limits of repeated measurement in fifteen out of sixteen dogs on HSI and in thirteen out of fourteen dogs on LSI.

The values of plasma volume and blood volume, unrelated to body weight in each dog, are shown in Figs. 6 and 7. The volumes of plasma and blood in the group on HSI were 1155·12 cm$^3$ ± 305·18 (mean ± s.d.) and 2003·92 cm$^3$ ± 569·77 and were significantly greater ($2P < 0·001$; $2P < 0·001$) than the respective volumes in the group on LSI of 668·61 cm$^3$ ± 118·82 and 1124·33 cm$^3$ ± 214·66.

The haematocrit in the group of dogs in HSI was 41·53% ± 5·34 (mean ± s.d.) and was not significantly different ($2P > 0·5$) from that in the group on LSI of 40·00% ± 7·75. Individual values of haematocrit in each dog are shown in Fig. 8.

**DISCUSSION**

*Measurement of blood volume.* The suitability of T-1824 as a plasma volume marker and the use of haematocrit and plasma volume in the estimation of blood volume have been well documented (Chien & Gregersen, 1962; Courtice, 1943; Gregersen, Boyden & Allison, 1950; Jones, 1970; Lawson, 1962; Mayerson, 1965). In the present investigation the same...
technique was used to measure these volumes in animal preparations during a steady state with respect to heart rate, arterial blood pressure and a constant dose of anaesthetic agent. This approach helps to minimize possible variation in the dynamics of the fluids measured; it could be argued that fluctuations of blood flow in the capillary bed could effect changes in the dynamics of T-1824 bound to albumin, and therefore changes in the volume of intravascular fluid (Lawson, 1962). Throughout the experiment, there was no significant change in the haematocrit value which could be attributed to significant fluid shifts or changes in blood volume. This was further confirmed by the adequate reproducibility of measurements of plasma and blood volume; the 95% confidence limits of repeated measurements were 1.1%.

To reduce differences between the two groups of dogs, strict experimental criteria were adhered to in both groups; the surgical procedure, sampling and replacement of fluids, and the rate of infusion of the anaesthetic solution relative to body weight were similar in both groups of animals.

In the present investigation, therefore, an adequate reproducibility in the estimation of plasma volume and blood volume by a standardized method was demonstrated. The method was considered of adequate accuracy in comparing volume measurements between two groups of dogs which were identical in respect of body weight, diet and experimental techniques and differed only in sodium intake which was low in one group and high in the other group.

*Effects of sodium intake.* The effects of a sustained diet, providing either a low or high sodium content, on the volume of plasma and blood are not clear. Most of the available information on this effect has been derived from indirect estimates. For example, O'Connor (1977) studied the effects of acute sodium loading in unanaesthetized dogs; the volume of plasma was calculated from changes in the concentration of plasma proteins and in the haematocrit. O'Connor (1977) showed that retention of sodium led to a small increase in the calculated volume of plasma. This method of calculating plasma volume assumes that total plasma protein and red cell volume remain unchanged and is therefore likely to underestimate actual increases in plasma volume. For instance, it has been shown that whilst increases in plasma volume caused by acute loading with sodium salts in man were associated with a decrease in the haematocrit value and the concentration of plasma protein, these decreases were less than those that could be accounted for by the process of dilution alone (Grant & Reischsman, 1946; Lyons, Jacobson & Avery, 1944).

The effect of sodium retention on blood volume in unanaesthetized dogs has been reported (Douglas et al. 1964). Sodium retention was brought about by the resection of 70% of the renal mass in dogs which were then made to drink 1.2% saline for five weeks. In that report, there was an increase in blood volume of 17.5% ± 2.8 (mean ± s.e.m.) during the first fourteen days. On the other hand, Reinhardt & Behrenbeck (1967) and Kramer et al. (1969) reported the effect of a daily diet providing a low sodium intake (0.5-0.8 mmol.kg⁻¹ body weight) on the volume of extracellular fluid (inulin space) in unanaesthetized dogs. Following the introduction of such a diet, measurements were made weekly for the first four weeks, at the sixth and at the fifteenth week. The inulin space in these dogs remained unchanged at about 22% of body weight during the first two weeks and progressively decreased during the subsequent four weeks to a steady state of 16% of body weight. In that report it was reasonable to assume that there was also a decrease in plasma and blood volume; studies in man and in the dog have shown that plasma volume constitutes about a third or a quarter of the volume of extracellular fluid (Lawson, 1962;
O'Connor, 1977). However, it is generally believed that in the presence of intact kidneys the net decreases or increases in plasma volume as a result of depletion or retention of sodium, respectively, are less marked than changes in the volume of extracellular fluid because of the osmotic function of the various components of body fluid and the postulated mechanisms responsible for the regulation of sodium balance and blood volume (e.g. O'Connor, 1977).

Finally it is interesting to mention data in another report on changes in plasma volume in unanaesthetized dogs kept on low sodium intake (about 0.4 mmol. kg\(^{-1}\). day\(^{-1}\)), normal intake (about 2.5 mmol. kg\(^{-1}\). day\(^{-1}\)) and on relatively higher sodium intake of about 4 mmol. kg\(^{-1}\). day\(^{-1}\) (Rocchini, Cant & Barger, 1977). Although it was concluded in the report that plasma volume was less in dogs on low sodium intake than in dogs on normal sodium intake, the presented data did not adequately support this conclusion. Firstly, the data on plasma volume were given as mean and s.e.m. of six estimations in each dog over six consecutive days (Table 1; Rocchini et al. 1977). Their table shows that although there was a difference in the mean data in between dietary regimes, this difference was less than two standard deviations around the same mean; the differences in plasma volume were within daily variability in the measurement of these volumes. Secondly, no information was given on the details of procedure used (Evans’ blue dye) and on the errors encountered. Thirdly, the study was concerned with assessing the carotid sinus reflex in relation to the level of sodium intake. Therefore, perhaps not unexpectedly, the data on plasma volume were selected; in five out of the eight dogs the plasma volumes during all three levels of sodium intake were not given. Finally, it is not clear how long these animals were kept on the mentioned diets, especially diets with low sodium content (cf. Reinhardt & Behrenbeck, 1967).

Therefore, there is no previous evidence demonstrating changes in blood volume, derived from direct measurements, e.g. of plasma volume, in standardized animal preparations subjected to either a high or a low sodium intake for a sustained period of time. In the present investigation, two groups of dogs were treated identically except with respect to salt intake; the first group received a high sodium intake (HSI) and the second group a low sodium intake (LSI). The animals were kept on such diets for a period of 6–10 weeks to ensure a steady state of sodium balance (cf. Reinhardt & Behrenbeck, 1967). In the present investigation measurements of plasma volume and blood volume were made under anaesthesia and during a steady state, allowing an adequate reproducibility of measurement with a confidence limit of 1.1 %. Both plasma and blood volume were shown to be distinctly greater in the group on HSI than in the group on LSI and the differences were considerably greater than the confidence limits of measurement. The plasma and blood volumes in the animals on HSI were respectively 65 and 70 % greater than in animals on LSI. The present results demonstrate that a greater plasma volume and red cell volume have resulted from HSI than from LSI, since the haematocrit was not significantly different in between these levels of sodium intake. Whilst it was not intended to study the mechanisms responsible for these findings, the difference in volumes could be explained by the available evidence on the control of plasma volume and red cell volume. For instance, first the differences in plasma volume may be attributed to retention of sodium in animals on HSI and to reduction in the body content of sodium in animals on LSI; changes in the body content of sodium have been shown to result in parallel changes in the volume of extracellular fluid (Douglas et al. 1964; Reinhardt & Behrenbeck, 1967). Secondly, the increase in plasma volume during the first two to four days of sodium retention results in haemodilution and reduction in the haematocrit (Grant & Reischman, 1946; Lyons et al. 1944). This decrease in
haematocrit would stimulate erythropoiesis resulting in a normal haematocrit again, but now at a higher red cell volume. Similarly a decrease in plasma volume during LSI would reduce the rate of production of red blood cells to maintain the haematocrit. It is widely accepted that the haematocrit is maintained within normal limits by changes in the rate of red cell production (DeGruchi, 1967; Grant & Root, 1952).

One implication of the present finding concerns the hypothesis advanced by Guyton that hypertension could be initiated by an increase in blood volume (Coleman & Guyton, 1969; Guyton & Coleman, 1969). For instance, a relationship between either high or low sodium intake and levels of blood pressure has been suggested (e.g. Dahl, 1972; Shaper, 1972; Freis, 1976) and it has been shown that in hypertensive subjects changes in plasma volume caused by either sodium loading or deprivation resulted in parallel changes in the level of blood pressure (e.g. Dustan, Bravo & Tarazi, 1973). Although a cause and effect relationship remains to be demonstrated, a sustained change in the level of sodium intake could be associated with increases in the level of blood pressure through changes in plasma volume.

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