J. Physiol. (1975), **244**, *pp.* 303–312
With 3 text-figures
Printed in Great Britain

THE RELATIONSHIP BETWEEN ELEVATED WATER INTAKE AND OEDEMA ASSOCIATED WITH CONGESTIVE CARDIAC FAILURE IN THE DOG

BY D. J. RAMSAY, BARBARA J. ROLLS AND R. J. WOOD

From the University Laboratory of Physiology and the Department of Experimental Psychology, University of Oxford, Oxford

(*Received 11 April 1974*)

SUMMARY

1. In the dog constriction of the thoracic region of the inferior vena cava increases water intake and extracellular and plasma fluid volumes.

2. Restriction of water intake to the pre-operative level for 2 weeks reduces the measured extracellular fluid volume to the pre-operative level.

3. Administration of the competitive angiotensin inhibitor, saralasin acetate, to two dogs in congestive cardiac failure following thoracic caval constriction markedly reduced their water intake.

4. These results suggest that increased fluid intake is probably important in the aetiology of the oedema associated with congestive cardiac failure, probably through the renin-angiotensin system.

INTRODUCTION

A decrease in the blood volume which leads to a reduced venous return stimulates the secretion of renin by the kidneys (Davis, 1967). This response is partly determined by activity in the renal sympathetic nerves (Hodge, Lowe & Vane, 1966). In the rat, acute reduction in venous return following ligation of the abdominal inferior vena cava increases water intake, an effect largely dependent on an elevated renin or angiotensin level since the drinking response is reduced by bilateral nephrectomy (Fitzsimons, 1969). Also, in the rat intravenous infusion (Fitzsimons & Simons, 1969) or intracranial injection of angiotensin (Epstein, Fitzsimons & Rolls, 1970) induces drinking.

In the dog the role of the renin–angiotensin system in thirst has not been elucidated. Hypovolaemia brought about by sodium depletion (Holmes & Cizek, 1951), or by haemorrhage (Szczepanska-Sadowska, 1973) increases water intake, but the role of the renin–angiotensin system

in this response is not clear. In the dog the administration of angiotensin intravenously lowers the threshold to an osmotic thirst stimulus (Kozlowski, Drzewiecki & Zurawski, 1972). In some preliminary experiments with M. Erskine we found that the injection of 6 ng of angiotensin II (Hypertensin, CIBA) into the third ventricle of the dog elicits drinking.

It therefore seemed reasonable to investigate the possibility that an increase in water intake may contribute to the oedema associated with the development of congestive cardiac failure following constriction of the thoracic inferior vena cava in the dog, a situation known to be associated with a raised plasma renin level (Davis, 1967). A preliminary report that thoracic inferior vena caval constriction leads to increased drinking has already been published (Ramsay, Rolls & Wood, 1973). These experiments have been repeated with measurement of some body fluid variables, and the effect of restriction of water intake on these variables investigated. In order to test the proposition that the increased drinking observed in dogs with thoracic caval constriction may be related to an elevated plasma angiotensin level, the effect on drinking of giving a competitive angiotensin inhibitor, saralasin acetate, was investigated.

METHODS

Animals

All experiments were carried out on mongrel dogs or bitches weighing between 10 and 22 kg, housed individually in kennels. Experience has shown it necessary to keep the animals to a strict routine of feeding and activity when investigating drinking. Typically, the kennels were cleaned out between 9.00 and 10.00 daily, during which time the dogs exercised. Each animal received a fixed amount of synthetic food (Dog Diet; Spratts Patent Ltd), based on its body weight, at 16.00. Chemical analysis had shown that the composition of synthetic food varied less than tinned meat. Lighting was on from 9.00 to 17.00 and temperature and humidity were controlled to 70° C and 60 % by an air-conditioning system. A float and kymograph apparatus in each kennel enabled continuous records of water intake to be made (Fig. 1).

Experimental procedures

Pre-operative measurements. For the main series of experiments 4 dogs were used, and the protocol was similar to that previously reported (Ramsay *et al.* 1973; Rolls & Ramsay, 1974) and is described in more detail here. Each dog was maintained under the regime described above for 7 days to establish the control levels of drinking. At the end of this period, a venous blood sample was taken via a leg vein cannulated under local anaesthesia, for the determination of haematocrit, plasma osmolality and plasma sodium, potassium and protein concentrations. The extracellular fluid volume was then determined using [1-^{14}C]mannitol (Rampton & Ramsay, 1974) and plasma volume with Evans Blue. Both space measurements were carried out on the unanaesthetized animal and involved the collection of a number of blood samples over 1 hr following a single I.V. injection of the marker.

Operative procedure. The day following these measurements, the animals were anaesthetized with sodium pentobarbitone and the chest opened via a right thoracotomy in the fifth intercostal space to give a good exposure of the inferior vena cava, which was then constricted to about one half its original diameter with a silk ligature. The chest was closed in layers and the pneumothorax reduced through a drainage tube and underwater seal before returning the animals to their kennels to recover.

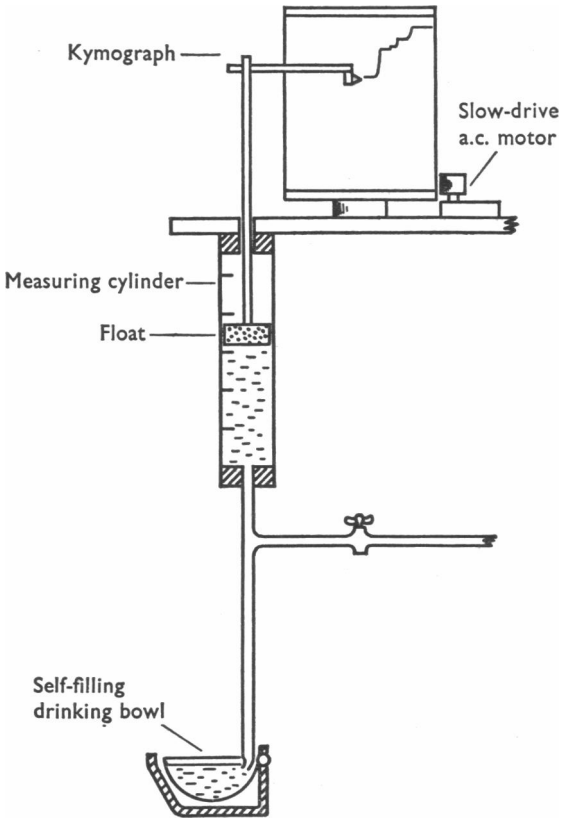

Fig. 1. The apparatus used for continuous recording of water intake.

Post-operative procedures. The animals recovered quickly from the operation and were eating normally by the following day. The daily fluid intake was recorded for seven days. At the end of this period, the measurements of extracellular fluid and plasma volumes, haematocrit, plasma osmolality, sodium, potassium and protein concentrations were repeated.

Restriction of water intake. The mean daily water intake for the pre-operative control period was calculated. The four dogs were then allowed this volume of water to drink, divided into two equal amounts given at 11.00 and 16.00. Intake was restricted over a period of 3 weeks for two of the dogs, and 4 weeks for the other two dogs, with the body fluid measurements repeated at weekly intervals. Following these periods of restricted intake, free access to water was allowed for 1 week and the body fluid measurements repeated on two of the dogs.

Saralasin acetate. Saralasin acetate (1-Sar-8 Ala-angiotensin II, Norwich Pharmacal), a competitive inhibitor of angiotensin, is most effective when administered by intravenous infusion because of its short half life in the body. So that the effect of the inhibitor could be studied over a period of several hours, a regime was devised under which access to water was allowed only during a restricted period in the day, namely 11.00 to 17.00. At all other times the dogs were left without water. After 6 days acclimatization to this procedure, the water intake during these 6 hr was similar to that when the dogs were allowed access to water for 24 hr.

These experiments were carried out on two bitches which had undergone caval constriction nineteen days previously, and were showing elevated levels of drinking (+34 and 94%), extracellular fluid volume (+21 and 40%) and plasma volume (+43 and 41%).

The experimental procedure was as follows. Each animal was placed on a table for the duration of the experiment which fell within the time period for which the dogs were normally allowed access to water. A hind-limb vein was cannulated, and an infusion of saline at a rate of 0·17 ml. min^{-1} began at 12.15. After 30 min, the dog was allowed access to water. Intake was measured for 1 hr, the animal was fed and drinking was recorded for a further 2 hr. The infusion was terminated at the end of this $3\frac{1}{2}$ hr period.

The following day, the dogs were allowed access to water in their kennels on the restricted schedule (11.00 to 17.00) and water intake was recorded. On the third day the experiment was repeated as above, with saralasin acetate in the saline infusate, dissolved to give a dose of 4 μg kg^{-1} min^{-1}, and the fluid intake measurements were repeated.

Chemical analyses. The activity of [1-^{14}C]mannitol in the timed plasma samples was estimated in a Unilux II scintillation counter (Nuclear Chicago) using Aquasol (NEN chemicals) as the scintillant, and counting to at least 10,000 counts except in the blank samples.

The concentration of Evans Blue in the same plasma samples was determined colorimetrically using a Unican ultraviolet spectrophotometer (S.P. 800 B) and reading from a calibration curve for Evans Blue in dog plasma.

Plasma osmolality was read with a semi-automatic osmometer (Precision Systems) and plasma sodium and potassium concentrations, using an automatic internal-standard-flame photometer (Instrumentation Laboratory 343).

Plasma protein concentration was measured using the falling-drop technique of Phillips, Van Slyke, Hamilton, Dole, Emerson & Archibald (1950) and the formula of Van Slyke, Hiller, Phillips, Hamilton, Dole, Archibald & Eder (1950), assuming the calibration curve of dog plasma to be the same as that for human plasma.

Blood samples for haematocrit determination were spun in capillary tubes of internal diameter 1·1 mm and 75 mm length (Biological Research, St Louis) for 3 min using a Hawksley Microhaematocrit Centrifuge. In reading the length of the red-cell layer, no correction was made for trapped plasma and the buffy coat was excluded from this measurement.

Statistics. Means and the S.E. of the means have been calculated. Tests of statistical significance have been carried out using the method of paired comparisons.

RESULTS

Effect of caval constriction on drinking and body fluids. In this series of experiments, as in that previously reported (Ramsay *et al.* 1973; Rolls & Ramsay, 1974), constriction of the thoracic inferior vena cava produced

an increase in drinking (Fig. 2). Control animals with a constriction of the abdominal inferior vena cava showed no such increase. The increase in drinking after thoracic caval constriction was a mean of 94% ($P < 0.02$, $n = 4$), associated with increases in extracellular fluid volume and plasma volume of 21% ($P < 0.05$) and 40% ($P < 0.05$) respectively, when these

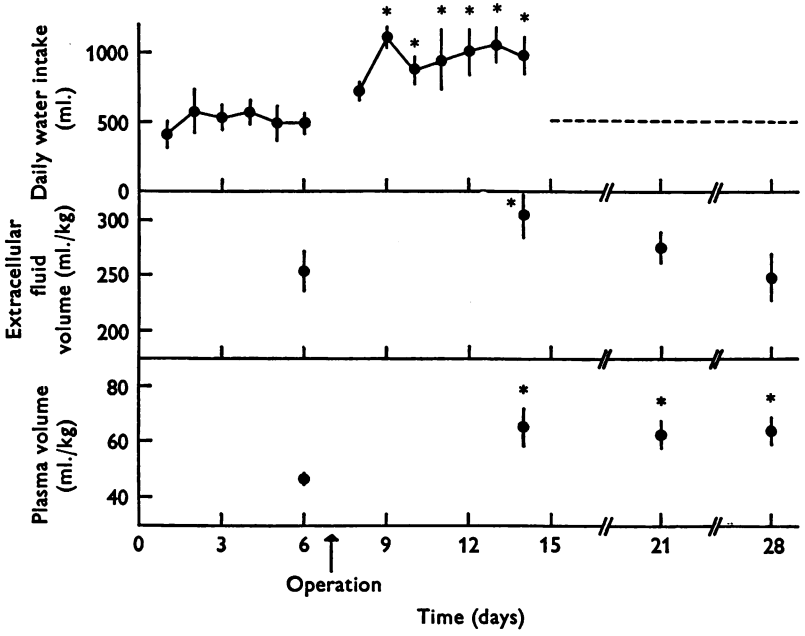

Fig. 2. The plasma volume, extracellular fluid volume and daily water Intake before and after thoracic inferior vena caval constriction. On days 8–14 the dogs were given *ad libitum* access to water. The first 2 weeks of fluid intake restriction are shown. On days 15–28, the dogs were restricted to their mean pre-operative water intake. Four dogs were used in the study. * Indicates a result which is significantly different from the pre-operative level. Vertical bars show the S.E. of the mean.

TABLE 1. Plasma composition (mean ± S.E. of mean) before and after thoracic caval constriction with and without water restriction ($n = 4$)

Variable	Pre-operative	1 week post-operative *ad lib.* water	Following 2 weeks of water restriction
Osmolality (m-osmole/kgH_2O)	300·0 ± 2·7	297·0 ± 3·3	301·0 ± 3·1
Na^+ (m-equiv/l.)	146·0 ± 1·1	148·0 ± 0·8	149·0 ± 1·7
K^+ (m-equiv/l.)	4·2 ± 0·1	4·2 ± 0·1	4·1 ± 0·1
Plasma protein (g%)	4·6 ± 0·08	3·4 ± 0·34	3·7 ± 0·31
Haematocrit	43·0 ± 1·3	32·0 ± 2·5	36·0 ± 2·0

variables were measured after 1 week. The changes in haematocrit, plasma osmolality, plasma protein and electrolyte concentrations are shown in Table 1. One week following caval constriction, there was a slight decrease

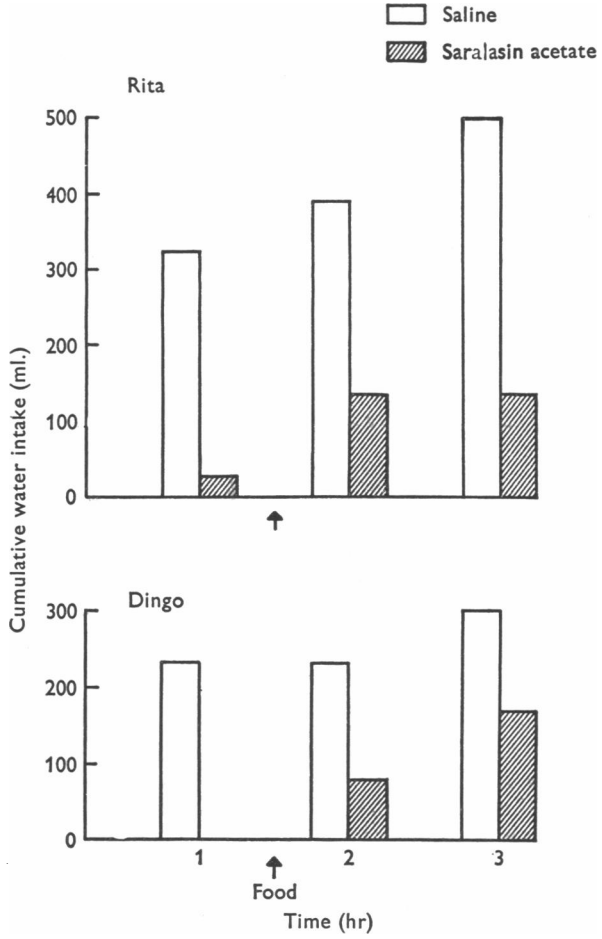

Fig. 3. The cumulative water intake for two dogs during a 3 hr infusion of either isotonic saline or saralasin acetate. Each block represents the cumulative volume of water drunk in ml. during 1 hr. The normal saline was infused at a rate of 0·17 ml. min^{-1}. The saralasin acetate was dissolved in the saline infusate to give a dose of 4 μg kg^{-1} min^{-1}. The dogs were fed 1 hr after the start of the infusion.

in osmolality, but this and any differences in the sodium and potassium levels were insignificant. Both the haematocrit and plasma protein concentration went down significantly ($P < 0{\cdot}02$ and $P < 0{\cdot}05$ respectively) by amounts consistent with the increase in plasma volume.

Effect of restricted water intake. The effect of the first 2 weeks of restricted water regime on the body fluids is shown in Fig. 2 and Table 1. The extracellular fluid volume was reduced by this procedure. One week following the commencement of fluid restriction the extracellular fluid volume had begun to decrease and after 2 weeks of restriction it had returned to control levels. The plasma volume, however, was not affected by water restriction and remained elevated. Reference to Table 1 shows that water restriction did not significantly alter plasma osmolality, protein, sodium or potassium concentrations nor the haematocrit. Continuing the period of restricted intake for another week (two dogs) or 2 weeks (two dogs) did not alter these variables further.

Effect of returning to an ad libitum water regime. After the dogs were allowed free access to water again for a period of 6 complete days, the daily records showed drinking to be elevated once more above the pre-operative level, but not to the same extent as formerly. The mean intakes (738 ± 82 ml.) lay about half way between the pre-operative and early post-operative levels. The body fluids, as measured in two dogs at this stage, did not differ significantly from the last measurements during restricted intake.

Experiments with saralasin acetate. The results for this experiment are presented in Fig. 3. During the control saline infusion on the table, water intake was similar to the normal pattern in the home kennel for the first hour of water access, and for the 2 hr following feeding. The infusion of saralasin acetate produced a marked decrease in drinking during the pre-prandial hour for one dog and complete abolition in the other.

DISCUSSION

These experiments show that following thoracic caval constriction in the dog, water intake increases significantly and extracellular fluid and plasma volumes are elevated. Thus increased fluid intake is probably an important factor in the development of the oedema which accompanies low-output heart failure.

In the dog, thoracic caval constriction is associated with increased drinking and abdominal caval constriction is not, while Fitzsimons (1964) reports that in the rat, ligation of the abdominal region of the inferior vena cava stimulates drinking. The difference may be the degree of constriction. The technique used by Fitzsimons in the rat was to ligate the vena cava so as to completely stop flow. Our technique in the dog is to reduce the diameter of the vena cava to about half its normal size to reduce flow. Such a technique when applied to the abdominal vena cava appears to be without measurable effect on either the circulation or fluid balance.

However, Davis, Kliman, Yankopoulos & Petersen (1958) reported that in two out of a number of dogs subjected to abdominal caval constriction similar changes in fluid balance were seen compared with a group of thoracic caval constriction dogs. In these two dogs the ligature had been tied too tightly around the abdominal vena cava, producing a more drastic reduction in diameter. It would appear that the difference between Fitzsimons' results and ours might depend upon the degree of obstruction to flow produced by ligation as opposed to constriction of the abdominal vena cava.

Restriction of water intake in dogs which had been allowed to drink *ad libitum* for a week after caval constriction reduces the measured extracellular fluid volume to pre-operative control values within two weeks. It is of some interest to note that the plasma volume was not reduced by water restriction. Possibly the continuance of the low output condition reflexly maintained an increased precapillary resistance and hence a balance of capillary Starling forces that did not favour filtration.

The reduction in extracellular fluid volume observed during water restriction reinforces the hypothesis that stimulation of water intake probably acts as a significant causative factor in the oedema which develops after caval constriction. It appears that if the dog is not allowed to increase its water intake, the oedema associated with congestive cardiac failure cannot occur. These findings may therefore be of some significance in the clinical treatment of congestive cardiac failure. Holmes (1960) has reported that patients with congestive cardiac failure sometimes show intense thirst. It would be of interest to determine whether restriction of fluid intake would be beneficial in the treatment of oedema in these patients. The relationship between water and sodium balance during the development of oedema, and during fluid restriction, should also be investigated.

The mechanism of stimulation of water intake following caval constriction in the dog is not clear. In the rat, the drinking after caval ligation is reduced by nephrectomy and would appear to be mediated by angiotensin (Fitzsimons, 1964, 1969). In the dog, angiotensin might also play a primary role in increased water intake following caval constriction, since caval constriction has been shown to elevate plasma renin levels (Schneider, Davis, Robb & Baumber, 1969) and since the competitive angiotensin inhibitor, saralasin acetate, decreased the drinking of two dogs in congestive cardiac failure. It is clearly of importance to evaluate this in more detail. In a further series of experiments we will measure plasma renin and angiotensin levels in dogs with elevated drinking.

From these results and others reported previously (Ramsay *et al.* 1973; Rolls & Ramsay, 1974) it appears that the renin–angiotensin

system could be important in the control of water intake in the dog. Drinking occurs after an elevation of endogenous angiotensin following acute constriction of the renal artery, when the blood pressure will be high, and following constriction of the thoracic part of the inferior vena cava, when initially the venous return and blood pressure will be low (Davis, 1965). In these two situations therefore, the increased drinking was correlated with high renin–angiotensin levels, not with cardiovascular baroreceptor or volume receptor reflexes. Furthermore, isoprenaline-induced drinking is abolished by the competitive angiotensin inhibitor, saralasin acetate, in the dog (Rolls & Ramsay, 1974). Thus in the various situations we have studied which lead to drinking, it may be inferred that the common feature has been increased levels of plasma angiotensin.

The results reported in this paper indicate that a stimulation of water intake may be important in the aetiology of the oedema associated with congestive cardiac failure, probably through the renin–angiotensin system.

This work was supported by the Medical Research Council. We wish to thank Dr A. W. Castellion of Norwich Pharmacal for supplying the saralasin acetate and Mrs B. Barber for technical assistance.

REFERENCES

DAVIS, J. O. (1965). The physiology of congestive heart failure. In *Handbook of Physiology*, section 2, vol. III, ed. HAMILTON, W. F., pp. 2071–2122. Washington, D.C.: American Physiological Society.

DAVIS, J. O. (1967). The regulation of aldosterone secretion. In *The Adrenal Cortex*, ed. EISENSTEIN, A. B., pp. 203–247. Boston, Massachusetts: Little, Brown and Company.

DAVIS, J. O., KLIMAN, B., YANKOPOULOS, N. A. & PETERSEN, R. E. (1958). Increased aldosterone secretion following acute constriction of the inferior vena cava. *J. clin. Invest.* **37**, 1783–1790.

EPSTEIN, A. N., FITZSIMONS, J. T. & ROLLS, B. J. (1970). Drinking induced by injection of angiotensin into the brain of the rat. *J. Physiol.* **210**, 457–474.

FIZTSIMONS, J. T. (1964). Drinking caused by constriction of the inferior vena cava in the rat. *Nature, Lond.* **204**, 479–480.

FITZSIMONS, J. T. (1969). The role of renal thirst factor in drinking induced by extracellular stimuli. *J. Physiol.* **201**, 349–368.

FITZSIMONS, J. T. & SIMONS, B. J. (1969). The effect on drinking in the rat of intravenous infusion of angiotensin, given alone or in combination with other stimuli of thirst. *J. Physiol.* **203**, 45–57.

HODGE, R. L., LOWE, R. D. & VANE, J. R. (1966). The effects of alteration of blood volume on the concentration of circulating angiotensin in anaesthetized dogs. *J. Physiol.* **185**, 613–626.

HOLMES, J. H. (1960). The thirst mechanism and its relation to edema. In *Edema: Mechanisms and Management*, ed. MOYER, J. H. & FUCHS, M., pp. 95–102. Philadelphia: Saunders.

HOLMES, J. H. & CIZEK, L. J. (1951). Observations on sodium chloride depletion in the dog. *Am. J. Physiol.* **164**, 407–414.

KOZLOWSKI, S., DRZEWIECKI, K. & ZURAWSKI, W. (1972). Relationship between osmotic reactivity of the thirst mechanism and the angiotensin and aldosterone level in the blood of dogs. *Acta physiol. pol.* **23**, 417–425.

PHILLIPS, R. A., VAN SLYKE, D. D., HAMILTON, P. B., DOLE, V. P., EMERSON, K. & ARCHIBALD, R. M. (1950). Measurement of specific gravity of whole blood and plasma by standard copper sulphate method. *J. biol. Chem.* **183**, 305–330.

RAMPTON, D. S. & RAMSAY, D. J. (1974). The effects of pentobarbitone anaesthesia on the volume and composition of the extracellular fluid of dogs. *J. Physiol.* **237**, 521–533.

RAMSAY, D. J., ROLLS, B. J. & WOOD, R. J. (1973). Increased drinking in dogs during congestive heart failure. *J. Physiol.* **234**, 48–50*P*.

ROLLS, B. J. & RAMSAY, D. J. (1974). The elevation of endogenous angiotensin and thirst in the dog. In *Control Mechanisms of Drinking*, Proceedings of a Symposium on Thirst held in Lugano, Switzerland, October 20 and 21, 1973. Ed. FITZSIMONS, J. T., PETERS, G. & PETERS, L. Heidelberg: Springer-Verlag (in the Press).

SCHNEIDER, E. G., DAVIS, J. O., ROBB, C. A. & BAUMBER, J. S. (1969). Hepatic clearance of renin in canine experimental models for low- and high-output heart failure. *Circulation Res.* **24**, 213–219.

SZCZEPANSKA-SADOWSKA, E. (1973). Plasma ADH level and body water balance in dogs after a moderate haemorrhage. *Bull. acad. Pol. Sci.* **21**, 89–92.

VAN SLYKE, D. D., HILLER, A., PHILLIPS, R. A., HAMILTON, P. B., DOLE, V. P., ARCHIBALD, R. M. & EDER, H. A. (1950). The estimation of plasma protein concentration from plasma specific gravity. *J. biol. Chem.* **183**, 331–347.