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VASOPRESSIN CLEARANCE AND SECRETION DURING HAEMORRHAGE IN NORMAL DOGS AND IN DOGS WITH EXPERIMENTAL DIABETES INSIPIDUS

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SUMMARY

1. The secretion of vasopressin in response to haemorrhagic shock has been investigated in anaesthetized dogs.

2. The changes in the plasma concentrations of vasopressin were followed over a period of 5 hr, during which the arterial blood pressure was kept constant at 40 mm Hg. It was found that vasopressin concentration in plasma rose to a high peak shortly after the onset of shock and gradually declined thereafter. Five hours later, it was still 3·5 times higher than control. Re-transfusion of blood was followed by a return to control levels.

3. The clearance of vasopressin was calculated before and during shock in normal dogs and in dogs with experimental diabetes insipidus. Soon after the onset of shock, the clearance rate dropped to one quarter of its normal level but slowly recovered, returning to near control values at the fifth hour of shock. Clearance rates did not vary as a function of infusion rates, suggesting that there is no maximal transport rate for the removal of the hormone over the entire secretory range found in normal and hypotensive dogs.

4. From the clearance rates and from the plasma concentrations of endogenously secreted vasopressin it has been possible to calculate the approximate secretory rates of the hormone in response to shock. Secretion rose to a very high level, some 40 times greater than control, at the onset of shock. This was followed by a fairly constant secretory plateau. At the fifth hour of shock secretion was 3·5 times higher than control.

5. The half-life of vasopressin was measured in normal and hypotensive dogs. Control measurements confirm the generally accepted value of approximately 5 min. The half-life was significantly higher in the early stage of shock, but returned to control values in the later stage.

6. Haemorrhage experiments performed in normal and diabetic dogs

suggest that vasopressin may play a part in the development of irreversible haemorrhagic shock: all normal animals died within a few hours of retransfusion, whereas four out of eight diabetic dogs similarly treated survived a 24 hr observation period. In a separate set of experiments, eight diabetic dogs were subjected to the haemorrhage procedure while receiving a constant infusion of vasopressin: only two of these survived. Surviving dogs showed none of the characteristic lesions of irreversible haemorrhagic shock.

INTRODUCTION

It has often been suggested (Weinstein, Berne & Sachs, 1960; Pickford, 1966; Somlyo & Somlyo, 1970) that the large amounts of circulating vasopressin found in response to haemorrhagic hypotension in various mammalian species may play a part in the vasomotor defence mechanism, but direct evidence in support of this suggestion is very fragmentary. It has been shown that the quantities of vasopressin released in haemorrhagic shock are sufficient to induce a rise in arterial blood pressure in a dog with denervation of the baroreceptor mechanisms (Rocha e Silva & Rosenberg, 1969).

It has been suggested that vasopressin secretion during maintained haemorrhagic hypotension is phasic, occurring in bursts of irregular frequency, duration and intensity (Gilmore, 1968; Vane, 1969), and not continuous and sustained throughout relatively long periods of hypotension as reported by various groups (Sachs, Share, Osinchak & Capri, 1967; Clark & Rocha e Silva, 1967; Share, 1967; Rocha e Silva & Rosenberg, 1969).

It should, however, be noted that no direct proof is available to show that the high blood concentration of vasopressin found during shock is entirely attributable to increased secretion; it could in fact be partly due to a reduced clearance rate of the hormone rather than to increased secretion. A reduced clearance would be a likely consequence of hypotension because the renal and hepatic circulations are severely decreased in this condition (Chien, 1967).

Ginsburg & Heller (1953) showed that haemorrhage in rats causes an increase in vasopressin concentration in the carotid artery and in the external jugular vein, but that the latter increase is much more pronounced than the former: this was the first unequivocal proof of vasopressin release in response to haemorrhage. The technique cannot, unfortunately, be used in dogs because the external jugular does not drain the neurohypophysis (and does not therefore contain more vasopressin than other peripheral vessels; Rocha e Silva & Rosenberg, 1969). The internal jugular, if present, does not yield sufficient amounts of blood for reliable sample collection. Moreover, the quantitative estimation of secretion rates would

require in addition to arteriovenous differences, the determination of the venous outflow of all vessels draining the neurohypophysis. The present paper describes an investigation on the plasma concentrations, the clearance rates and the estimation of secretory rates during long lasting haemorrhagic hypotension. Clearance measurements were performed in normal dogs and in dogs with experimental diabetes insipidus. The results indicate that the secretion of vasopressin rises to an intense peak, 40 times greater than control, at the onset of shock. After this, it decreases to a secretory plateau, 3·5 times higher than control which is maintained throughout the entire period of shock. Further, it was found that plasma concentrations of the hormone alone are not a true indicator of the secretory levels because clearance rates vary continuously throughout the period of hypotension.

The problem of the half-life of vasopressin in the circulatory system of the dog has also been re-examined, in view of recent results (Gilmore & Vane, 1970) suggesting that it may be much shorter than was originally supposed (Lauson, 1967).

Observations on some aspects of haemorrhagic shock in the absence of endogenous vasopressin secretion indicate that the hormone may play an important part in the development of irreversible haemorrhagic shock, although much more detailed work is required before such an indication can be finally accepted.

METHODS

Experiments were performed on forty Beagle dogs, thirty-eight males, two females, weighing between 9 and 18 kg and anaesthetized with pentobarbitone sodium (25 mg/kg), given I.P. In four experiments, a single supplementary dose of a fifth of the initial dose was subsequently given, intravenously. In all other experiments, no more anaesthetic was used. A tracheal tube was inserted to ensure a clear airway and blood pressure was recorded from a cannulated femoral artery by means of a strain gauge transducer (Statham P23Db) connected to a potentiometric recorder (Smith's Servoscribe) or to one channel of a galvanometric recorder (Devices M2). Heart rate was read directly from the e.c.g., displayed continuously on a Tektronix oscilloscope (Type 502). Body temperature was kept between 36·5 and 38·5° C, by means of an adjustable heater contained in the operating table. An interval of at least 60 min was allowed between the end of the surgical procedure and the start of the experiment. In haemorrhage experiments, bleeding was not begun until the depth of anaesthesia had reached such a level as to allow the presence of a clear cut bilateral leg stretch reflex in response to very gentle compression of the quadriceps surae muscle of one leg.

Haemorrhage procedure. Haemorrhage was produced by the method of Lamson & de Türk (1945): a Y-shaped segment was adapted to the femoral artery cannula and one of its branches connected to a reservoir which could be moved vertically with respect to the heart of the dog. Blood clotting was prevented by a single I.V. injection of heparin 500 u./kg. Haemorrhage was produced by opening the femoral cannula into the reservoir; blood pressure was lowered to 40 mm Hg over a period of 15 min and kept at this level for a further period of 5 hr. Zero time was taken to

be the moment when blood pressure first fell to 40 mm Hg. At the end of the fifth hour, all blood remaining in the reservoir was re-transfused intra-arterially, over 15 min. The volume of blood in the reservoir was noted at 15 min intervals over the entire period of hypotensive shock. Following retransfusion, blood-clotting time was brought back to normal by an I.V. injection of a suitable amount (usually 5 mg/kg) of protamine sulphate. The arterial cannula was removed, the skin sutured and the animal placed in a heated post-operative kennel and observed for 24–72 hr. Twenty such experiments were performed on normal dogs and sixteen on dogs with chronic diabetes insipidus, induced by a surgical lesion of the median eminence of the hypothalamus.

Induction of chronic diabetes insipidus. Experimental diabetes insipidus was induced by a transverse cut of the median eminence of the hypothalamus. Under pentobarbitone sodium anaesthesia (25–30 mg/kg), given I.P., the anterior part of the *Sella turcica* of the sphenoid was exposed through a transbuccal approach. The intersphenoid suture and the occipitosphenoid suture were identified, as well as the position of the emissary vein which lies caudal to the intersphenoid suture (McLean, 1928). The bone was removed from the region just ahead of the emissary vein. The dura mater was slit open and the anterior pole of the hypophysis identified. A transverse surgical lesion was placed in the region limited by the optic chiasma, anteriorly and the pole of the hypophysis, posteriorly. This ensures that minimal damage is caused to the function of the anterior hypophysis and that the whole of the hypothalamo-neurohypophysial system is sectioned. Drinking water was made freely available and the intake of these animals was measured daily for the first 17 days following the operation. An interval of at least 22 days (mean 35 days; maximum 90 days) was allowed between this operation and the experiment. A post-mortem anatomical and histochemical check of the hypothalamo-hypophysial system was carried out at the end of such experiments (see below).

Determination of the clearance and half-life of vasopressin. The total clearance of vasopressin was estimated in twenty haemorrhage experiments, ten in normal dogs, ten in diabetic dogs, before and during hypovolaemic shock. The control (pre-haemorrhage) clearance was determined by measuring the plasma concentration of vasopressin in a venous blood sample collected at the end of a 20 min constant-rate intravenous infusion of 1–10 m-u. min^{-1} vasopressin (Pitressin, Parke-Davis). The total clearance of vasopressin, C, is the ratio $C = Ex/P$ where Ex is the extraction rate of the hormone (u./min) and P the plasma concentration (u./ml.). Assuming that the I.V. infusion has produced a steady-state concentration, the infusion rate I must be equal to the extraction rate Ex and one can therefore write $C = I/P$.

Clearances during hypotensive shock were determined in a similar manner, but in this case the infusion was kept going throughout the entire period of shock. The assumption of steady-state concentrations cannot be made for these measurements, because it was never possible to produce a constant plasma concentration with a constant infusion. Values here are therefore only approximate estimations of the true clearance rates. In normal dogs, clearance values were estimated only from the second to the fifth hour after the onset of shock, because of the high endogenous levels of vasopressin in blood in the early stages of shock; in diabetic dogs, clearances were estimated over the entire period of shock. In seven experiments, performed on diabetic dogs, an additional sample of blood was collected 3·5 min after the start of the pre-haemorrhage infusion. The concentration of vasopressin in plasma in this sample was compared to that in the 20 min sample to determine whether a steady-state plasma concentration had been reached at this early stage.

The half-life of vasopressin was determined, in the pre-haemorrhage phase (control half-life), in normal and diabetic dogs, by measuring vasopressin concentrations in

serial blood samples collected after stopping of the pre-haemorrhage infusions. Samples collected at the end of 20 min infusions (for the estimation of control clearances) were used as first samples in the series for half-life determinations. The second sample was collected 5 or 10 min later. In some experiments, a third sample was collected after a similar interval. In normal dogs, the half-life was also determined following single injections of vasopressin. This was done in the pre-haemorrhage period, as well as at the second and fourth hour following the onset of shock. In these experiments, an interval of 10 min was allowed between the injection and the first sample of each series.

Vasopressin determination. Blood sampling, extraction and assay. Blood samples (5 ml.) were collected from a femoral vein, with simultaneous replacement of an equal volume of Dextran Solution (Glaxo, Intradex, B.P.). Samples for the estimation of half-lives were collected at a steady rate, over an exactly measured period of 30 sec and collection time was taken to be the mid-point of the collection interval. In the first eleven experiments, blood samples were extracted according to the method of Bisset, Hilton & Poisner (1967), and the concentration of vasopressin expressed per ml. of blood. In the remaining experiments, a slight modification was introduced: following centrifugation of blood, 2 ml. plasma was separated and added to 6 ml. absolute ethanol; the mixture was centrifuged and the supernatant concentrated *in vacuo* at 55° C; vasopressin concentration was expressed per ml. of plasma. Blood concentrations, measured in the first experiments, were converted to plasma concentrations, taking into account mean haematocrit values determined in a series of eight experiments performed in strictly comparable conditions.

Antidiuretic activity was measured by I.V. injections into water-loaded ethanol-anaesthetized rats, according to the method of Dicker (1953), with the modifications described by Bisset (1962) and Clark & Rocha e Silva (1967). All samples except subthreshold and near-threshold samples were assayed by means of a 2×2 assay; during a series of eight consecutive experiments, thirty-eight samples were selected for which a second 2×2 assay was performed; the ratio between first and second duplicate assays of a sample (R1/R2) was used as a measure of the variability of the assay procedure. The following rules were set for the selection of samples to be replicated:

a Samples were assayed in a strictly defined sequence, in each experiment.

b At least four samples from each experiment were assayed twice.

c Samples assayed first on a newly established rat were assayed twice.

d Samples related to the measurement of the half-life were assayed twice.

e Whenever possible, duplication was carried out on a different rat, two being set up simultaneously.

Post-mortem examination. The lungs, kidneys, liver, stomach and intestines were systematically examined in newly killed dogs. Samples of these tissues were fixed in 10% formol saline. Frozen or paraffin sections, cut 5–10 μm, were stained with haematoxylin and eosin or with Mallory aniline blue.

At the end of each experiment, the head of the animal was perfused with 0·9% NaCl solution, followed by 10% formol saline. The brain, with the pituitary gland attached, was removed and stored in 10% formol saline to ensure complete fixation. Frozen sections of 20 μm were cut in the coronal plane and stained with Luxol fast blue and Cresyl violet (Klüver & Barrera, 1953) or for neurosecretory material with performic acid–Victoria blue (J. C. Sloper, personal communication). This is a modification of the method described by Adams & Sloper (1956).

Statistical analysis. The significances of differences between means were estimated by *t* tests. The variances for vasopressin secretory rates ($Var\ (S)$) were estimated from the variances for vasopressin clearance rates ($Var\ (C)$) and from the variances

for endogenous vasopressin plasma concentrations ($Var\ (P)$), according to the following relation:

$$Var\ (S) = Var\ (C \times P) = C^2\ Var\ (P) + P^2\ Var\ (C),$$

where C and P are respectively mean values for clearance rates and endogenous plasma concentrations. $Var\ (S)$ was used to estimate the S.E. of mean for vasopressin secretion.

Materials. All cannulae, glassware and needles which were to be in contact with blood were siliconed (Silicone Repelcote, Hopkin & Williams) to prevent kinin formation in blood samples and to retard clotting in haemorrhage experiments. Extracts related to Pitressin infusions were assayed against the infused solution. All other blood extracts, as well as every batch of Pitressin to be used for infusion, were assayed against synthetic arginine vasopressin (Sandoz) of standardized antidiuretic activity. There was generally good agreement between the potency of the arginine-vasopressin standard and the nominal potency of Pitressin batches.

RESULTS

Precision of the vasopressin assay

Thirty-eight samples, selected as described in the Methods section, were assayed twice in order to estimate the variance of individual assays. The R1/R2 duplicate ratio was found to be $0{\cdot}99 \pm 0{\cdot}08$ (S.D. of an observation), indicating that the 95% confidence limit of the assay procedure is in the region of 15% on either side of measured values.

Water intake of diabetic dogs

Fig. 1 shows the average daily water intake of all diabetic dogs used in the present experiments during the first 17 days following the establishment of the hypothalamic lesion. The dogs are divided into two groups: continuous lines show the intakes of dogs subsequently used for simple haemorrhage experiments; interrupted lines show intakes of dogs subsequently used for haemorrhage experiments with vasopressin infusions. There is no significant difference between the curves, both groups showing the typical pattern of intake following the destruction of the hypothalamo-hypophysial tract (Fisher, Ingram, Hare & Ranson, 1935; Fisher, Ingram & Ranson, 1937): after an initial phase of high water intake (the transient polyuria) comes the normal interphase, in turn followed by permanent polyuria. As the water intakes of animals in Groups A and B are essentially similar, it has been assumed that virtually no endogenous secretion of vasopressin occurred in Group B during haemorrhage, since virtually none could be detected in Group A.

Haemorrhage experiments

Normal dogs. Fig. 2, continuous lines, shows the results of haemorrhage experiments, performed on normal dogs. A standard shock procedure was

employed, leading to irreversible shock: the arterial blood pressure was lowered to 40 mm Hg and kept constant at this level for 5 hr, by means of the blood reservoir connected to the femoral artery. Although all the blood still remaining in the reservoir was then re-transfused intra-arterially, blood

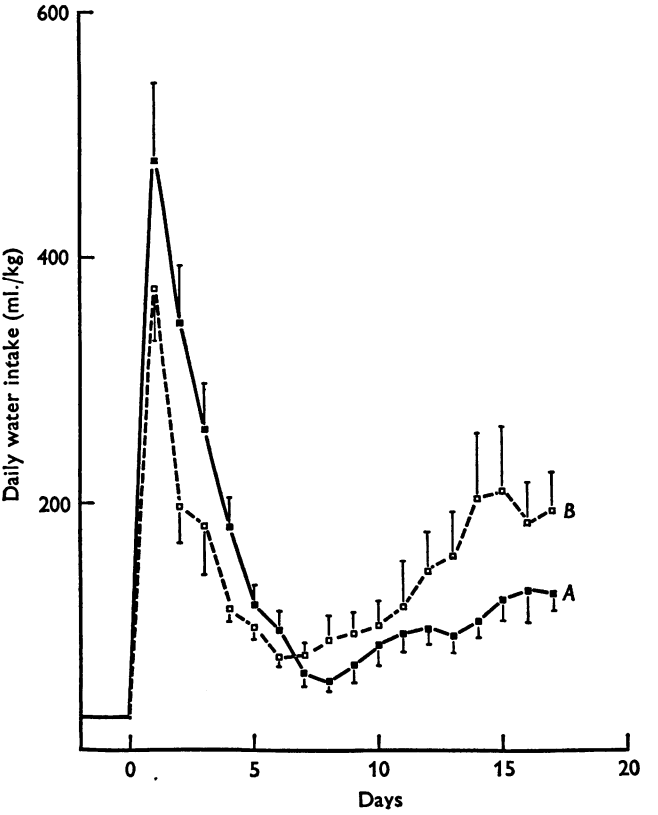

Fig. 1. Daily water intake (ml./kg) of sixteen dogs following surgical interruption of the hypothalamo-neurohypophysial tract by an incision in the median eminence, between the optic chiasma and the hypophysis, performed on day 0. The continuous line shows intakes of eight dogs subsequently used for simple haemorrhage experiments (Group *A*) and the interrupted lines show the same for eight dogs used for haemorrhage experiments with vasopressin infusions (Group *B*). Means and S.E. of means are shown in this and in all subsequent Figures. The horizontal section of the tracing, before day 0, represents the average water intake over a period of 3 days (24 ± 2 ml./kg; S.E. of mean; $n = 16$).

pressure only recovered partially; it gradually declined thereafter until death occurred, 6–10 hr later. The volume of blood in the reservoir (middle tracing) rose steeply as the blood pressure was brought to 40 mm Hg; it continued rising during the first hour of shock, although the blood pressure

was kept constant, because of the development of the general vasoconstrictor response to shock. The maximum bleeding volume (MBV), once reached, was maintained for approximately 1–1·5 hr and followed by a gradual uptake of blood from the reservoir as the general vasoconstrictor reaction slowly began to fade. No animals survived in this group of eight experiments. The lower tracing shows plasma concentrations of vaso-

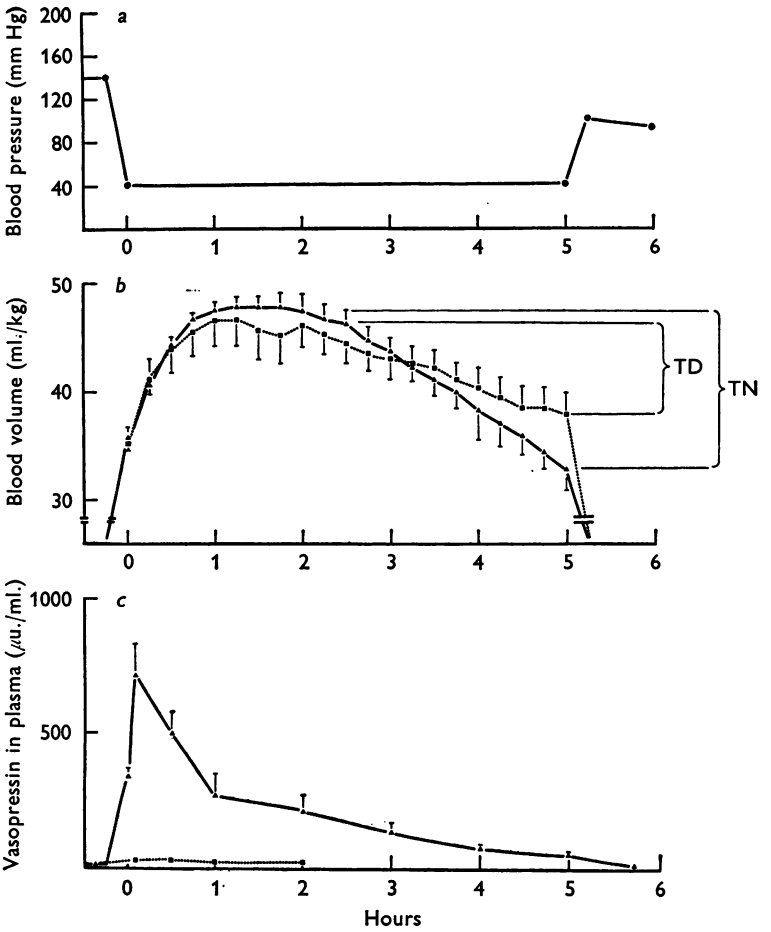

Fig. 2. Recordings of blood pressure, blood volume in the reservoir and plasma concentrations of vasopressin before, during and after haemorrhage experiments. Bleeding into the reservoir was started at −15 min. At zero time blood pressure first reached 40 mm Hg. All the blood remaining in the reservoir was retransfused 5 hr later. Continuous lines represent eight experiments in normal dogs, interrupted lines eight experiments in diabetic dogs. TN, total blood volume taken up in normal dogs. TD, total blood volume taken up in diabetic dogs. Control levels of vasopressin were 14 ± 2 μu./ml. in normal dogs, 9 ± 2 μu./ml. in diabetic dogs.

pressin, which rose abruptly, from a low control ($14{\cdot}5 \pm 1{\cdot}8$ μu./ml.), to a peak, 5 min after zero time (713 ± 119 μu./ml.). The vasopressin concentration gradually declined thereafter, but was still three times higher than control level at the fifth hour; re-transfusion was followed by a return to control levels.

Diabetic dogs. Fig. 2 (interrupted lines) shows the results of similar experiments performed on diabetic dogs. The basic pattern of the volume-in-reservoir curve is similar to that of the normal group, but the total volume of blood returned to the animal by the fifth hour, expressed as a percentage of MBV, was significantly smaller (see Table 1). In this series, four

TABLE 1. Haemorrhage experiments; Summary of data on maximum bleeding volume (MBV), time at which it was reached, start of uptake of blood from reservoir, uptake at the fifth hour of shock and survival rates for normal and diabetic dogs

	n	MBV (ml./kg)	Time MBV reached (hr)	Start of blood uptake (hr)	Uptake* (%)	Survival (%)
I Normal dogs	8	$48{\cdot}4 \pm 1{\cdot}3$	$1{\cdot}00 \pm 0{\cdot}12$	$2{\cdot}18 \pm 0{\cdot}16$	$32{\cdot}1 \pm 2{\cdot}4$	0
II Diabetic dogs	8	$47{\cdot}6 \pm 1{\cdot}9$	$1{\cdot}25 \pm 0{\cdot}31$	$2{\cdot}59 \pm 0{\cdot}36$	$19{\cdot}7 \pm 3{\cdot}9$	50
P**		$> 0{\cdot}7$	$> 0{\cdot}4$	$> 0{\cdot}3$	$< 0{\cdot}02$	—
III Diabetic dogs + infusion	8	$53{\cdot}2 \pm 3{\cdot}1$	$1{\cdot}15 \pm 0{\cdot}14$	$2{\cdot}15 \pm 0{\cdot}18$	$26{\cdot}4 \pm 3{\cdot}1$	25

* Expressed as a % MBV.

** Significance level for comparison between normal and diabetic dogs.

dogs out of eight were found to be alive at the end of a 24 hr observation period. One was unconscious but the other three were conscious, capable of walking and alert to sensory stimuli; they accepted food and drink and passed urine and faeces. Vasopressin was detectable in control samples in three out of eight dogs and at some stage during shock in seven out of eight experiments. The highest concentration of vasopressin found in any individual sample was 68 μu./ml., found in the 5 min sample of one of these animals. This individual value is less than one tenth of the average vasopressin concentration found in the 5 min samples of normal dogs.

Diabetic dogs with vasopressin infusions. Eight other haemorrhage experiments were performed on diabetic dogs, but in these a constant I.V. infusion of vasopressin was maintained throughout the period of shock (Fig. 3). Infusions were started simultaneously with the start of bleeding and continued until the start of re-transfusion at the fifth hour. Three different infusion rates (infusions A, B and C) were successively given in an attempt to mimic the pattern of the changes in blood concentration found following haemorrhagic shock in non-diabetic dogs. The solid horizontal

bars at the bottom of Fig. 3 show the duration of each infusion. Infusion *A* ranged from 2 to 10 m-u./min.kg, infusion *B* was 2·0–2·1 m-u./min.kg and infusion *C* ranged from 1 to 2 m-u./min.kg. The middle tracing shows the volume-in-reservoir curve which is again similar to those in the preceding groups. The total uptake of blood, expressed as a percentage of MBV, is intermediate between the normal group and the diabetic group without infusion (see Table 1). The lower tracing shows the mean values for vasopressin concentration in plasma. The curve is very similar to the

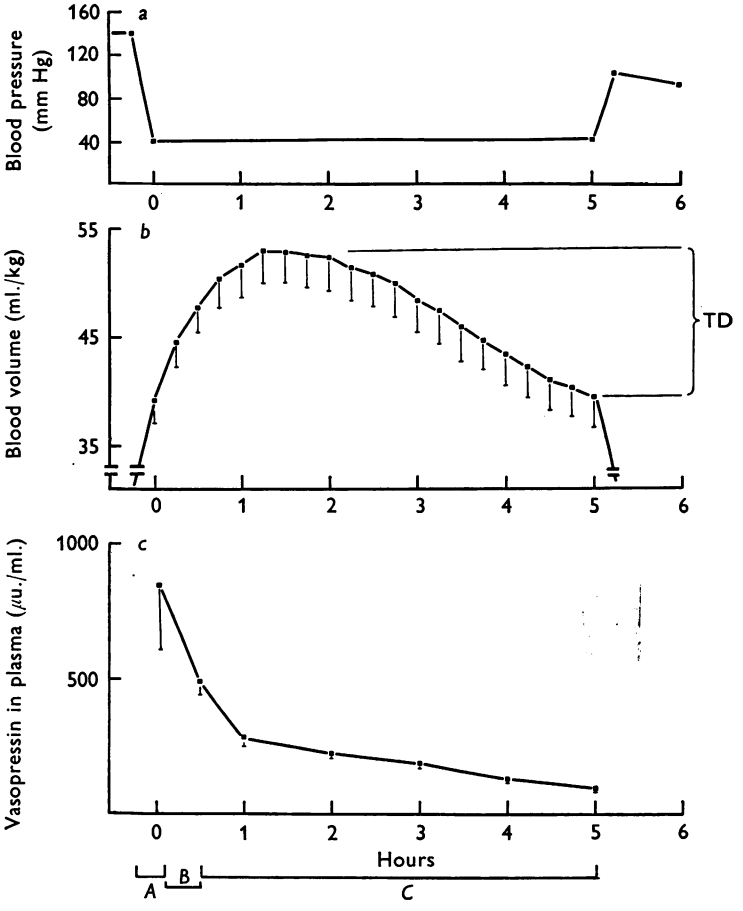

Fig. 3. Recordings of blood pressure, blood volume in the reservoir and plasma concentrations of vasopressin in eight haemorrhage experiments performed on diabetic dogs. Vasopressin was infused from −15 min to +5 min (infusion *A*), from 5 to 30 min (infusion *B*) and from 30 min to 5 hr (infusion *C*). TD, total blood volume taken up in diabetic dogs with vasopressin infusion. Individual values for infusion rates and measured plasma concentrations are given in Table 1.

TABLE 2. Infusion rates, plasma concentrations and clearance of vasopressin in diabetic dogs

Dog no.		Time after blood pressure reaching 40 mm Hg						
		5 min	30 min	1 hr	2 hr	3 hr	4 hr	5 hr
1	Infusion rate (m-u./min.kg)	2·1	2·1	2·1	2·1	2·1	2·1	2·1
	Plasma concn. (μu./ml.)	166	356	335	327	248	178	154
	Clearance (ml./min.kg)	13·2	6·2	6·6	6·7	8·8	12·4	14·4
2	Infusion rate (m-u./min.kg)	5·0	2·0	1·0	1·0	1·0	1·0	1·0
	Plasma concn. (μu./ml.)	328	368	185	179	137	114	93
	Clearance (ml./min.kg)	15·7	5·4	5·4	5·6	7·3	8·0	10·7
3	Infusion rate (m-u./min.kg)	10·0	2·0	1·0	1·0	1·0	1·0	1·0
	Plasma concn. (μu./ml.)	1000	490	238	232	156	112	97
	Clearance (ml./min.kg)	10·0	4·1	4·2	4·3	6·4	8·9	10·3
4	Infusion rate (m-u./min.kg)	10·0	2·0	1·0	1·0	1·0	1·0	1·0
	Plasma concn. (μu./ml.)	1300	406	229	150	185	91	78
	Clearance (ml./min.kg)	7·6	4·9	4·1	6·4	5·0	10·4	12·1
5	Infusion rate (m-u./min.kg)	10·0	2·0	1·0	1·0	1·0	1·0	1·0
	Plasma concn. (μu./ml.)	675	505	180	165	156	95	50
	Clearance (ml./min.kg)	14·8	4·0	5·5	6·1	6·4	10·5	20·0
6	Infusion rate (m-u./min.kg)	10·5	2·1	1·05	1·05	1·05	1·05	1·05
	Plasma concn. (μu./ml.)	1860	800	376	228	164	127	114
	Clearance (ml./min.kg)	5·7	2·6	2·8	4·6	6·4	8·3	9·2
7	Infusion rate (m-u./min.kg)	10·0	2·0	1·0	1·0	1·0	1·0	1·0
	Plasma concn. (μu./ml.)	960	650	425	270	212	117	75
	Clearance (ml./min.kg)	11·5	3·4	2·6	4·1	5·2	9·4	14·7
8	Infusion rate (m-u./min.kg)	5·0	2·0	2·0	2·0	2·0	2·0	2·0
	Plasma concn. (μu./ml.)	458	400	325	305	290	200	102
	Clearance (ml./min.kg)	10·9	5·0	6·1	6·5	6·9	10·0	19·6

one shown for normal dogs in Fig. 2, although it was obtained from a series of experiments which were heterogeneous with regard to infusion rates. Data on individual experiments in this group are shown in Table 2. Although the rates of infusion vary from experiment to experiment, it is clear that clearance values were similar from dog to dog, at any given time after the onset of shock. Two out of eight dogs in this group survived a 72 hr observation period: they were both conscious, alert to sensory stimuli and capable of walking; they accepted food and drink and passed urine and faeces. Five dogs died within 8 hr of re-transfusion and one within 30 hr of re-transfusion.

Table 1 summarizes values for the MBV, time at which it was reached, time of start of uptake of blood, total uptake and survival. When group I (normal dogs) and II (diabetic dogs without infusion) are compared there is a significant difference between uptakes. All other comparisons produce non-significant differences. Results in group III (diabetic dogs with vasopressin infusions) show uptake and survival rates which are intermediate between groups I and II.

Post-mortem findings

General. a. All non-surviving dogs exhibited characteristic lesions of irreversible haemorrhagic shock, namely widespread haemorrhagic necrosis of the mucosae in the large and small intestine, intense hepatic congestion with centro-lobular necrosis and renal congestion, especially in the medullary layer.

b. All conscious surviving dogs were essentially normal, with occasional patches of congestion in the mucosae of the small and large intestine; liver and kidneys were normal.

c. The dog which was alive but unconscious at the end of the observation period was found to have an essentially normal intestinal mucosa; liver and kidneys were normal, but an intense congestion of the lungs was found.

Hypothalamo-hypophysial system. Microscopical examination of brain sections confirmed the position of the surgical lesions in the median eminence of the anterior hypothalamus. Sections stained with performic acid-Victoria blue revealed accumulation of neurosecretory matter in the paraventricular and supraoptic nuclei and their associated fibres coursing down to the median eminence in normal dogs. In diabetic dogs, the number of neurosecretory cells and intensity of staining was reduced. In some of the diabetic dogs, however, there was an accumulation of neurosecretory matter proximal to the surgical lesion.

TABLE 3. Clearance, half-life and apparent distribution space of vasopressin in normal and diabetic dogs

	Control				Haemorrhage	
	Clearance (ml./min.kg)	ADS (ml./kg)	$t_{\frac{1}{2}}$ (after infusion) (min)	$t_{\frac{1}{2}}$ (after injection) (min)	$t_{\frac{1}{2}}$ 2 hr (min)	$t_{\frac{1}{2}}$ 4 hr (min)
Normal dogs	$14{\cdot}7 \pm 1{\cdot}2$ (5)	112 ± 16 (5)	$5{\cdot}2 \pm 0{\cdot}4$ (5)	$5{\cdot}0 \pm 0{\cdot}5$ (4)	$10{\cdot}1 \pm 1{\cdot}2$ (5)	$4{\cdot}7 \pm 0{\cdot}2$ (5)
Diabetic dogs	$15{\cdot}1 \pm 0{\cdot}9$ (9)	137 ± 13 (9)	$6{\cdot}8 \pm 0{\cdot}4$ (12)	—	—	—
P	$> 0{\cdot}7$ (*a*)	$> 0{\cdot}2$ (*a*)	$< 0{\cdot}02$ (*a*)	$> 0{\cdot}7$ (*b*)	$< 0{\cdot}01$ (*c*)	$< 0{\cdot}01$ (*d*) $> 0{\cdot}5$ (*e*)

$t_{\frac{1}{2}}$, half-life of vasopressin measured following the stopping of a constant infusion and after a single injection during the control period and after single injections 2 and 4 hr after the onset of shock.

ADS, apparent distribution space of vasopressin = $(C \times t_{\frac{1}{2}}) \log_e 2$.

P, t test applied between

(*a*) normal and diabetic dogs,

(*b*) single injection and infusion measurements in normal dogs,

(*c*) $t_{\frac{1}{2}}$ (after injection) and $t_{\frac{1}{2}}$ (2 hr),

(*d*) $t_{\frac{1}{2}}$ (2 hr) and $t_{\frac{1}{2}}$ (4 hr),

(*e*) $t_{\frac{1}{2}}$ (after injection) and $t_{\frac{1}{2}}$ (4 hr).

Half-life of vasopressin

In normal dogs, the half-life of vasopressin during the control period was 5·2 min following constant infusions and 5·0 min, following single injections, the difference being statistically insignificant. In diabetic dogs, the half-life of vasopressin during the control period was 6·8 min, which

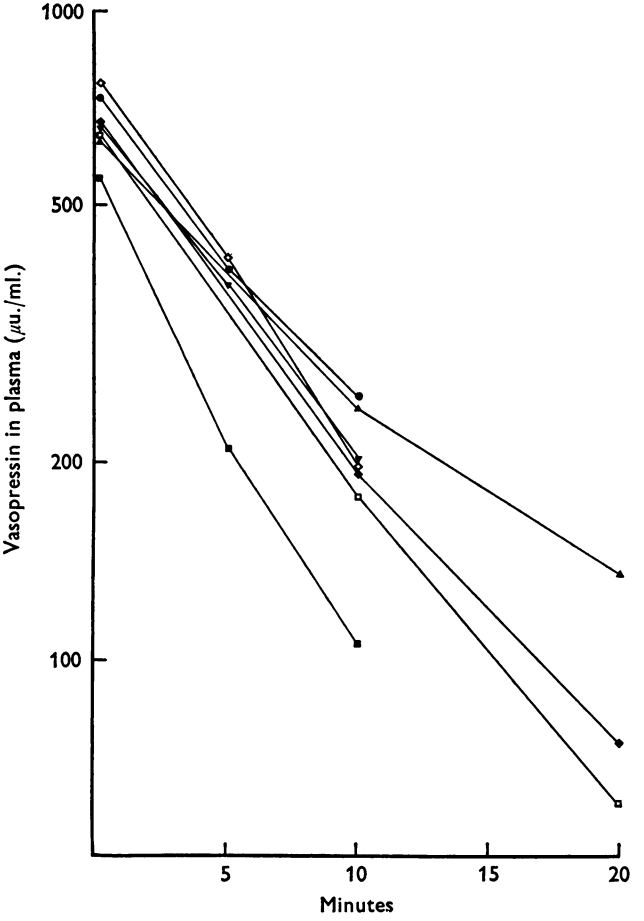

Fig. 4. The decay of vasopressin concentration in circulating plasma following termination of constant intravenous vasopressin infusions in normal dogs. Each different symbol represents a separate experiment.

is significantly higher than the control value found in normal dogs. In normal dogs the half-life rose to 10·1 min, 2 hr after the onset of shock, but dropped to 4·7 min at the fourth hour; both the rise and the fall are statistically significant. The 4-hr value is not significantly different from

the control value. These results are summarized in Table 3, which also shows the calculated values for the apparent distribution space of vasopressin. These agree with generally reported values and it is interesting to note that the apparent distribution space is greater than the vascular but smaller than the extracellular compartment.

Fig. 4 shows individual measurements of vasopressin concentration when three samples were serially collected at 5 min intervals in four experiments and at 10 min intervals in three experiments: they indicate that vasopressin clearance is apparently a first order reaction, with a simple exponential decay curve.

The clearance of vasopressin

The total clearance of vasopressin was measured in normal and diabetic dogs before and during haemorrhagic shock. Measurements during haemorrhagic shock are subject to the reservation made in the Methods section: they could not be measured at steady-state conditions, because no such steady state can be produced during shock, by a constant I.V. infusion (see Table 2: in Expt 1, vasopressin was infused for the duration of shock at a constant rate of 2·1 m-u./min.kg. In all other experiments the rate of infusion was varied through the shock period, but was constant for the last 4 hr of shock). Clearance rates given below are therefore only approximate estimates of the true clearances. Measurements in normal dogs were confined to the control period and to the last 3 hr of shock, because of the high endogenous vasopressin concentrations found in early shock. Results are summarized in Fig. 5. Infusion rates ranged from 5 to 10 m-u./min.kg in the control periods of normal and diabetic dogs and in the last 3 hr of shock in normal dogs; infusion rates during shock in diabetic dogs have been shown above (Table 2). In normal dogs the clearance of vasopressin dropped from a control 14·7 ml./min.kg to 5·9 ml./min.kg at the second hour of shock, but gradually recovered thereafter to reach 13·9 ml./min.kg at the fifth hour. Clearance measurements in diabetic dogs closely follow those from normal dogs, where the latter are available. In addition, measurements in diabetic dogs show that the clearance drops only slightly from the control to the 5 min sample. At 30 min, the clearance is at its lowest value and gradually rises to reach the same 13·9 ml./min.kg found at the fifth hour in normal animals. The variance of experiments in normal dogs is notably larger than in diabetic ones and the difference must be attributed to endogenous vasopressin secretion.

In seven experiments, performed in diabetic dogs, an additional blood sample was collected 3·5 min after the start of the control infusion. Vasopressin concentration in this sample was compared to that of the sample taken 20 min after the start of infusion. The ratio between the 3·5 min

and the 20 min vasopressin concentrations was found to be $0{\cdot}35 \pm 0{\cdot}03$ (S.E. of mean; $n = 7$). This therefore indicates that vasopressin concentration in plasma does not reach a steady state 3·5 min after the onset of a constant intravenous infusion.

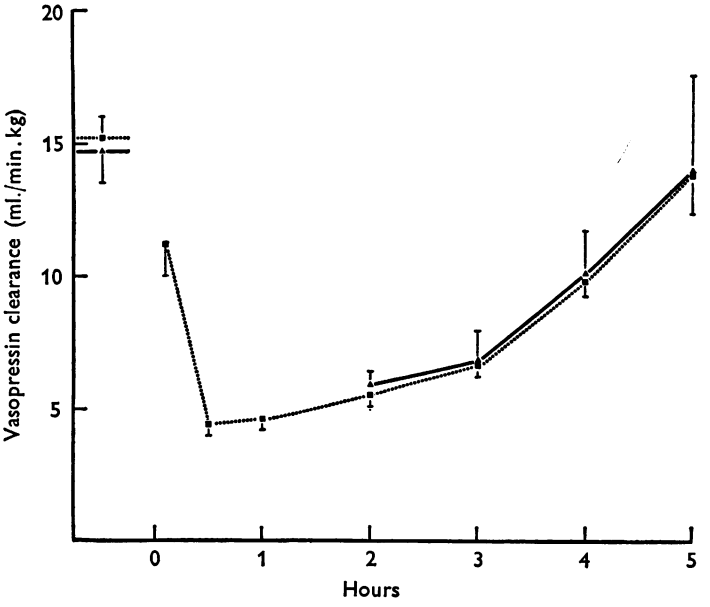

Fig. 5. The total clearance of vasopressin from plasma before and during haemorrhagic shock of the type shown in Fig. 2. Continuous lines represent measurements in normal dogs and interrupted lines in diabetic dogs. Control measurements were performed in five normal and nine diabetic dogs; clearances during haemorrhage were performed on five normal dogs and eight diabetic dogs.

Vasopressin secretion

From the calculated clearance values (Fig. 5) and the measured concentrations of endogenously secreted vasopressin in normal and diabetic dogs (Fig. 2), the secretory rates for vasopressin during shock can be calculated. It is necessary here to assume that endogenous secretion and intravenous infusions are equivalent and that the secretory rate S is equal to the extraction rate Ex. One can therefore rewrite the clearance equation

$$C = Ex/P$$

to

$$C = S/P,$$

where C is the clearance, P is the plasma concentration of secreted vasopressin, Ex the extraction rate and S the secretory rate. The assumption $Ex = S$ is again subject to the same reservation made before with regard

to clearance measurements. There is never a steady-state plasma concentration and secretory rates given below are only approximate estimations of secretion. Fig. 6 summarizes such estimations for normal and diabetic dogs: in the normal group the secretory rate rose from a control $0{\cdot}2 \pm 0{\cdot}02$ m-u./min.kg to a peak, 5 min after zero time of about 8 m-u./min.kg. At 30 min, it was down to about a quarter of the initial peak, at 2 m-u./min.kg. Over the next half hour secretion was halved again to 1·2 m-u./min.kg; over the next 4 hr, however, the secretion rate dropped

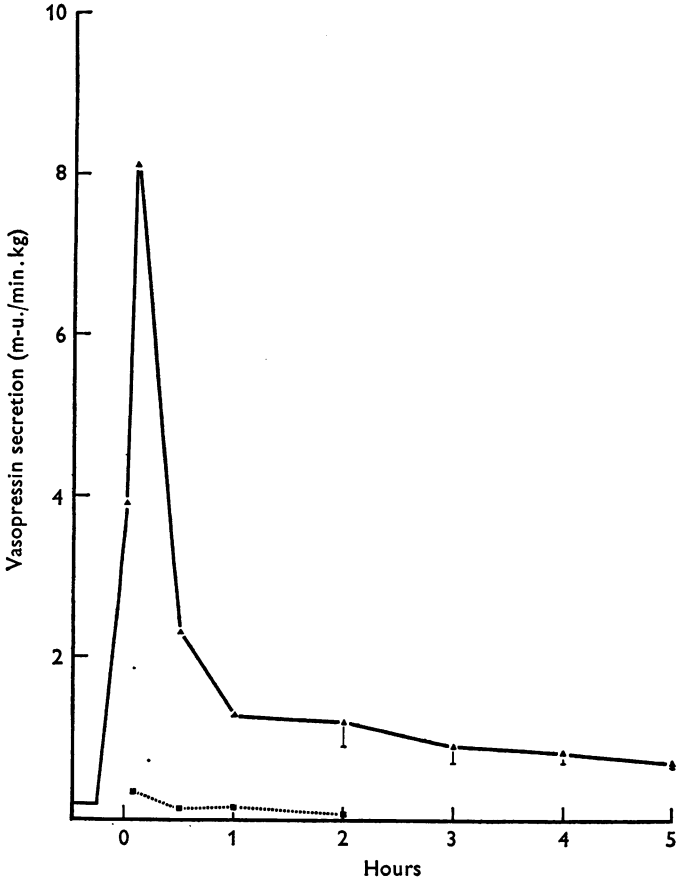

Fig. 6. Vasopressin secretion before and during haemorrhagic shock of the type shown in Fig. 2. Secretion was calculated as the product of clearance values, obtained in diabetic dogs (Fig. 5) and plasma concentrations of endogenously secreted vasopressin measured in normal and diabetic dogs (Fig. 2). The continuous line represents secretion in normal dogs and the interrupted line in diabetic dogs. Control secretion was $0{\cdot}2 \pm 0{\cdot}02$ m-u./min.kg for normal dogs. No S.E. of mean are shown for estimates of secretion during the first hour because of the approximate nature of the estimates.

very slowly, to 0·7 m-u./min.kg at the time of re-transfusion. Over this same 4 hr period the plasma levels of vasopressin dropped much more steeply from 270 μu./ml. at 1 hr to 47 μu./ml. at the fifth hour. It is clear, therefore, that the plasma concentration of vasopressin, taken alone, is not a true indicator of vasopressin secretion during shock. In diabetic dogs secretion was estimated at 0·21 m-u./min.kg for the three dogs with measurable vasopressin in their control samples. It rose slightly to 0·34 m-u./min.kg 5 min after zero time but dropped below the control value in all remaining samples. It should, however, be noted that no inhibition of secretion is hereby demonstrated, because the control values refer only to three dogs, presumably the ones with highest secretory rates.

Incubation of vasopressin with blood

The experiments were performed to check whether the rise in vasopressin clearance during shock could be attributed to inactivation of the hormone by blood. Known amounts of vasopressin were added to blood samples collected from normal dogs and from dogs 4 hr after the onset of shock. Each sample was divided in two parts; one was immediately extracted and the other was incubated at 37° C, for 10 min and then extracted. Two experiments were carried out. In normal blood, incubation caused a loss of 20 and 17% of antidiuretic activity; in blood collected after 4 hr of shock, a loss of 8 and 10 % of activity. It is clear, therefore, that inactivation of vasopressin by blood late in shock cannot be the cause of the increased clearance because it was, if anything, smaller than that occurring in normal blood.

DISCUSSION

The main object of this study was the estimation of the secretory rates of vasopressin during long-lasting haemorrhagic shock. The changes in the plasma concentrations of the hormone have been extensively studied in short-lasting shock in several mammalian species and there is general agreement about their pattern. The reduction in arterial blood pressure causes an immediate large increase in the plasma concentration of vasopressin. If blood pressure remains low this concentration falls to a lower value and remains more or less constant at this plateau. Re-transfusion of blood, whenever described, is always followed by a return of vasopressin to control values. Experiments of this kind have been carried out in the dog (Sachs *et al.* 1967; Schrier, Verroust, Jones, Fabian, Lee & de Wardener, 1968; Rocha e Silva & Rosenberg, 1969), the cat (Beleslin, Bisset, Haldar & Polak, 1967; Clark & Rocha e Silva, 1967) and the rat (Ginsburg & Brown, 1956; Fabian, Forsling, Jones & Lee, 1969), with the duration of the hypotensive period extending up to 1 hr. Such results have led to the

conclusion that vasopressin secretion in response to haemorrhage is a continuous two-phased process, with an initial intense burst of secretion followed by a less intense secretory plateau. In contrast, Gilmore (1968) suggested that vasopressin is released during a maintained period of shock in bursts of irregular frequency duration and intensity. These results were obtained with the superfused rabbit rectum as a vasopressin bioassay preparation (Gilmore & Vane, 1970). The rabbit rectum relaxes in the presence of vasopressin and the preparation was proposed as a method for the continuous monitoring of blood levels of vasopressin. During haemorrhagic shock, the rectum superfused with blood from a femoral artery was found to go through irregular periods of relaxation separated from one another by partial returns to base line. Gilmore therefore concluded that the usual method of collecting blood samples at discrete intervals was not a good method for the detection of the true variations in vasopressin secretion which occur during hypotension. In the present experiments the blood levels of vasopressin were monitored at fixed intervals during the five hours of haemorrhagic hypotension. No blood sample (from a total of seventy-two, collected from eight dogs during shock) was found to contain control amounts of vasopressin. This does not of course prove that vasopressin secretion proceeds in a continuous manner during shock; it seems unlikely, however, that samples collected at regular intervals would show any meaningful pattern if secretion were irregularly phasic. The discrepancy could be explained by a difference between collecting blood from a femoral artery, as opposed to a femoral vein, assuming that, during the passage of blood through the vascular bed drained by the femoral vein, some 'smoothing' of fluctuation in blood level by transcapillary movement of vasopressin could have occurred; alternatively the discrepancy might be explained by assuming that the response of the rabbit rectum to vasopressin might be interfered with by unidentified factors present in blood during haemorrhagic shock.

There is little doubt that the initial peak of vasopressin in blood actually reflects increased secretion, but there has been no direct proof that the moderately high plateau which follows is likewise a reflexion of increased secretion. It could be argued that the clearance of vasopressin must be dramatically reduced during shock and that the plateau of vasopressin in blood might be due, not to increased secretion, but to normal secretion in the presence of a reduced clearance rate.

In the present experiments, the total clearance of vasopressin has been measured in normal dogs and in dogs with experimental diabetes insipidus. Clearance measurements in the early stages of shock could only be performed on diabetic dogs. Measurements in normal and diabetic dogs, where the former are available, are closely parallel, but the variance in the normal

group is predictably larger as a consequence of endogenous secretion. The clearance rate during shock does not seem to depend on the rate of infusion. Infusions in normal dogs, during the last 3 hr of shock, ranged from 5 to 10 m-u./min.kg, whereas in diabetic dogs the range was 1–2 m-u./min.kg. One can therefore assume that there is no limiting rate of transport over a range of 1–10 m-u./min.kg. A similar assumption can be safely made for the control situation: present results agree with other findings, where infusions ranged from 0·5 to 18 m-u./min.kg (Vorherr, Bradbury, Hoghoughi & Kleeman, 1968; Lauson & Bocanegra, 1961).

The total clearance of vasopressin was predictably reduced during early shock, falling to about one quarter of its control value. From the second hour onwards, however, it rose continually to reach control values at the fifth hour. The latter, unexpected finding is not discussed in this paper. The rise in clearance is not, however, due to increased inactivation of the hormone in blood.

The half-life of vasopressin was also measured before and at selected times during shock. Before shock it was found to be 5·2 min in normal and 6·8 min in diabetic dogs. The difference is statistically significant, but it is difficult to imagine any functional significance for such a difference. It cannot be attributed to endogenously secreted vasopressin in normal dogs because this would tend to produce a higher half-life in the normal group, as opposed to what was effectively found; in any case endogenously secreted vasopressin produced plasma concentrations of 14 ± 2 μu./ml., too low to influence clearance or half-life measurements (performed at plasma concentrations of 100–1000 μu./ml.). During shock, in non-diabetic dogs, the half-life was high 2 hr after the onset of shock, but returned to control levels at the fourth hour, a result of which is consistent with the clearance findings. The control half-life values agree with most findings in the literature (see Lauson, 1967). However, it has been recently proposed (Gilmore & Vane, 1970) that the half-life of vasopressin may be in the region of 1 min; these results were obtained by estimating the rate of disappearance of vasopressin over 1 circulation time (CT), by means of the superfused rabbit rectum bio-assay method. Measurements were made after infusion periods of 3 and 4 min (three experiments in each case) and infusion rates ranged from 3·2 to 19 m-u./min.kg. The estimated disappearance rate, over 1 CT was 26·9 %, with a range of 12–47 %. There are several possible explanations for the rather wide discrepancy. After 3–4 min of infusion, a steady state may not have been reached, because equilibration with the interstitial compartment is still not complete. Such equilibration is an essential prerequisite for the estimation of the rate of disappearance of secreted hormone. In the present experiments, blood samples were collected 3·5 and 20 min after the start of vasopressin in-

fusions. The 3·5 min sample was found to contain only $35 \pm 3\%$ (S.D. of mean; $n = 7$) of the corresponding amount in the 20 min sample, which shows a steady state is not reached 3·5 min after the start of an infusion. The rate of disappearance over 1 CT is not a satisfactory method of estimating a long half-life. It requires an extrapolation far beyond the actual experimental data and from a small arteriovenous difference. In order to determine a half-life of 5 min by this method, a 3% arteriovenous difference must be detected, a discriminative power beyond the capabilities of bioassay procedures. Our present observations do not support the suggestion made by Gilmore & Vane (1970) that the longer half-lives reported by most workers may be caused by the long duration of infusions leading, in time, to a saturation of an enzymic or binding inactivation mechanism. We have shown that the transport system for vasopressin does not exhibit a maximal transport rate, over the infused rates and that the half-life is the same, whether measured following single injections or the stopping of constant infusions. Published data show measurements following the stopping of infusions of up to 3 hr, with basically similar results (Lauson & Bocanegra, 1961).

From the clearance rates and from the plasma concentrations of endogenously secreted vasopressin it is possible to estimate the secretory rates of the hormone in normal and diabetic dogs, but the following reservations must be borne in mind:

a. I.V. infusions of Pitressin and endogenous secretion of vasopressin have been assumed equivalent; Pitressin was used because the only information originally required concerned the rate of removal of the hormone from circulation during an I.V. infusion; both samples and infused solutions were always assayed for antidiuretic activity and the rates of removal of arginine and lysine vasopressin are essentially identical (Lauson, 1967);

b. It has been also assumed that vasopressin is removed in a similar manner in normal and diabetic dogs; this was shown to be the case in the pre-haemorrhage situation and in the late stages of shock (see Fig. 5);

c. Clearance values can only be determined at a steady-state plasma concentration; but in this paper, with the reservations given, an estimate for clearance values has been obtained; the same applies to secretory levels. It is thus obvious that both clearance and secretory rates are only approximate estimates of true clearance and secretion, and that the error is greater towards the early stages of shock because it is at this time that the rate of change in plasma concentrations is greatest; nevertheless, clearance rates at any given time during shock were fairly constant from animal to animal and varied throughout shock in a similar pattern in every dog; finally, the assumption is supported by the fact that intravenous infusions designed to mimic the effects of endogenous secretion, on the basis of preliminary

clearance calculations did in fact very effectively mimic such secretion, as far as plasma concentrations of the hormone are concerned (compare the curves in Figs. 2*C* (endogenously secreted vasopressin) and 3*C* (I.V. infused vasopressin)).

Estimations for secretion show that there is effectively a very intense secretory peak at the onset of haemorrhagic shock, some 40 times greater than the control rate of secretion. It rapidly declines to a sustained secretory plateau, which is maintained throughout the entire duration of shock. At the fifth hour, secretion is still 3·5 times higher than the control rate. In diabetic dogs, secretion remained at the low, control level over the shock period. These findings clearly show that the secretion of vasopressin in response to haemorrhagic shock cannot be quantitatively described without the measurement of clearance rates. Plasma concentrations alone produce a very misleading picture of the secretory pattern, probably leading to the assumption that secretion is back to control levels early after the onset of shock. The paradoxical rise of the clearance rate late during shock means that vasopressin secretion remains high throughout the entire period of hypovolaemia and that a progressively increasing clearance rate is partially responsible for the rapid fall in vasopressin concentration in plasma shown in Fig. 2.

The role of vasopressin in the mechanism of blood pressure control during haemorrhagic shock is still uncertain. Conclusions from the effects of vasopressin in normal animals cannot be extended to animals in haemorrhagic shock because of the blood pressure regulating mechanisms, which tend to antagonize the effects of vasopressin in the normal condition, but not during hypotensive shock. The large variations in clearance rates, throughout shock, which have been described here, make comparisons more difficult. But it has been shown that blood concentrations ranging from 50 to 400 μu./ml. will cause a rise in blood pressure if the afferent input from cardiovascular receptors is cut off (Rocha e Silva & Rosenberg, 1969); such levels, which are approximately equivalent to plasma levels of 100–800 μu./ml., occur throughout most of the duration of the shock experiments reported in this paper.

It has been recently shown that the pressor action of vasopressin during shock may be exerted mainly in the mesenteric vascular territory (McNeill, Stark & Greenway, 1970) and it is known that mesenteric vasoconstriction is the most important factor in the development of irreversible shock in the dog. Lillehei (1957) reported that a very high percentage of dogs survive an otherwise lethal shock procedure if their mesenteric territories are selectively perfused with donor blood at normal arterial pressure during shock.

In the present experiments, it has been found that four out of eight

diabetic dogs submitted to the shock procedure survived a 24 hr observation period, whereas all normal dogs died within a few hours of re-transfusion. When vasopressin infusions were given to diabetic dogs only two out of eight survived. Surviving dogs showed none of the characteristic lesions normally associated with irreversible haemorrhagic shock. It may therefore be suggested that in the absence of vasopressin secretion, mesenteric vasoconstriction is either absent or attenuated, allowing a sufficient flow of blood in the mesenteric territory to prevent ischaemic lesions from developing. The pattern of uptake of blood from the reservoir, in diabetic dogs, as compared to normal dogs (Fig. 2), supports this view: it is generally thought that the uptake of blood late in shock is caused by the transition from ischaemic anoxia to stagnant anoxia. At this stage there is an increase in the capacitance of the vascular territories most severely shut off during early shock, accompanied by a shift, first of fluid, later of whole blood, from the vascular to the interstitial compartment (Chien, 1967); both phenomena are attenuated if the mesenteric territory is not allowed to go into the early phase of ischaemic anoxia; as a consequence, uptake of blood from the reservoir is reduced (Lillehei, 1957). It may therefore be suggested that vasopressin secretion during haemorrhagic shock is a factor in the development of irreversible shock, although a detailed analysis of mesenteric circulatory dynamics during shock with and without vasopressin is essential before such a suggestion may be regarded as being established.

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