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THE CLEARANCE AND ANTIDIURETIC POTENCY OF NEUROHYPOPHYSIAL HORMONES IN MAN, AND THEIR PLASMA BINDING AND STABILITY

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SUMMARY

1. Bio-assay techniques have been used to measure plasma levels of neurohypophysial hormones in man, following either a single injection or a continuous infusion.

2. The median half-life of oxytocin after a single injection of 2 u. was 3·2 min (2·0–5·7, 95% confidence limits); this increased significantly ($P < 0·01$) to 4·8 min (4·4–6·1) when the hormone was infused at a rate of 500 m-u./min. The vasopressins had appreciably longer half-lives. After a single injection of 1 or 1·5 u. 8-lysine vasopressin (LVP), the half-life was 5·7 min (3·6–6·0). Continuous infusions of the hormones at a rate of 120 m-u./min yielded half-lives of 5·5 min (5·0–7·1) for LVP, and 5·6 min (3·9–9·5) for 8-arginine vasopressin (AVP).

3. The apparent volumes of distribution of the hormones were all of the order of two thirds the extracellular volume.

4. In accordance with its shorter half-life, the clearance of oxytocin was greater than that of the vasopressins (1·5 l./min, compared with 1·0 l./min).

5. The antidiuretic potencies of the hormones were studied in overhydrated subjects, by measuring the rate of urine excretion following an I.V. injection. Duration of antidiuretic action increased in the order: oxytocin, LVP, AVP. A 5:1 mixture of oxytocin and AVP was not as long-lasting as AVP alone. 8·5% (4–22) of an administered dose of AVP was excreted in the urine, and this amount was significantly correlated with urine volume ($r = +0·67$, $P < 0·05$).

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6. Ultrafiltration of human plasma containing exogenous hormones showed that 30 % (13–50) of AVP was bound, the degree of binding being independent of concentration over the range used (50–400 μ-u./ml.) In contrast, oxytocin was completely unbound.

7. Exogenous oxytocin was more stable than exogenous AVP in human plasma. At 4° C there was no significant loss of oxytocin until 7 days, whereas 20 % of AVP was inactivated in 2 days. At 37° C a 20 % loss of AVP occurred within 4 hr, and a 50 % loss within 24 hr; corresponding times for oxytocin were 24 and 48 hr.

INTRODUCTION

In man, there is still uncertainty as to whether the neurohypophysial hormones circulate in a bound form, and their half-lives in the blood are as yet unsatisfactorily determined. Czaczkes, Kleeman & Koenig's (1964) original results for arginine vasopressin (AVP), using an extremely sensitive preparation, must be regarded with caution, in view of the subsequent statement by the senior author (Vorherr, Kleeman & Hoghoughi, 1968). The half-life of oxytocin has been measured in pregnant women, but with high dosage (Gonzalez-Panizza, Sica-Blanco & Mendez-Bauer, 1961). Furthermore, most workers have used the single injection technique, which is less reliable than continuous infusion. In the present study, small amounts of the hormones were infused intravenously, following a preliminary investigation with single injections. Determinations were also made of the stability of the hormones in human plasma, as there is still confusion on this issue. Some of these findings have been reported in brief previously (Fabian, Forsling, Ibbitson, Jones & Stone, 1968).

Since lysine vasopressin (LVP) is the vasopressin commercially available, there is interest whether this hormone is equipotent to AVP in man. Some investigators have failed to realize that the essential difference may be only of duration of effect, and not of intensity. The antagonistic action of oxytocin on the antidiuretic activity of the vasopressins has been cited mainly in the German literature, with isolated findings emanating from different laboratories. An investigation was undertaken by this department in order to clarify the situation.

METHODS

Injections and infusions of the hormones

Blood level determinations

Normal male and female volunteers, 20–48 years of age, were studied. The hormones were injected into a forearm vein (usually antecubital) either as a single dose, or by continuous infusion from a syringe-driven pump (1 ml./min). For the infusion

experiments a polyethylene catheter (Intracath '1936', 36 in. long) was introduced under local anaesthesia, and the subjects remained in the supine position throughout. No adverse effects were recorded; some subjects showed minor reactions such as flushing with oxytocin and pallor with vasopressin. Venous blood samples were collected from the opposite arm.

Oxytocin. Most of the subjects were in the age range 20–25 years; one was 37 and one 48. Nine subjects (eight ♂, one ♀) were given an injection of 2 u. oxytocin, and blood samples were taken at 2, 4 and 6 min. Seven subjects (five ♂, two ♀) were given an infusion at a rate of 500 m-u./min for 25 min, and blood was withdrawn 3 min before, and about 3, 6 and 9 min after stopping the infusion.

Lysine vasopressin. All subjects were 20–22 years old. Six ♂ subjects were given an injection of 1 or 1·5 u. LVP, and blood samples were taken at either 2 and 8, 2 and 9, or 4 and 8 min. Four subjects (two ♂, two ♀) were given an infusion at a rate of 120 m-u./min for 18 min, and blood was withdrawn 2 min before, and either 3 and 6, or 4 and 8 min after stopping the infusion.

Arginine vasopressin. All subjects were 20–22 years old. Six subjects (five ♂, one ♀) were given an infusion at a rate of 120 m-u./min for 20 min, and blood was withdrawn 2 min before, and either about 4 and 8, or 5 and 10 min after stopping the infusion.

Since the single injection studies indicated that the $t_{\frac{1}{2}}$ was unlikely to be more than 6 min, the infusions were continued for about 20 min, so that equilibration would be about 90 % complete. Only one sample was collected before stopping the infusion, because the accuracy of the bio-assay would not allow such a small error in equilibration to be detected.

Antidiuretic potency of the hormones

Thirty-five male subjects, 18–21 years of age, were studied. The quantities of hormone administered were standardized to the following doses per 70 kg: AVP 100 m-u.; LVP 100 m-u.; oxytocin 1 u.; AVP + oxytocin mixture 100 m-u. + 500 m-u.; these doses were given in a volume of 1 ml. 0·9 % NaCl (w/v) was used as a control, and the experiment was performed as a double blind.

After emptying the bladder, the subjects were hydrated with an oral water load standardized at 1 l./70 kg. Thirty minutes after the initial drink the urine voided was measured. An equal volume of water was drunk to maintain the water load, and as soon as possible after this the hormones were administered. This procedure of measuring the urine passed every half hour and drinking an appropriate volume of water was continued for another 2½ hr. The subjects were ambulant during the experiment.

After an interval of 1 week the procedure was repeated on thirty-four of the subjects, who were now injected with a different substance. The total number of subjects receiving each treatment was as follows: control thirteen; AVP twenty-one; LVP fifteen; oxytocin thirteen; mixture seven. Each of ten of the subjects given 100 m-u. AVP pooled the five samples of urine passed over 2½ hr, and each specimen of pooled urine was subsequently assayed for antidiuretic activity.

Collection of blood

Blood was collected into plastic syringes containing about 0·1 ml. heparin (5000 u./ml.), and centrifuged in plastic containers for 20 min at 3000 rev/min. The plasma was stored at 4° C, and assayed within 48 hr of collection.

Binding

Oxytocin or AVP was added to eleven samples of plasma to give concentrations of 50–400 μ-u./ml. Ultrafiltration was carried out by the method of Bocanegra & Lauson (1961), as described in detail by Fabian, Forsling, Jones & Lee (1969*a*).

Stability

Oxytocin or AVP was added to plasma samples to give concentrations of 50–1000 μ-u./ml. The samples were maintained at either 4° C (sixteen oxytocin, eighteen AVP) or 37° C (ten oxytocin, eighteen AVP), and aliquots were taken for assay up to 15 days.

Bio-assay

(*a*) Antidiuretic activity was determined in the water-loaded, ethanol-anaesthetized rat (Dicker, 1953), with the modifications introduced by Forsling, Jones & Lee (1968).

(*b*) Oxytocin-like activity was measured either on the isolated strip of rat mammary gland (Rydén & Sjöholm, 1962), or by the milk-ejection response in the lactating rat (Bisset, Clark, Haldar, Harris, Lewis & Rocha e Silva, 1967). The relative advantages of these two methods have been discussed previously (Fabian, Forsling, Jones & Lee, 1969*b*).

Hormones

The oxytocin and 8-lysine vasopressin used were both synthetic hormones (Sandoz). For the arginine vasopressin experiments, a partially purified extract of bovine pituitaries (40 u./mg.) was made up into sterile ampoules containing 10 u./ml. (Parke-Davis: arginine Pitressin (Vasopressin), Lot 274927). The hormone preparation used for injection or infusion was also used for bio-assay of the plasma samples.

Statistics

All results are expressed in terms of the median and its 95% confidence limits. Wilcoxon's test (Mainland, 1963) was used to assess the significance of the difference between groups of data. A worked example using these methods has already been published (Forsling *et al.* 1968).

The use of arginine vasopressin was approved by the Ethical Committee of Charing Cross Hospital. All experiments were carried out under the supervision of Dr J. Lee.

RESULTS

Half-life, clearance and volume of distribution

In Figs. 1 to 4, plasma concentrations of the hormones have been plotted against time after injection or cessation of infusion. Individual measurements of half-life were made from such graphs. The method of determining clearance and 'apparent' volume of distribution in a continuous infusion experiment has been described in detail elsewhere (Fabian *et al.* 1969*a*). In the single injection experiments, volume of distribution was calculated by dividing the amount injected by the extrapolated zero time concentration, and clearance, by dividing this volume by the time constant of decay.

The results are summarized in Table 1. The half-lives of the vasopressins,

following either a single injection or a continuous infusion, were essentially the same, viz. about 5·5 min. Oxytocin had an appreciably shorter half-life, and there was a significant difference between the injection and infusion values ($P < 0{\cdot}01$). The apparent volumes of distribution of the

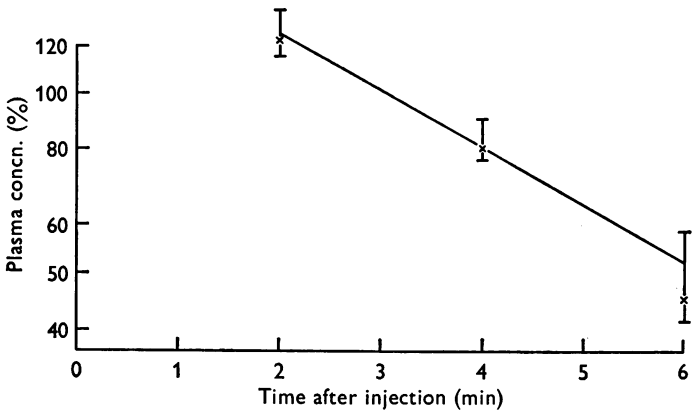

Fig. 1. Concentration of oxytocin in the plasma, following an injection of 2 u. at time zero. Results are plotted on a semi-log scale, and expressed as percentages of the 3 min value. Each point represents the median of nine experiments; the bars indicate the 95 % confidence limits. The median absolute concentration at 2 min was 170 μ-u./ml. (110–470, 95 % confidence limits).

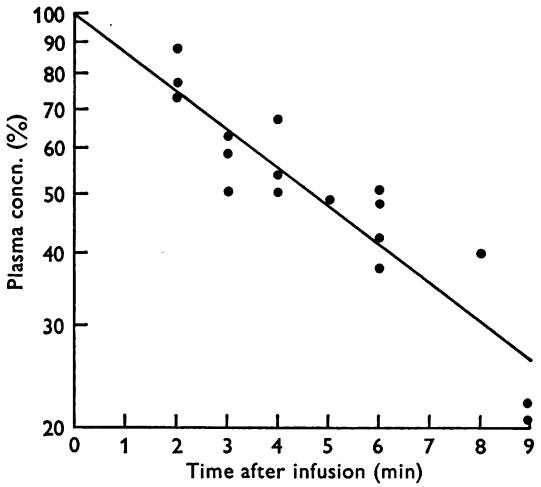

Fig. 2. Concentration of oxytocin in the plasma, following an infusion at the rate of 500 m-u./min. Results are plotted on a semi-log scale, and expressed as percentages of the value at equilibrium, i.e. before stopping the infusion. Points from individual experiments (seven) have been plotted, as the samples were all taken at different times. The median absolute concentration at equilibrium was 360 μ-u./ml. (280–560, 95 % confidence limits).

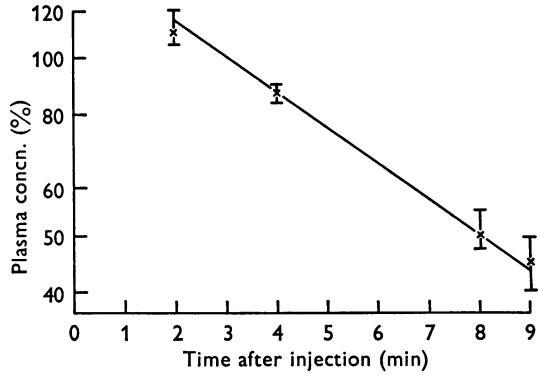

Fig. 3. Concentration of LVP in the plasma, following an injection of 1 or 1·5 u. at time zero. Results are plotted on a semi-log scale, and expressed as percentages of the 3 min value. Each point represents the median of three to six experiments; the bars indicate the range. The median absolute concentration at 2 min was 110 μ-u./ml. (80–150, range).

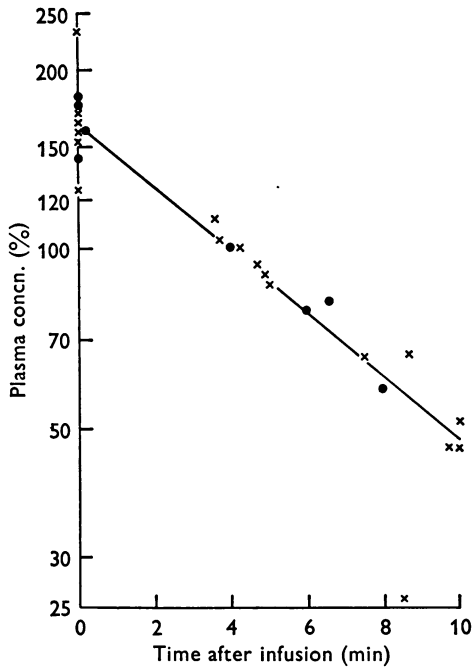

Fig. 4. Concentration of LVP (●) and AVP (×) in the plasma, following an infusion at the rate of 120 m-u./min. Results are plotted on a semi-log scale; they are expressed as percentages of the value at 4 min to obtain the best fit for the data from all ten subjects. Points from individual experiments have been plotted (four LVP, six AVP), as the samples were all taken at different times. The median absolute concentrations at equilibrium were: LVP-110 μ-u./ml. (75–240, range); AVP-120 μ-u./ml. (110–300, 95% confidence limits).

three neurohypophysial hormones did not differ greatly; all were of the order of two thirds of the extracellular volume. In accordance with its shorter half-life, oxytocin was cleared more rapidly than LVP or AVP.

TABLE 1. Half-life ($t_{\frac{1}{2}}$), apparent volume of distribution (V) and clearance (C) of neurohypophysial hormones after injection or infusion

Hormone	Dose	Median $t_{\frac{1}{2}}$ (min)	95% confidence limits	Median V (l.)	95% confidence limits	Median C (l./min)	95% confidence limits	No.
Oxytocin	2 u.	*3·2	2·0–5·7	7·4	2·7–11·4	1·6	—	9
	500 m-u./min	4·8	4·4–6·1	10·5	7·0–13·5	1·4	0·9–1·8	7
LVP	1·0–1·5 u.	5·7	3·6–6·0	9·0	6·2–12·0	1·1	—	6
	120 m-u./min	5·5	†5·0–7·1	8·3	†5·1–11·6	1·1	†0·5–1·6	4
AVP	120 m-u./min	5·6	3·9–9·5	8·5	5·2–11·0	1·0	0·4–1·1	6

* $P < 0·01$. † Range.

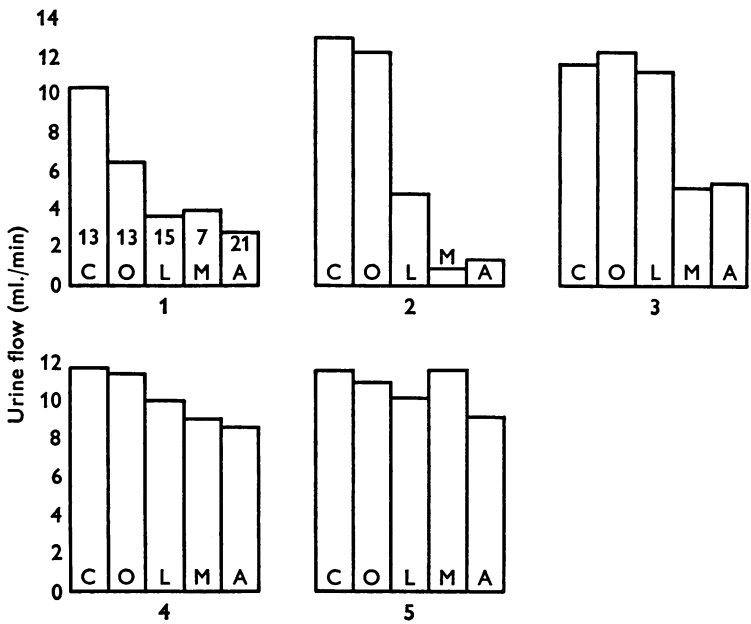

Fig. 5. Antidiuretic activity of neurohypophysial hormones in sixty-nine overhydrated subjects, following an I.V. injection at time zero. Nos. 1–5 represent successive half-hour periods of urine collection. Median volumes excreted are plotted in ml./min. C = control (0·9% NaCl), O = oxytocin 1 u.), L = 8-lysine vasopressin (100 m-u.), M = mixture of oxytocin and 8-arginine vasopressin (500 m-u. + 100 m-u.), A = 8-arginine vasopressin (100 m-u.), all doses/70 kg. Numbers in blocks denote number of subjects in each group. Wilcoxon's test gave the following results: A1, L1 < C1 ($P < 0·01$); A2, L2 < C2 ($P < 0·01$); L3 > A3 ($P < 0·01$); C5, M5 > A5 ($P < 0·02$).

Antidiuretic potency

Figure 5 shows the effect of the injections on the rate of urine excretion. Oxytocin had a transient antidiuretic action, which was over within half an hour. LVP induced a large antidiuresis, but its action was complete within 60 min. Both AVP alone, and the mixture of oxytocin and AVP produced a very marked inhibition of urine flow, the peak of which occurred within the second half hour. The effect of the mixture had disappeared by the last half hour period, whereas AVP alone was still causing a significant reduction in urine flow at this time (C 5, M 5 > A 5, $P < 0{\cdot}02$). Following the injection of AVP, a total of 8·5 % (4·0–22·0) was excreted in the urine, and this fraction was significantly correlated with the urine volume ($r = +0{\cdot}67$, $P < 0{\cdot}05$, for volumes of 172–1015 ml./2½ hr).

Binding

Some 30 % (13–50) of AVP was bound, and the degree of binding was independent of concentration. No binding of oxytocin could be detected.

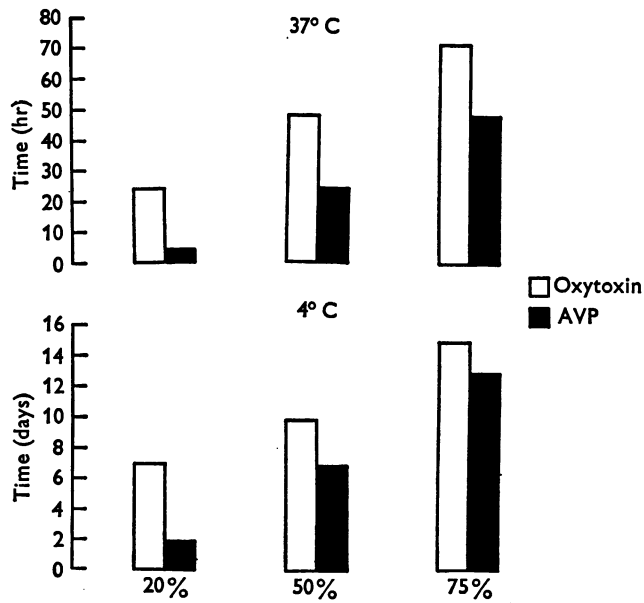

Fig. 6. Inactivation of exogenous oxytocin and 8-arginine vasopressin (AVP) by human plasma at 4 and 37° C. Percentages denote the amount lost. The number of samples were as follows: 4° C, sixteen oxytocin, eighteen AVP; 37° C, ten oxytocin, eighteen AVP; concentration ranged from 50 to 1000 μ-u./ml. Values plotted are the medians. Note that upper scale is in hr, lower scale in days.

Stability

The results are summarized in Fig. 6. Oxytocin lost only 20 % of its activity after 7 days at 4° C, or 24 hr at 37° C, whereas AVP lost 50 % under the same conditions; these differences were significant ($P < 0{\cdot}02$ at 4° C, $P < 0{\cdot}001$ at 37° C).

DISCUSSION

While it is generally agreed that neurohypophysial hormones are rapidly inactivated in plasma from pregnant women (50 % of oxytocin disappears in 5 min at 38° C from plasma obtained at term; Mendez-Bauer, Carballo, Cabot, Negreiros de Paiva & Gonzalez-Panizza, 1961), reports on their stability in plasma from non-pregnant subjects vary widely. Although Dieckmann, Egenolf, Morley & Pottinger (1950) showed that Pitressin suffered no loss of antidiuretic activity after 1 hr at 38° C, Czaczkes *et al.* (1964) found that, even at 4° C, there was an appreciable loss (15 %) of AVP in this time. In the present experiments the stability of AVP at 4° C was much greater, only 20 % being inactivated in 2 days. Oxytocin decayed even more slowly, though the difference in the stabilities of the two hormones in human plasma was not as marked as in rat plasma (Fabian *et al.* 1969*a*).

About one third of AVP was bound in the ultrafiltration experiments, and this fraction was independent of concentration over the range used (50–400 μ-u./ml.), indicating that the binding protein was present in relatively large quantities. If there were a simple equilibrium between the bound and unbound forms of the hormone:

$$HP \rightleftharpoons H + P,$$

where H denotes free hormone, P denotes protein, and HP denotes the protein–hormone complex, the

$$K_{\text{equil.}} = \frac{[H][P]}{[HP]} \text{ or } \frac{[H]}{[HP]} = \frac{K_{\text{equil.}}}{[P]},$$

i.e. the fraction of the total hormone bound is constant when the quantity of protein present is much greater than the amount of hormone. Whether human plasma also contains small quantities of a specific binding protein such as neurophysin has not yet been determined. In contrast to AVP, oxytocin was completely unbound over the same range of concentrations.

Several other workers have investigated the question of binding of neurohypophysial hormones, and have obtained results ranging from no binding of AVP (Holliday, Burstin & Hurrah, 1963; Czaczkes *et al.* 1964; Vorherr *et al.* 1968), to 'some' binding of both oxytocin and AVP (Heller & Lederis, 1957), to complete binding of AVP (Ahmed, George, Gonzalez-

Auvert & Dingman, 1967). The methods used to demonstrate binding in these studies were all *in vitro* ones involving dialysis or ultrafiltration. Brook & Share (1966) attempted a definitive experiment by using an intact dog in their dialysis experiments. They also collected fluid from lymph ducts and the thoracic and peritoneal cavities, and showed that, in the dog, AVP could cross capillary membranes. However, as they point out, their data did not preclude a small degree of binding at low concentrations. We are at present re-investigating this problem to determine the influence of such factors as pH and temperature on the ultrafiltration of the hormones.

While no evidence could be found for the binding of oxytocin, its apparent volume of distribution was not very different from that of the vasopressins, viz. about two thirds of the extracellular volume. Schröder & Rott (1959), Holliday *et al.* (1963), and Klein & Roth (1967) found the distribution volume of injected vasopressin to lie between the plasma and extracellular volumes, but Czaczkes *et al.* (1964) obtained a value less than that of the plasma. The apparent discrepancy between binding and distribution volume has been considered by these and other authors (Brook & Share, 1966; Lauson, 1967), but at present it remains unsolved.

When considering an equilibrium state, the problem is relatively simple if the substance remains within the vascular system, for virtually complete equilibration will be attained after a period of time equal to $4 . \tau$. In the case of the neurohypophysial hormones, whose volume of distribution exceeds the plasma volume, diffusion presents an additional complicating factor. However, our major consideration in the vasopressin experiments was that the total quantity administered should not exceed what we regarded as a safe single dose (viz. 2·5 u.), since the biological half-life for pressor activity may be relatively long. In calculating the rate of infusion required, we assumed a half-life in the blood of about 5 min, and a volume of distribution of the order of the extracellular volume. An equilibrium concentration of 100 μ-u./ml. was chosen, so that the 10 min samples would still be easily measurable. With all these pre-conditions, we were therefore limited in the period of time for which we could infuse, and it is fully appreciated that the half-lives obtained in these experiments may be only approximations. In the case of oxytocin, where much larger doses can be administered with impunity, our initial results with single injections led us to believe that the half-life after infusion would be only about 4 min; hence a 25 min period should have been sufficient to reach equilibrium. As the half-life subsequently proved to be only marginally longer, we felt it unnecessary to modify the established procedure for a negligible gain in accuracy.

Gonzalez-Panizza *et al.* (1961) obtained a much shorter half-life for

oxytocin in their experiments on pregnant women, viz. 1·2–4·0 min, compared with 4·4–6·1 min in the present study. While this may simply have been due to the fact that their subjects were in a late stage of pregnancy (immediately before, or just after, parturition), another explanation is that, because the quantities of hormones administered were unphysiologically high (8–16 u./min), a more rapid turnover occurred. This phenomenon has been observed in the rat (Fabian *et al.* 1969*a*) and attributed to some less specific inactivating process, which is called into play at very high blood concentrations. It is difficult to account for the very short half-life of 15 sec recorded by Fitzpatrick (1961) following a single injection, since we found the half-life to be 3·2 min with this technique.

Czaczkes *et al.* (1964) reported that the half-life of exogenous AVP depended on the degree of hydration, ranging from 20 to 44 min. However, problems of assay make the interpretation of these data questionable. Considerably earlier, Schröder & Rott (1959) obtained a value of 7·8 min, following an injection of Tonephin (probably AVP). Single injections were also used by Silver, Schwartz, Fong, Debons & Dahl (1961), who reported a half-life of 2·3–5·5 min for [^{3}H]AVP and by Klein & Roth (1967), who obtained a value of 2 min for the unlabelled hormone. Our median value of 5·6 min for the continuous infusion of AVP is in approximate agreement with these authors.

As the distribution volumes of the neurohypophysial hormones include the interstitial volume, the determination of their half-life in the blood, following an infusion, may be influenced by diffusion across the capillary membrane. Vane (1969) has considered *back* diffusion to be the overriding problem, but his technique of infusing for a very short period of time (5–10 min) may have the same disadvantages as a single injection, viz. inadequate mixing in the volume of distribution. In support of this criticism is the observation that thiocyanate (mol. wt. 81) requires 20 min to reach 90 % equilibration (Gaudino & Levitt, 1949). An additional problem, not considered in the present investigation, is the possibility that inactivation may occur in the interstitial fluid. It would therefore appear that, with the experimental procedures currently available, a definitive half-life is virtually unattainable. Nonetheless, it is important to realize that, when the neurohypophysis secretes, both inactivation and diffusion take place simultaneously, so that non-equilibrium conditions may not be unphysiological. Notwithstanding the difficulties of interpreting a half-life measured in the blood, it is interesting that the fall in concentration with time was linear on a semi-log scale over the entire 10 min period of measurement. Ideally, one should continue for a longer time to see whether there is a second component to the curve, such as has been found in the rat (Fabian *et al.* 1969*a*).

The half-life of LVP in the plasma, and its clearance from the plasma, were essentially the same as those of AVP; however, its biological half-life, as measured by its effect on the rate of urine excretion, was considerably shorter. This shorter duration of action of LVP has been noted in the rat, and the different pattern of response employed as an aid to identification (Jones & Lee, 1967). It has also been observed in the human. Miller, Fisch & Kleeman (1967) infused LVP or AVP into subjects who had been overhydrated, until a steady antidiuresis was induced, and measured the time taken for the free water excretion to return to the overhydrated level, once the infusion was stopped. They found that AVP was more potent than LVP, and that the biological half-lives were 24 and 15 min, respectively. It should be emphasized that, despite Miller's statement that certain data are strongly suggestive of a close correlation between degree of antidiuresis and plasma hormone concentration, a comparison of our experimental results using LVP and AVP suggests that the two concepts of half-life may be quite unrelated. This view is supported by findings in the rat (Sawyer, 1963), in which LVP has a shorter biological half-life, but a longer plasma half-life, than AVP.

Guhl (1961) states that 'the antidiuretic effect of Lys-VP and Arg-Vp is virtually identical'; however, the data are not convincing that LVP is equipotent to AVP. LVP would therefore appear to be less suitable than AVP as a therapeutic agent in diabetes insipidus, since duration of action is obviously of importance. With increasing dosage of LVP the intensity of the effect could be increased, but duration would be only marginally lengthened. Furthermore, the two vasopressins are equipotent as pressor agents, and there is a limit to the amount of pressor material that can be administered.

It has been generally recognized that the threshold dose for antidiuresis in man is about 1–10 m-u., and that oxytocin has antidiuretic activity of the order of 1 % of its oxytocic activity. Our finding that 1 u. oxytocin produced a minimal antidiuretic effect confirms this. Hence, only when hundreds of units of oxytocin are administered would an unphysiological antidiuresis occur. Somewhat surprisingly, such large doses have been given in clinical medicine, over a period of 24 hr, with the result that serious water retention occurred, leading to a state of water intoxication (Pittman, 1963; Silva & Allan, 1966).

Frey, Kerp & Reichardt (1959) observed a reduction of the antidiuretic effect of I.V. Tonephin (probably AVP) in water-loaded subjects, when oxytocin was administered simultaneously in twice the dose. This inhibitory action of oxytocin on AVP was not detectable until the final period in our experiments (500 m-u. oxytocin: 100 m-u. AVP). Whether the ratio of the neurohypophysial hormones is critical has not yet been

ascertained; neither is it known whether these findings are of any significance physiologically.

The fraction of administered hormone which was excreted, following a single injection of AVP, was 8·5 % (4–22 %). This does not differ greatly from the results previously obtained by other workers (Burn & Singh Grewal, 1951; review by Lauson, 1967). Theoretically, the amount of AVP appearing in the urine, assuming 30 % binding, is

$$\frac{0{\cdot}7 \times \text{G.F.R.} \times I \times t_{\frac{1}{2}}}{V \times 0{\cdot}693},$$

where G.F.R. denotes the glomerular filtration rate, I the amount injected, V the volume of distribution, and $t_{\frac{1}{2}}$ the half-life. Thus,

$$\text{Filtered AVP} = \frac{0{\cdot}7 \times 130 \times 100 \times 5{\cdot}6}{8500 \times 0{\cdot}693}$$

$$= 8{\cdot}6 \text{ m-u.}$$

$$= 8{\cdot}6\,\% \text{ of the administered dose (100 m-u.)}$$

Values for half-life and distribution volume used in this calculation are derived from the experiments on the infusion of AVP. It would therefore appear that the whole of the AVP excreted in the urine had been filtered. If the human kidney clears half the injected hormone, as occurs in many species (Lauson, 1967), and only 8·5 % appears in the urine, a considerable quantity of AVP must be inactivated by the kidney tissue.

It should be emphasized that the values used in the calculation of filtered AVP are all medians for subjects in a normal state of hydration. However, in this experiment, all subjects were given an excess of water, and it is possible that, under these conditions, a variable increase in both G.F.R. (O'Connor, 1962) and half-life (Czaczkes *et al.* 1964) may have occurred. Hence, the calculated value may be an under-estimate and some hormone may in fact have been inactivated during its passage down the kidney tubule.

There was a very wide range in the volumes of urine excreted by both the control subjects, and those receiving AVP. This large variation (six- to tenfold) was probably due to variation in the degree of hydration before the experiment. Those subjects who were already normally or over-hydrated would tend to pass larger volumes, drink more water, and thus maintain their body fluids at a more expanded level. It is well known that the responsiveness of the kidney to injected AVP is decreased under these circumstances (de Wardener, 1967). In addition, G.F.R. may have increased relatively more in these subjects. The apparent correlation between volume of urine and quantity of AVP excreted can also be explained in

terms of the state of hydration of the subjects. If the half-life and the G.F.R. increase progressively as the degree of overhydration increases, then those subjects who pass the largest urine volumes because they are in the most overhydrated state will also filter the greatest quantity of AVP. This somewhat surprising finding is therefore readily explicable.

The value of clearance measurements is to assess both the rate of inactivation of a hormone and the rate of its removal from the circulation, these processes being of equal importance to the rate of secretion in the determination of its concentration in the blood. It is impossible accurately to measure the clearance following a single injection, since the estimates of both apparent volume of distribution and half-life are subject to considerable errors. On the other hand, when a steady state is achieved following an infusion, the total clearance of the hormone can be unequivocably calculated. For all three hormones, the total clearance was found to be about 1 l./min; assuming that inactivation occurs predominantly in the liver and kidneys (Lauson, 1967), most of the activity must be removed from the blood in a single passage through these organs.

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