Br. J. Pharmac. Chemother. (1968), **33**, 523–530.

THE CLEARANCE OF NEUROHYPOPHYSIAL HORMONES FROM THE CIRCULATION OF NON-MAMMALIAN VERTEBRATES

BY

S. H. HASAN* AND H. HELLER

From the Department of Pharmacology, University of Bristol

(*Received March* 8, 1968)

Many workers (for references, see Heller & Ginsburg, 1966; Lauson, 1967) have shown that after intravenous injection the activities of vasopressin and oxytocin rapidly disappear from the circulation of mammals. Other peptide hormones such as insulin (Elgee, Williams & Lee, 1954) and corticotrophin (Richards & Sayers, 1951; Sayers, Burns, Tyler, Jager, Schwartz, Smith, Samuels & Davenport, 1949; Greenspan, Li & Evans, 1950) have similarly short half-lives. So far, however, no work seems to have been reported on the fate of neurohypophysial hormones in non-mammals. It was therefore decided to investigate the fate of 8-arginine oxytocin (vasotocin) in chickens and toads—that is, in a warm-blooded and a cold-blooded species in which this active peptide is known to be elaborated (see Heller, 1966). The fate of oxytocin—another hormone which also occurs both in birds (Acher, Chauvet & Lenci, 1960) and amphibians (Follett & Heller, 1964; Munsick, 1966)—was also investigated.

METHODS

Hens and cockerels (White Leghorns and Rhode Islands obtained commercially), weighing from 1.0 to 2.0 kg, were used. The birds were anaesthetized with pentobarbitone sodium (55 mg/kg) and received heparin 1,000 u./kg. Intravenous injections were given into the brachial vein and the blood samples were collected from a common carotid artery. *Bufo marinus* of either sex, weighing 150–300 g, obtained from the West Indies were used. Anaesthesia was produced by immersing the toads in a 0.2% solution of MS222 (Sandoz). Intravenous injections were given into the femoral vein and the blood samples were collected from the carotid arch after heparin (1,000 u./kg) had been administered. Adult albino rats of both sexes, weighing from 150 to 200 g were used for assays.

Assay of antidiuretic activity

The original technique of Jeffers, Livezey & Austin (1942) was employed with slight modifications. The rats were fasted overnight but were allowed to drink water. On the day of the assay warm tap water 50 ml./kg body weight was given by stomach tube and 45 min later the same volume of 12% ethanol by the same route. If the depth of anaesthesia produced by ethanol was not adequate for surgical operations, 3 mg of pentobarbitone sodium was administered intraperitoneally. As soon as surgical anaesthesia had been obtained a jugular vein was cannulated, as were the stomach and the urinary bladder, and the penile urethra was ligatured. Tracheotomy was also performed to ensure a free airway. The rat was then laid on its back

* Present address: Department of Pharmacology, University of Ibadan, Nigeria.

and the urine flow was recorded with the apparatus described by Dyball, Lane & Morris (1966). The assay was started when the urine flow reached 60 μl./min or more and remained at approximately the same level for 9 min. Before administration of the hormones, 0.4 ml. of 0.9% NaCl solution was given intravenously to ensure that the injection of a small volume of fluid as such did not produce an inhibition of diuresis. Assays of standard (2+2) design were performed.

Assay of milk ejection activity

The method used was that of Tindal & Yokoyama (1962). Lactating guinea-pigs, weighing 500–800 g, were employed. Pentobarbitone sodium, approximately 50 mg/kg, was given intraperitoneally and 1 mg atropine sulphate subcutaneously. A cannula was then inserted into the trachea and connected to a Palmer miniature respiration pump. After ligature of the femoral and the caudal superficial epigastric artery an internal saphenous artery was cannulated. The duct of a mammary gland was then cannulated and connected to a Sanborn transducer. Assays of standard (2+2) design were performed.

Assay of pressor activity

The method used was that of Landgrebe, Macauley & Waring (1946) as modified by Dekanski (1952). A male rat, weighing 180–250 g, was anaesthetized with urethane (25%, w/v) by the intraperitoneal injection of 0.6 ml./100 g body weight. A femoral vein was cannulated and tracheotomy was performed. A carotid artery was then cannulated and connected to a Condon manometer. Dibenyline, 0.1 mg/100 g, was given intravenously to block effects caused by sympathetic activity. Four (2+2) assays were usually performed for each determination of pressor activity.

Assay of oxytocic activity

Holton's (1948) method was employed. Mature virgin female rats, weighing 180–200 g, were injected with 100 μg of stilboestrol for 3 days before the assay (Follett & Bentley, 1964). The rats were killed by decapitation. The middle part of one uterine horn was suspended in a 3 ml. organ bath containing magnesium-free Munsick (1960) solution and connected to a frontal writing lever with a load of 2 to 3 g. The bath temperature was kept constant at 33° C and a slow stream of 95% oxygen plus 5% carbon dioxide was bubbled through the solution. Assays of standard design of four (2+2) blocks were performed.

Extraction of oxytocin from chicken and toad plasma

The method described by Ginsburg & Smith (1959) was used. Blood was withdrawn from the cannulated artery of anaesthetized and heparinized chickens and toads. The blood was collected in polythene centrifuge tubes surrounded by ice and care was taken to avoid contact with glass to avoid the formation of kinins (Armstrong, Jepson, Keele & Stewart, 1957). The blood was centrifuged at 35,500 rev/min for 30 min at 5° C, the plasma removed with a plastic syringe into a chilled polythene centrifuge tube; 10 volumes of acetone were then added, mixed quickly, and allowed to stand for 10 min to complete the flocculation of the proteins. The mixture was again spun for 10 min at 2,500 rev/min and the supernatant was transferred to another polythene tube. The precipitate was washed with 0.5 ml. of 90% (v/v) acetone in water, centrifuged again at the same speed for 5 min and the supernatant added to the first supernatant. Air was then allowed to pass through this mixture at 40° C. The resultant material was brought to the original volume with saline.

Hormone preparations used

Oxytocin (Syntocinon, Sandoz), arginine vasopressin (Tonephin, Hoechst) and arginine vasotocin (synthetic vasotocin, Sandoz) were used.

RESULTS

Withdrawal of blood beyond a certain volume has been shown in mammals to lead to the release of neurohypophysial hormones (Ginsburg & Heller, 1953; Ginsburg & Brown, 1957; Ginsburg & Smith, 1959; Weinstein *et al.*, 1960; Share, 1961; Heller, Hasan

& Saifi, 1968). In chickens, when several samples of blood (in the aggregate equivalent to less than 1 % of body weight) were withdrawn from either a wing vein under local anaesthesia or from a carotid artery in pentobarbitone anaesthesia at intervals of 30 min, the plasma had some antidiuretic activity (range 25–56 μ-u./ml.) but this activity was much the same in the first and the last sample taken 2 hr later. Similar control experiments were performed in toads in MS222 anaesthesia but no antidiuretic activity could be demonstrated in any of the plasma samples. Weak milk-ejection activity (equivalent to less than 25 μ-u./ml.) was usually obtained with both chicken and toad plasma, but there was no relationship between the intensity of these effects and the duration of the anaesthesia or the number of blood samples taken.

Disappearance of injected 8-arginine oxytocin from the circulation of laying and non-laying hens and cockerels

8-Arginine oxytocin (vasotocin) in a dose of 500 m-u./100 g body weight was injected intravenously into laying and non-laying hens and into cockerels anaesthetized with pentobarbitone and injected with heparin. A control blood sample of 1.5 ml. was collected 5 min before the injection of the hormone. When the hormone concentration at different intervals of time was plotted on a logarithmic scale a curve with two exponential components was obtained (Fig. 1). Each point on the graphs is the average of the results of three or four experiments. The half-lives calculated from the slower component by extrapolating to zero time were 18.3 ± 2.0 (S.E.), 20.7 ± 2.2 and 24.3 ± 2.2 min in non-laying hens, laying hens and cockerels respectively. The fall in pressor activity was considerably quicker in the first 20 min than in subsequent periods of 20 min. When straight lines were fitted (by eye) the half-lives were 14.2 ± 1.0, 12.1 ± 0.5 and 13.4 ± 1.6 min respectively.

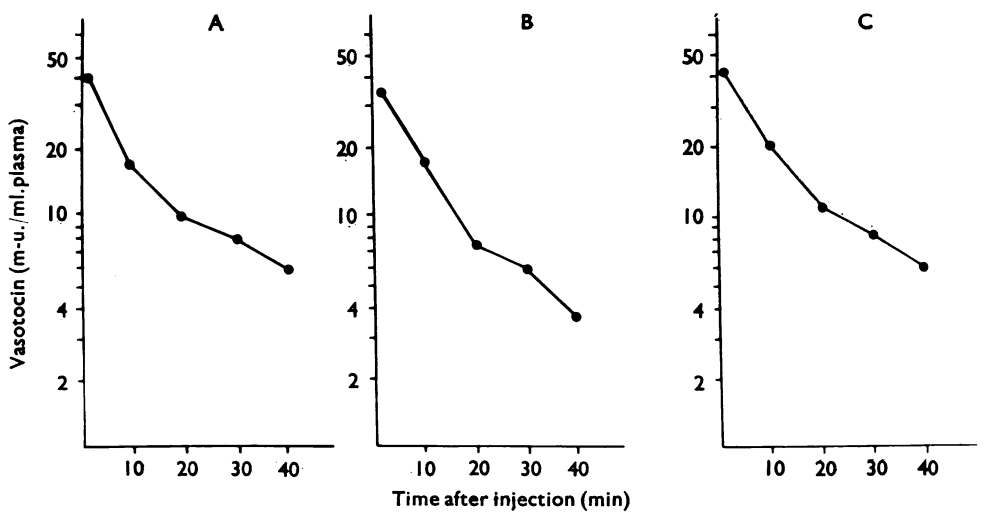

Fig. 1. Disappearance of the pressor activity of arginine vasotocin from the circulation of the domestic fowl. A single dose of 500 m-u./100 g body weight was injected intravenously into birds anaesthetized with pentobarbitone sodium. A, Laying hens; B, non-laying hens; C, cockerels. Note: ordinate logarithmic. Mean of three or four experiments.

Disappearance of 8-*arginine oxytocin from the circulation of male and female* Bufo marinus

Vasotocin, 300 m-u./100 g body weight, was injected intravenously into anaesthetized and heparinized *Bufo marinus*. A control sample of 1 ml. blood was taken 5 min before the injection of vasotocin, 4–5 ml. of blood from another toad was given intravenously after the withdrawal of the second sample and the blood deficit in the animal was kept at 2–3 ml. As in chickens, two-component exponential curves were obtained for both male and female toads when the concentration of vasotocin in plasma was plotted on a logarithmic scale. The half-lives calculated from the slower component were 31.7 ± 2.2 min for males and 33.0 ± 2.6 min for females. Half-lives calculated on the assumption of a single exponential curve were 20.9 ± 1.1 and 19 ± 1.3 min respectively.

Disappearance of injected arginine vasopressin from the circulation of chickens and toads

Arginine-vasopressin, 500 m-u./100 g body weight, was injected intravenously into anaesthetized and heparinized non-laying hens. When the levels of pressor activity in plasma were plotted semilogarithmically a pattern similar to that for vasotocin was obtained. The half-life calculated by extrapolating the slower component was 20.8 ± 1.5 min; the half-life calculated from a straight line was 7.8 ± 0.1 min.

Intravenous injections of arginine vasopressin, 300 m-u./100 g body weight, were given to anaesthetized and heparinized female toads. A diphasic curve was again obtained. The half-life calculated from the slow component was 23.7 ± 0.9 min and that calculated on the assumption of a straight line was 18.2 ± 3.0.

Disappearance of injected oxytocin from the circulation in laying and non-laying hens and cockerels

Oxytocin, 500 m-u./100 g, was injected intravenously into laying and non-laying chickens and cockerels under the same conditions as in the experiments with the other neurohypophysial hormones. The plasma was treated as described in METHODS before being assayed on the isolated rat uterus. When the values for oxytocic activity were plotted, all the points except the first fitted a straight line. The half-lives calculated by extrapolating to zero time were 9.0 ± 0.3, 10.9 ± 0.3 and 10.3 ± 0.7 min in the three types of bird. Calculated for a straight line the half-lives were 8.1 ± 0.2, 8.9 ± 0.6 and 8.9 ± 0.2 min.

Disappearance of injected oxytocin from the circulation of Bufo marinus

Experiments in which 300 m-u. of oxytocin was injected intravenously into male and female toads were performed in similar conditions as those for vasotocin in the same species. The half-lives calculated on the assumption of a single exponential function were 8.0 ± 0.1 and 10.6 ± 1.0 min in male and female toads respectively. The half-lives calculated by extrapolating the line to zero times were 9.0 and 12.0 ± 0.7 min.

Tests for inactivation of neurohypophysial hormones by chicken and toad plasma

In order to obtain an indication whether the decay of activity of injected neurohypophysial hormones in the circulation of the birds and toads was due, or partially due, to inactivation by blood arginine vasotocin, arginine vasopressin and oxytocin were incubated in chicken and toad plasma *in vitro*.

Table 1 shows that when chicken or toad plasma was incubated with arginine vasotocin for up to three hr at 42.5° or 26.5° C, no inactivation of the hormone could be demonstrated.

TABLE 1

RECOVERY OF BIOLOGICAL ACTIVITY OF NEUROHYPOPHYSIAL HORMONES AFTER INCUBATION WITH CHICKEN OR TOAD PLASMA AT 42·5° AND 26·5° C RESPECTIVELY

Rat pressor activity was assayed in the case of arginine vasotocin and arginine vasopressin and rat uterus activity in the case of oxytocin. Controls: hormone added to Munsick solution. *P* for difference between all values from non-laying and all values from laying birds $>0{\cdot}05$.

A. Vasotocin. Plasma of heparinized blood was diluted with an equal volume of Munsick solution containing vasotocin, 100 m-u./ml.

Provenance of plasma	% recovery		
	1 hr	2 hr	3 hr
Non-laying hens	87, 85, 92	95, 113, 100	95
Controls	100, 92	118, 88	102
Toads	92, 106, 100, 92, 90	99, 105, 88, 92, 92	96, 107
Controls	91, 102	98	91

B. Vasopressin. Plasma of heparinized blood was diluted with an equal volume of Munsick solution containing vasopressin 100 m-u. ml.

Provenance of plasma	% recovery		
	1 hr	2 hr	3 hr
Non-laying hens	103, 85	88, 96	—
Laying-hens	96	94	—
Controls	84	96	—
Toads	98, 106	100, 100	—

C. Oxytocin. 0·1 Syntocinon (50 m-u. oxytocin) was added to 0·9 ml. plasma of heparinized blood

Provenance of plasma	% recovery		
	1 hr	2 hr	3 hr
Non-laying hens	93, 90, 96, 83, 71, 77	84, 75, 82	77, 82, 80
Laying-hens:			
Blood obtained 1 hr after oviposition	63, 86, 91	—	77, 86, 85
Blood obtained 3 hr after oviposition	73, 67, 67	—	82, 67, 67
Cockerels	70, 72, 75, 96	—	—
Controls	96	—	103
Toads	98, 96, 89, 72, 96	94	—

DISCUSSION

Because vasotocin is probably concerned with oviposition in birds (Munsick, Sawyer & van Dyke, 1960; Tanako & Nakajo, 1962; Gilbert & Lake, 1963) and has also been shown (Heller, Ferreri & Leathers, 1967) to affect the amphibian oviduct, it seemed worth while to investigate the fate of this hormone in both sexes. Table 2, however, shows that the half-lives of both arginine vasotocin and oxytocin were very similar in male and female birds or toads. In view of the finding of Smith (1963) that Ca^{2+} inhibited the inactivation of vasopressin by rat kidney slices, it seemed surprising that the changes in calcium metabolism in the laying hen do not seem to have an influence on the fate of the hormone in the circulation. The explanation may be that while total concentration of calcium in the

laying hen changes considerably, that of free Ca^{2+} does not (S. H. A. Talukder, private communication). Table 2 shows also that the half-lives of the three active peptides used were longer in birds and toads than those of arginine vasopressin (Ginsburg & Heller, 1953) and oxytocin (Ginsburg & Smith, 1959) in rats. There seems to be a rough correlation between the size of the animal and the half-life of neurohypophysial hormones in the blood, but even if this is taken into account, the half-life of oxytocin in the rabbit (Chaudhury & Walker, 1957) was found to be 3.3 min as compared with about 9 min in chickens with a somewhat lower body weight. The weight of the marine toads used was of the same order as that of adult rats.

TABLE 2

HALF-LIFE (MIN) OF NEUROHYPOPHYSIAL HORMONES IN NON-MAMMALIAN SPECIES

The birds received a single intravenous injection of the hormones of 500 m-u./100 g. body weight. The toads were injected intravenously with 300 m-u./100 g. Means ±S.E. of three to four experiments. The figures in parentheses were calculated on the assumption of a straight-line relationship between time and log blood clearance values.

	Arginine vasotocin	Arginine vasopressin	Oxytocin
Domestic fowl			
Non-laying hens	18·3±2·0	20·8±1·5	10·9±0·2
	(12·2±0·5)	(7·8±0·1)	(8·9±0·6)
Laying hens	20·7±2·2	—	9·0±0·3
	(14·2±1·0)	—	(8·1±0·2)
Cockerels	24·3±2·2	—	10·3±0·7
	(13·4±1·6)	—	(8·9±0·2)
Bufo marinus			
Female	33·0±2·6	23·7±0·9	12·0±0·7
	(19·9±1·3)	(18·2±3·0)	(10·6±1·0)
Male	31·7±2·2	—	9·0±0·1
	(20·9±1·1)	—	(8·0±0·1)

Most of the decay curves obtained in the experiments reported show two exponential components, which is not unusual in clearance experiments of this type (Czaczkes & Kleeman, 1964). It is commonly assumed that the steeper, initial part of such curves reflects the mixing of the intravenously injected hormone and that the second part represents the true rate of decay. The half-lives of vasotocin and vasopressin were therefore calculated from the second slope. The pattern of disappearance of oxytocin was somewhat different from that of vasotocin and vasopressin in the chicken and toad and was similar to the pattern in mammals. All the points on a semi-logarithmic scale fitted a straight line, with the exception of the first point in the chicken results; this deviation may have been caused by insufficient mixing of the hormone.

It seems from the papers of Ginsburg & Heller (1953) and Czaczkes & Kleeman (1964) that the dose of neurohypophysial hormones injected influences the decay curve which, in the case of vasotocin and vasopressin, may be due to differences in the intensity of their vascular effects. This factor may also be concerned in differences between the half-lives of the vaso-active peptides and those of oxytocin found in the present investigation. Table 2 shows that the half-life of oxytocin in both the domestic fowl and in the toads was considerably shorter than that of vasotocin and vasopressin. It shows also that the half-lives of oxytocin were very similar in the chickens and toads, which is in contrast to the results for vasotocin. The half-life of this peptide in the toad, a cold-blooded vertebrate, was considerably longer than that in the chicken with its high body temperature. The

similarity of the half-lives of oxytocin in the two species may at first glance suggest that the difference in the body temperature between chickens and toads did not matter in the case of oxytocin, but if a relationship between the body weight and half-life of the neurohypophysial hormones holds, the figures obtained in the birds with their much greater body weight would still indicate that oxytocin disappears more quickly at the higher body temperature.

It must be emphasized that the half-lives given refer to the decay of the biological activity of the hormones in the circulation and not to the disappearance of half the dose in terms of the weight of hormone injected. Differences from the application of these two parameters might be expected if the mechanisms for the removal or inactivation of the hormones were saturated, which seems unlikely.

The results reported give no information about the mechanism(s) by which the biological activities of the neurohypophysial hormones tested disappeared from the circulation of the non-mammalian vertebrates except that it could be shown that the hormones are not inactivated by incubation with chicken or toad plasma. Gilbert & Lake (1964) have reported that the plasma of egg-laying hens contains an " oxytocinase," but this conclusion is not supported by our experiments *in vitro* with the plasma of laying hens even though the blood was obtained from birds within 1 hr after oviposition. It therefore seems likely that, as in mammals, the neurohypophysial hormones in the chicken and toad are partly excreted and partly inactivated in the tissues. The latter assumption is supported by unpublished results of M. S. A. Talukder (private communication), who found that neurohypophysial hormones are inactivated by kidney and liver slices of the chicken and toad.

SUMMARY

1. Both 8-arginine oxytoxin (vasotocin) and oxytocin were found to disappear rapidly from the circulations of birds (*Gallus domesticus*) and toads (*Bufo marinus*) but at a slower rate than arginine vasopressin and oxytocin in mammals with similar body weights.

2. The rate of disappearance of vasotocin was uniformly longer than that of oxytocin and longer in *Bufo marinus* than in the chicken.

3. With the doses used, the semi-logarithmic curve of decay of the pressor activity of vasotocin in circulating blood showed a diphasic pattern both in the chicken and the toad. The pattern of disappearance of oxytocin from the circulation of both species, like that in mammals, was that of a single exponential curve.

4. No clear difference in the rate of clearance of the neurohypophysial hormones studied was found between male and female chickens or toads or between laying and non-laying hens.

5. No inactivation of vasotocin, arginine vasopressin or oxytocin could be demonstrated when these peptides were incubated with plasma of non-laying or laying hens at 42.5° C or with toad plasma at 26.5° C.

REFERENCES

ACHER, R., CHAUVET, J. & LENCI, M. T. (1960). Isolement de l'ocytocine du poulet. *Biochim. biophys. Acta*, **38**, 344–345.

ARMSTRONG, D., JEPSON, J. B., KEELE, C. A. & STEWART, J. W. (1957). Pain-producing substance in human inflammatory exudates and plasma. *J. Physiol., Lond.*, **135**, 350–370.

CHAUDHURY, R. & WALKER, J. M. (1959). The fate of injected oxytocin in the rabbit. *Endocrinology*, **19**, 189–192.

CZACZKES, J. W. & KLEEMAN, C. R. (1964). The effect of various states of hydration and the plasma concentration on the turnover of antidiuretic hormone in mammals. *J. clin. Invest.*, **43**, 1649–1958.

DEKANSKI, J. (1952). The quantitative assay of vasopressin. *Br. J. Pharmac. Chemother.*, **7**, 567–572.

DYBALL, R. E. J., LANE, G. J. & MORRIS, R. G. (1966). A simplified automatic device for the performance of antidiuretic assays. *J. Physiol., Lond.*, **180**, 43–44*P*.

ELGEE, N. J., WILLIAMS, R. H. & LEE, N. D. (1954). Distribution and degradation studies with insulin. *J. clin. Invest.*, **33**, 1252–1260.

FOLLETT, B. K. & BENTLEY, P. J. (1964). The bioassay of oxytocin: increased sensitivity of the rat uterus in response to serial injections of stilboestrol. *J. Endocr.*, **29**, 277–282.

FOLLETT, B. K. & HELLER, H. (1964). The neurohypophysial hormones of lungfishes and amphibians. *J. Physiol., Lond.*, **172**, 92–106.

GILBERT, A. B. & LAKE, P. E. (1963). The effect of oxytocin and vasopressin on oviposition in the domestic hen. *J. Physiol., Lond.*, **169**, 52–53*P*.

GILBERT, A. B. & LAKE, P. E. (1964). Plasma aminopeptidase (oxytocinase) activity in the domestic fowl. *Proc. V° Congr. Intern. Per La Riproduzione Animale E La Fecondazione Artificiale, Trento*, pp. 317–320.

GINSBURG, M. & BROWN, L. M. (1957). The effects of haemorrhage and plasma hypertonicity on the neurohypophysis. In *The Neurohypophysis*, ed. Heller, H. London: Butterworths.

GINSBURG, M. & HELLER, H. (1953). The clearance of injected vasopressin from the circulation and its fate in the body. *J. Endocr.*, **9**, 282–291.

GINSBURG, M. & SMITH, M. W. (1959). The fate of oxytocin in male and female rats. *Br. J. Pharmac. Chemother.*, **14**, 327–333.

GREENSPAN, F. S., LI, C. H. & EVANS, H. M. (1950). Disappearance rate of adrenocorticotropic hormone from rat plasma after intravenous injection. *Endocrinology*, **46**, 261–264.

HELLER, H. (1966). The hormone content of the vertebrate hypothalamo-neurohypophysial system. *Br. med. Bull.*, **22**, No. 3, 227–231.

HELLER, H., FERRERI, E. & LEATHERS, D. G. H. (1967). The effect of neurohypophysial hormones on the amphibian oviduct. *J. Endocr.*, **37**, xxxix.

HELLER, H. & GINSBURG, M. (1966). Secretion, metabolism and fate of the posterior pituitary hormones. In *The Pituitary Gland*, ed. Harris, G. W. & Donovan, B. T., vol. 3, pp. 330–373. London: Butterworths.

HELLER, H., HASAN, S. H. & SAIFI, A. Q. (1968). Antidiuretic activity in the cerebrospinal fluid. *J. Endocr.*, in the Press.

HOLTON, P. (1948). A modification of the method of Dale and Laidlaw for standardization of posterior pituitary extract. *Br. J. Pharmac. Chemother.*, **3**, 328–334.

JEFFERS, W. A., LIVEZEY, M. M. & AUSTIN, J. H. (1942). A method for demonstrating an antidiuretic action of minute amounts of Pitressin. Statistical analysis of results. *Proc. Soc. exp. Biol. Med.*, **50**, 184–186.

LANDGREBE, F. W., MACAULEY, M. H. I. & WARING, H. (1946). The use of rats for pressor assays of pituitary extracts with a note on response to histamine and adrenaline. *Proc. R. Soc. Edinb.*, **62**, 202–210.

LAUSON, L. D. (1967). Metabolism of antidiuretic hormones. *Am. J. Med.*, **42**, 713–744.

MUNSICK, R. A. (1960). Effect of magnesium ion on the response of the rat uterus to neurohypophysial hormones and analogues. *Endocrinology*, **66**, 451–457.

MUNSICK, R. A. (1966). Chromatographic and pharmacologic characterization of neurohypophysial hormones of an amphibian and reptile. *Endocrinology*, **78**, 591–599.

MUNSICK, R. A., SAWYER, W. H. & VAN DYKE, H. B. (1960). Avian neurohypophysial hormones. Pharmacological properties and tentative identification. *Endocrinology*, **66**, 860–871.

RICHARDS, J. B. & SAYERS, C. (1951). Fate and excretion of adrenocorticotrophic hormones. *Proc. Soc. exp. Biol. Med.*, **77**, 87–93.

SAYERS, G., BURNS, T. W., TYLER, F. H., JAGER, B. V., SCHWARTZ, T. B., SMITH, E. L., SAMUELS, L. I. & DAVENPORT, H. W. (1949). Metabolic actions and fate of intravenous administered adrenocorticotropic hormones in man. *J. clin. Endocr.*, **9**, 593–614.

SHARE, L. (1961). Acute reduction in extracellular fluid volume and the concentration of antidiuretic hormone in blood. *Endocrinology*, **69**, 925–933.

SMITH, M. W. (1963). The effect of calcium on the inactivation of vasopressin by rat kidney slices. *J. Physiol., Lond.*, **166**, 22–23*P*.

TANAKO, K. & NAKAJO, S. (1962). Participation of neurohypophysial hormones in oviposition in the hen. *Endocrinology*, **70**, 453–458.

TINDAL, J. S. & YOKOYAMA, A. (1962). Assay of oxytocin by the milk-ejection response in the anaesthetized lactating guinea-pig. *Endocrinology*, **71**, 196–202.

WEINSTEIN, H., BERNE, R. H. & SACHS, W. (1960). Vasopressin in blood: effect of haemorrhage. *Endocrinology*, **66**, 712–718.