J. Physiol. (1968), **196**, *pp.* 495–505
With 1 *text-figure*
Printed in Great Britain

FACTORS INFLUENCING THE SENSITIVITY OF THE RAT TO VASOPRESSIN

BY MARY L. FORSLING,* J. J. JONES AND J. LEE
From the Department of Physiology, Charing Cross Hospital Medical School, London, W.C. 2

(*Received* 31 *January* 1968)

SUMMARY

1. Indirect evidence suggests that the concentration of arginine vasopressin in the plasma of normally hydrated man is about 1 μ-u./ml., but this is usually considered to be below the limit of sensitivity of the standard assay preparation, the water-loaded Wistar rat under ethanol anaesthesia.

2. It was found that there was a surprising variation in sensitivity to vasopressin between batches of Wistar rats, and that other varieties of rat (including those with diabetes insipidus) were no more sensitive.

3. Three modifications of the standard assay procedure produced an increase in sensitivity:

(*a*) using Wistar rats weighing 100–150 g, rather than larger animals;

(*b*) commencing the assay shortly after surgery, i.e. as soon as the urine flow reached 25 μl./min;

(*c*) infusing vasopressin intravenously (0·5–3 μ-u./min). By using modification (*a*) with either (*b*) or (*c*) it was possible to detect as little as 0·5 μ-u.

4. With these modifications antidiuretic activity equivalent to 0·5–2·0 μ-u./ml. of arginine vasopressin was measured in nine samples of plasma from a normally hydrated subject.

5. It is suggested that the frequent reports of enhanced sensitivity may have been due to the fortuitous use of a particularly sensitive batch of rats, or to a high endogenous secretion of vasopressin due to operative trauma.

INTRODUCTION

Evidence, both direct and indirect, which indicates that the concentration of vasopressin in the plasma of man in normal water balance is of the order of a few μ-u./ml. has been reviewed by Lauson (1967). The standard preparation used for the assay of vasopressin is the rat under

* Supported by a grant from the Medical Research Council.

ethanol anaesthesia given an excess of water, but it had been generally accepted that about 10 μ-u. was the minimum dose for an adequate response in this preparation (Jeffers, Livezey & Austin, 1942; Dicker, 1953). Thus, in order to detect the minute quantities in plasma, the sensitivity of the preparation had to be markedly improved. In 1959 Heller & Štulc introduced modifications which, they reported, enabled them to detect as little as 0·625 μ-u. Czaczkes, Kleeman & Koenig (1964) agreed with this, Tata & Gauer (1966) detected 0·2 μ-u. and Gupta, Chaudhury & Chuttani (1967), 0·1 μ-u. Nevertheless, the majority of workers still report a sensitivity which is some twenty-fold less.

The present work sets out to examine the possible causes for the conflicting results concerning the sensitivity of the rat preparation, and to examine various procedures which affect its responsiveness. With the introduction of modifications which increase the sensitivity of the rat preparation to vasopressin, it has become possible to carry out a preliminary investigation into the concentration of antidiuretic activity in human plasma.

METHODS

The female Wistar rat of weight 150–250 g, allowed free access to water and on a diet of 41.B pellets, was the standard preparation used. The method used for over-hydration and anaesthesia was to administer a 12% ethanol solution by stomach tube; the total volume amounted to 6% of the animal's weight, given in two doses 30 min apart. The external jugular vein was catheterized (cannula P.P 50 Portex), the urinary bladder exposed and a catheter inserted (cannula P.P. 240 Portex). All wounds were sutured. Hydration was maintained by intravenous infusion of 1·5% ethanol in 0·4% saline at a rate of 120 ± 20 μl./min, this composition of intravenous fluid having been found to be the most suitable. After an interval of 30–60 min, when the urine flow had reached about 100 μl./min, the assay procedure was commenced. Materials to be assayed were injected into the catheterized jugular vein and washed in by perfusion fluid. The volumes injected were usually between 0·25 and 0·5 ml.; on rare occasions 1 ml. was given, in which case it was injected slowly over 1 min. Every fifth rat was weighed at the end of the assay procedure to ensure that excessive overhydration had not occurred. Urine flow was measured by displacing silicone fluid (M.S. 200 3 ctS Midland Silicone Co.) through a photo-electric cell. Each drop was recorded on paper using a staircase integrator set for 1 min (Devices of Welwyn Garden City). The electrolyte content of the urine was estimated by measuring urine conductivity in the bladder catheter between two platinum electrodes (Devices conductivity assembly). The percentage inhibition of urine flow was calculated by comparing the pre-injection level with that passed in the 3rd–7th min after injection. To compare the sensitivities of the different preparations, the dose of vasopressin producing a 50% antidiuretic response was determined from a log dose–response curve constructed for each preparation. The hormone standards were synthetic arginine vasopressin, lysine vasopressin and oxytocin (Sandoz).

The variables used

Variation in the rat preparation

(*a*) Weight of rats and batch. Two groups of Wistar rats were compared: (i) weighing 100–150 g; (ii) weighing 151–250 g. One consignment of rats constituted one batch, and three batches of rats were studied.

(*b*) Different strains. Besides the standard Wistar rat, the following were investigated: Sprague–Dawley, hooded Long Evans, non-diabetic insipidus (NDI) Brattleboro, Merionides and specific pathogen free (SPF) Wistar rats.

(*c*) Rats with diabetes insipidus. Rats with diabetes insipidus (DI), in which the disease was either congenital (Brattleboro strain), or produced by destruction of the median eminence using ultrasound or electrolysis, were studied. Dr John Pond produced ultrasonic lesions of the median eminence of female Wistar rats (weighing 165–200 g) though a 1 cm diameter hole in the skull at the lambda. The ultrasonic beam was at a frequency of 3 MHz and an intensity of 1·5 kW/cm^2 for an exposure of 1–4 sec. Diabetes insipidus was also induced in Wistar rats by electrolytic destruction of the median eminence, as described by Kennedy, Lipscomb & Hague (1963).

Variation in the procedure

(*a*) No-protein diet. A diet devoid of protein and consisting of sucrose, olive oil and Marmite was given to the rats for 14 days.

(*b*) Urea-enriched diet. Two rats were given their normal diet plus urea (3 g/day) by gavage.

(*c*) Repeated intragastric loading. Rats were given intragastric water loads equivalent to 4% of their body weight every day for 3 or 4 days before assay.

(*d*) Tracheotomy. The effect of a tracheotomy on sensitivity was determined.

(*e*) Respiratory infection. The responses of rats with respiratory infections were recorded.

(*f*) Variation in the method of hydration. Instead of the initial intragastric loading, the fluid was given intravenously through a cannula inserted 24 hr previously. In these circumstances only 2 ml. of 12% ethanol in 0·4% saline was given, the remaining volume consisting of 1·5% ethanol in 0·4% saline. Another variation was to use the intragastric route both for the initial hydration and for its maintenance 1·5% (v/v) ethanol was dissolved in water and administered at the same rate as intravenously (100–140 μl./min). In one group of rats all access to water was withdrawn 12–16 hr before the assay.

(*g*) Reduction of the surgical trauma. All the operative procedures were carried out several days before assay to avoid the effects of trauma. This technique has been described previously and these rats are referred to as pre-operated (Jones & Lee, 1965). Bladder catheterization was also used as an alternative to the routine suprapubic incision, so as to reduce surgical manipulations before assay.

(*h*) Administration of phenoxybenzamine. Sixteen hours before using the rat for assay 10 mg/kg was injected sub-cutaneously.

(*i*) Use of local anaesthetics. The suprapubic and inguinal areas (before femoral vein cannulation) were infiltrated with lignocaine hydrochloride (0·5 g/100 ml.).

(*j*) Varying the route of the administered hormone. Intra-arterial injections were made into a polyethylene (PP.25) catheter which had been passed down the carotid artery to the abdominal aorta, to just above the level of the renal arteries. Alternatively either the tail vein or femoral vein was used.

(*k*) Time of commencement of assay. The assay procedure was commenced soon after completing the surgery, when the urine flow reached 25 μl./min.

(*l*) Intravenous infusion of neurohypophysial hormones. Sufficient hormone was infused to reduce the urine flow to about half. The amount of arginine vasopressin required to achieve this varied between 0·5 and 3 μ-u./min, the smaller dose frequently proving sufficient. A similar quantity of lysine vasopressin was needed, whereas with oxytocin it had to be raised to 250–500 μ-u./min. Sensitivity to the neurohypophysial hormones was determined before, during and after the infusion.

Each animal served as its own control in procedures *j*, *k* and *l*. In the remaining experiments (*a–i*) each rat was compared with a litter mate of the same weight, and whenever possible this comparison was carried out on the same day.

In some experiments the number of observations was small and their non-Gaussian (e.g. skew) distribution makes the median the most suitable statistic to represent each group. The precision that one can attach to the median is indicated by the 'limits' of the median when $P = 2{\cdot}5\,\%$ (single tail). The groups were compared with each other by the signed rank test (Wilcoxon & Wilcox, 1964) or the two-sample rank test (White, 1952; Wilcoxon & Wilcox, 1964). The method used to calculate the 'limits' is illustrated in the Appendix together with a worked example of the signed rank test.

Assay of human plasma

Only one subject was investigated and blood samples were taken at different times of the day. Blood was collected into a 20 ml. plastic disposable syringe containing 500 u. of heparin. Immediately after collection, the blood was centrifuged at 50 sec^{-1} for 5 min. The plasma was divided into two portions: one was assayed at once for its antidiuretic activity and the osmolality of the other was determined cryoscopically (Advance Instruments, Fisons, of Loughborough). In order to assess the reliability of the assay preparation arginine vasopressin was added to samples of plasma to bring the concentration up to 2 μ-u./ml. before assay.

RESULTS

The results may be considered under two main headings: (*a*) where the procedure was standard and the rat preparation altered (Table 1), and (*b*) where the rat preparation was standard and the procedure altered (Tables 2 and 3).

Standard procedure varying the preparation (*Table* 1). As sensitivity varied so widely between different batches of standard rats, comparison between different strains of rat was difficult, and it was not possible to detect any change of sensitivity in response to arginine vasopressin with the Sprague-Dawley, hooded Long Evans, Brattleboro NDI and SPF rats, although the Merionides showed a marked decrease in sensitivity.

The antidiuretic response to vasopressin by rats of weight 100–150 g was significantly greater than that by heavier rats of the same batch even when the dose was expressed on a body weight basis.

The sensitivity to arginine vasopressin of rats with congenital DI was about twice that of their NDI litter mates but they were no more sensitive than the normal Wistar rats of the most sensitive batch (III). Comparing normal Wistar rats with their litter mates who had DI induced experimentally also failed to reveal any change in sensitivity to the hormone. An attempt was made to induce diabetes insipidus by ultrasonic lesions in eighteen rats and by electrolytic lesions in twelve: ten of these rats (ultrasound six, electrolytic four) died within 24 hr. Hypotonic urine and polyuria developed in nine rats but only five rats (ultrasound four, electrolytic one) fulfilled the criteria for complete loss of neurohypophysial function (Jones & Lee, 1967).

Standard rat varying the procedure (*Tables* 2 *and* 3). The only two variations which significantly increased the sensitivity of the preparation were

infusion of vasopressin (Table 3) and commencement of the assay as soon as the urine flow reached 25 μl./min (Table 2). The latter preparation was essentially similar to the infused one, but the enhanced sensitivity lasted

TABLE 1. The dose of arginine vasopressin in μ-u./100 g producing 50 % inhibition of water diuresis in various strains of rat under standard assay conditions

Strain of rat	Median dose	'Limits' of median*	Number of rats
(*a*) Wistar, batch I < 150 g body weight	7	4–10	21
(*b*) Wistar, batch I > 150 g body weight	10	7–12	87
(*c*) Wistar, batch II > 150 g body weight	14	11–20	16
(*d*) Wistar, batch III > 150 g body weight	5	3–9	29
(*e*) Sprague–Dawley	6	3–10	6
(*f*) Merionides	100	20–250†	4
(*g*) Hooded Long Evans	11	9–19†	5
(*h*) SPF rats	—	20 and 30†	2
(*i*) NDI Brattleboro	7	6–20	20
(*j*) DI Brattleboro	4	2–9	7
(*k*) DI Wistar	4‡	3–9†	5

* See Appendix.

(*a*) < (*b*), $P < 0{\cdot}02$; (*j*) < (*i*), $P < 0{\cdot}01$

† = range.

‡ The litter mates of these rats are included in batch III.

The weight of rats *e*–*h* was between 150 and 250 g.

TABLE 2. The effect of modifying the assay procedure on the dose of arginine vasopressin in μ-u. producing 50 % inhibition of water diuresis in the standard Wistar rat

Procedure	Median dose for control	Median dose (procedure –control)	'Limits' of median		Number of rats
(*a*) No-protein diet	9	+5	+15	−5	12
(*b*) Urea-enriched diet	9	+10	+11 and	+8	2
(*c*) Repeated intragastric water load	11	+4	+8	−3	8
(*d*) After tracheotomy	10	+8	+12	−5	18
(*e*) Infection	—	†	—		10
(*f*) Variation in method of hydration:					
(1) Intragastric and intragastric	17	+6	+15	−5	30
(2) Intravenous and intravenous	12	+8	+11	−6	16
(3) Deprivation of water prior to assay	8	+5	+7	−3	16
(*g*) Reduction of surgical trauma:					
(1) Pre-operation	18	−0·5	+3	−7	11
(2) Bladder catheterization	8	−5	+4	−8	7
(*h*) Phenoxybenzamine	16	+5	+13	−4*	5
(*i*) Local anaesthesia	12	+1	+6	−4	14
(*j*) Varying the site of hormone injection:					
(1) Intra-aortic	10	0	+3	−6	6
(2) Tail vein	16	−3	+3	−8	12
(3) Femoral vein	18	−3	+4	−10	20
(*k*) Assay as soon as flow = 25 μl./min	14	−6‡	−12	−4	16

* = range.

† These rats died after a few injections; at least 100 μ-u. were required to induce a minimal antidiuretic response.

‡ This difference is significant, $P < 0{\cdot}01$. None of the other differences is significant.

for not more than four or five injections, and only very occasionally would this preparation respond to 0·5 μ-u. (25% inhibition of urine flow). In two out of twenty-four rats infused with arginine vasopressin a similar sensitivity was achieved.

TABLE 3. The effects of background infusions of vasopressin or oxytocin on the dose of arginine vasopressin in μ-u. producing a 50% inhibition of water diuresis in the standard Wistar rat

Infusion	Median dose for control	Median dose (procedure-control)	Limits of median		P	Number of animals
Arginine vasopressin	16	−4	−2	−10	< 0·01	24
Lysine vasopressin	9	−4	0	−8	< 0·01	11
Oxytocin	13	+8	0	+12	< 0·05	7

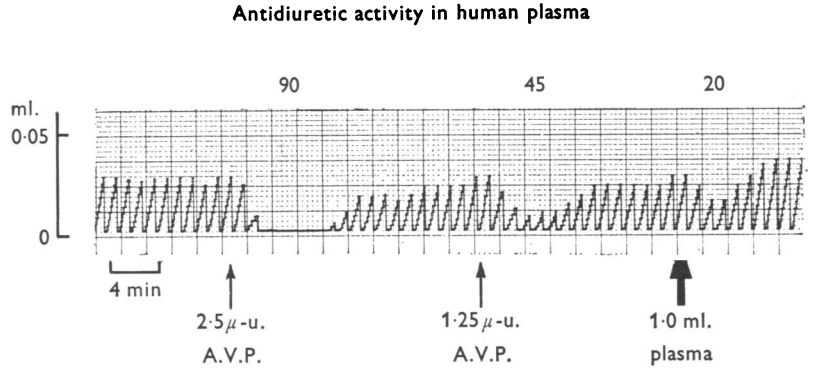

Fig. 1. Urine flow in a water loaded rat under ethanol anaesthesia. The tracing shows the antidiuretic response of one of the highly sensitive rats (female Wistar weight 130 g) where the bio-assay was commenced as soon as the urine flow reached 25 μ-l./min. A.V.P. = arginine vasopressin. All injections are given intravenously. The numbers above the tracing represent the percentage inhibition of urine flow as described in Methods.

Intravenous infusion of lysine vasopressin also increased the sensitivity of the preparation. In contrast, an infusion of oxytocin reduced the antidiuretic activity of vasopressin and also the antidiuretic activity of oxytocin itself.

Estimation of antidiuretic activity in human plasma. With the modifications introduced it became possible to detect 0·5 μ-u. of vasopressin and thus feasible to attempt to measure the antidiuretic activity of human plasma. Both methods (intravenous infusion of vasopressin and commencement of the assay as soon as the urine flow reached 25 μl./min) were employed in the assay of the nine samples of human plasma. The anti-

diuretic activity was equivalent to 0·5–2 μ-u./ml. of vasopressin and the osmolality range was from 285 to 310 m-osmole/kg. The tracing shows the response of the preparation to 1 ml. of plasma and to vasopressin. The plasma to which hormone had been added gave the expected increase in antidiuretic activity.

DISCUSSION

Despite extensive investigation it was not possible to determine why different batches of the same strain of rat should vary so widely in their sensitivity to arginine vasopressin (Table 1). Consequently a change in responsiveness with variation of the strain of rat would have to be considerable to be detected, and in fact, apart from Merionides, no such difference was found. Even the rat with diabetes insipidus, whether of genetic or surgical origin, was not significantly more sensitive than some standard Wistar rats. However, one positive fact did emerge: the smaller animals were more sensitive, although if they were too small there were technical difficulties and their responses became irregular. Rats weighing 100–150 g proved to be the most suitable and of particular value when the quantity of hormone available was small.

Alterations of the procedures either allowed the rats to serve as their own controls or allowed the sensitivity of litter mates to be compared; thus relatively small changes could be detected. Contrary to the suggestion of Gupta *et al.* (1967) changes in either the method of hydration or the nitrogen intake did not enhance the sensitivity to vasopressin. A possible reason for the difference in sensitivity between groups could be a variation in the degree of adrenergic stimulation induced by operative trauma. However, the prior administration of phenoxybenzamine did not influence the responses of the preparation. When the route of administration of vasopressin was altered each animal served as its own control, but despite the claim by Holliday, Burstin & Harrah (1963) intra-aortic injection showed no advantage.

It was suggested by Tata & Buzalkov (1966) and implied by Heller & Štulc (1959) and Czaczkes *et al.* (1964) that their remarkable sensitivity was achieved by the virtual absence of endogenous vasopressin, a condition induced by minimizing the surgical trauma. Their explanation is difficult to accept because the various procedures designed to limit the effect of surgical trauma (e.g. pre-operation, use of local anaesthetics, bladder catheterization) failed to produce the expected advantages in our experiments. Indeed, the speculation is untenable since the Brattleboro DI rat, while twice as sensitive as its NDI litter mate (Sawyer & Valtin, 1967; Vierling, Little & Radford, 1967), is no more sensitive than the standard Wistar rat (Jones & Lee, 1967). Furthermore, in the present

work DI induced by surgical intervention did not enhance the sensitivity of the Wistar rat.

An inspection of the records of Heller & Štulc (1959), Czaczkes *et al.* (1964) and Tata & Buzalkov (1966) showed a low urine flow and this suggested that their rats had an excess of endogenous vasopressin and not an absence of the hormone. It was decided, therefore, to determine the effect of a background infusion of arginine vasopressin and it was found possible with this procedure to obtain a preparation that would occasionally respond to as little as 0·5 μ-u.; these results have been reported briefly (Forsling, Jones & Lee, 1967*a*, *b*). This finding applies equally to lysine vasopressin and each hormone sensitizes the preparation to the other; the antidiuretic effect of oxytocin is also enhanced. The increased sensitivity to vasopressin during a background infusion of the hormone is not simply due to reduced urine flow, as lowering the urine flow by decreasing the infusion rate did not alter sensitivity. Nevertheless, itis true that standard preparations with relatively low urine flows, soon after operation, were occasionally much more sensitive to arginine vasopressin, but this occurred presumably because there was endogenous secretion of the hormone or a low glomerular filtration rate.

It was found that an intravenous infusion of oxytocin had the opposite action, decreasing the antidiuretic effect of oxytocin and of vasopressin. It is interesting to note that the infusion of oxytocin into the lactating rat has been found to cause a five–twenty fold increase in the threshold dose of oxytocin which is required to produce a milk ejection response on intravenous injection (M. Fabian, M. L. Forsling, J. J. Jones & J. Lee, unpublished work). A similar effect has been described in the guinea-pig by Beránková-Ksandrová, Bisset, Jošt, Krejčí, Pliška, Rudinger, Rychlík & Šorm (1966). The failure of the congenital DI rat to be more sensitive to vasopressin may be due to its endogenous secretion of oxytocin, which appears to be unimpaired, although the inability to secrete oxytocin, as exemplified by DI induced in the Wistar rat, did not improve its sensitivity.

The value of vasopressin infusion in increasing the sensitivity of the rats must not be over emphasized: for example, when considering the results it is of interest that the median response of these rats was similar to batch III of standard Wistar rats. The positive contribution is small, but it can provide a preparation that sometimes gives an adequate response to 0·5 μ-u. (25% inhibition of urine flow). In addition, a background infusion of vasopressin is useful to increase the responsiveness of a relatively insensitive preparation (i.e. responding to 20–30 μ-u.), although ineffective in highly sensitive or insensitive animals. It must be pointed out that these preparations are not suitable for prolonged assays as the threshold rises and the responses become irregular.

It would therefore appear that those workers claiming a sensitivity of less than 1 μ-u. were probably employing a preparation with a high level of endogenous vasopressin, induced by surgical trauma, and were starting the assay soon after completing the surgery; also, they may have used a particularly sensitive batch of rats. Although it is thus possible to offer some explanation which may account for these claims of high sensitivity, this in no way vindicates their results. While these investigators reported that not all the rats were satisfactory, they failed to appreciate that at this low sensitivity the preparation may respond in an unreliable and capricious way, rendering any findings suspect. Despite the frequently quoted findings of Heller & Štulc (1959) in clinical work, their results have to be accepted with caution. It was therefore necessary to determine the basal concentration of vasopressin in plasma of normal man; plasma was used, as methods which involve considerable concentration of plasma and subsequent extraction of the hormone appear to introduce inherent errors.

In order to achieve a preparation which will respond satisfactorily to 0·5 μ-u., whether by infusing vasopressin or by using the assay preparation soon after operative procedure, many preparations may have to be discarded. Furthermore, several of these sensitive preparations are essential for assaying each sample of plasma, since it is seldom possible to give more than 1 ml. of plasma to each rat without loss of sensitivity. A small pilot experiment has been carried out to estimate the quantity of antidiuretic activity in human plasma from one subject at varying stages of hydration, as determined by the plasma osmolality. It is of interest that the predicted concentration of vasopressin of about 1 μ-u./ml. is very close to the observed antidiuretic activity, equivalent to 0·5–2·0 μ-u./ml. of vasopressin. The level of activity appeared to be related to the osmolality, but the inaccuracies of the assay at these low concentrations preclude any definite conclusions. It will not be possible to establish that this activity is due to arginine vasopressin until all the criteria of identification described by Barraclough, Jones & Lee (1966) have been satisfied.

We wish to thank Dr John Pond for producing the ultrasonic lesions, Professor R. Warwick for the hospitality of the Ultrasonic Laboratory at Guy's Hospital and Miss P. Woodward, of the National Institute for Medical Research, for help with the statistics. We are grateful to Dr H. Valtin for supplying the Brattleboro rats and to Sandoz for the synthetic hormones.

APPENDIX

A worked example to illustrate the statistical calculations

The example is taken from Table 3: the effect of a background infusion of lysine vasopressin on the dose of arginine vasopressin (μ-u.) producing a 50% inhibition of water diuresis.

Dose for control	Dose (procedure − control)	Signed rank
11	+2	+1·5
10	0	—
3	−2	−1·5
4	−3	−3·5
5	−3	−3·5
5	−4	−5·5
6	−4	−5·5
9	−6	−7
13	−7	−8
19	−8	−9
25	−10	−10
	Rank sum	−52

The median is the $\frac{1}{2}(n+1)$ item, i.e. the 6th. The median control is 9 μ-u. and the median pair difference is -4 μ-u.

The 'limits' of the median of the paired differences (i.e. the limits within which the median of the population represented by these data is expected to lie) are obtained using table I from Mainland (1963). In this table for $P = 2{\cdot}5\%$ (single tail) when $n = 11$ and $X = 1$, the upper limit is about 40% (by interpolation), and when $n = 11$ and $X = 2$, the upper limit is 52%. Therefore the expected median might be the second item (i.e. 0) since $X = 2$ includes the median (i.e. the 50th percentile) when $P = 2{\cdot}5\%$ (single tail). The expected median is unlikely to be the first item (i.e. +2) since $X = 1$ excludes the median. Similarly, the lower limit of the expected median is the tenth item (i.e. −8) and not the eleventh. The limits of the expected median in the parent population of paired differences are 0 and −8 with a probability of 2·5% (single tail). The control and procedure groups were compared by the signed rank test (Wilcoxon & Wilcox, 1964). The zero difference was dropped and the paired differences were arranged in ascending order disregarding sign. They were numbered in order of rank, giving ties the mean rank. Each rank was given a plus or minus sign according to the sign of the difference. The algebraic sum of the ranks was −52.

In Mainland's (1963) table VI, when $n = 10$ (since the zero difference was excluded), and $P = 0{\cdot}01$ (both tails), the minimum rank sum is 49. The observed rank sum was greater than 49 so that the probability of these pair differences being due to random sampling from an infinite parent population in which positive and negative items were equally common was less than 0·01.

REFERENCES

BARRACLOUGH, M. A., JONES, J. J. & LEE, J. (1966). Production of vasopressin by anaplastic oat cell carcinoma of the bronchus. *Clin. Sci.* **31**, 135–144.

BERÁNKOVÁ-KSANDROVÁ, Z., BISSET, G. W., JOŠT, K., KREJČÍ, I., PLIŠKA, V., RUDINGER, J., RYCHLÍK, I. & ŠORM, F. (1966). Synthetic analogues of oxytocin acting as hormonogens. *Br. J. Pharmac. Chemother.* **26**, 615–632.

CZACZKES, J. W., KLEEMAN, C. R. & KOENIG, M. (1964). Physiologic studies of antidiuretic hormone by its direct measurement in human plasma. *J. clin. Invest.* **43**, 1625–1640.

DICKER, S. E. (1953). A method for the assay of very small amounts of antidiuretic activity with a note on the antidiuretic titre of rats' blood. *J. Physiol.* **122**, 149–157.

FORSLING, MARY L., JONES, J. J. & LEE, J. (1967*a*). A change of sensitivity to neurohypophysial hormones induced by their intravenous infusion in the rat. *J. Physiol.* **191**, 127–128*P*.

FORSLING, MARY L., JONES, J. J. & LEE, J. (1967*b*). Intravenous infusion of vasopressin to increase the sensitivity of its assay in the water-ethanol loaded rat. *Nature, Lond.* **215**, 433–434.

GUPTA, K. K., CHAUDHURY, R. R. & CHUTTANI, P. N. (1967). Plasma antidiuretic hormone concentrations in normal subjects and in persons with oedema of cardiac and renal origin, and in normal pregnancy. *Indian J. med. Res.* **55**, 643–651.

HELLER, J. & ŠTULC, J. (1959). Physiology of the antidiuretic hormone. 1. A simple titration method. *Physiologia bohemoslov.* **8**, 558–564.

HOLLIDAY, M. A., BURSTIN, C. & HARRAH, JEAN (1963). Evidence that the antidiuretic substance in the plasma of children with nephrogenic diabetes insipidus is antidiuretic hormone. *Pediatrics, Springfield* **32**, 384–388.

JEFFERS, W. A., LIVEZEY, MARY M. & AUSTIN, J. H. (1942). A method for demonstrating antidiuretic action of minute amounts of pitressin; statistical analysis of results. *Proc. Soc. exp. Biol. Med.* **50**, 184–188.

JONES, J. J. & LEE, J. (1965). The value of the pre-operated rat in the bioassay of vasopressin. *J. Endocr.* **33**, 329–330.

JONES, J. J. & LEE, J. (1967). The value of rats with hereditary hypothalmic diabetes insipidus for the bioassay of vasopressin. *J. Endocr.* **37**, 335–344.

KENNEDY, G. C., LIPSCOMB, H. S. & HAGUE, P. (1963). Plasma corticosterone in rats with experimental diabetes insipidus. *J. Endocr.* **27**, 345–353.

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

MAINLAND, D. (1963). *Elementary medical statistics*, pp. 253, 266, 273, 283, 358, 371, 373, 2nd edn. London: Saunders.

SAWYER, W. H. & VALTIN, H. (1967). Antidiuretic responses of rats with hereditary hypothalamic diabetes insipidus to vasopressin, oxytocin and nicotine. *Endocrinology* **80**, 207–210.

TATA, P. S. & BUZALKOV, R. (1966). Vasopressin studies in the rat. III. Inability of ethanol anaesthesia to prevent ADH secretion due to pain and haemorrhage. *Pflügers Arch. ges. Physiol.* **290**, 294–297.

TATA, P. S. & GAUER, O. H. (1966). Vasopressin studies in the rat. I. A sensitive bioassay for exogenous vasopressin. *Pflügers Arch. ges. Physiol.* **290**, 279–285.

VIERLING, A. F., LITTLE, J. B. & RADFORD, E. P. JR. (1967). Antidiuretic hormone bioassay in rats with hereditary hypothalamic diabetes insipidus (Brattleboro strain). *Endocrinology* **80**, 211–214.

WHITE, C. (1952). The use of ranks in a test of significance for comparing two treatments. *Biometrics* **8**, 33–42.

WILCOXON, F. & WILCOX, ROBERTA, A. (1964). *Some Rapid Approximate Statistical Procedures*. New York: Lederle Laboratories.