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ANTIDIURETIC SUBSTANCES IN HUMAN URINE AFTER HAEMORRHAGE, FAINTING, DEHYDRATION AND ACCELERATION

By R. L. NOBLE and N. B. G. TAYLOR*

From the Departments of Physiology and Medical Research, University of Western Ontario, London, Canada

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Gilman & Goodman (1937) reported the presence of an antidiuretic substance in the urine of dehydrated rats. Evidence was presented that the antidiuretic substance was identical with the secretion of the posterior lobe of the pituitary gland. This work has been confirmed by others (Boylston & Ivy, 1938; Bundschuh & Kuschinsky, 1939; Hare, Hickey & Hare, 1941; Ingram, Ladd & Benbow, 1938). In contrast to these results, Walker (1939) found an antidiuretic substance in the urine of normal rats but was unable to confirm that it was increased after dehydration or absent after hypophysectomy. Noble, Rinderknecht & Williams (personal communication) observed that when male or female human subjects were dehydrated by the exclusion of fluid from the diet, the urine contained an antidiuretic substance which they believed to be the posterior lobe hormone. No antidiuretic substance was found in the urine of subjects with a normal fluid intake. Similar findings have been reported for normal female subjects, both pregnant and non-pregnant (Teel & Reid, 1939). Heller & Urban (1935) have shown that the rat can excrete injected posterior lobe extract in the urine; there are similar reports for other animals (Heller, 1937; Larson, 1938). Considerable evidence has been presented by Brun, Knudsen & Raaschou (1945*a–d*, 1946) that the antidiuresis following postural fainting is due to the liberation of hormone from the neurohypophysis.

The experiments reported in this paper describe the presence or absence of an antidiuretic substance in the urine in a variety of conditions. A preliminary report of this work has appeared (Taylor & Noble, 1950), and our observations concerning the results of the intravenous infusion of vasopressin in man have since been confirmed by Burn & Singh Grewal (1951).

* Medical Research Fellow of the National Research Council of Canada.

METHODS

Dehydration of rats

The rats were deprived of food, but had free access to water in the 18 hr period preceding the experiment. At the start of the experiment they were deprived of water and placed over funnels in clean dry metabolism cages with wire-grid bottoms. There were four rats in each of eight cages. The urine was collected in beakers containing 5 ml. of 1% (v/v) acetic acid and a small amount of toluene as preservative. Precautions were taken to prevent the contamination of the urine with faeces. At the end of each 24 hr period the rats were transferred to clean dry cages and funnels and the used cages were carefully washed with a fine jet of distilled water. The urine and washings from all cages were pooled (forming one sample for each 24 hr period), strained to remove any foreign matter, concentrated *in vacuo* at low temperature to a specific gravity of 1·020, and then extracted. The final volume of extract was such that 1 ml. extract represented the daily urine excretion of one rat. The experiment was terminated after 72 hr.

Dehydration of man

Human male volunteers were dehydrated by the exclusion from the diet of liquids and foods of high fluid content. The urine was voided at will, the time of voiding and the amount of urine being recorded. Three experiments were done, the period of dehydration being 48 hr in two of the experiments and 65 hr in the other. The specific gravity of the urine ranged from 1·030 to 1·040 when voided. Each specimen was adjusted to a specific gravity of 1·010 with distilled water and extracted. The extracts were concentrated to 1% of the volume of urine at sp.gr. 1·010.

Intravenous infusion of vasopressin

The urine specimens were obtained during the course of experiments on the effect of vasopressin (Pitressin, Parke Davis) on the peripheral blood flow of healthy young adult male human subjects (Sunahara, Duncanson & Edholm, 1949). At the beginning of the experiment the subject emptied his bladder and this specimen was kept as a control. A continuous-drip intravenous infusion of physiological saline was started, and the vasopressin was injected into the tubing of the infusion apparatus during a period of 10 min. The subject again emptied his bladder 4–5 hr after completion of the infusion of vasopressin. Both specimens of urine were adjusted to a specific gravity of 1·010 with distilled water and extracted. The extracts were concentrated to 10% of the volume of urine at sp.gr. 1·010.

Venesection experiments

Opportunity for the study of urine after fainting arose out of experiments on the effect of venesection on the peripheral blood flow (Edholm, 1949). The following scheme outlines the experimental procedure and the collection of urine specimens.

Before the experiment. The subject voided (control, specimen A).

At 0 *hr.* The subject lay on a bed, legs horizontal, the trunk supported at an angle of 60° from the horizontal. He remained in this position until the blood pressure and heart rate were constant.

At 2–2½ *hr.* Venesection started. Blood was withdrawn from the right median basilic vein. If the subject fainted, venesection was discontinued immediately. The amounts of blood taken ranged from 359 to 1270 ml.

At 2½–3 *hr.* Venesection ended. The subject remained quietly on the bed until the blood pressure and heart rate again became constant.

At 3½–6 *hr.* The subject voided (specimen B) and the previously withdrawn blood was re-infused.

At 7–8 *hr.* The experiment was ended. The subject voided (specimen C). The control specimen of urine was not obtained in all experiments. In one experiment, two control specimens were obtained. The first was voided before the experiment and a second was collected in the 24 hr period after the experiment. All urine specimens were adjusted to a specific gravity of 1·010 with distilled water. The extracts were concentrated to 5% of the volume of urine at sp.gr. 1·010.

Non-haemorrhagic faints

During the period when the venesection experiments were in progress urine specimens were obtained from three subjects who had fainted from causes other than haemorrhage. These faints resulted from comparatively minor physical or psychic trauma and were not related to the venesection experiments. One subject fainted from a purely psychic stimulus, one as a result of venepuncture and one after an accidental cut of the finger. Urine specimens were obtained at the first voiding after the faint and in two of the subjects we obtained a control specimen at a time remote from the faint. The dilution of the urine and the concentration of the extracts were the same as in the venesection experiments.

'Black-out' due to acceleration

The urine for this study was obtained from the Institute of Aviation Medicine, Royal Canadian Air Force, Toronto.

The subjects were prospective air-crew recruits whose tolerance to acceleration was being tested. They were placed in a seat which could be revolved around a point in a horizontal plane. The seat was so suspended that the centripetal acceleration was applied in the long axis of the subject's body in a direction toward the head. The speed of rotation of the apparatus could be controlled and was slowly increased from zero to the point at which the subject became unconscious ('blacked out'). After this point the subject was decelerated and the test ended. Urine specimens were obtained from each subject before and after blackout. The dilution of the urine and the concentration of the extracts were the same as in the venesection experiments.

Extraction of urine

Noble, Rinderknecht & Williams (1939) reported a new method for the extraction of pituitary (posterior lobe) hormone from urine. This method seemed superior to those previously employed for the purpose and has been used throughout the experiments described in this paper. The hormone is adsorbed on zinc ferrocyanide from which it is then eluted with 1% (v/v) ammonia in 80% (v/v) alcohol. The ammonia and alcohol are removed by vacuum distillation and the dry residue is suspended in distilled water to form an extract of the desired volume. The advantages of the zinc ferrocyanide adsorption method are its reliability, the high recovery rate and the increased sensitivity occasioned by the factor of augmentation (*vide infra*). In agreement with Noble *et al.* (1939) we have found the method to recover consistently from 70 to 90% of the antidiuretic activity of pituitary (posterior lobe) extract added to urine.

Test for antidiuretic activity

The method of Burn (1937) has been used. A diuresis was produced in rats by the administration of water through a stomach tube. The material to be tested was injected into the animal subcutaneously or intraperitoneally immediately after the administration of the water. The results are expressed as the time at which 50% of the total urine excretion occurred and an increase in the 50% time indicates an antidiuresis.

Male rats of the Sprague-Dawley strain, maintained on a diet of Masters Fox Chow cubes and water *ad lib.*, have been the test animals throughout. When they weighed about 150 g they were given a dose of water (5% of body weight) by stomach tube to accustom them to the procedure. The urine output was not recorded on this occasion because it has been observed frequently that the diuresis after the administration of water for the first time is irregular. The tests were carried out, starting 1 week after the preliminary procedure, at intervals of not less than 1 week until the rats weighed between 225 and 250 g and had been used 3 or 4 times. At this time they were discarded.

Injection of extracts. Under certain conditions of assay the apparent antidiuretic activity of the

posterior lobe antidiuretic hormone may be increased. The factors governing this augmentation have been studied by Noble *et al.* (1939). When extracts of urine, made by adsorption on zinc ferrocyanide and previously shown not to exhibit antidiuretic activity when injected subcutaneously, were mixed with known amounts of commercial pituitary (posterior lobe) extract and tested by subcutaneous injection, the apparent antidiuretic activity of the mixture was 2–3 times as great as that found when the pituitary (posterior lobe) extract was diluted in 0·9% (w/v) sodium chloride solution and tested under similar conditions. We have confirmed this observation repeatedly. Augmentation of antidiuretic activity may be avoided by the use of intraperitoneal rather than subcutaneous injections. The intraperitoneal injection of extracts sometimes, however, causes peritoneal irritation and a non-specific suppression of diuresis of sufficient degree to interfere with the detection of small amounts of antidiuretic activity. The non-specific suppression of diuresis has not been seen after subcutaneous injection of either 0·9% sodium chloride solution or non-active urine extracts. For this reason, non-injected animals have served as controls for those tests in which the extracts have been injected subcutaneously. In the experiments reported here, injections have been made subcutaneously to detect small amounts of antidiuretic substance, intraperitoneally for quantitative assays and by both methods when the quantity of extract was sufficient to permit it. When a test of an extract by subcutaneous injection failed to demonstrate antidiuretic activity, this was taken as the true result even when intraperitoneal injection caused an increase in the 50% time. Such an increase was never great and was attributed to non-specific effects of the intraperitoneal injection.

Presentation of results. Results expressed as milliunits (mU) of pituitary (posterior lobe) antidiuretic hormone have been derived from the dose-response curve (Fig. 1) made by plotting known amounts of the hormone against the corresponding 50% times found after intraperitoneal injection. In these experiments the hormone was mixed with a urine extract previously shown by subcutaneous injection not to exhibit antidiuretic activity. When applicable, results have been given as: mean in minutes $\pm$ standard error, followed by the number of determinations in brackets. In all cases, four rats were used in making each determination.

The number of determinations in any one extract has been limited by the amount of extract (i.e. urine) available. The same limiting factor has precluded any further attempt to characterize the antidiuretic substance by estimation of its stability to heat and alkali.

Differences between means have been tested for significance by Student's t test for small and unequal series (Brownlee, 1949).

RESULTS

Dehydration in rats and in man

Fig. 2 shows the results obtained by the *intraperitoneal* injection of extracts of the urine from rats after dehydration for 24, 48 and 72 hr. The volume of extract injected was equal to 0·25 ml./100 g rat body weight. The total volume of each extract was such that 1·0 ml. represented the daily excretion of urine for one rat. The mean 50% times for 24, 48 and 72 hr extracts were, respectively, 99 min $\pm$ 1·4 (3), 135 min $\pm$ 5·0 (2) and 161 min $\pm$ 13·0 (4). The results for the 48 and 72 hr extracts both differ significantly from that for the 24 hr extract, P in each case being $< 0{\cdot}001$. Comparison of these results with Fig. 1 indicates that no detectable antidiuretic substance was excreted in the first 24 hr period, whereas in the second and third 24 hr periods there was excreted the approximate equivalent, respectively, of 15 and 25 mU vasopressin per rat.

The results for the dehydration of human subjects are given in Fig. 3. It should be noted that in these tests all the injections (0·5 ml. extract/100 g rat

body weight) were given subcutaneously and that the results are not strictly quantitative.

Subject 2 excreted antidiuretic substance in the first period of dehydration, but otherwise the results in the human subjects were similar to those in the rats. An extract made from urine voided by subject 2 when he was known to

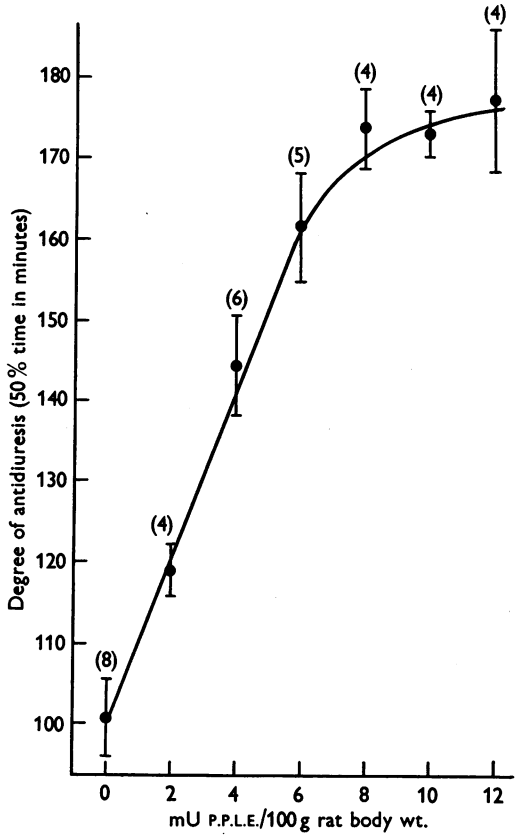

Fig. 1. Dose-response curve for the intraperitoneal injection of pituitary (posterior lobe) extract (P.P.L.E.) mixed with an extract of urine previously shown not to exhibit antidiuretic activity when tested by subcutaneous injection. The volume of each injection was 0·5 ml./100 g rat body weight. Vertical lines: ±S.E. Figures in brackets: no. of determinations.

be hydrated failed to cause an antidiuresis in the test animals [50% time 78·5 min ± 8·5 (2)].

In the calculation of means for statistical analysis, the first specimen voided during dehydration has been excluded. The mean for all other specimens from any one subject has been compared with the mean 50% time for an uninhibited (non-injected) water diuresis in the same rats as were used for the tests. The experimental results are given below, followed by the control values in brackets. Subject 1: 120·8 min ± 10·2 (8) [85·0 min ± 2·7 (12)]; subject 2:

194·0 min ± 19·7 (9) [81 min ± 3·9 (12)]; subject 3: 127·0 min ± 3·6 (3) [90·0 min ± 3·3 (12)]. In each case, the experimental value is significantly greater than the control ($P < 0{\cdot}001$). From separate assays (not shown in Fig. 3) by intraperitoneal injection, it has been estimated that the total excretion of antidiuretic substance in subject 3 was the equivalent of approximately 40 mU of vasopressin in the period from 21 to 33 hr and 70 mU in the period from 33 to 48 hr.

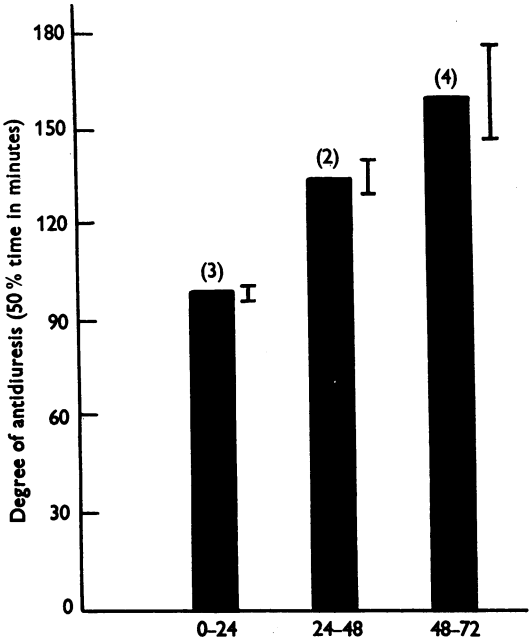

Fig. 2. Antidiuretic effects of extracts of urine from dehydrated rats. Intraperitoneal injection of 0·25 ml. of extract per 100 g body weight of the assay rat. This amount of extract represents 25 % of the daily urine excretion of one dehydrated rat. Figures in brackets: no. of determinations. Vertical lines: ±S.E. Figures beneath bars: period of dehydration in hours.

Infusion of vasopressin in man

In each of seven experiments the subject became very pale and had an antidiuresis lasting from 1½ to 3 hr following the intravenous infusion of vasopressin in doses ranging from 3–5 units. The *subcutaneous* injection into rats of extracts of the urine voided by the subjects before the infusion produced no antidiuresis in the test animal, whereas the *subcutaneous* injection of extracts of the urine voided after the infusion invariably caused a marked antidiuresis. In Fig. 4 are presented the results of the *intraperitoneal* injection of the extracts. Some of the extracts made from urine voided before the infusion caused an antidiuresis when injected intraperitoneally, but in each

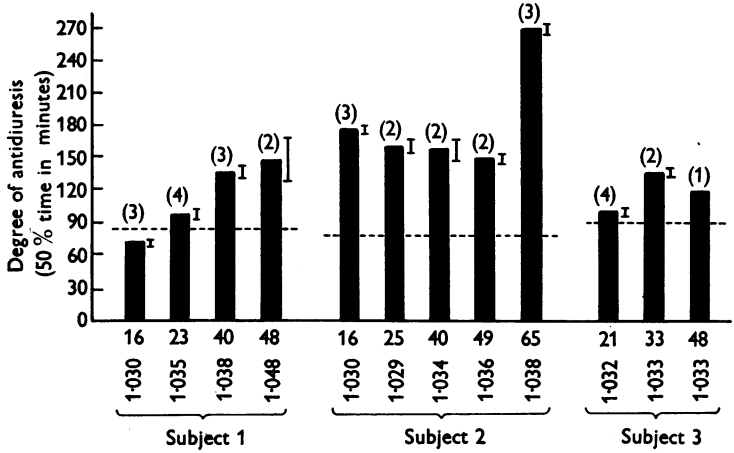

Fig. 3. Antidiuretic effects of extracts of urine from dehydrated human subjects. Subcutaneous injection of 0·5 ml. extract (representing 50 ml. of urine adjusted to specific gravity 1·010) per 100 g rat body weight. Interrupted lines: 50% time for an uninhibited (non-injected) water diuresis in the same rats used for the tests (see text). Vertical lines: ±S.E. Figures over bars: no. of determinations. Figures under bars: no. of hours dehydration and specific gravity of urine before dilution to 1·010 for extraction. The subcutaneous injection of an extract of urine from subject 2 voided when he was hydrated caused no antidiuresis in the test animals [50% time: 78·5 min±8·5 (2)].

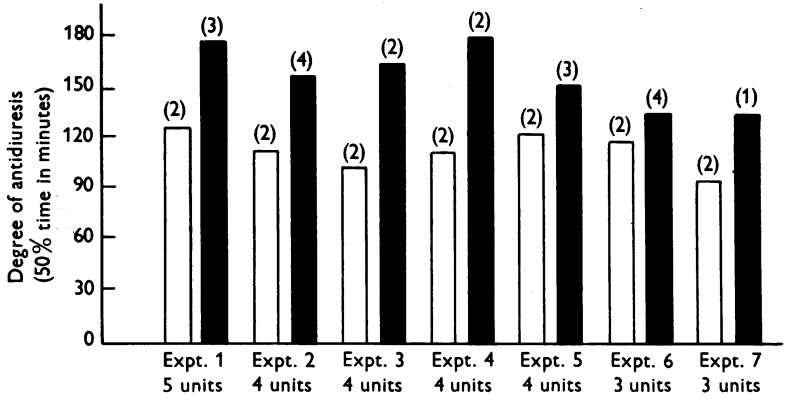

Fig. 4. Antidiuretic effects of extract of urine from human subjects before and after the intravenous infusion of vasopressin. The effects follow the intraperitoneal injection of 0·5 ml. of extract (representing 50 ml. of urine at a specific gravity of 1·010) per 100 g rat body weight. Open bars: extract of urine voided before infusion. Solid bars: extract of urine voided after infusion. Figures over bars: no. of determinations. Amount of vasopressin infused is shown under each experiment.

case the 50% time of the extracts of urine voided after infusion was greater than the 50% time of the corresponding extract of urine voided before the infusion. The apparently increased 50% times following the intraperitoneal injection of extracts of urine voided before the infusion have been rejected as false for the reasons given earlier.

In assessing the statistical significance of these experiments, the mean of all the results from the tests made on the extracts of urine voided before the infusion of vasopressin has been compared with that of the specimens voided after the infusion. The mean for the pre-infusion extracts is 115·9 min ± 3·9 (14) and for the post-infusion extracts is 156·4 min ± 4·2 (19). These means differ significantly ($P < 0{\cdot}001$).

On the assumption that the excreted antidiuretic substance was vasopressin, it has been calculated that the mean excretion was approximately 13% of the amount infused, with a range 4·5–30·0% (Table 1).

TABLE 1. Excretion of antidiuretic substance after infusion of vasopressin

The inhibition of diuresis in the rat following the intraperitoneal injection of extracts of urine from human subjects who had received vasopressin by intravenous infusion. Dose: 0·5 ml. extract/100 g rat body weight. Activity expressed as equivalents of vasopressin per 0·5 ml. extract. *Subcutaneous* injection of each of above extracts resulted in a 50% time >225 min.

		Assay of extract of post-infusion urine specimen			Total activity excreted $\frac{[(4) \times (5)]}{0{\cdot}5}$	
Subject (1)	Amount infused (units) (2)	Degree of antidiuresis (50% time in min) (3)	Activity (mU) (4)	Volume extract (ml.) (5)	(mU) (6)	Excretion as % of dose given (7)
1	5	179±4·0 (3)	>12·0	35	>840	>16·8
2	4	155±2·4 (4)	5·5	43	473	11·8
3	4	165±3·0 (2)	6·5	42	546	13·6
4	4	180±3·0 (2)	>12·0	50	>1200	>30·0
5	4	151±3·6 (3)	4·5	20	180	4·5
6	3	133±4·9 (4)	3·5	27	189	6·3
7	3	135 (1)	3·5	28	196	6·5
Mean percentage excretion						12·8

Venesection experiments

The results of fourteen experiments are presented. In seven of these the subjects fainted and in the remaining seven there was no faint. When a subject fainted as a result of either venesection or venepuncture, the urine voided subsequently always contained an antidiuretic substance. Fig. 5 presents graphically the results of these experiments. The extracts of urine were tested for antidiuretic activity by the subcutaneous injection of 0·5 ml. extract/100 g body weight of the test animal.

Subject 6 fainted during venesection but was unable to void until after the re-infusion of blood. The specimen represents excretion during the whole experiment, with no differentiation possible between the urine excreted after the venesection and that excreted after the infusion. Subject 11 fainted during

the venesection and again approximately 2 hr later during venepuncture for the re-infusion of blood. Subject 9 also fainted during venepuncture for the re-infusion of blood, although he had failed to do so during the previous venesection when 1100 ml. of blood were withdrawn. With the exception of subject 10, the antidiuretic substance was found in the first specimen voided after fainting. The specimen voided by subject 10 immediately after fainting (after venesection) contained little or no antidiuretic substance, whereas the

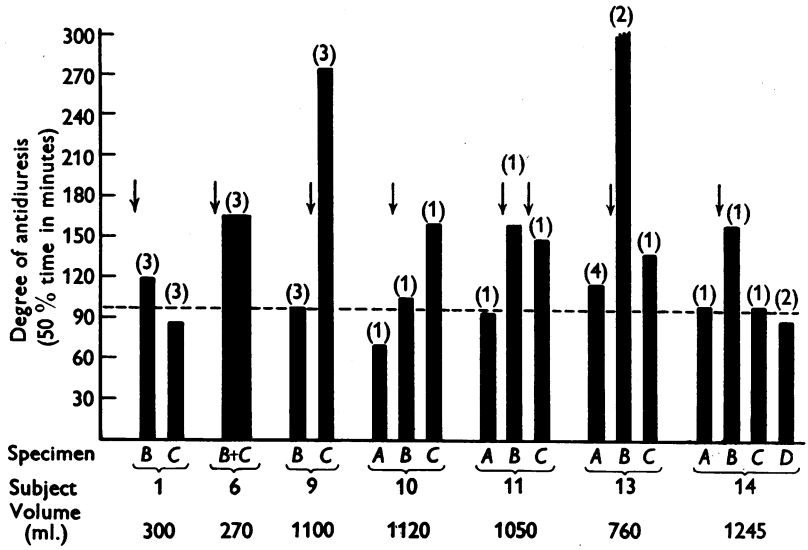

Fig. 5. Antidiuretic effects in rats following the subcutaneous injection of extracts of urine from human subjects who fainted as a result of venesection. The volume of all injections was equal to 0·5 ml. of extract/100 g of rat body weight. Letters beneath the bars refer to time specimen was voided: *A*, before experiment; *B*, after venesection; *C*, after re-infusion of blood; *D*, after experiment. Lowest row of figures indicates volume of blood withdrawn by venesection. Interrupted line drawn at the level of the mean 50% time for an uninhibited (non-injected) water diuresis in the same rats as used in the tests [97·4 min ± 3·4 (15)]. Figures over bars: no. of determinations. Arrows indicate time of fainting relative to the times of voiding the urine specimens.

next specimen voided 2 hr later, after the re-infusion of blood, had antidiuretic activity. The significance of this experiment will be discussed later. In assessing the results of these experiments the mean for all determinations performed on urine voided immediately before fainting has been compared with that for all determinations on the urine specimens voided first after fainting. In the cases of subjects 1 and 6 there were no 'pre-syncopal' urine specimens. The mean 50% time of the assays of 'pre-syncopal' urine specimens is 100·8 min ± 4·5 (10) and of 'post-syncopal' specimens, 189·6 min ± 5·2 (15). These means differ significantly ($P < 0·001$). The mean 50% time for the uninhibited (non-

injected) water diuresis of the same rats as were used in the tests is 97·4 min ± 3·4 (15). This mean does not differ significantly from that resulting from the tests of 'pre-syncopal' urine specimens ($P > 0{\cdot}1$). From assays by intraperitoneal injection (subjects 11, 13 and 14), it has been estimated that the equivalent of 85–372 mU of vasopressin were excreted in the urine after a faint (Table 2). On the assumption of 13% excretion in the urine, this corresponds to a liberation of 0·7–3·0 units of pituitary (posterior lobe) hormone. Fig. 6 presents the results of the experiments in which the subjects did not faint. The tests were made by the subcutaneous injection of 0·5 ml.

TABLE 2. Excretion of antidiuretic substance after haemorrhagic fainting

The inhibition of diuresis in the rat following the intraperitoneal injection of extracts of urine from human subjects who fainted as a result of venesection. Dose: 0·5 ml. extract/100 g rat body weight.

Subject	Urine specimen	Degree of antidiuresis (50% time in min)	Activity* (mU)	Volume extract (ml.)	Total activity excreted $\frac{[(4)\times(5)]}{0{\cdot}5}$ (mU)	Calculated liberation of posterior lobe hormone $\frac{[(6)\times 100\times 1]}{13{,}1000}$ (units)
11	*B*	145± 9·0 (2)†	5	22·0	220	1·7
	C	136±13·5 (2)	2·5	28·0	140	1·1
13	*B*	186±15·0 (2)	>12	>15·5	>372	>2·9
14	*B*	136± 7·5 (2)	2·5	17·0	85	0·7

* Activity expressed as equivalents of vasopressin per 0·5 ml. extract.

† Results given as mean 50% time±S.E. (no. of determinations); mean for an uninhibited water diuresis, 100 min±4·8 (8).

extract/100 g rat body weight. In the calculation of means, the results for subjects 2 and 12 have been excluded. The mean 50% time following the injection of extracts of urine voided after venesection (86·6 min ± 3·8 (15)) differs significantly from that of the subjects who fainted (189·6 min ± 5·2 (15); $P < 0{\cdot}001$) but does not differ significantly either from the mean of the results for the 'post-infusion' specimens of subjects who did not faint (90·1 min ± 3·5 (15); $P > 0{\cdot}1$) or from the mean 50% time of an uninhibited (non-injected) water diuresis (92·7 ± 2·4 (12); $P > 0{\cdot}1$) in the same animals as were used in the tests.

It can be seen (Figs. 5, 6) that there was no correlation between the amount of blood withdrawn and the occurrence of fainting.

Fainting not associated with venesection or appreciable blood loss

Table 3 gives the results obtained in three subjects who fainted with little or no blood loss. In each case, antidiuretic substance was excreted after fainting. Specimens of urine voided at a time remote from the faint were obtained from two of the subjects. Neither of these exhibited antidiuretic activity. Subject 17 did not lose consciousness, although he had other symptoms of fainting (pallor, faintness and nausea).

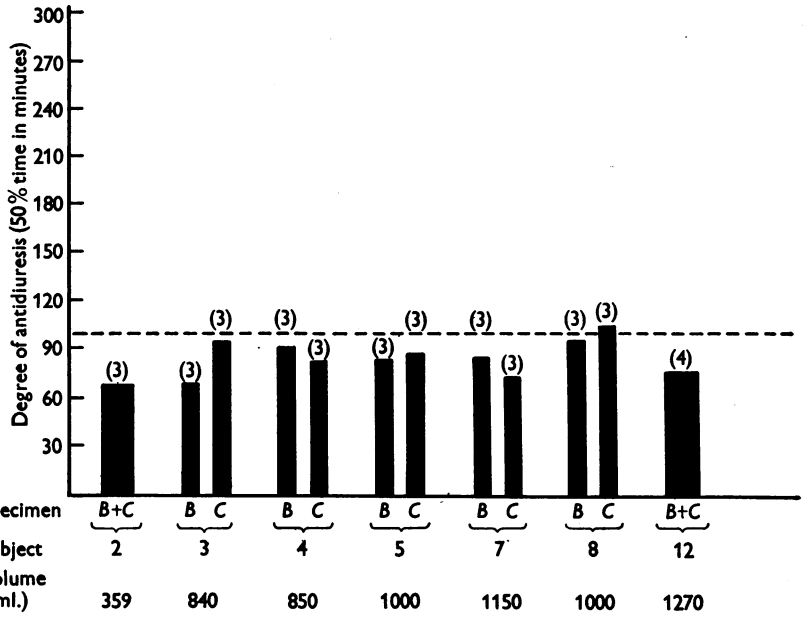

Fig. 6. Results of the intraperitoneal injection into rats of extracts of urine from human subjects who did not faint as a result of venesection. Conventions as in Fig. 5.

TABLE 3. Excretion of antidiuretic substance after non-haemorrhagic fainting

The inhibition of diuresis in the rat following the subcutaneous injection of 0·5 ml. extract of urine from human subjects after faints not associated with venesection.

Subject	Cause of faint	Time of voiding urine specimen	Degree of antidiuresis (50% time in minutes)	Interpretation
15	Conditioned reflex (fear)	1 hr after fainting	177 (1)	Marked antidiuresis
		Remote from fainting (control)	84 (1)	No antidiuresis
16	Venepuncture (before withdrawal of blood)	3 hr after fainting	250 (1)	Very marked antidiuresis
		8 hr after fainting	143·5±1·5 (2)*	Moderate antidiuresis
17	Cut of finger (minimal loss of blood)	1 hr after fainting	196±2·0 (2)	Marked antidiuresis
		Remote from fainting (control)	87±4·8 (3)	No antidiuresis

* Results given as mean 50% time±S.E. (no. of determinations); mean for an uninhibited water diuresis, 97 min±2 (21).

'Black-out' due to acceleration

The urine from nine subjects has been tested. The acceleration at which the subjects became unconscious ranged between 4·0 and 6·5 *g* (acceleration due to gravity). In no case did an extract of urine voided before or after blackout cause significant antidiuresis in the test animal ($P > 0{\cdot}3$ in all tests).

DISCUSSION

An antidiuretic substance has been found in the urine of human subjects following the intravenous infusion of vasopressin, and we have assumed that the antidiuretic substance is vasopressin. The results indicate that 4·5–30% of the vasopressin infused is excreted in the urine and that the mean excretion is 13%. These results are analogous to those reported in the experimental animal (Heller & Urban, 1935; Heller, 1937; Larson, 1938; O'Connor, 1950), and suggest that the proportion of vasopressin excreted increased with the amount infused in agreement with Heller (1937).

The finding of an antidiuretic substance in the urine of dehydrated rats and human subjects confirms previous reports. Convincing evidence has been presented in these reports that the antidiuretic substance is the pituitary (posterior lobe) hormone (Gilman & Goodman, 1937; Boylston & Ivy 1938; Ingram *et al.* 1938; Bundschuh & Kuschinsky, 1939; Teel & Reid 1939; Hare *et al.* 1941).

The observation that roughly 13% of infused vasopressin is excreted in the urine has been applied to estimate rate of liberation of pituitary (posterior lobe) hormone during dehydration. In subject 3 (110 kg), the calculated rate was 4·1 μU/kg/min in the second, and 5·3 in the final period of dehydration. In the rats (average weight 250 g), the calculated rate was 300 μU/kg/min in the second, and 515 in the final period of dehydration. The calculated rate of liberation of hormone by the rats is of the same order as that reported by O'Connor (1950) following the administration of hypertonic saline by stomach tube (120–1400 μU/min in dogs weighing 8–15 kg). The calculated rate of liberation of hormone in the rats was much greater per unit weight than in the human subject and may have been due to more severe dehydration of the rats. On the other hand, the total amount liberated was greater in the human subject, and the calculated rate for this subject was in agreement with those reported for the non-hydrated dog (Shannon, 1942, 0·2–1·0 μU/kg/min; Verney, 1946, 1·0 μU/sec).

The results in 'black-out' and in venesection without fainting are unequivocal in that no antidiuretic substance was found in the urine at any time during the experiments. Conversely, when the subject fainted from any cause there was always antidiuretic substance in the urine voided subsequently, and except in the case of subject 10, the antidiuretic substance was found in the

first specimen of urine voided after the faint. According to Brun *et al.* (1946) the post-syncopal oliguria lasted from 30 to 90 min. The failure to find antidiuretic substance in the first post-syncopal urine specimen of subject 10 may be explained by assuming that the oliguria lasted until after the first voiding. This specimen would be composed mainly of urine secreted before the faint and would not exhibit antidiuretic activity. Under these circumstances, antidiuretic substance liberated at the time of fainting would appear in the second post-syncopal urine specimen, as was observed.

Antidiuretic substance was excreted for a variable period of time after fainting. In the case of two subjects (nos. 1 and 14), the excretion was complete at the first post-syncopal voiding 1½–4 hr after fainting since no antidiuretic substance was found in the second specimen voided. In the case of four other subjects (nos. 9–11 and 13), the excretion was sufficiently prolonged that antidiuretic substance was found in the second post-syncopal specimen of urine voided 5–6 hr after fainting. This variability may represent prolonged production or delayed excretion, or may be due to the range of time after fainting at which the first post-syncopal specimens of urine were voided.

The urine voided by subject 13 before the experiment had slight antidiuretic activity when tested by subcutaneous injection. This finding was unusual because the urine of many normal subjects has been tested and found not to exhibit antidiuretic activity under ordinary circumstances. The experiment on subject 13 was done on a hot summer day and he may have been dehydrated.

Subject 17 did not lose consciousness, although he had other signs and symptoms of fainting, including faintness, nausea, pallor and sweating, and subsequently excreted an antidiuretic substance in the urine. Another subject (not reported in the present series) excreted antidiuretic substance in the urine after a severe psychic trauma. He did not lose consciousness nor did his urine contain antidiuretic substance at other times. These results indicate that loss of consciousness may not be an integral part of the vaso-vagal syndrome.

We have no direct evidence as to the identity of the antidiuretic substance found in the urine of our experimental subjects after fainting. The intravenous infusion of vasopressin in doses of from 3 to 5 units during 10 min is followed by pallor, sweating, abdominal discomfort, nausea (Sunahara *et al.* 1949), an inhibition of diuresis and the excretion of an antidiuretic substance in the urine. These signs and symptoms also accompany a vaso-vagal attack and our findings, therefore, support the hypothesis of Brun *et al.* (1945*a–c*, 1946) that there is a reflex stimulation of the neurohypophysis during fainting. A consideration of the various other procedures that have been reported to cause stimulation of the neurohypophysis may elucidate the mechanism by which such stimulation is effected in fainting.

Acetylcholine (Pickford, 1939, 1947; Chalmers & Lewis, 1951), di*iso*propylfluorophosphonate (DFP) (Duke, Pickford & Watt, 1950), nicotine (Burn,

Truelove & Burn, 1945; Chalmers & Lewis, 1951), cigarette-smoking (Burn *et al.* 1945; Walker, 1949; Chalmers & Lewis, 1951; Taylor & Walker, 1951), exercise (Rydin & Verney, 1938), emotional stress (Rydin & Verney, 1938; O'Connor & Verney, 1942, 1945), suckling (Cross, 1951), pain (Kelsall, 1949, 1951; Chalmers & Lewis, 1951), postural fainting (Brun *et al.* 1945*c*, *d*, 1946), arterial haemorrhage (Rydin & Verney, 1938), electrical stimulation of the central end of the divided vagus nerve (Lim and collaborators; see Chang, Chia, Hsu & Lim, 1937; Chang, Chia, Huang & Lim, 1939), and direct electrical stimulation of the supraoptic nuclei (Haterius & Ferguson, 1938; Haterius, 1940; Ferguson, 1941; Harris, 1948*a*, *b*) have all been reported to cause liberation of hormone from the neurohypophysis. The studies of Rechnitzer & Noble (1950) and Taylor & Noble (1950) indicate that the neurohypophysis may also be stimulated during the electro-convulsive shock therapy of human subjects. The action of acetylcholine, nicotine, cigarette-smoking and DFP is directly upon the supraoptic nuclei, and there is no counterpart to these stimuli, or to direct electrical stimulation of the neurohypophysis, in our experiments. Emotional stress and pain correspond to the psychic stress and venepuncture which caused fainting and the liberation of antidiuretic substance in some of our experiments. These results and the response of the neurohypophysis to suckling indicate that the primary stimulus to cause a liberation of hormone from the neurohypophysis may arise in the cerebrum or in peripheral sensory nerves.

The postural fainting studied by Brun *et al.* (1945*c*, *d*, 1946) was produced by seating the subject so that blood accumulated in the dependent legs. The subjects literally 'bled into their own veins', the vascular effects of which are very similar to haemorrhage resulting from arterial puncture (Rydin & Verney, 1938) or from venesection. It is difficult to explain the liberation of hormone after these procedures as being due to either peripheral sensations or psychic influences, and it may be that the responses are initiated by the fall in blood pressure which follows a loss of blood or a decreased venous return to the heart. It is known that sensory fibres of the vagus respond to changes in blood pressure and the experiments of Lim *et al.* have shown that the stimulation of vagal afferents causes a liberation of pituitary (posterior lobe) hormone. These facts suggest that the liberation of hormone after postural fainting, arterial haemorrhage, or fainting due to venesection may result from the stimulation of visceral sensory fibres of the vagus.

Rydin & Verney (1938) first drew attention to the inhibitory effect of the sympathetic nervous system on reflex stimulation of the neurohypophysis. In experiments with dogs, they found that the withdrawal of small amounts of blood by arterial puncture caused a pituitary type antidiuresis only if a previous operation had been done to decentralize the abdominal sympathetic system and section the splanchnic nerves. Subsequently, O'Connor & Verney

(1945) showed in normal dogs that emotional stress *sometimes* caused a pituitary type antidiuresis, whereas after abdominal sympathectomy and section of the splanchnic nerves emotional stress *invariably* caused a pituitary type antidiuresis. The antidiuresis could be prevented, in both normal and 'sympathectomized' dogs, by the administration of adrenaline by vein in doses of approximately 1·5 μg/kg. The effect of adrenaline on the antidiuresis resulting from the pain of ischaemic muscle has been studied in man by Kelsall (1949) and by Chalmers & Lewis (1951). In Kelsall's experiments, adrenaline prevented the antidiuresis in one subject, gave an equivocal response in another, and was without effect in the remaining three subjects. Chalmers & Lewis gave adrenaline to two subjects before and during the period of pain. In neither subject was the antidiuresis prevented. Before these results are interpreted as refutation of the work of O'Connor & Verney, it should be noted that the conditions of the experiments, apart from species, have not been duplicated by the subsequent workers. Either the route of injection was different or the dose of adrenaline was smaller. Furthermore, the dose of adrenaline used by O'Connor & Verney was sufficient to cause a transitory inhibition of urine flow, whereas no such effect was reported by Kelsall or by Chalmers & Lewis. Evidence that the dose of adrenaline was insufficient may also be adduced from the notation by Kelsall that the one subject in whom adrenaline prevented an antidiuresis also showed more marked effects from the adrenaline injection than any other subject.

The observation of O'Connor & Verney on the relationship between adrenaline and sympathetic activity on one hand, and the liberation of hormone from the neurohypophysis on the other, may be pertinent to the experiments here reported. The variable responses of our experimental subjects to venesection might be explained on the basis of a higher level of sympathetic activity in those who did not excrete antidiuretic substance, and a lower level in those who did. Similarly, it is suggested that a liberation of adrenaline as a result of stimulation of the carotid sinus during centripetal acceleration could prevent any liberation of pituitary (posterior lobe) hormone which might otherwise occur.

SUMMARY AND CONCLUSIONS

1. The urine of human subjects, voided after venesection, fainting and 'black-out' due to centripetal acceleration, has been extracted by a method employing adsorption on zinc ferrocyanide and the extracts tested for antidiuretic activity by the rat method of Burn.

2. In fourteen venesection experiments, seven subjects fainted and seven did not. There was no quantitative correlation between the amount of blood withdrawn and the occurrence of fainting.

3. Three additional subjects fainted following physical or psychic trauma not associated with venesection or more than slight blood loss.

4. All subjects who fainted subsequently excreted an antidiuretic substance in the urine.

5. No antidiuretic substance was found: (i) in the urine of subjects who did not faint; (ii) in the pre-syncopal urine of the subjects who fainted; and (iii) in the urine voided before or after 'black-out'.

6. The failure to detect antidiuretic substance in the urine of normal subjects should not be taken as a statement that such urine contains no antidiuretic substance. The point to be made is that, under standardized conditions of extraction and assay, antidiuretic substance was found under experimental conditions and not in the controls.

7. The excretion of antidiuretic substance appears to be related directly to fainting and only indirectly to the stimulus which initiates the faint or to cerebral anoxia.

8. Fainting and 'black-out' are basically different reactions.

9. Our observations are in agreement with the hypothesis of Brun *et al.*, that there is a liberation of hormone from the neurohypophysis during or after fainting.

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