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RENAL EFFECTS OF VERATRIDINE

BY

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WITH AN APPENDIX BY P. W. TAILBY AND D. A. GILBERT

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Veratridine hydrochloride injected subcutaneously into unanaesthetized rats inhibited water diuresis. A linear relationship between log dose and antidiuretic effect could be established over the dose range 50 to 200 μg./100 g. of body weight. When veratridine hydrochloride was injected intravenously in doses from 10 to 30 μg./100 g., this relationship was also linear. In terms of its antidiuretic action, the alkaloid was approximately five times as effective when given intravenously. Rats anaesthetized with urethane responded to an intravenous injection with a more pronounced inhibition than unanaesthetized animals. Protoveratrine injected intravenously into unanaesthetized rats showed no clear relationship between dose and magnitude of antidiuretic effect. Veratridine hydrochloride injected intravenously had a pronounced hypotensive effect in both anaesthetized and unanaesthetized rats. Treatment with atropine did not affect this hypotensive action significantly. Atropine given subcutaneously 30 min. before an intravenous injection of veratridine hydrochloride abolished or diminished the inhibitory effect of veratridine on water diuresis. Veratridine hydrochloride injected intravenously into unanaesthetized rats caused a marked depression of the clearance of inulin and *p*-aminohippurate. In unanaesthetized rats with an osmotic diuresis, veratridine hydrochloride produced its usual antidiuretic effect. The urine of rats injected with veratridine hydrochloride produced an antidiuretic effect when injected intravenously into other animals. The antidiuretic potency of such urines was not affected by treatment with thioglycollate. Animals injected with veratridine excreted small amounts of a veratridine-like substance in the urine. These results do not suggest that veratridine in antidiuretic and hypotensive doses stimulated the neurohypophysis in the rat.

Ginsburg and Heller (1952, 1953a) and Ames and van Dyke (1952) found that blood obtained by heart puncture from rats contained more antidiuretic activity than blood taken from a carotid artery. The rats were anaesthetized, which made it unlikely that the neurohypophysis was stimulated by pain. Since, however, relatively large volumes of blood were rapidly withdrawn, stimulation of receptors in or near the heart is likely to have occurred.

It is known that, in certain mammalian species, nerve impulses originating from cardio-aortic receptor areas reach the central nervous system by way of the vagus. It has also been reported (Chang, Chia, Huang, and Lim, 1939; Andersson, 1951) that stimulation of the central end of a divided vagus causes liberation of posterior pituitary hormones. Brun, Knudson, and Raaschou (1945), Noble and Taylor (1953), Andersson and Larson (1954), and Smith (1957) have suggested that, following a fall in blood pressure, impulses arriving by way of such connexions stimulate the posterior pituitary, and Henry, Gauer, and Reeves (1956) have shown that stimulation of left atrial stretch receptors in the dog influenced water diuresis. It seemed of interest, therefore, to investigate whether stimulation of cardio-aortic baro- or stretch-receptors by veratrum alkaloids (Cerletti, Li, Alanis, and Aviado, 1951; Rothlin and Cerletti, 1954) produced a stimulation of the neurohypophysis. That this occurs is suggested by the work of Blackmore (1955), who found that the injection of protoveratrine into unanaesthetized dogs produced an inhibition of water diuresis which was not found in dogs with diabetes insipidus due to stalk section. In contrast are the results of Meilman (1952), who reported that the oliguria after the intravenous injection of protoveratrine into hypertensive patients was accompanied by a striking decrease in excretion of sodium and chloride in the urine. This would not be

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expected if the antidiuretic effect of protoveratrine were mainly due to the release of posterior pituitary hormones, since increased secretion of vasopressin and oxytocin would either leave the electrolyte output unchanged (Crutchfield and Wood, 1948; Barclay, Kenny, and Nutt, 1949; Chalmers, Lewis, and Pawan, 1951) or would possibly increase it (Dicker and Heller, 1946; Brooks and Pickford, 1957).

In view of the results of Ginsburg and Heller (1952, 1953) and Ames and van Dyke (1952) already mentioned, rats were used in the present investigation.

Methods

Adult male albino rats of the Wistar strain were used. Those used for diuresis experiments weighed 160 to 230 g., and those for blood pressure experiments, 300 to 390 g.

Measurements of Blood Pressure. — With anaesthetized rats the method of Dekanski (1952) was used, but no dibenamine was given. With conscious rats the animal was anaesthetized with ether and short polythene cannulae were inserted into a carotid artery and a jugular vein. The rat was then given heparin and both cannulae were brought out through the skin at the back of the neck. When the rat regained consciousness extensions were fitted to the cannulae. The venous cannula was closed with a removable cap and the arterial cannula was connected with a manometer (Condon, 1951).

Tests for Antidiuretic Action.—The method of Heller and Zaidi (1957) was followed when test substances were injected intravenously, and that of Ginsburg (1951) when the test substances were injected subcutaneously; only two doses of water were given. The same animals were used again after an interval of two days, but were "crossed over."

Renal Clearance Estimations.—The method of Dicker and Heller (1945) was modified in that the bladder was cannulated on the day before the experiment. Immediately before and at the end of the clearance period the bladder was rinsed with tepid 0.9% NaCl solution. The animal was then anaesthetized with ether and blood was collected from a carotid artery. Ginsburg (1957) has shown that inulin clearances estimated in this manner are in good agreement with the results of experiments in which conscious rats received a constant intravenous infusion of inulin and in which several blood samples were taken. Inulin clearance (C_{IN}) and *p*-aminohippurate clearance (C_{PAH}) were determined simultaneously. Inulin was estimated according to the method of Schreiner (1950) and *p*-aminohippurate according to that of Smith, Finkelstein, Aliminosa, Crawford, and Graber (1945).

Inactivation Experiments with Thioglycollate.— The modification of the method of Ames and van Dyke (1951) due to Vogt (1953) was used.

Materials

Veratridine. — A stock solution was prepared from the pure substance supplied by Sandoz Laboratories, Basle. The base was converted into veratridine hydrochloride by adding a few drops of N-HCl. About four drops of the acid were needed to dissolve 2 mg. of the base. The solution was then neutralized with N-NaOH and made up to volume with 0.9% NaCl solution. The stock solution contained 20 mg. of veratridine hydrochloride/100 ml.

Puroverine. — Puroverine, a mixture of protoveratrine A and B, was supplied by Sandoz Laboratories, Basle. The stock solution contained 10 mg. of the preparation in 100 ml. 0.9% NaCl solution.

Other preparations used were: atropine sulphate (B.D.H.), Pularin (Evans), Pitressin (Parke, Davis), inulin (B.D.H.), *p*-aminohippuric acid (B.D.H.), and thioglycollic acid (B.D.H.).

Results

The Antidiuretic Action of Subcutaneous Injections of Veratridine Hydrochloride in Unanaesthetized Rats

Fig. 1 shows the relationship between the dose of the alkaloid injected and its antidiuretic effect. The solutions of veratridine were dilute, neutral, and isotonic, but the rats resented the injection. They started to salivate profusely about 15 min. after the injection.

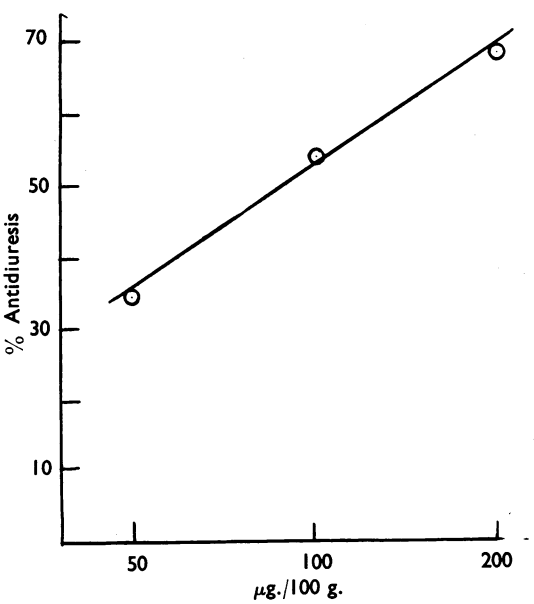

FIG. 1.—The antidiuretic action of veratridine hydrochloride injected subcutaneously in unanaesthetized male rats. Each point represents the mean of results in 12 animals.

The Antidiuretic Action of Intravenous Injections of Veratridine Hydrochloride

Fig. 2 shows the inhibitory effect of intravenous injections of the alkaloid on the water diuresis of conscious rats. The rats showed no resentment during the injection, but almost immediately after the injection had been completed the animals became restless; clonic convulsions occasionally occurred after large doses had been given. Gross changes in respiration were also noted in some instances. About 5 min. after the injection, the rats began to salivate profusely and continued to do so for about 10 min.

A comparison of Fig. 2 with Fig. 1 shows that the alkaloid was much more potent when injected intravenously. The antidiuretic effect of 20 μg./100 g. of body weight injected intravenously was approximately equal to the effect of 100 μg./100 g. injected subcutaneously.

Since unanaesthetized animals showed excitement shortly after the intravenous injection of veratridine and excitement itself is known to favour the release of antidiuretic hormone, the effect of intravenous injections of veratridine on water diuresis was also tested in anaesthetized animals. Pronounced antidiuretic effects were observed in rats anaesthetized with a 25% solution of urethane (0.7 ml./100 g.). The mean % antidiuresis in three such animals was 82.6 ± 7.2 (s.e.) which compares with $52.4 \pm 4.2\%$ (s.e., $n=18$) in conscious rats which had received the same dose (20 μg. of veratridine hydrochloride/100 g.). Thus veratridine was slightly more inhibitory in the anaesthetized rats ($P<0.001$).

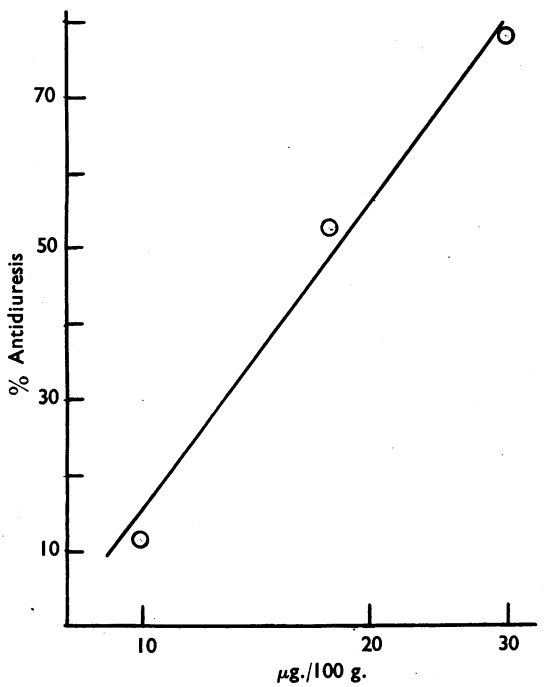

FIG. 2.—The antidiuretic action of veratridine hydrochloride injected intravenously in unanaesthetized male rats. Each point represents the mean of results in 6 to 18 animals.

Effect of Veratridine Hydrochloride on the Water Diuresis of Rats Treated with Atropine

Table I shows that atropine prevented the inhibitory effect of 10 μg. of veratridine hydrochloride/100 g. and that it diminished the antidiuretic effect produced by 20 μg. of veratridine/100 g. The antidiuretic effect of 30 μg. of veratridine was not much effected. Atropine itself in the dose and by the route given in these experiments had no effect on water diuresis: the mean % water load excreted in 3 hr. by 12 conscious rats which had been subcutaneously injected with 10 mg. atropine sulphate/100 g. was 63.5 ± 5.0 (s.e.) and that of 12 animals with control injections of 0.9% NaCl solution was 69.5 ± 5.6 ($P>0.4$).

TABLE I

EFFECT OF VERATRIDINE HYDROCHLORIDE ON THE WATER DIURESIS OF RATS AFTER ATROPINE

The animals were unanaesthetized; 10 mg. of atropine sulphate/100 g. was given subcutaneously 30 min. before veratridine was injected intravenously. Mean % antidiuresis is given with ± s.e. Number of animals used is given in parentheses.

Veratridine (μg./100 g.)	Veratridine Alone	Veratridine with Atropine	*P*
10	11·5±4·1 (6)	0·0 (6)	—
20	54·2±5·2 (15)	18·6±6·4 (15)	<0·001
30	78·2±4·1 (8)	59·6±8·6 (3)	>0·05

Effect of Puroverine Injected Intravenously on the Water Diuresis of Unanaesthetized Rats

Table II shows that the antidiuretic effect of puroverine was much less uniform than that of veratridine. There was less salivation, but the animals were much more excited and showed respiratory distress. Some animals injected with the highest dose of puroverine used (30 μg./100 g.) died in respiratory failure. The experiments with puroverine were therefore discontinued.

Effect of Veratrine Hydrochloride on the Systemic Blood Pressure of Rats

In view of the well-known hypotensive action of veratrum alkaloids in other mammalian species and in man, it seemed desirable to investigate

TABLE II

EFFECT OF INTRAVENOUS INJECTIONS OF PUROVERINE ON THE WATER DIURESIS OF UNANAESTHETIZED RATS

Animals marked with asterisk died shortly after conclusion of the experiment.

Animal No.	% Antidiuresis
7·5 μg./100 g.	
1	0
2	0
3	0
4	0
10 μg./100 g.	
1	0
2	7·5
3	80·5
4	0
15 μg./100 g.	
1	0
2	52·5
3	0
4	9·4
5	0
6	84·0
7	5·0
8	11·2
20 μg./100 g.	
1	0
2	0
3	48·0
30 μg./100 g.	
1	23·3
2	60·7*
3	55·8*
4	44·6*
5	0
6	33·7
7	0
8	20·8
9	0

the effect of antidiuretic doses of veratridine on the blood pressure of the rat. Fig. 3*a* shows the marked depressor effect of 10 μg. of veratridine hydrochloride/100 g. injected intravenously in an unanaesthetized rat, and Fig. 3*b* shows that this effect was not inhibited by atropine. Hypotensive effects in rats anaesthetized with urethane were somewhat less pronounced and were hardly influenced by atropine. On some occasions veratridine injected after atropine gave a pure pressor response (Fig. 4).

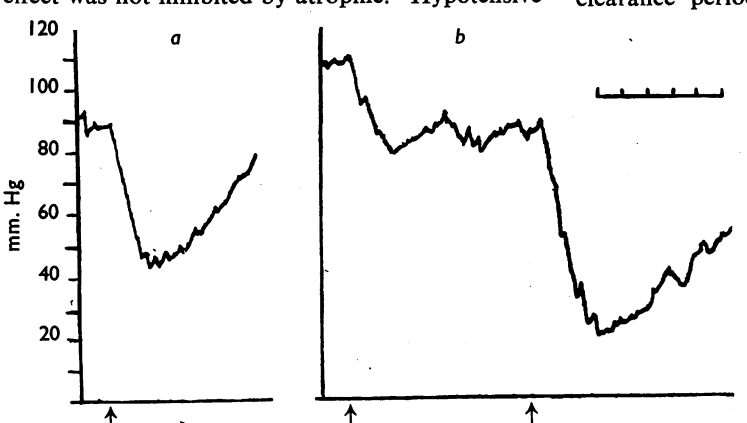

FIG. 3.—Arterial blood pressure of a conscious rat. *a:* At arrow, veratridine hydrochloride (10 μg./100 g.) intravenously. *b:* At first arrow, atropine sulphate (1 mg./100 g.) intravenously; at second arrow, veratridine hydrochloride (10 μg./100 g.) intravenously. Time, 1 min.

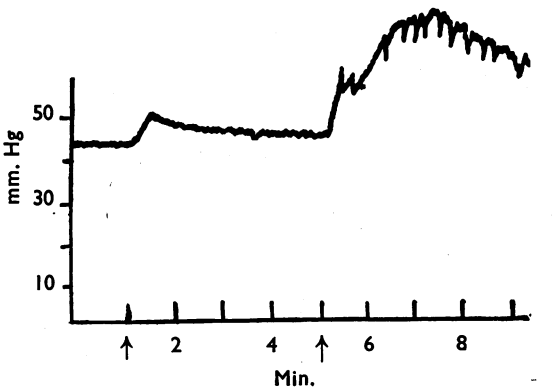

FIG. 4.—Pressor effect of veratridine: rat anaesthetized with urethane. Atropine sulphate (0·5 mg./100 g.) intravenously 10 and 5 min. before start of record. At first arrow, acetylcholine (1 μg./100 g.) intravenously; at second arrow, veratridine hydrochloride (20 μg./100 g.) intravenously.

Effect of Veratridine Hydrochloride on the Inulin and p-Aminohippurate Clearance of Unanaesthetized Rats

Since veratridine had a pronounced hypotensive effect in unanaesthetized rats (Fig. 3), it might be expected to modify glomerular filtration rate and renal blood flow. Inulin clearance (C_{IN}) was therefore estimated in conscious rats injected with 20 μg. of veratridine hydrochloride/100 g. The clearance period lasted 30 min. from the time when the intravenous injection was completed. C_{IN} (mean ± s.e.) in 12 such animals was 0.17 ± 0.026 ml. / 100 g. / min., and mean C_{IN} in 20 controls injected with 0.9% NaCl solution was 0.60 ± 0.037 ($P<0.001$). Simultaneous inulin and *p*-aminohippurate clearances (C_{PAH}) in another group of 7 rats injected with the same dose of veratridine gave the following mean results: C_{IN}, 0.17 ± 0.079, C_{PAH}, 0.49 ± 0.134. C_{IN} in 10 controls was 0.59 ± 0.035 and C_{PAH} 3.0 ± 0.452 ml. Thus veratridine lowered both clearances significantly ($P<0.001$).

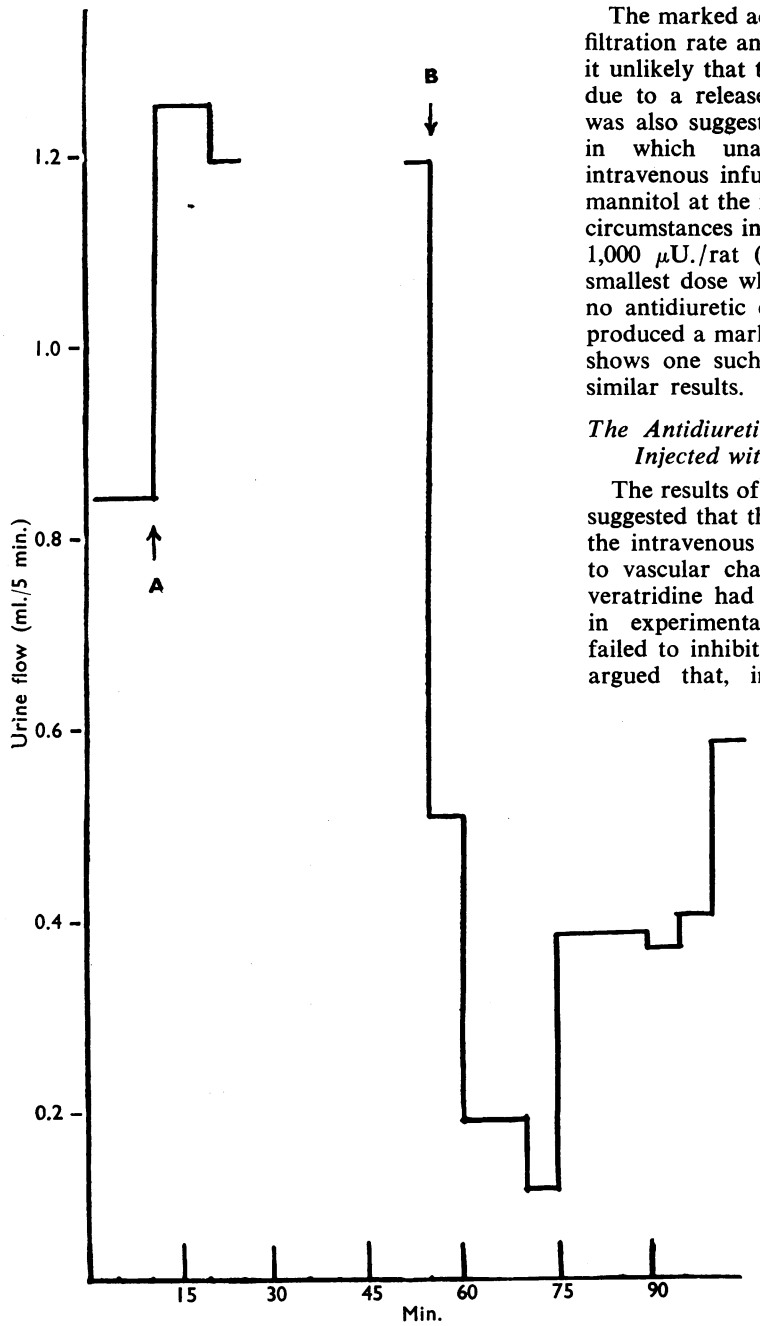

FIG. 5.—Antidiuretic effect of veratridine during osmotic diuresis. Unanaesthetized rat (315 g.) given intravenous infusion of 20% mannitol (0·1 ml./min.). A: pitressin (160 μU./100 g.) intravenously; B: veratridine hydrochloride (20 μg./100 g.) intravenously.

Effect of Veratridine Hydrochloride on Osmotic Diuresis

The marked action of veratridine on glomerular filtration rate and effective renal blood flow made it unlikely that the antidiuretic effects were solely due to a release of antidiuretic hormone. This was also suggested by the results of experiments in which unanaesthetized rats received an intravenous infusion of a solution of 20% (w/v) mannitol at the rate of 0.1 ml./min. Under these circumstances intravenous doses of pitressin up to 1,000 μU./rat (about 50 times as much as the smallest dose which inhibited water diuresis) had no antidiuretic effect. However, veratridine still produced a marked decrease in urine flow. Fig. 5 shows one such experiment. Three others gave similar results.

The Antidiuretic Activity in the Urine of Rats Injected with Veratridine Hydrochloride

The results of the clearance estimations strongly suggested that the antidiuretic effects produced by the intravenous injection of veratridine were due to vascular changes in the kidneys. Moreover, veratridine had a pronounced antidiuretic action in experimental circumstances when pitressin failed to inhibit urine flow. But it could still be argued that, in addition to having a direct antidiuretic action, veratridine caused the liberation of such large amounts of vasopressin that the renal vascular effect of this hormone became apparent. If this were so one would expect some of the released hormone to be excreted by the kidneys (Heller and Urban, 1935; Heller, 1952; Ginsburg and Heller, 1953b; Dicker, 1954; Heller and Zaidi, 1957). The urines of rats injected with veratridine were therefore tested for antidiuretic activity. The volume of urine voided 20 min. before an intravenous injection of 20 μg. veratridine hydrochloride/100 g. was measured and the urine excreted in the 20 min. after the injection was made up to the same volume with distilled water. Equal volumes of each urine were then injected into another rat. The urine collected after the injection of veratridine inhibited the water

diuresis of the recipient animal (Table III). However, Table III also shows that veratridine, added either to saline or to rat urine is not inactivated by thioglycollate. The antidiuretic substance excreted after an intravenous injection of veratridine was likewise not inactivated. The antidiuretic potency of such urines remained the same ($P>0.7$) before and after treatment with thioglycollate, suggesting that their inhibitory effect was solely due to the excretion of veratridine or some metabolic product of this alkaloid.

TABLE III

THE ACTION OF THIOGLYCOLLATE ON VERATRIDINE RESPONSE AND ON THE ANTIDIURETIC POTENCY OF URINE COLLECTED AFTER THE INTRAVENOUS INJECTION OF VERATRIDINE

All injections were made intravenously. Volume injected was 0·2 ml./100 g. throughout. Mean % antidiuresis is given with ± s.e. Number of animals used is given in parentheses.

Soln. No.		Mean % Antidiuresis
1	Urine collected for 20 min. *after* injection of veratridine hydrochloride (20 μg./100 g.) ..	39·9±6·1 (8)
2	Urine collected for 20 min. *before* injection of veratridine hydrochloride (20 μg./100 g.) ..	7·8±3·6 (8)
3	100 μg. of veratridine hydrochloride + 9 mg. of NaCl/ml. distilled water	61·5±6·3 (7)
4	As 3, but treated with thioglycollate ..	67·8±8·6 (7)
5	250 μU. of pitressin and 9 mg. NaCl/ml. distilled water	58·9±4·6 (7)
6	As 5, but treated with thioglycollate ..	8·1±3·8 (7)
7	Urine + 100 μg. added veratridine hydrochloride/ml.	78·8±6·3 (3)
8	As 7, but treated with thioglycollate ..	81·6±7·7 (3)
9	Urine + 250 μU. added pitressin/ml. ..	50·2±7·8 (3)
10	As 9, but treated with thioglycollate ..	13·6±6·8 (3)
11	Urine collected for 20 min. after i.v. injection of veratridine hydrochloride (20 μg./100 g.)	44·6±4·1 (6)
12	As 11, but treated with thioglycollate ..	42·8±4·5 (6)
13	Urine collected for 20 min. after the i.v. injection of pitressin (50 μU./100 g.) ..	37·4±5·8 (5)
14	As 13, but treated with thioglycollate ..	4·1±1·8 (5)

DISCUSSION

Fries, Stanton, Culbertson, Litter, Halperin, Burnett, and Wilkins (1949) investigated the oliguria caused by the intramuscular injection of veratrone (protoveratrine) into normotensive and hypertensive patients, and found that this preparation caused a fall in inulin and *p*-aminohippurate clearance. They noted, however, that the decrease in urinary volume persisted when renal plasma flow had returned to pre-injection values. During the period of depressed urine output, there was a marked rise of the inulin urine/plasma ratio. They stress that oliguria could also be produced by doses of protoveratrine which did not cause a significant reduction of systemic blood pressure. Likewise, Meilman (1953), who gave protoveratrine intravenously to similar groups of patients, emphasized that oliguria persisted in some patients after the glomerular filtration rate and renal plasma flow had returned to control values. During the period of oliguria, inulin urine/plasma ratios were increased and there was a striking diminution in the excretion of sodium and chloride.

The results of Nungesser and Hiatt (1954) in anaesthetized dogs injected with protoveratrine resemble, on the whole, those in man; the drug caused a decrease in urine volume with an increase of creatinine urine/plasma ratios which lasted longer than the decreases in blood pressure, glomerular filtration rate and renal plasma flow. The rise in inulin urine/plasma ratio, or in other words the increase in the tubular reabsorption of water, does not necessarily signify that the oliguria observed in these experiments was due to an increased release of antidiuretic hormone, since we know (del Greco and de Wardener, 1956; Berliner and Davidson, 1956) that a reduction of glomerular filtration rate may produce this effect by itself. However, the persistence of the oliguria for longer than the changes of blood pressure, glomerular filtration rate, and renal plasma flow is compatible with an antidiuretic effect due to the release of vasopressin. Blackmore (1955) injected protoveratrine into unanaesthetized dogs in doses which were probably not hypotensive (Maison, Gotz, and Stutzman, 1951) and obtained antidiuretic effects which were not seen when the same dose was administered to dogs with diabetes insipidus.

These findings can be compared with the results of the present investigation. It was possible to produce antidiuretic effects in rats with veratridine and protoveratrine, but the doses of protoveratrine needed to produce these effects were much higher than those required in the other species. Given by single intravenous injection, 3 μg. of protoveratrine/kg. reduced the urine flow in unanaesthetized dogs (Blackmore, 1955) and about 2 μg./kg. injected intravenously was needed in man (Meilman, 1953) whereas 300 μg. of the same alkaloid/kg. given by the same route to unanaesthetized rats failed to produce antidiuresis regularly. A similar comparison with veratridine cannot be made since this substance has apparently not been used to investigate renal effects in dogs and man. However, it could be shown that the inhibition of water diuresis in rats by veratridine resembles the oliguric effect of protoveratrine in other species in so far as a decrease in the clearances of inulin and *p*-aminohippurate was produced.

The blocking of parasympathetic effects by atropine had little or no effect on the fall of blood pressure produced by the intravenous injection of veratridine into rats, which is in accordance with the findings of Moe, Bassett, and Krayer (1944) in the dog. Occasionally a pure pressor response was seen in anaesthetized rats after atropine, which resembled that observed by Krayer and Acheson (1946) when large doses of veratridine had been given to dogs after vagotomy or atropinization. Atropine decreased the antidiuretic effect of small doses of veratridine but had little effect on the antidiuresis produced by large doses.

The marked effect of the intravenous injections of veratridine on the inulin and *p*-aminohippurate clearances made it unlikely that the antidiuretic effects produced in rats were due solely to stimulation of the neurohypophysis.

To establish whether veratridine releases vasopressin after the intravenous injection of antidiuretic doses in rats, it would have been desirable to investigate the effect of the alkaloid in neurohypophysectomized rats. However, since complete neurohypophysectomy in this species is notoriously difficult, another type of experiment was substituted. It is well known that the antidiuretic hormone ceases to depress urine flow during an osmotic diuresis (Adolph and Ericson, 1927; Melville, 1936; Paine and Nelson, 1940). Unanaesthetized rats were therefore given an infusion of 20% mannitol solution. In this condition, veratridine still produced its usual antidiuretic effect, whereas doses up to 1,000 μU. of pitressin had lost their inhibitory action. But it could still be argued that the amounts of vasopressin released by veratridine were so large (and it has been shown that very large amounts of vasopressin can be released by "noxious" stimuli from the rat pituitary (Ginsburg and Brown, 1956)) that the vascular effect of the hormone had contributed to the antidiuresis. However, if such large amounts were released, antidiuretic activity of neurohypophysial origin should appear in the urine (Heller and Urban, 1935; Heller, 1952; Ginsburg and Heller, 1953b; Dicker, 1954; Heller and Zaidi, 1957). Samples of urine excreted up to 20 min. after the intravenous injection of veratridine were therefore collected and injected into other rats in water diuresis. Such urines invariably had an antidiuretic action, but their antidiuretic activity was not inactivated by treatment with thioglycollate and the depression of urine flow did not differ significantly ($P>0.7$) before and after treatment with thioglycollate. It could also be shown by means of ultraviolet spectroscopy (see appendix) that after the injection of veratridine, the alkaloid or a chemically closely similar substance was excreted in the urine.

Assuming that veratridine did not suppress the renal excretion of vasopressin, and it will have been noted that it did not prevent its own excretion into the urine during the critical period, there is no evidence from these results in rats that veratridine caused a stimulation of the posterior pituitary. It may well be that veratridine differs in this respect from protoveratrine, but it is also possible that dogs and rats differ in their response to veratrum alkaloids for anatomical reasons. The afferent pathway for the aortic baroreceptor (and perhaps also coronary chemoreceptor) impulses (Dawes, 1947) appears to be peculiar in the rat (Andrew, 1954); and Blood, Kosman, and d'Amour (1955) have shown that, although pulmonary depressor reflexes can be elicited in rats, they are not abolished by vagal section as in other species. It would also seem that, in the rat, the receptors stimulated to release antidiuretic hormone by removal of blood following heart puncture are not identical with those that are activated by veratridine.

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APPENDIX

By P. W. TAILBY AND D. A. GILBERT

The antidiuretic substance excreted in the urine of rats after the intravenous injection of veratridine hydrochloride was investigated.

First, precipitation of the active substance was attempted by adding 2% phosphotungstic acid in 5% (v/v) H_2SO_4 to urine, and a colour reaction with concentrated sulphuric acid was sought. However, control experiments with urines to which veratridine hydrochloride had been added showed that concentrations of more than 500 μg. of veratridine/ml. urine were needed to give a positive result.

Next, extraction of veratridine from the urine samples was tried. By adding solid Na_2CO_3, the urine was brought to *p*H 10, extracted with a mixture of ether (3 vol.) and chloroform (1 vol.), and the extract shaken with 2N-HCl. A few drops of this acid extract were then put on a filter paper, dried in air, and sprayed with Dragendorff's reagent. When veratridine hydrochloride was added to rat urine, concentrations of 100 μg./ml. could be demonstrated by this method, but the results with the urines of rats which had been injected with small amounts of the alkaloid were negative. A more sensitive method had therefore to be adopted.

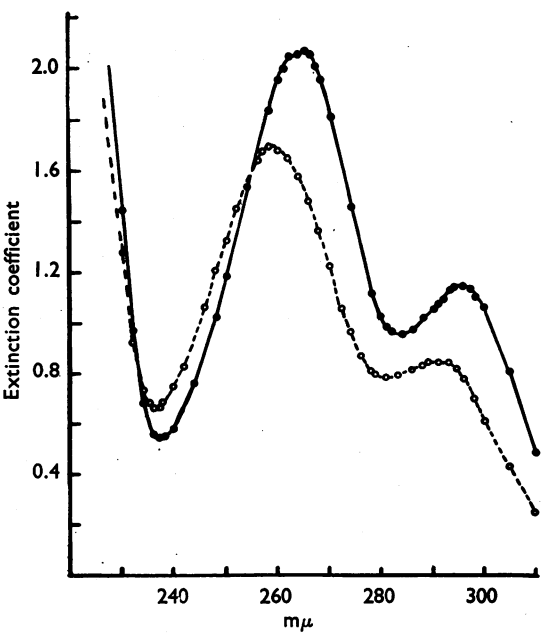

FIG. 1.—Ultraviolet absorption spectrum of veratridine hydrochloride dissolved in 0·9% NaCl solution. O - - - O: *p*H 5. ●——●: *p*H 3·6.

TABLE I

ESTIMATION BY ULTRAVIOLET SPECTROSCOPY OF THE VERATRIDINE-LIKE SUBSTANCE IN THE URINE OF A RAT INJECTED SUBCUTANEOUSLY WITH 100 μG. VERATRIDINE HYDROCHLORIDE

Urine which had been collected before the injection and to which 10 μg. veratridine/ml. had been added served as the standard. The collecting period in min. after the injection was given.

Collecting Period (Min.)	Urine Vol. Excreted (ml.)	Veratridine-like Substance Excreted (μg.)
0– 30	0·75	0·42
30– 60	0·40	1·40
60– 90	0·30	0·61
90–120	0·30	0·41

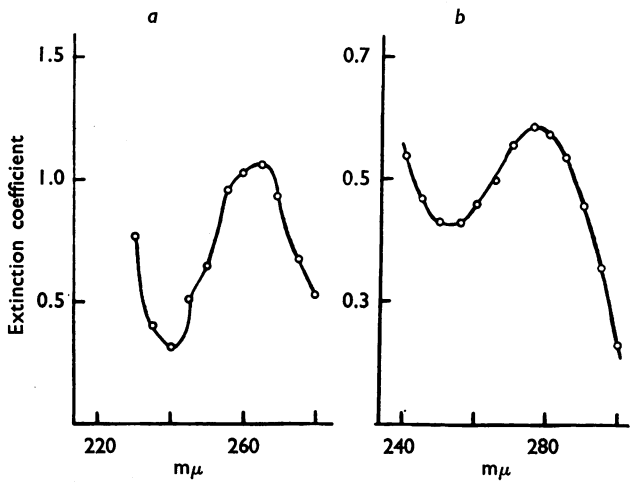

FIG. 2.—*a*: Ultraviolet absorption spectrum of veratridine hydrochloride added to rat urine. *b*: Spectrum of the urine of a conscious rat injected intravenously with veratridine hydrochloride (50 μg./100 g.). Urine was collected from a bladder cannula for 30 min. after the injection.

Preliminary experiments with solutions of pure veratridine hydrochloride showed that the alkaloid gave a characteristic absorption curve in the ultraviolet range (Fig. 1): there was a maximum at 265 mμ and the extinction coefficient at this value changed markedly (from $E\ ^{1\ cm.}_{265} = 1.553$ to $E\ ^{1\ cm.}_{265} = 2.077$) when the *p*H of the solution was altered from 5 to 3.6. Next, 50 μg. of veratridine hydrochloride/100 g. was injected subcutaneously into unanaesthetized rats (weighing about 200 g.) with bladders cannulated. Urine was collected for half an hour before and for 120 min. after the injection. The urine samples were then extracted with ether/chloroform as already described. Fig. 2 shows the ultraviolet absorption spectrum of these extracts. The spectrum of the urinary extract resembled that of pure veratridine very closely. In Table I the quantitative changes in the spectrogram show that the excretion of the veratridine-like substances reached a peak during the second 30 min. after the injection. The recovery of these substances in 2 hr. was 2.84% of the injected dose in terms of veratridine base. We are unable to say whether the urinary excretion of the veratridine-like factor proceeded beyond 2 hr. after the injection.

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