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# A PRESSOR SUBSTANCE IN URINE

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The gonadotrophins in human urine may be concentrated by adsorption on kaolin (Scott, 1940; Dekanski, 1949, 1950). A substance resembling vasopressin is present in the supernatant fluid, after the gonadotrophins have been removed, and may be concentrated by the method developed by Grollman and Woods (1949) for the concentration of the urinary antidiuretic factor. The pressor activity of these concentrates was assayed in comparison with vasopressin on rats' blood pressure (Landgrebe, Macaulay, and Waring, 1946), the method being eventually modified in certain details.

## METHODS

*Extraction of urine.*—The total amount of urine collected during 24 hours was first treated with kaolin. The acidic supernatant fluid was partly siphoned off when the kaolin had settled down and partly poured off after the kaolin suspension had been centrifuged. This acid fluid was filtered through a Buchner funnel under reduced pressure and treated by the method used by Grollman and Woods (1949) for the concentration of the antidiuretic fraction.

*Assay methods.*—Assays were carried out for pressor, antidiuretic, and oxytocic effects.

The pressor activity of urinary concentrates was quantitatively assayed on rats' blood pressure by a method similar to that of Landgrebe, Macaulay, and Waring (1946). The best results were obtained with rats weighing between 300 and 350 g., anaesthetized with urethane, injected subcutaneously, and treated with dibenamine (Nickerson, 1949; Nickerson and Goodman, 1947). The animals were kept warm and were ready for the experiment 45–60 minutes after the injection of urethane. The trachea was cannulated in the usual fashion and one carotid artery ligated. The femoral vein was then cannulated just below the inguinal ligament, the corresponding artery separated, and the posterior division of the femoral nerve cut. A blunted needle (No. 1) was used for venous cannulation, and heparin was injected intravenously. All injections were made through a two-way stopcock fitted with a tuberculin syringe, and all solutions were washed in with 0.2 c.c. normal saline from a microburette (with attached thistle funnel) connected with a piece of rubber tube. The carotid cannula was connected with a mercury manometer by a column of normal saline. The C.N.S. was left intact, in order to avoid excessive bleeding after heparin, the vagi and associated sympathetics being cut at the end of the operation. Dibenamine was injected intravenously just before the beginning of assay.

On two occasions the antidiuretic activity of the same concentrates was assayed by measuring the average antidiuretic response in groups of rats (Burn, 1931), Pitressin

(Parke, Davis and Co.) being used as the standard preparation for both the pressor and antidiuretic assays. Oxytocic activity was assayed on the virgin guinea-pig's uterus (Dale and Laidlaw, 1912) suspended in a bath containing a salt solution with no Mg (Burn, 1937), Pitocin (Parke, Davis and Co.) being used as the standard preparation.

*Drugs used :*

*Intraperitoneally :* Allobarbitone—Dial Liquid Comp. (Ciba)—0.03 c.c./100 g. body weight (followed by urethane injections).

*Subcutaneously :* Urethane (B.D.H.)—25 g. per cent (w/v) solution in distilled water—0.7 c.c./100 g. body weight.

*Intravenously :* Heparin *B.P.* (Boots), sterile, 109.3 u./mg.—2 mg./100 g. body weight dissolved in 1–2 c.c. normal saline. *l*-adrenaline base (B.W. and Co.) and *l-nor*adrenaline bitartrate (Bayer). Doses given in mμg. of the base dissolved in normal saline containing ascorbic acid ($5 \times 10^{-3}$). Piperidine acid tartrate (B.D.H.)—100 mg. per cent (w/v) solution in normal saline (≡36.3 mg. base per 100 c.c.). Ergotoxine ethanesulphonate (B.D.H.)—25 mg. per cent (w/v) suspension in faintly alkaline saline. Piperoxane HCl (May and Baker) (piperidylmethylbenzodioxane hydrochloride, 933F)—0.2 mg./c.c. solution in normal saline. Dibenamine HCl (Smith, Kline, and French) (N : N-dibenzyl-β-chloroethylamine)—2.5 mg./c.c. solution in faintly acid saline; 12.5 mg. dibenamine HCl dissolved in 0.5 c.c. 95 per cent ethanol made acid (0.05 N) with concentrated $H_2SO_4$ and then diluted up to 5 c.c. with normal saline.

## RESULTS

The pressor equivalents are given in Table I. When the standard preparation was injected in small doses (4–20 mu.) there was always a simple pressor effect, as seen in other anaesthetized mammals, but with very high doses a diphasic response may occur when coronary vasoconstriction is severe enough to affect the heart. There was never enough of the pressor factor in a 24-hour specimen to cause such a diphasic response. The intravenous injections of the concentrates were interposed between the injections of the standard, doses producing effects

TABLE I

Individual results. Pressor equivalents (mu.) per 24-hour urinary specimens. Concentrates diluted with normal saline.

| Subject—Age, Sex (M) = male (F) = female | 24-hour urinary output (c.c.) | Final volume of concentrate (c.c.) | Volume (c.c.) of concentrate equivalent to 0.004 i.u. pitressin | Total pitressin equivalent (mu.) |
|---|---|---|---|---|
| P. 67 (F) .. .. | 1,640[1] | 10 | 0.35 | 114 |
| F. 26 (F) .. .. | 1,800[1] | 10 | 0.4 | 100 |
| D. 52 (M) .. .. | 1,550 | 5 | 0.2 | 100 |
| D. 52 (M) .. .. | 1,200 | 5 | 0.25 | 80 |
| B. 30 (M) .. .. | 1,560 | 5 | 0.25 | 80 |
| B. 23 (F) .. .. | 1,400 | 5 | 0.25 | 80 |
| D. 52 (M) .. .. | 1,100 | 5 | 0.25 | 80 |
| D. 52 (M) .. .. | 580[2] | 5 | 0.15 | 133 |
| S. 22 (F) .. .. | 1,120[3] | 20 | 0.25 | 320 |
| S. 22 (F) .. .. | 1,200 { 600 | 2.5 | 0.25 | 40 |
| | { 600[4] | 50 | 0.25 | 800 |

[1] Concentrate tested also for antidiuretic effect. [2] Urine collected during 36 hours' withdrawal of food and water. [3] 0.5 i.u. pitressin added to supernatant urine (*p*H 4.0). [4] 1 i.u. pitressin added to 600 c.c. of supernatant urine (*p*H 4.7), the other 600 c.c. being used as control.

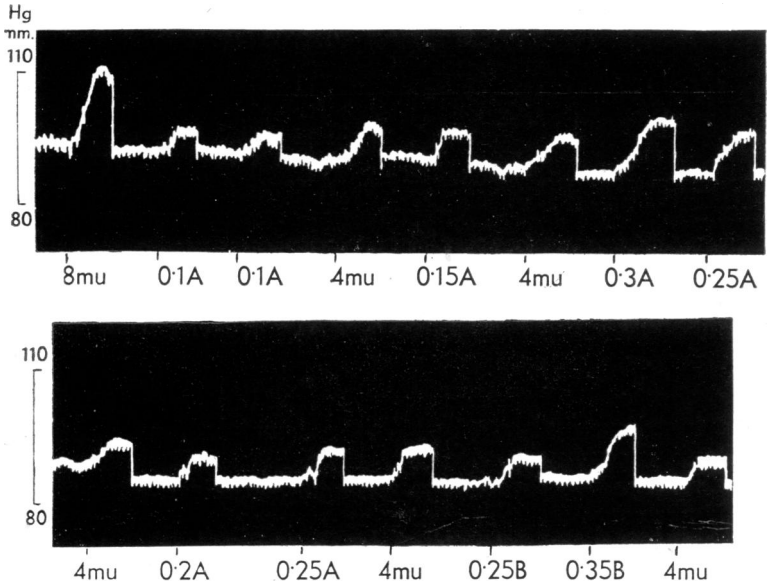


FIG. 1.—Effect of urinary pressor fractions A and B compared with that of pitressin on rat's carotid blood pressure. 10-minute intervals. Doses in mu. (4 mu. in 0.1 c.c.) and c.c. A = a concentrate (× 12) of urine with added pitressin (1,000 mu. per 600 c.c.). B = a concentrate (× 240) of the same urine without pitressin.

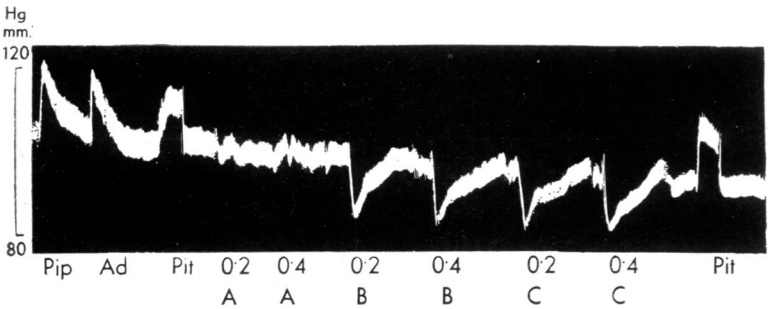


FIG. 2.—Rat's blood pressure. Pip = piperidine (110 μg. base). Ad = adrenaline (0.6 μg.). Pit = pitressin (10 mu.). A, B, and C—concentrates (× 200) made by method of Grollman and Woods. Doses in c.c. A = piperidine (90 mg.) in 1 l. water. B = piperidine (90 mg.) in 1 l. decomposed urine. C = same urine alone. If piperidine had survived the concentration A and B would have contained 18 mg. per c.c.

above and below that of the standard being determined (see Fig. 1). The concentrates of unpreserved urine which had been left for a few days showed a definite depressor effect (see Fig. 2), and it was found necessary to extract fresh specimens only.

When one unit of pitressin was added to 600 c.c. of the supernatant fluid after removal of the kaolin, 76 per cent of it was recovered by the methods used (Table I, Fig. 1). In another experiment the recovery was about 50 per cent. When piperidine was similarly added to water or the supernatant fluid, none of it was recovered (Fig. 2).

The pressor effect of urinary concentrates may be due to the presence of nicotine, piperidine, and other pressor bases. Therefore, the identification of the urinary pressor factor was carried out by testing it on rats treated with ergotoxine (30–40 μg./100 g. body weight), piperoxane (5–10 μg./100 g. body weight), or dibenamine (1–2 mg./100 g. body weight). Both ergotoxine and piperoxane reverse the pressor action of adrenaline in the rat, as they do in the cat, but the urinary pressor fraction, like pitressin, still caused a rise of blood pressure after these drugs had been given. However, these results were complicated by the toxic effects of ergotoxine and by the short duration of the action of piperoxane, and often by the incompleteness of the blocking action of those compounds. Dibenamine hydrochloride, on the other hand, was found to have definite advantages of specificity, potency, and prolonged action.

The total dose of 1–2 mg. dibenamine hydrochloride per 100 g. body weight was injected intravenously into the rat in five equal portions, spread over 25–30 minutes. Rapid injection caused a marked fall in arterial pressure; even a slow rate of injection usually produced a progressive fall in blood pressure, but this was not fatal. If necessary, artificial respiration was used. The blood pressure soon established itself on a new basal level and remained constant for seven to nine hours.

After the administration of dibenamine, the usual pressor effects of small doses of adrenaline, *nor*adrenaline, and piperidine were always abolished, but never changed to a pure depressor response even when the total dose of injected dibenamine was as much as 4 mg./100 g. body weight. The pressor response to pitressin and the urinary pressor fraction, on the other hand, still occurred after

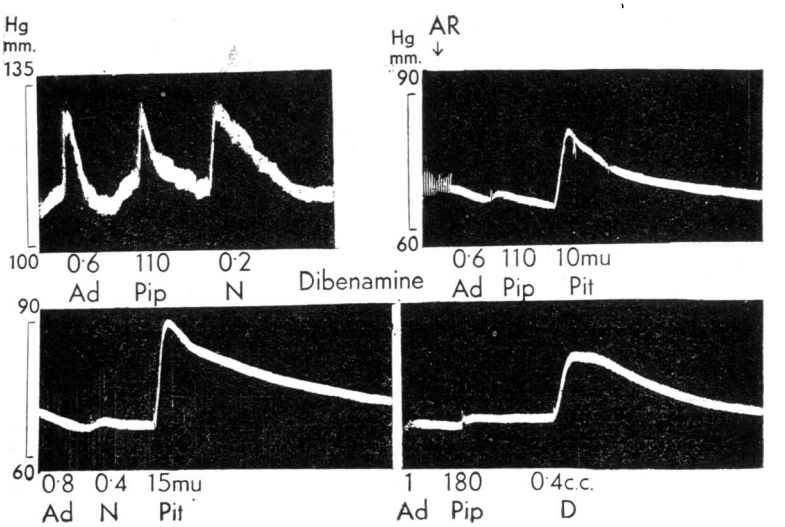


FIG. 3.—Rat's blood pressure. Dibenamine (1 mg./100 g. body weight) injected after the first three records had been taken caused a fall of B.P.; artificial respiration was started at this time. The effects of adrenaline (Ad) *nor*adrenaline (N), and piperidine (Pip) were abolished by dibenamine (doses in μg.), but the effects of pitressin (Pit) or of a concentrate (× 240) of urine (D) were not.

dibenamine (Fig. 3), and in good preparations 40 or more satisfactory responses were obtained. It was found that useful discrimination for the assay of pituitary pressor hormone was obtained with doses of about 4 to 20 mu. With doses of 60 mu. and over the responses were less regular, even with intervals of 15 minutes between injections. The maximum simple pressor response occurred with a dose of 200 mu. of pitressin.

The antidiuretic effect of urinary concentrates was quantitatively assayed on two occasions in comparison with the pressor effect. When injected subcutaneously into the rats, two fractions produced antidiuretic responses equivalent to about 50 mu. and 80 mu. of pitressin per 24-hour specimen. These figures were 50 and 70 per cent of the estimates of the pressor activity in the same solutions.

The average amount of pressor activity recovered from the urine excreted in 24 hours was equivalent to about 90 mu. of pitressin (Table I). The withdrawal of water appeared to be associated, in one experiment, with a small increase in the amount of pressor activity found in urine.

No significant oxytocic activity was detected in the concentrates examined. In one experiment the concentrate from 240 c.c. urine contained the equivalent of less than 3 mu. pitocin. The pressor activity of this fraction was equivalent to 80 mu. pitressin.

## Discussion

Previous work has shown that several pressor substances may be present in extracts of urine. Fresh urine has been shown to contain adrenaline, *nor*adrenaline, and hydroxytyramine (Holtz, Credner, and Kroneberg, 1947 ; Holtz, 1950 ; Euler, 1950), nicotine (Dingemanse and Freud, 1933 ; Helmer, Kohlstaedt, and Page, 1939 ; Lockett, 1944a, 1944b), and piperidine (Euler and Sjöstrand, 1943 ; Euler, 1944a, 1944b, 1945). Bacterial action may cause the formation of *iso*amylamine (Bain, 1915 ; Bohn and Hahn, 1933 ; Bohn and Coester, 1934 ; Lockett, 1944a) and tyramine (Enger and Arnold, 1937). All these bases have pressor actions, but the experiments with ergotoxine, piperoxane, and dibenamine have shown that the pressor substance described here is not identical with any of them. Direct experiment has shown that any piperidine present originally would not be present in the final concentrates. The substance studied in these experiments is probably the pressor principle from the posterior pituitary, vasopressin, which has indeed been previously found in urine. For example, Dale (1909) showed that when posterior pituitary extracts were injected intravenously in cats the pressor principle could be detected in the urine by its effect on the cat's blood pressure. There has also been much recent work on the excretion of the antidiuretic principle in the urine. This substance is thought (Waring and Landgrebe, 1950) to be identical with the pressor principle, and assays based on the antidiuretic effect should therefore give the same result as assays based on the pressor effect. The results of preliminary experiments on these lines, with the urinary concentrates obtained by the method described, are suggestive but by no means conclusive. The test for oxytocic activity, on the other hand, gave negative results and showed that oxytocin was not present in comparable amounts.

SUMMARY

1. Human urine, from which gonadotrophins had been removed with kaolin, was treated by the method of Grollman and Woods for the preparation of antidiuretic concentrates. These concentrates were then tested for other forms of activity.

2. They had a pressor action in rats which was not due to adrenaline, *nor*adrenaline, *iso*amylamine, tyramine, piperidine, or nicotine, since it survived the injection of dibenamine, ergotoxine, or piperoxane (933F). It is thought that the effect was due to vasopressin.

3. The urine concentrates appeared to have no action on the guinea-pig's uterus. Their oxytocic activity in terms of posterior pituitary extracts was certainly less than 1/20th of their pressor activity.

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