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THE ACTION OF THE ANTIDIURETIC PRINCIPLE OF POSTERIOR PITUITARY EXTRACTS ON THE URINE EXCRETION OF ANAESTHETIZED ANIMALS

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Extracts of the posterior pituitary gland are known to have two actions on the secretion of urine. Very small doses inhibit the water diuresis of normal mammals. There is substantial evidence that this action is produced by a specific effect on the tubular epithelium. The second—historically earlier—known action of post-pituitary extracts is an increase of the urine flow in anaesthetized animals. This increase is produced by doses which are larger than those used for the production of the antidiuretic effect, it lasts from 10 to 30 min. and in the experience of the majority of observers is not followed by an inhibition of the urine flow.

The diuretic action of post-pituitary extracts in anaesthetized animals has been explained in various ways. Some authors [Magnus & Schäfer, 1901; Hoskins & Means, 1913; Schäfer & Herring, 1908] thought it due to "a specific stimulation of the renal cells" [Sharpey-Schäfer, 1926]. They arrived at this conclusion because (1) the increased urinary output in some cases outlasts the rise of blood pressure, (2) tachyphylaxia is more pronounced for the blood pressure than for the diuretic effect, (3) the increase in urine flow is sometimes accompanied by an increase in kidney volume and sometimes not.

Other workers [Houghton & Merrill, 1908; Richards & Plant, 1922; Knowlton & Silverman, 1918; Nelson, 1934] postulate a vascular origin of the diuretic effect. They base their opinion on (1) the close parallel between blood pressure and diuresis, (2) the similarity between the action of post-pituitary extracts and the diuretic action of small doses of adrenaline, (3) the absence of an increase of oxygen consumption during

pituitrin diuresis, and (4) the increase of glomerular filtration after an intravenous injection of pituitrin into an anaesthetized animal.

The experimental support for an essentially vascular origin of the diuretic action of post-pituitary extracts in the anaesthetized animal seems, on the whole, stronger. The matter could clearly be much advanced, if not decided, if a post-pituitary preparation could be used which, while retaining its antidiuretic activity, no longer influences the blood pressure. A preparation which fulfils these conditions to a high degree has recently become available. The antidiuretic and the vasopressor factors have not been separated chemically, but heat inactivation of the two activities proceeds at different rates [Heller, 1939]. By making use of the different stability of the antidiuretic and vasopressor principles preparations have been obtained which contain only about 8 parts of pressor activity to 100 parts antidiuretic activity.

The object of the present enquiry was the investigation of the urinary action of such antidiuretic but non-pressor post-pituitary preparations in the anaesthetized animal.

Methods

The post-pituitary extracts employed in this investigation were Messrs Parke, Davis and Co.'s pitressin, pitocin and "specially prepared" pitressin. The term milliunit (mU.) is used to denote the activity of 0·0001 c.c. of pituitary (posterior lobe) B.P. extract.

Assay of post-pituitary extracts. Spinal cats were used for the estimation of the pressor potency and Burn's [1931] rat method for that of the antidiuretic activity.

Diuresis experiments. Rabbits weighing between 2 and 4 kg. were used. Urine samples of unanaesthetized animals were obtained by bimanual expression of the bladder. Ethylurethane was used as anaesthetic. 1·5 g./kg. rabbit were given intraperitoneally or 2·5 g./kg. subcutaneously. These doses ensure a steady "surgical" anaesthesia (complete abdominal relaxation, spontaneous respiration, absent or very sluggish corneal reflex, blood pressures over 60 mm. Hg) for many hours. Urine samples of such animals were collected from a cannula tied into the distal end of one ureter. The other ureter was clamped. To reduce the "dead space" of the urine collecting system special cannulae blown from capillary tubes were used. The "dead space" of these cannulae equalled 0·2–0·3 c.c. water. Urine flow was recorded with Condon's drop counter and measured by subsequent collection in small measuring cylinders. Urinary osmotic pressure was estimated with a Beckmann apparatus for freezing-point determinations of small quantities. The same apparatus

was used to determine the depression of the freezing point of normal rabbit's blood. Fourteen estimations on four animals gave an average of $\Delta = -0{\cdot}548 \pm 0{\cdot}0215°$ C. for defibrinated venous blood.

Results

The preparation of an antidiuretic non-pressor extract

It was reported in a previous paper [Heller, 1939] that heating of a commercial posterior pituitary extract for 90 min. at *p*H 10·0 and 99° C. resulted in a preparation which contained $11{\cdot}5 \pm 0{\cdot}85\%$ of its initial pressor action, i.e. the ratio of antidiuretic to pressor activity was approximately 100 to 8. For the purpose of this investigation it seemed desirable to obtain an extract which contained even less pressor activity. For this reason the pressor and antidiuretic potencies of extracts were compared which had been heated for 105 and 120 min. respectively. The following curves resulted (Fig. 1). It will be seen that traces of pressor activity are still present after 120 min. of heating. However, this is relevant only to injections of comparatively large amounts of such extracts, the small amounts used in the present investigation had usually no noticeable pressor effect (Fig. 3).

Clark and Lubs buffers were used to maintain the reaction while heating at *p*H 10·0. This buffer contains potassium in a concentration which, when injected into spinal cats might, to a certain extent, counteract the pressor effect of post-pituitary extracts [Dawes, 1940]. An isotonic potassium free buffer consisting of boric acid and sodium hydroxide was, therefore, used in some experiments but no essential differences of pressor action were found.

Fraser [1937] and Kuschinsky & Bandschuh [1939] published experiments indicating that post-pituitary extracts contain a diuretic substance which seemed to be identical with the oxytocic factor. The objection could, therefore, be raised that the different strength of the pressor and antidiuretic activities after heating at *p*H 10·0 is only apparent, viz. due to the inactivation of an antagonistic substance which masked the initial concentration of the antidiuretic principle. This possibility would seem to be supported by the observation that the oxytocic factor is undoubtedly inactivated at *p*H 10·0 [Gaddum, 1930; Heller, 1939]. However, the post-pituitary extract used to obtain a "non-pressor antidiuretic" preparation contained originally only about 5% of oxytocic activity or, in terms of the doses actually employed in the antidiuretic assay, only amounts of the order of 0·1 of a mU. (oxytocic) per 100 g. rat. Using the

technique of Kuschinsky & Bundschuh [1939] twenty-one groups of rats were injected with 0·15 mU. (oxytocic) and 0·30 mU. (oxytocic)/100 g. rat but no diuretic effects were observed. It seems, therefore, justifiable

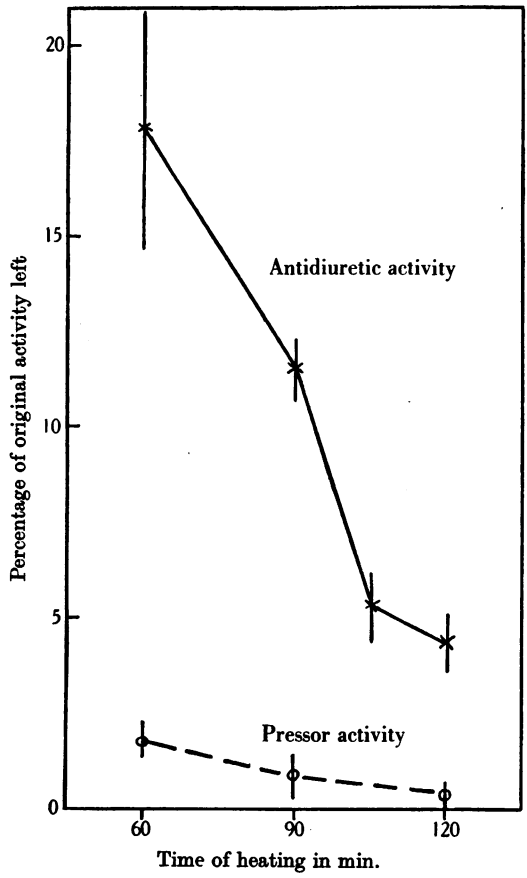

Fig. 1. The inactivation of the antidiuretic and the pressor activity of post-pituitary extracts (pitressin) at 99° C. and *p*H 10·0. X—X, antidiuretic activity; O----O, pressor activity. The vertical lines indicate the standard error.

to assume that the antidiuretic activity of our heated extracts is that of the stated percentage of the initial antidiuretic hormone concentration, and is not due to an apparent increase of antidiuretic activity caused by the inactivation of a diuretic factor.

The effect of an "antidiuretic non-pressor" preparation on the urine flow of the unanaesthetized and the anaesthetized rabbit

Fig. 2 shows the effect of an intravenous injection of 2000 mU. of "pitressin" heated for 90 min. at *p*H 10·0 on the water diuresis of an unanaesthetized rabbit. This dose equals approximately 200 mU. of an untreated extract.

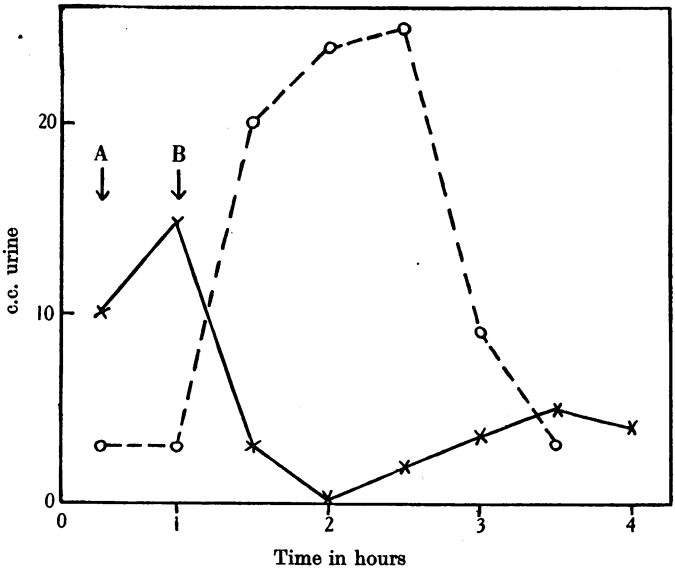

Fig. 2. The action of a non-pressor antidiuretic preparation of post-pituitary extract on the water diuresis of an *unanaesthetized* rabbit (♀, 2100 g.). 3 and 4 % of body weight of water by stomach tube 4·5 and 0·5 hr. prior to A. O----O, normal water diuresis. X—X, same rabbit, two days later, water and pitressin. B = intravenous injection of 2000 mU. pitressin heated for 90 min. at *p*H 10·0.

The marked and lasting inhibition of the urinary output will be noted compared with the diuresis following the same amount of water (without pitressin) in an experiment performed two days previously. The *same animal* and the *same dose* of extract, prepared in an identical manner, were used two days later to compare the action in the anaesthetized animal of a pressor and a non-pressor post-pituitary preparation of equal antidiuretic potency. Fig. 3*b* shows the typical effect of a dose of the post-pituitary pressor principle on the systemic blood pressure and the urine flow of a rabbit anaesthetized with urethane. The pronounced increase and the long duration of the increase of urine flow after the injection will be noted. The increase is preceded by a short (40 sec.)

cessation of urinary secretion. This (primary) inhibition of urine flow is known to occur—not with regularity however—in anaesthetized animals and has been attributed to a brief ureter spasm immediately after the

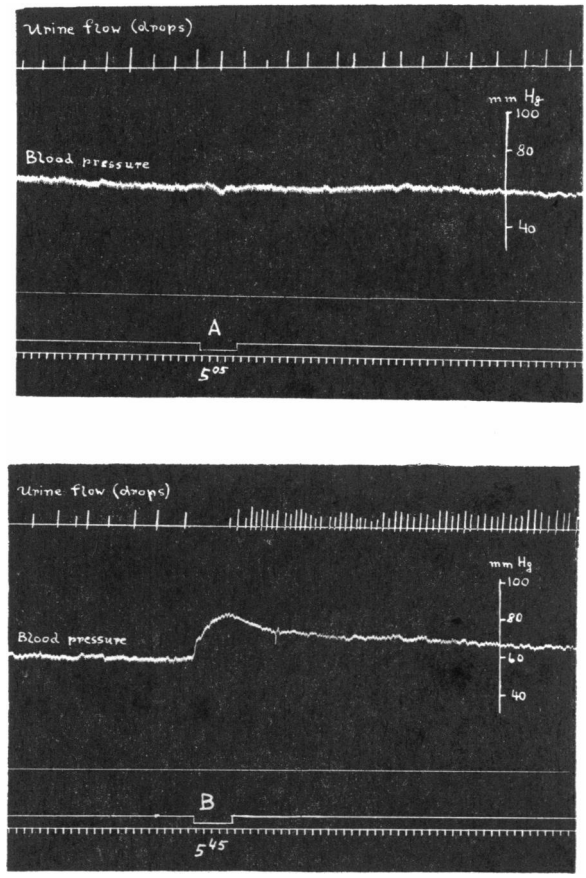

Fig. 3. Comparison of the effect on B.P. and urine flow of an anaesthetized (urethane) rabbit of an intravenous injection of a post-pituitary extract containing pressor and antidiuretic activities in equal proportion with the effect of a non-pressor preparation of the same antidiuretic activity. *Same* rabbit as in Fig. 2. A = intravenous injection of 2000 mU. pitressin heated for 90 min. at 99° C. and *p*H 10·0. B = intravenous injection of 200 mU. pitressin not heated. Time marker = 5 sec.

intravenous injection [Molitor & Pick, 1924; Mackersie, 1925]. In the present series it was observed in the majority of experiments with pressor post-pituitary extracts. Neither this immediate cessation nor the

subsequent rise in urine flow followed upon an injection of a non-pressor preparation (Fig. 3*a*) which had been administered 40 min. previously. The non-pressor extract was given first and before the rabbit had received any other post-pituitary extract on that day. This excludes the possibility that the absence of a rise of blood pressure was due to the refractoriness (tachyphylaxia) of the animal after a previous injection of post-pituitary extract. The lack of a diuretic effect concurrent with the absence of a rise of blood pressure is obvious. The short primary inhibition is equally absent. Nor was it found to occur in other experiments with non-pressor extracts.

The experiment recorded in Fig. 3 was performed on an animal which had received 147 c.c. of water by stomach tube before urethane anaesthesia started. Sufficient time (2 hr.) had been allowed for the intestinal absorption of the bulk of this amount [Heller & Smirk, 1932*a*]. The urinary excretion rate in this and other experiments of the same series was nevertheless low. The same results regarding the action of pressor and non-pressor antidiuretic preparations on blood pressure and urine flow were obtained when a diuresis was produced by intravenous infusion of hypertonic (10%) solutions of sucrose or sodium sulphate during the anaesthesia.

Neither in these experiments nor where water had been given by stomach tube did a (secondary) inhibition follow the diuretic response. The significance of this finding will be discussed in the following section.

It is clear from these results that the primary inhibition and the increase in the rate of urine flow following upon an intravenous injection of an untreated post-pituitary extract is due to its pressor activity, and not to the antidiuretic principle proper.

The relation of the urinary osmotic pressure to the action of the antidiuretic hormone

It is well known that the post-pituitary antidiuretic factor ceases to have an inhibitory action on the urine flow of unanaesthetized animals if a certain height of urinary osmotic pressure is reached. The evidence for this rests on two groups of experiments: (1) The antidiuretic hormone does not inhibit a diuresis produced by concentrated solutions of a variety of organic and inorganic substances. The high osmotic pressure of the urine excreted under these conditions has not actually been demonstrated, but it can be deduced from the analytical data given by various authors. (2) Injection of post-pituitary extracts does not decrease the urine flow of "unhydrated" animals. The urinary (morning)

osmotic pressure (expressed as freezing point depressions) of a series of ten unhydrated rabbits on a standard diet of oats, bran and cabbage was estimated, and the following figures found: $\Delta = -3{\cdot}556$, $-2{\cdot}958$, $-2{\cdot}155$, $-2{\cdot}170$, $-2{\cdot}380$, $-1{\cdot}735$, $-2{\cdot}205$, $-2{\cdot}380$, $-3{\cdot}065$, $-2{\cdot}825$, average $\Delta = -2{\cdot}543 \pm 0{\cdot}556°$ C., that is to say the urine was in every case strongly hypertonic.

It has been mentioned that injections of a non-pressor but antidiuretic preparation of post-pituitary extracts failed to reduce the urine flow of deeply anaesthetized rabbits which had received large doses of water prior to the injection of the anaesthetic or which during anaesthesia received intravenous infusions of concentrated (10%) solutions of sodium sulphate or sucrose. It can be shown that under these experimental conditions also the antidiuretic principle fails to act because the urinary osmotic pressures are too high. This has been implicitly assumed by numerous authors [Fee, 1929; Melville, 1936; Smith, 1937; and others]. The present investigation offered the opportunity of a quantitative enquiry into the relation between the antidiuretic action of posterior pituitary extracts and the osmotic pressure of the urine. It seemed of interest to determine the "critical urinary osmotic pressure", i.e. that at which the antidiuretic principle ceases to act.

The effect of anaesthesia and operative measures on the flow and osmotic pressure of the urine is shown in Fig. 4, excretion rates per 10 min. falling from 3·8 to 0·28 c.c. and freezing-point depressions falling from $\Delta = -0{\cdot}345°$ C. to $-2{\cdot}910°$ C. The urine flow increases somewhat half an hour after the operation but still remains on a low level, in spite of the "extra water" (=water load)[1] present in the body. 200 mU. pitressin injected at this stage had no antidiuretic effect. Infusion of a hypertonic solution of sodium sulphate or sucrose considerably increases the excretion rate under deep anaesthesia but has little effect on the osmotic pressure of the urine. That is to say the urine remains hypertonic and the antidiuretic hormone does not act. This result is in agreement with Melville's [1936] finding that the rate of urine flow does not affect the action of the antidiuretic hormone.

An unsuccessful attempt was made to produce a "hypotonic diuresis" in four experiments on rabbits under deep urethane anaesthesia. Fig. 5

[1] The water load in this and the following experiments was calculated as the difference between the volume of fluid administered and the volume of urine excreted. Extrarenal losses, about 10 c.c./hr. [see Heller & Smirk, 1932*b*], do not affect the results appreciably and are not accounted for. In this and the following experiments of this series the blood pressure was recorded during anaesthesia to ensure that a decrease of urine flow was not due to a fall in blood pressure.

represents the one of those experiments, in which the lowest urinary osmotic pressures were observed. The animal received 50 c.c. of tepid water per kg. body weight by stomach tube 3 hr., and again 1 hr. before

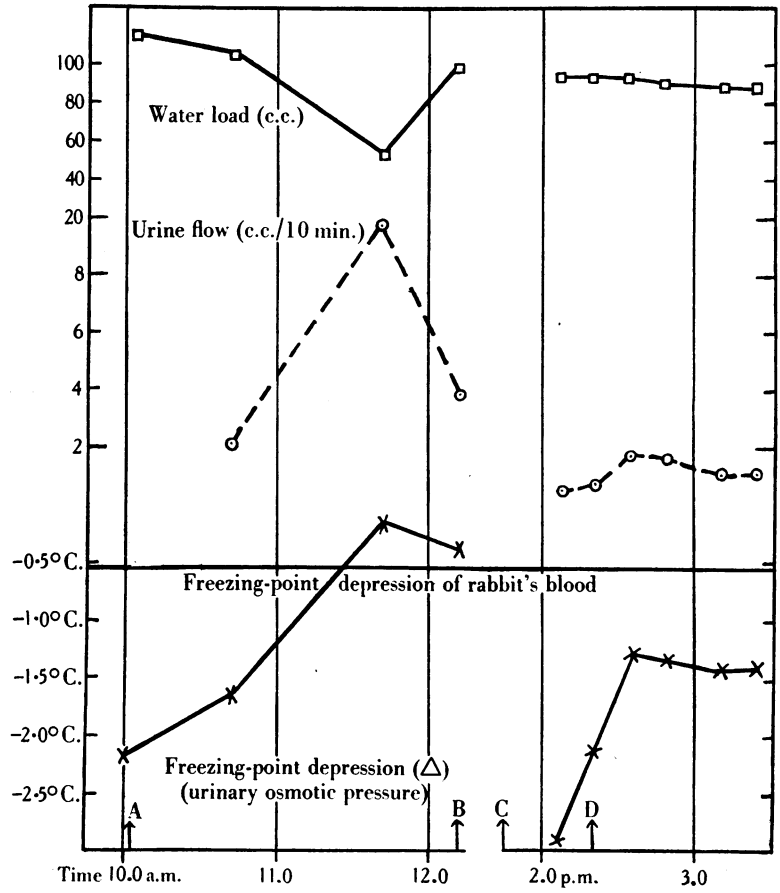

Fig. 4. The effect of anaesthesia and operative procedures on urinary osmotic pressure and urine flow (animal given water prior to anaesthesia. Rabbit ♀, 2900 g. At A 3% of body weight of water by stomach tube. Note that urine becomes hypotonic. At B 2% of body weight of water by stomach tube and 1·5 g. urethane/kg. intraperitoneally. C = operative procedures finished. Note decrease of urinary excretion rate and increase of urinary osmotic pressure. D = intravenous injection of 200 mU. pitressin. Note absence of antidiuretic effect. Urine remains hypertonic. The water load decreases little during anaesthesia, thus denoting the inhibition of diuresis.

anaesthesia started. The osmotic pressure of the urine fell to a minimum value of $\Delta = -0{\cdot}085$ after this large quantity of water. Anaesthesia and operative measures increased the osmotic pressure to $\Delta = -0{\cdot}790$. This

level was more or less maintained even during a rapid intravenous infusion (6 c.c./min.) of a hypotonic solution ($\Delta = -0{\cdot}280$) of sucrose. The urine flow under these conditions was quite as high as that reached

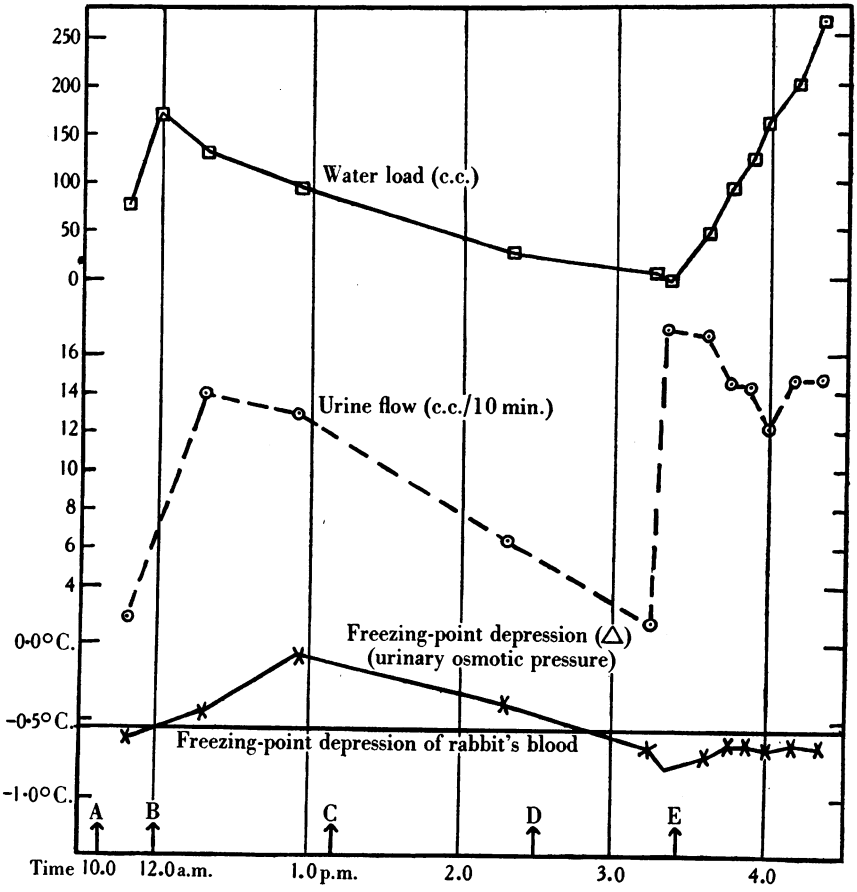

Fig. 5. The effect of anaesthesia and operative procedures on the urinary osmotic pressure and the urine flow of an animal receiving a rapid infusion of a *hypotonic* solution of sucrose. Rabbit ♂, 2150 g. At A and B 5% of body weight of water by stomach tube. Note low osmotic pressure of urine. At C 1·5 g. urethane/kg. intraperitoneally. D = operation finished. E = intravenous infusion of 4% sucrose started (6·0 c.c./min.). Note hypertonic urine in spite of excretion rate which surpasses that at height of water diuresis.

during the water diuresis of the unanaesthetized rabbit. In spite of the high water load, however, the urine of the anaesthetized animal was not hypotonic.

These results show that once the inhibitory action of the anaesthetic is elicited it can not, or can only to a small degree, be counteracted by blood dilution with a hypertonic or even hypotonic fluid. They suggest that the mechanism underlying the inhibition of water diuresis in anaesthesia is at least in part a renal one.

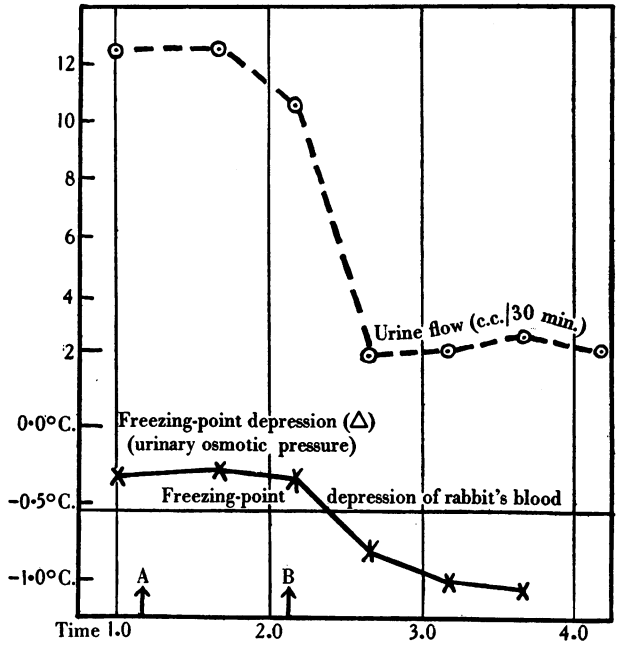

Fig. 6. The effect of an injection of pitressin on the urine flow and the urinary osmotic pressure of a rabbit under the influence of a dose of urethane insufficient to inhibit a water diuresis. Rabbit ♀, 1750 g. Not operated (urine obtained by expression of bladder). 5% of body weight of water by stomach tube at 10.0 a.m. and again at 12.0. A = subcutaneous injection of 1·5 g. urethane/kg. Note hypotonic urine after this dose of urethane. See text for description of depth of anaesthesia. B = intravenous injection of 100 mU. pitressin. Note lasting inhibition of water diuresis.

Intraperitoneal injections of the amount of urethane used in the foregoing experiments (1·5 g./kg.) inhibit the water diuresis of rabbits to the same extent if the animals are not operated upon after the anaesthetic has been given. The average urine volume of four such animals in the 2 hr. preceding the injection of the anaesthetic amounted to $56{\cdot}1 \pm 22{\cdot}2$ c.c. as compared with $5{\cdot}6 \pm 2{\cdot}8$ c.c. in the 2 hr. following it. The water load at the beginning of anaesthesia averaged $125 \pm 26{\cdot}9$ c.c. The inhibition of the water diuresis after subcutaneous application of urethane (2·5 g./kg.)

was somewhat less pronounced in the small number of animals tested. The figures for three rabbits were: $34{\cdot}3 \pm 7{\cdot}3$ c.c. in the 2 hr. before injection, $14{\cdot}1 \pm 1{\cdot}4$ c.c. in the 2 hr. after injection. Water load: $148 \pm 24{\cdot}3$ c.c.

Smaller subcutaneous doses of urethane (1·4 to 1·7 g./kg.) failed to inhibit the water diuresis of unoperated rabbits to any marked extent (Fig. 6), the urine was consequently hypotonic, the antidiuretic principle retained its inhibitory action. Such "smaller doses" of urethane had nevertheless a pronounced anaesthetic effect. For example, the rabbit which yielded the data presented in Fig. 7 showed, at the time of the injection of the post-pituitary extract: a loss of position reflexes, a sluggish corneal reflex, no resentment when its ear was pricked for the intravenous injection of pitressin, and a respiration rate of 34 per min.

The number of experiments performed in these series is not sufficiently great to permit the definition of the "critical urinary osmotic pressure" (i.e. that at which the antidiuretic factor ceases to act) with accuracy. It can, however, be stated that post-pituitary extracts retained a pronounced antidiuretic action at urinary osmotic pressures below $\Delta = -0{\cdot}360$ and failed to decrease the urine flow at osmotic pressure values above $\Delta = -0{\cdot}800$.

Discussion

The experimental evidence presented in this paper shows that non-pressor but antidiuretic preparations of post-pituitary extracts exert no *diuretic* action in the anaesthetized animals. In fact, such preparations produce no significant change of the urine flow of rabbits under deep urethane anaesthesia in spite of previous ample hydration or infusion of sucrose or sodium sulphate solutions during the experiment. Two main conclusions can be drawn from these results:

(1) The diuretic action in anaesthetized animals of post-pituitary extracts containing both the antidiuretic and the vasopressor activity is due to the latter. The post-pituitary pressor principle can, therefore, be grouped with other pressor substances like adrenaline [Richards & Plant, 1922; Toth, 1937] or veritol [Springorum, 1938] which under similar experimental conditions show a similar diuretic action.

These findings do not necessarily mean that the pressor principle causes the diuretic effect which has been repeatedly observed in badly hydrated, unanaesthetized animals. This diuretic action is usually explained by the increase of the urinary salt excretion caused by post-pituitary extracts [Stehle, 1927], i.e. as an osmotic diuresis. The fact that the antidiuretic principle proper augments the chloride excretion

in the unanaesthetized organism [Heller, 1939] is consistent with this view. However, the results of the present investigation suggest the possibility of an alternative explanation, viz. the diuretic action of the pressor principle under experimental conditions which prevent the action of the antidiuretic factor.

(2) The action of the antidiuretic principle proper is not "reversed", i.e. changed into a diuretic one under the influence of anaesthesia. It is merely abolished if the doses of anaesthetic given are large enough to influence urinary excretion to an extent which raises the osmotic pressure of the urine to values equivalent to freezing-point depressions below $\Delta = -0{\cdot}800°$ C.

The antidiuretic hormone may in certain circumstances fail to act even in unanaesthetized animals, for example in badly hydrated animals, and in animals in which diuresis had been produced by concentrated solutions of salts or certain organic substances like urea or sucrose. In both these instances the urine excreted is hypertonic. It can be said, therefore, that in all circumstances whether the animal is anaesthetized or not, the antidiuretic principle ceases to act at high urinary osmotic pressures. This conclusion agrees with the present concept of the physiological role of the antidiuretic hormone in the water metabolism of the mammal. This concept, formed on experimental and morphological evidence [Starling & Verney, 1925; Burgess, Harvey & Marshall, 1933; Smith, 1937] regards the antidiuretic hormone as the means by which a hypertonic urine is produced by the mammalian and probably avian kidney, that is to say, the hormone acts as an agent which in these groups of vertebrates renders the ratio of water to solids excreted more economical. The fact that the antidiuretic hormone ceases to act at a certain osmotic pressure of the urine can, therefore, be regarded as a self-regulating mechanism which prevents the urine to become too concentrated under the influence of the hormone.

Summary

1. A non-pressor but antidiuretic extract of the posterior pituitary lobe has been prepared (Fig. 1) and its action on the excretion of urine has been tested.

2. Such preparations have no diuretic action on the anaesthetized animal (Figs. 2, 3). The diuretic effect of post-pituitary extracts containing both the antidiuretic and the vasopressor principle is, therefore, due to the pressor activity.

3. The action of the antidiuretic principle on the urine flow of anaesthetized rabbits depends on the osmotic pressure of the urine elaborated and this in turn on the dose of anaesthetic (urethane) used (Figs. 4, 6). It was found that post-pituitary extracts retained a pronounced antidiuretic action above an osmotic pressure equivalent to $\Delta = -0{\cdot}360$ and failed to decrease the urine flow at freezing-point values below $\Delta = -0{\cdot}800$.

I wish to express my thanks to Prof. J. H. Burn for providing facilities in his department. I am indebted to Messrs Parke, Davis and Co. for a generous supply of posterior pituitary extracts.

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