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THE RENAL ACTION OF POSTERIOR PITUITARY EXTRACT AND ITS FRACTIONS AS ANALYSED BY CLEARANCE EXPERIMENTS ON RATS

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Renal clearance estimations, after injection of posterior pituitary extracts, have been performed by Poulsson (1930) with creatinine on dogs, by Burgess, Harvey & Marshall (1933) with sucrose and xylose on man and dogs, by Walker, Schmidt, Elsom & Johnson (1937) with creatinine on rabbits and dogs, and by Corcoran & Page (1939) with inulin and phenol red on dogs. Measurements of the renal blood flow by means of a thermostromuhr after the injection of posterior pituitary extract, or its vasopressor fraction, have been recorded by Janssen & Rein (1928), Geiling, Herrick & Essex (1934), Handovsky & Samaan (1937), Walker *et al.* (1937) and Wakim, Herrick, Baldes & Mann (1942). Recently developed clearance methods are likely to yield a more complete and more accurate picture of renal changes after an injection of posterior pituitary extract, since they permit the simultaneous measurement of glomerular filtration rate, effective renal blood flow and tubular activity in unanaesthetized and intact animals. A further clarification of the action of posterior pituitary extracts on the kidney, particularly with reference to the effect on the urinary excretion of chloride, can be expected from the results of clearance experiments made after the injection of the vasopressor and oxytocic fractions. Kuschinsky & Bundschuh's (1939) and Fraser's (1942) finding that the oxytocic fraction is more active than the vasopressor fraction in increasing the urinary chloride excretion will be remembered in this connexion. However, the mechanism of this effect was not investigated by these authors.

METHODS

Male adult albino rats were used throughout. The animals were kept on a standard diet (Vitamin A Test Diet, *U.S. Pharmacopoeia*, 11, revised 1937, with the addition of cod-liver oil and tocopherol) for some time before and during the period of experimentation.

All injections of posterior pituitary extract, or its fractions, were made subcutaneously and immediately after a dose of water, equal to 5% of the animal's body weight, had been given by

stomach tube. The posterior pituitary preparations were pituitrin, pitressin and pitocin, supplied by Messrs Parke, Davis and Co. The clearance estimations were performed when the rate of urine flow was sufficiently high to ensure short urine collecting periods (15–20 min.), i.e. about 45 min. after injection of pitocin, about 120 min. after injection of pitressin and about 60 min. after injection of pituitrin.

The experimental procedures for the determination of simultaneous inulin and diodone clearances in rats have been described in a previous paper (Dicker & Heller, 1945). Inulin in plasma and urine was determined by the method of Smith, Goldring & Chasis (1938). Diodone iodine in plasma and urine was determined by Alpert's (1941) method. Inulin (Kerfoot and Co.) and Per-Abrodil (Bayer Products Ltd.) were used. Chloride in plasma was estimated by Whitehorn's (1921) method, chloride in urine by that of Volhard-Arnold and expressed as NaCl. Definition and method of calculation of glomerular filtration rate (GFR = inulin clearance = C_{IN}), effective renal plasma flow (RPF), and total tubular excretory mass (Tm_D) conform to those outlined in a previous paper (Dicker & Heller, 1945). The fraction of plasma filtered through the glomeruli (filtration fraction = FF) was determined by dividing the filtration rate by the renal plasma flow. The rate of the tubular reabsorption of chloride (T_{Cl}) was calculated as follows:

$$T_{Cl} = (P_{Cl} \times C_{IN}) - (U_{Cl} \times V),$$

where P_{Cl} = concentration of plasma chloride in mg./100 ml., U_{Cl} = concentration of urinary chloride in mg./100 ml. and V = urine flow in ml./min. In order to permit the comparison of T_{Cl} values obtained at different values of C_{IN}, T_{Cl} was expressed as the percentage of chloride filtered.

Statistical treatment of results. Fisher's 't' test was applied to estimations of the significance of differences of means. 'Small sample' methods were used for the calculation of 't' and the correlation coefficient (r) for populations smaller than twenty (Mainland, 1938). Allowance for the number of samples in any one series of experiments was made by determining the probability (P) of 't' or 'r' from the tables of Fisher & Yates (1943).

RESULTS

Inulin, diodone and chloride clearances in normal rats. To provide a basis of comparison for the experiments with the posterior pituitary fractions, inulin, diodone and chloride clearances were estimated in a series of normal rats. The animals received 5% of their body weight of water by stomach tube. The following mean values and standard deviations were obtained (some previously published results (Dicker & Heller, 1945) of inulin and diodone clearance determinations were included): $GFR = 0{\cdot}347 \pm 0{\cdot}0432$ ml./100 g./min. (134 observations), $RPF = 2{\cdot}222 \pm 0{\cdot}2812$ ml./100 g./min. (28), $FF = 0{\cdot}17 \pm 0{\cdot}037$ (28), $Tm_D = 0{\cdot}1324 \pm 0{\cdot}01848$ mg. I/100 g./min. (84), $T_{Cl} = 97{\cdot}1 \pm 1{\cdot}51\%$ (41). There was no significant correlation between T_{Cl} and rate of urine flow ($r = +0{\cdot}012$, S.E. $= \pm 0{\cdot}156$, $P > 0{\cdot}1$), in other words the decrease in the concentration of urinary chloride which accompanied an increase of urine flow was not due to a change of the chloride reabsorption but solely due to the increase of urinary volume.

The plasma chloride concentration (P_{Cl}) varied little from animal to animal (mean $P_{Cl} = 356{\cdot}2$, S.E. $= \pm 18{\cdot}40$ mg./100 ml.).

The effect of injections of pitocin on inulin, diodone and chloride clearance. A series of twenty-one rats received 5% of their body weight of water by stomach tube and 3 mU./100 g. pitocin by subcutaneous injection. The

clearance estimations showed a significant increase of *GFR* and *RPF* (Fig. 1 A) but no change in the filtration fraction. The mean and standard deviation for *GFR* was $0{\cdot}624 \pm 0{\cdot}2373$ ml./100 g./min. A statistical comparison of this figure with the mean *GFR* of normal rats gave $t = 4{\cdot}461$, $P < 0{\cdot}001$, i.e. a highly significant difference. The mean and standard deviation for *RPF* was

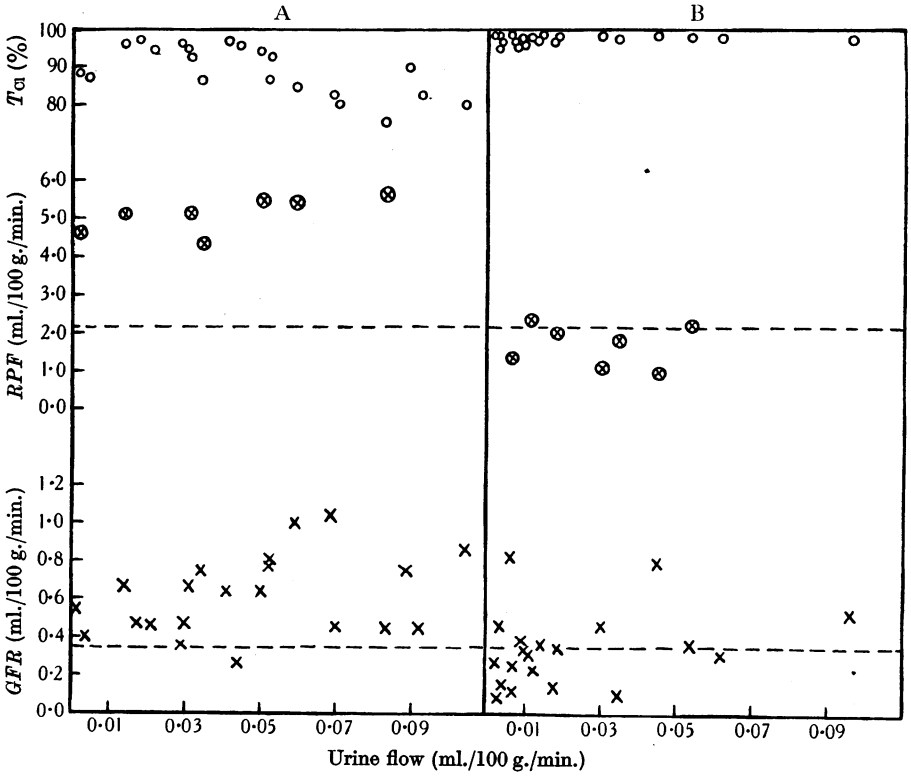

Fig. 1. A, renal effects of single subcutaneous injections of 3 mU./100 g. 'pitocin' on unanaesthetized rats hydrated with 5% of their body weight of water. B, renal effects of single subcutaneous injections of 3 mU./100 g. 'pitressin' on unanaesthetized rats hydrated with 5% of their body weight of water. × = glomerular filtration rate (*GFR*); ⊗ = effective renal plasma flow (*RPF*); ○ = rate of tubular chloride reabsorption as percentage of chloride filtered (T_{Cl}). The broken lines indicate the mean values obtained in non-injected controls.

$5{\cdot}230 \pm 0{\cdot}4679$ ml./100 g./min.; *t* between this figure and that for the mean *RPF* of the control animals was 16·683 ($P < 0{\cdot}001$). The mean value for *FF* was $0{\cdot}17 \pm 0{\cdot}053$. The figures for Tm_D (mean $Tm_D = 0{\cdot}1471$ mg. I/100 g./min., S.D. = $\pm 0{\cdot}02890$) were much the same as those obtained in the normal rats (*t* between the mean Tm_D of the 'pitocin animals' and that of the controls = 1·425, $P > 0{\cdot}1$).

The results of the determinations of the rate of tubular chloride reabsorption (T_{Cl}) gave a mean of $91{\cdot}5 \pm 2{\cdot}15\%$, a figure which differed very significantly

from that obtained in the normal series ($t=10{\cdot}470$, $P<0{\cdot}001$). The rate of tubular chloride reabsorption in the pitocin animals was significantly correlated to the rate of urine flow ($r=-0{\cdot}581$, S.E. $=\pm 0{\cdot}235$, $P<0{\cdot}01$). The mean plasma chloride concentration in the experiments with pitocin was $379{\cdot}2\pm 10{\cdot}09$ mg./100 ml.; it did not differ significantly from that of the normal rats ($t=1{\cdot}197$, $P>0{\cdot}2$). There is, therefore, no evidence to ascribe the decreased T_{Cl} after pitocin to anything but a renal effect.

The interpretation of the results obtained with the dose of pitocin used (3·0 mU./100 g. rat) is complicated by the fact that this preparation contains appreciable amounts (5–10%) of the vasopressor-antidiuretic factor (Kamm, Aldrich, Grote, Rowe & Bugbee, 1928; Moir, 1944). Control experiments with 0·3 mU./100 g. pitressin were therefore performed.

The effect of injections of pitressin on inulin, diodone and chloride clearance. Twelve rats received 5% of their body weight of water by stomach tube and 0·3 mU./100 g. pitressin by subcutaneous injection. Inulin and diodone clearance estimations in these animals gave the following results (means and S.D.): $GFR=0{\cdot}735\pm 0{\cdot}2226$ ml./100 g./min., $RPF=4{\cdot}670\pm 0{\cdot}9141$ ml./100 g./min., $FF=0{\cdot}20\pm 0{\cdot}036$. The means for *GFR* and *RPF* are significantly different from the respective mean values obtained in the control animals (t for $GFR=6{\cdot}933$, $P<0{\cdot}001$; t for $RPF=8{\cdot}023$, $P<0{\cdot}001$), showing that a dose of pitressin as small as 0·3 mU./100 g. rat had a pronounced renal effect.

A comparison of the results obtained with 0·3 mU./100 g. pitressin with those obtained with 3·0 mU. pitocin showed that there was no significant difference between the mean values for *GFR* ($t=1{\cdot}441$, $P>0{\cdot}1$) and *RPF* ($t=1{\cdot}488$, $P>0{\cdot}1$) [the values for *FF* of the two series were also of similar magnitude $t=1{\cdot}282$, $P>0{\cdot}2$], i.e. injections of 0·3 mU./100 g. pitressin and 3·0 mU./100 g. pitocin had much the same effect on glomerular filtration rate and renal plasma flow. It would appear from this that an admixture of about 10% of the vasopressor-antidiuretic factor to the oxytocic principle could account for the increase of *GFR* and *RPF* observed after the injection of 'pitocin'.

The renal effect of injection of 0·3 mU. pitressin differed from that following injection of 3·0 mU. pitocin in that pitressin failed to have an action on the rate of chloride reabsorption by the tubules (t between T_{Cl} of the 0·3 mU. pitressin series and that of the control series $=0{\cdot}300$, $P>0{\cdot}8$). It could further be shown that this absence of a significant effect on the rate of tubular chloride reabsorption applied also when much higher doses of pitressin were injected (Fig. 1 B). The mean value for the rate of chloride reabsorption in twenty rats injected with 3·0 mU./100 g. pitressin was $98{\cdot}3\pm 1{\cdot}35$%. This result was not significantly different from that obtained in the control series ($t=1{\cdot}650$, $P>0{\cdot}2$), showing again that the vasopressor-antidiuretic fraction had no effect on the rate of tubular chloride reabsorption.

Determinations of inulin and diodone clearances in the rats injected with 3·0 mU./100 g. pitressin gave the following results (means and S.D.): $GFR = 0{\cdot}352 \pm 0{\cdot}2010$ ml./100 g./min., $RPF = 1{\cdot}775 \pm 0{\cdot}5330$ ml./100 g./min., $FF = 0{\cdot}32 \pm 0{\cdot}249$, $Tm_D = 0{\cdot}0973 \pm 0{\cdot}03793$ mg. I/100 g./min. Considering the pronounced increase of *GFR* after the injection of 0·3 mU./100 g. pitressin it seemed surprising, at first, that the mean value for *GFR* of animals which had received a dose ten times higher was much the same as that of normal controls. However, a comparison of the coefficient of variation of *GFR* of the 3·0 mU./100 g. pitressin series (= 57·1 %, S.E. = ± 9·03) with that of the control series (= 12·4 %, S.E. = ± 0·76) showed a highly significant difference (44·7 %, S.E. = ± 9·06), implying that 3·0 mU./100 g. pitressin did have an effect on the rate of glomerular filtration. Furthermore, the difference between the coefficient of variation of *RPF* in this series (= 30·0 %, S.E. = ± 8·05) and that of the control animals (= 2·6 %, S.E. = ± 0·04) was also significant (difference between coefficients of variation = 27·4 ± 8·06 %). From this figure it would seem likely that the increased variability of *GFR* after the injection of 3·0 mU./100 g. pitressin was, partly at least, due to a vascular effect.

The effect of injections of pituitrin on inulin, diodone and chloride clearance. Next it seemed of interest to compare these effects of the posterior pituitary fractions with the effects of the undifferentiated posterior pituitary extract. The standard mammalian extract contains the oxytocic and vasopressor-antidiuretic factors in equal proportions. The dose of 3·0 mU./100 g. 'pituitrin' was, therefore, chosen. The following results were obtained in a series of forty clearance experiments (means and S.D.): $GFR = 0{\cdot}379 \pm 0{\cdot}1460$ ml./100 g./min. (coefficient of variation = 38·5 %, S.E. = ± 4·30), $RPF = 1{\cdot}705 \pm 0{\cdot}6427$ ml./100 g./min. (coefficients of variation = 31·8 %, S.E. = ± 4·8), $FF = 0{\cdot}23 \pm 0{\cdot}131$, $Tm_D = 0{\cdot}1047 \pm 0{\cdot}04278$ mg. I/100 g./min.

The difference between the coefficient of variation of the mean *GFR* of this series and that of the animals injected with 3·0 mU./100 g. pitressin was not significant (18·6 %, S.E. = ± 9·99), nor was there any significant difference between the coefficients of the mean *RPF* of the two series (1·8 %, S.E. = ± 9·37). These results suggest that the changes of variability of *GFR* and *RPF* after the injection of 3·0 mU./100 g. pituitrin were essentially due to the pitressin content of this preparation.

The mean value of T_{Cl} for the pituitrin series was 94·9 %, S.D. = ± 4·44. This figure differs significantly from that obtained in the controls ($t = 2{\cdot}983$, $P < 0{\cdot}01$). Since it has been shown that pitocin lowers the rate of tubular chloride reabsorption whereas pitressin does not influence it significantly, it seems justifiable to assume that the effect of pituitrin on the tubular chloride reabsorption was due to the oxytocic principle contained in the undifferentiated posterior pituitary extract.

DISCUSSION

A substantial separation of the posterior pituitary principles has been effected in the preparations 'pitocin' and 'pitressin', but it should be noted that the differentiation is not complete and that each of these extracts contains 5–10% of the other, reckoned in units of respective activity. 'Pitocin' and 'pitressin' are therefore not synonymous with the pure oxytocic or vasopressor-antidiuretic principles.

A comparison of the effects of small subcutaneous doses of pitocin and pitressin (Table 1) shows that pitocin decreased the rate of tubular reabsorption

TABLE 1. Renal effects of injections of posterior pituitary extract and its fractions. The figures are mean results with their standard deviations

	GFR (inulin clearance) ml./100 g./min.	*RPF* (diodone clearance) ml./100 g./min.	*FF* (*GFR/RPF*)	Tm_D mg. I/100 g./min.	T_{Cl} (as % of chloride filtered)
Controls	0·347±0·0432	2·222±0·2812	0·17±0·037	0·1324±0·01848	97·1±1·51
3·0 mU./100 g. pitocin	0·624±0·2373	5·230±0·4679	0·17±0·053	0·1471±0·02890	91·5±2·15
0·3 mU./100 g. pitressin	0·735±0·2226	4·670±0·9141	0·20±0·036	—	98·6±0·73
3·0 mU./100 g. pitressin	0·352±0·2010	1·775±0·5330	0·32±0·249	0·0973±0·03793	98·3±1·35
3·0 mU./100 g. pituitrin	0·379±0·1460	1·705±0·6427	0·23±0·131	0·1047±0·04278	94·9±4·44

of chloride (T_{Cl}) by the rat kidney while pitressin had no effect on this process. It may, therefore, be concluded that the depression of the rate of tubular chloride reabsorption was due to the posterior pituitary oxytocic principle.

However, it is more difficult to decide whether the increase of glomerular filtration rate (*GFR*) and of effective renal plasma flow (*RPF*), found after the injection of 3·0 mU./100 g. pitocin, was due to the oxytocic factor or to the residual vasopressor-antidiuretic activity contained in pitocin. Table 1 shows that injections of a very small dose of pitressin (0·3 mU./100 g. rat) produced rises of *GFR* and *RPF* which were statistically comparable with those produced by 3·0 mU./100 g. pitocin. It would seem, therefore, that the oxytocic factor, in the doses given, did not affect *GFR* and *RPF* (compare also the results of injections of 3·0 mU./100 g. pituitrin with those of 3·0 mU./100 g. pitressin). Had the effect on *GFR* and *RPF* of injections of 3·0 mU. pitocin been due to the oxytocic principle, why did it occur after the injection of 0·3 mU. pitressin which contains presumably not more than 0·03 mU. of the oxytocic factor, and why did it not occur after the injection of 3·0 mU. pituitrin which contains 3·0 mU. of the oxytocic substance?

It may be concluded from this comparison that any effects on *RPF* and *GFR* were due to the vasopressor principle, but it will be seen that these

effects varied with the amount of the vasopressor substance injected. Injections of the lower dose (0·3 mU./100 g.) of pitressin produced an increase of *RPF* and *GFR* (Table 1) explainable, for instance, by a dilator action on the glomerular arterioles. The response to the higher dose (3·0 mU./100 g.) lacked uniformity: the values for *GFR* and *RPF* in individual animals were frequently either significantly lower or significantly higher than those of normal rats. It is understandable that such differences from the normal did not find an expression in the mean values for *GFR* and *RPF* of the series. However, they appear in a highly significant difference between the coefficients of variation of the means. There is a similarity between these results on rats and those of Wakim *et al.* (1942), who measured the renal blood flow in dogs after the injection of pitressin. Their measurements, which were made with a thermostromuhr, showed decreases of the renal blood flow after intravenous injections, but increases were observed when pitressin was administered intramuscularly. These findings agree with the results of the present clearance experiments on rats in suggesting that the renal vascular effect of pitressin may vary in character according to the pitressin concentration in the blood.

SUMMARY

1. Glomerular filtration rate (*GFR*), effective renal plasma flow (*RPF*) and rate of tubular chloride reabsorption were measured by simultaneous inulin, diodone and chloride clearances in unanaesthetized rats.

2. Subcutaneous injections of 3 mU./100 g. pitocin produced a significant depression of the rate of tubular chloride reabsorption and a significant increase of *GFR* and *RPF*.

3. Subcutaneous injections of 3 mU./100 g. pitressin had no effect on the rate of tubular chloride reabsorption. However, they produced a significantly increased variability of *GFR* and *RPF*.

4. Subcutaneous injections of 3 mU./100 g. of undifferentiated posterior pituitary extract (pituitrin) produced changes of *GFR* and *RPF* of the same magnitude as those observed after 3 mU./100 g. pitressin, and a significant decrease of the rate of chloride reabsorption similar to that observed after the injections of pitocin.

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