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# THE METABOLISM OF EXOGENOUS AND ENDOGENOUS ANTIDIURETIC HORMONE IN THE KIDNEY AND LIVER *IN VIVO*

BY

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Negligible antidiuretic activity (less than 0.17 mU./g.) was found in extracts of the kidneys either of unanaesthetized adult rats in normal water balance or of rats in whose blood a rise of the level of endogenous antidiuretic hormone had been induced by ether anaesthesia. Extracts of the livers of unanaesthetized rats had negligible antidiuretic activity (less than 0.06 mU./g.), but liver extracts from rats anaesthetized with ether showed antidiuretic effects equivalent to $0.74 \pm 0.24$ mU. Pitressin/g. liver. When Pitressin was injected intravenously into unanaesthetized rats, small amounts of antidiuretic activity were occasionally found in the livers and the kidneys of animals killed up to 3 min. after the injection but none in animals killed later. Some 3% of the antidiuretic activity of an injected dose of Pitressin was found in the urine and the "dead space" of the kidneys in rats decapitated 3 min. after the intravenous injection. When Pitressin was added to rat kidney homogenate and the mixture was incubated at 38°, only 0.75% of the initial antidiuretic activity was recovered after 30 min. and less than 0.40% after 60 min. Experiments with "glomerular" and "tubular" fractions of rat kidney indicated that the inactivation was essentially due to tubular tissue. It is suggested that, in the rat, the kidneys and perhaps the liver are not only sites of clearance of the antidiuretic hormone but also sites of irreversible inactivation.

Ginsburg and Heller (1953a) and Crawford and Pinkham (1954) showed that, after intravenous injection into intact rats, the antidiuretic activity of doses of Pitressin (Parke Davis) ranging from 5 to 100 mU./100 g. body weight disappeared rapidly from the circulation: 5 min. after the injection less than 5% of the injected antidiuretic activity could be recovered from the blood. Similar experiments were done by Dicker (1954), who infused rats with pitressin at rates ranging from 30 to 100 $\mu$U./min./100 g. In 4 of his animals, killed 2 to 3 min. after the end of the infusion, no antidiuretic activity could be demonstrated in the plasma; in two others the recovery was equivalent to about 1% of the infused dose. However, when pitressin was injected or infused into nephrectomized rats (Ginsburg and Heller, 1953a; Crawford and Pinkham, 1954; Dicker, 1954), it was found that the antidiuretic activity was "cleared" at a considerably lower rate, the recovery after 2 to 5 min. varying from 37 to 63% of the administered dose. Sham-nephrectomized rats (Crawford and Pinkham, 1954) or animals with both ureters tied (Dicker, 1954) cleared the antidiuretic hormone at about the same rate as intact rats. It seems therefore that the kidney of the rat plays an important rôle in the removal of antidiuretic hormone from the circulation. But it is not the only organ so concerned. Ginsburg and Heller (1953a) found also that considerable amounts of the antidiuretic principle were removed in the splanchnic vascular bed, and Ginsburg (1957) subsequently found that little or no pressor activity was cleared by the intestine, suggesting that the liver was another organ of vasopressin clearance. These experiments fail to give any information about the fate of the hormone *in* these organs. There are obviously several possibilities: the active peptide may be temporarily stored or reversibly bound in these tissues, it may be excreted into the urine or the bile, it may be irreversibly inactivated, or a combination of these processes may apply. To investigate these possibilities the antidiuretic potency of extracts of the kidneys and the liver was estimated after raising the amount of circulating hormone either by injection of pitressin or by induced increase of the endogenous antidiuretic hormone.

## METHODS

Adult albino rats of the Wistar strain (200 to 250 g.) were used. They were kept on a diet containing 25.6 g. protein (crude) and 0.3 g. NaCl/100 g.

*Aqueous Extracts.*—In preliminary experiments, aqueous extracts of the kidneys or the liver were prepared. Each rat was decapitated and bled, and the kidneys or liver removed as quickly as possible. The organs were weighed, cut into small pieces, transferred immediately into a test-tube containing boiling Krebs-Ringer solution and boiled for 3 min. The tube was then cooled, and the contents quantitatively transferred into a glass homogenizer and made up to the required volume. The mixture was centrifuged at 2,500 rev./min. and the supernatant used for assay. Other extracts of kidneys and liver were prepared in the same way using 0.25% acetic acid in 0.8% NaCl solution instead of Krebs-Ringer.

However, these methods of extraction were abandoned after some time because liver extracts prepared in either manner often produced antidiuretic effects. A pronounced but transient increase of urine flow preceding the antidiuretic effect was also seen in some rats. Similar effects were obtained with aqueous extracts of kidney although they occurred more rarely. When such aqueous extracts were intravenously injected into rats anaesthetized with urethane, it was found that they regularly lowered the blood pressure. These depressor effects were quite marked, for example a volume of extract equivalent to 20 mg. of kidney (wet weight) lowered the blood pressure by 21 mm. Hg. They were abolished neither by atropine nor antihistamines and may have been responsible for the "unspecific" renal effects just described.

*Acid Ethanol Extracts.*—Procedures used by Vogt (1953) for the extraction of vasopressin from the hypothalamus and by Bisset and Walker (1954) for the extraction of oxytocic and antidiuretic activity from blood, which are based on the method of Melville (1937), were therefore modified as shown on Table I. The interval between decapitation of the animal and immersion of the liver or the kidneys in ice-cold acid ethanol was approximately 3 min. Shortly after this method of extraction was instituted it was found that extracts neutralized to *p*H 7.2 in one stage frequently produced undesirable renal effects similar to those produced by aqueous extracts. This was probably due to the fact that part of the precipitate which forms at *p*H 4 to 5 redissolves on further addition of NaOH solution. The neutralization was therefore done in two stages. First, the precipitate formed at *p*H 4 to 5 was removed by centrifugation and, second, the clear supernatant was then adjusted to *p*H 7.2 and dialysed as described by Bisset and Walker (1954). Flamephotometric control estimations showed that 30 min. of dialysis reduced the potassium concentration of such liver and kidney extracts to less than 0.1 mg./ml. The freezing point depressions of some of the extracts were determined. They were found to have been hypotonic to rat plasma (mean and S.E. of 8 extracts, $0.40 \pm 0.07$).

TABLE I

EXTRACTION OF VASOPRESSIN FROM TISSUES WITH ACID ETHANOL

Tissue ground in glass mortar with ice-cold acid ethanol (1 ml. conc. HCl/l. of 98% ethanol, 10 ml. of this mixture/g. tissue)

↓

Transferred to centrifuge bottle with 30 ml. ethanol/g. tissue and centrifuged at 1,500 rev./min. for 15 min.

↓ Supernatant decanted

↓ 10 ml. acid ethanol/g. added to residue, again centrifuged at 1,500 rev./min. for 15 min.

↓ Supernatant decanted

↓ Combined supernatants concentrated *in vacuo* at 48° almost to dryness

↓ Residue taken up with 0.45% NaCl solution

↓ NaOH solution added until heavy flocculent precipitate appears, centrifuged at 2,500 rev./min. for 30 min.

↓ Supernatant dialysed against running water for 30 min. and adjusted to *p*H 7.0 to 7.2

*Preparation of Kidney Homogenates for* in vitro *Experiments with Pitressin.*—Kidneys from decapitated and bled animals were homogenized in a Potter-Elvehjem homogenizer with 10 or 20 ml./g. of Krebs-Ringer phosphate buffer (*p*H = 7.4.). Having been incubated for a given time, one volume of homogenate was mixed with 12 volumes of ice-cold acid ethanol and extracted as described on Table I.

Glomerular and tubular fractions of rat kidney were prepared by differential centrifugation in Krebs-Ringer solution (Lowell, Greenspon, Krakower and Bain, 1953). All procedures necessary for fractionation (including centrifugation) were done in a "cold room" at about +5°. Microscopical inspection showed slight contamination of the glomerular fraction with tubular elements. In order to make comparable incubation experiments with the two fractions, their content of solids had to be determined. Three samples of 2 ml. of each fraction were put into tared weighing bottles, dried at 105° for 37 hr., transferred to a desiccator containing phosphorus pentoxide, weighed, kept a further 12 hr. in the desiccator and reweighed.

*Antidiuretic Assay.*—Antidiuretic potency was assayed by intravenous injection of the extracts into unanaesthetized rats following the technique of Ginsburg and Heller (1953b) with the modification that not only a jugular vein but also the bladder was cannulated on the day before the experiment. Urine volumes were read at 5 min. intervals. The water load was kept at 6 to 8% of the body weight. Except when expressly mentioned, each assay consisted of the intravenous injection of four doses, two of the standard and two of the test material. The ratio of high to low doses was usually two. The order of injections was varied at random. Whenever possible each extract was assayed simultaneously on several rats. The volumes injected were always the same for standard and unknown. The antidiuretic effect of each dose was calculated in terms of % antidiuresis

as described by Ginsburg and Heller (1953b) with the difference that 5 min. collecting periods were used.

*Estimation of Antidiuretic Activity in Urine.*—To make the collection of urine as complete as possible, a fine polythene tube was inserted through the cannula and the bladder washed three times with small volumes of warm 0.9% NaCl solution. Emptiness of the bladder was verified by post-mortem examination.

The action of extracts on the blood pressure was tested in rats anaesthetized with urethane as described by Dekanski (1952). Pitressin (Parke, Davis) was used as the standard preparation.

## Results

*Antidiuretic Assays of Kidney Extracts of Normal Rats.*—Normal rats are defined as animals which had been allowed food and water up to the time they were decapitated. In most instances acid ethanol extracts of the kidneys of such animals, in doses equivalent to 20 mg. fresh kidney, did not influence the water diuresis of the assay rats. Occasionally an antidiuretic effect was observed which, in contrast to the action of pitressin in the same animals, disappeared on repeated injection (Fig. 1). Such transient effects were therefore disregarded in this and all following experiments and it was assumed that renal extract of "normal animals" contained no demonstrable amount of vasopressin (Table II). In terms of the liminal sensitivity of the assay animals both kidneys contained less than the equivalent of about 0.23 mU. pitressin or less than 0.17 mU./g. kidney.

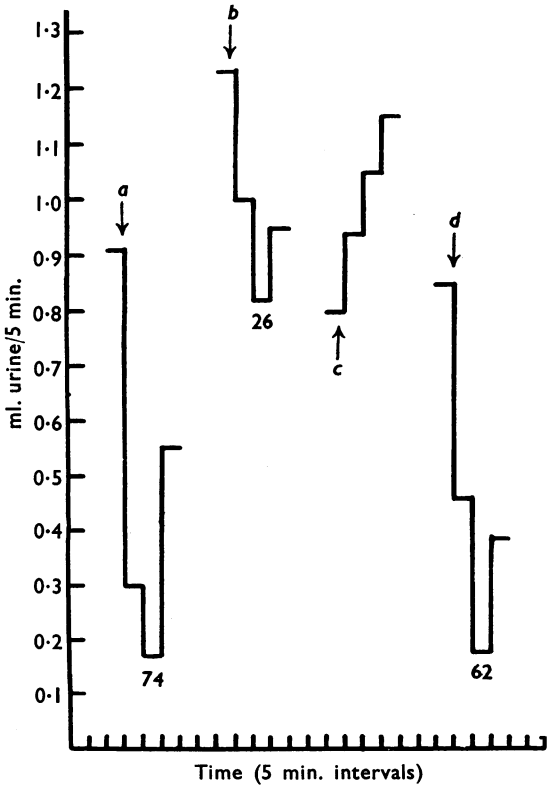


FIG. 1.—Transient antidiuretic effect of an acid ethanol kidney extract. Rat, 220 g. Intravenous injections. *a*, 100 μU. pitressin in 0.5 ml. of 0.9% NaCl solution. *b*, 0.1 ml. kidney extract (equivalent to 5 mg. wet weight of kidney) diluted to 0.5 ml. with 0.9% NaCl solution. *c*, 0.2 ml. kidney extract diluted to 0.5 ml. *d*, 50 μU. pitressin in 0.5 ml. of 0.9% NaCl solution. The numerals below each response indicate % antidiuresis.

TABLE II

ANTIDIURETIC ASSAYS OF ACID ETHANOL EXTRACTS OF KIDNEYS OF NORMAL RATS

Column 2 gives the dose of pitressin producing at least 25% inhibition of diuresis. The total volume of each extract (of both kidneys) was 30 ml.

| No. of Extract (1) | Sensitivity of Rat (μU.Pitressin/Rat) (2) | Volume of Extract Injected Without Antidiuretic Effect (3) | Maximum Antidiuretic Activity in Both Kidneys in Terms of mU. Pitressin (4) |
|---|---|---|---|
| 1 | 6·25 | 0·8 | <0·23 |
| 2 | 6·25 | 0·8 | <0·23 |
| 3 | 6·25 | 0·4 | <0·46 |
| 4 | 6·25 | 0·3 | <0·62 |
| 5 | 6·25 | 0·3 | <0·62 |
| 6 | 6·25 | 0·3 | <0·62 |
| 7 | 6·25 | 0·4 | <0·46 |
| 8 | 6·25 | 0·4 | <0·46 |
| 9 | 3·13 | 0·4 | <0·23 |

TABLE III

ANTIDIURETIC ACTIVITY OF ACID ETHANOL EXTRACTS OF KIDNEYS FROM UNANAESTHETIZED RATS INJECTED WITH 100 mU. PITRESSIN/100 G.

| Min. Killed After Injection | % Recovery of Injected Dose |
|---|---|
| 1 | <0·12, <0·12, <0·24, <0·24 |
| 3 | 0·20, 0·26, <0·12, <0·12 |
| 6 | <0·45, <0·20, <0·45 |
| 12 | <0·50, <0·25, <0·25 |
| 15 | <0·50, 0·09 |

*Antidiuretic Assays of Kidney Extracts of Rats Injected with Pitressin.*—Table III shows the results of assays with renal extracts of unanaesthetized rats which had received intravenous injections of 100 mU. pitressin in 0.2 ml. saline/100 g. rat during 20 sec. It will be seen that antidiuretic activity was sometimes demonstrable in the renal extracts of animals killed up to 3 min. after the injection (counted from 10 sec. before the completion of the injection) but none in animals killed later. Similar results were obtained in rats anaesthetized with ether. The animals were anaesthetized for 10 min. and then decapitated. The standard dose of pitressin was injected during the 7th or 9th min. of anaesthesia.

No antidiuretic effects were obtained with kidney extracts of these animals. In two experiments the extract of both kidneys contained less than the equivalent of 0.45 mU. and 0.23 mU. pitressin respectively.

Before these results could be considered valid, it had to be shown that extracts of normal kidneys did not modify the action of added vasopressin, namely that they contained neither antagonistic nor potentiating substances. Fig. 2 (one of 9 similar experiments) shows that this did not apply. Furthermore, it was necessary to know how much of the antidiuretic activity of a dose of pitressin added to ground kidney could be recovered by our method of extraction. In 10 such experiments, in which 100 mU. was added to both finely divided kidneys, the mean recovery was $75.8 \pm 2.6\%$. Other recovery experiments were done by injecting 40 mU. pitressin contained in 0.02 ml. of 0.9% NaCl solution (the amount cleared by one kidney after the intravenous injection of 100 mU. pitressin/100 g. (Ginsburg and Heller, 1953a)) with an "Agla" micrometer syringe into the parenchyma of a kidney whose ureter was ligated immediately after decapitation. Recoveries in four such experiments were 86.0, 96.6, 68.4, and 100.0%. In three similar experiments, 5 mU. pitressin was injected into a kidney; the recoveries were 70.5, 55.0, and 70.0%.

There are reasons (see below) for believing that the true recovery was higher than is indicated by these results. However, even with recovery as low as about 75% it is evident from the result, shown on Table III, that, after an intravenous injection of pitressin, little of the large amounts of antidiuretic hormone cleared by the kidneys (Ginsburg and Heller, 1953a; Crawford and Pinkham, 1954; Dicker, 1954) can be extracted from kidney tissue. Is the disappearance of the hormone due to inactivation, or to excretion into the urine, or to both?

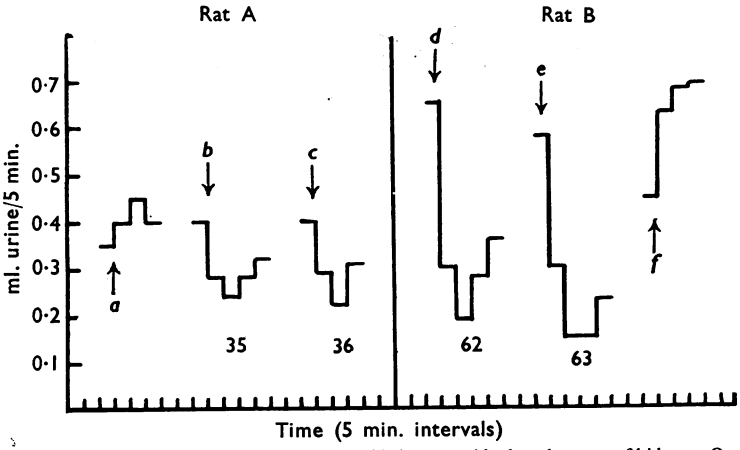


FIG. 2.—The antidiuretic effect of pitressin added to an acid ethanol extract of kidney. One ml. of extract was equivalent to 75 mg. wet weight of kidney. Intravenous injections. Rat A, 220 g. *a*, 0.4 ml. kidney extract. *b*, 0.4 ml. extract containing 6.25 μU. pitressin. *c*, 6.25 μU. pitressin in 0.4 ml. of 0.9% NaCl solution. Rat B, 270 g. *d*, 100 μU. pitressin in 0.3 ml. of 0.9% NaCl solution. *e*, 100 μU. pitressin in 0.3 ml. kidney extract. *f*, 0.3 ml. kidney extract. The numerals below each response indicate % antidiuresis.

*Excretion of Antidiuretic Activity in the Urine.*—The antidiuretic activity excreted by conscious rats in the first 3 min. after an injection of 100 mU./100 g. was estimated. All the urines, obtained by emptying and washing the bladder (9 experiments), inhibited the water diuresis of test animals. Assays were satisfactory in four of these experiments; the mean amounts excreted were equivalent to $0.70 \pm 0.17\%$ of the injected dose. Since the rate of urine flow in the donor rats was low and the urine collecting period very short, it is to be expected that antidiuretic activity would be present in the "dead space" of the renal pelves and the ureters which, in the experiments reported in Table III, had been carefully removed. Unanaesthetized rats were therefore injected with 100 mU. pitressin/100 g., killed after 3 min., the ureters tied close to the bladder and the kidneys removed together with the ureters. Extracts of such kidneys contained antidiuretic activity equivalent to $2.71 \pm 0.70\%$ of the injected dose, amounts which were much greater than those found in the parenchyma alone (Table III).

These results showed that about 3.4% of the antidiuretic activity of the injected pitressin was excreted by the kidney very rapidly within the first 3 min. after intravenous injection. However, it can be calculated from the results of Ginsburg and Heller (1953a) that the amounts of antidiuretic activity cleared by the kidneys of the rat in the same time (the same dose of pitressin was injected as in the present experiments) were 25% or more of the injected dose, suggesting that inactivation of the hormone in the kidney plays a more important rôle than renal excretion. Since little antidiuretic activity was recovered from the kidney parenchyma of rats killed 3 min. after injection and none from the kidneys of animals killed later (Table III) the rate of inactivation would appear to be high. Rapid inactivation may also partly

account for the incomplete recovery of antidiuretic hormone added to kidney tissue or injected into the kidney.

*Antidiuretic Assays of Kidney Extracts of Rats Anaesthetized with Ether.*—In order to obtain some information about the fate of endogenous vasopressin in rat kidney, the hormone level was raised by ether anaesthesia (Ginsburg and Heller, 1952, 1953c; Ames and van Dyke, 1952; Ever-

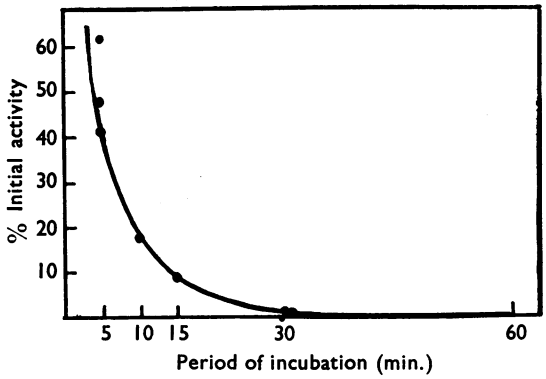


FIG. 3.—Inactivation of pitressin by rat kidney homogenate in Krebs-Ringer phosphate buffer (without Ca and Mg) incubated at 38°. Extraction by the acid ethanol method. (2+2) antidiuretic assays. For further details see text. Curve fitted by eye. At 60 min., the % activity remaining was <0.4%.

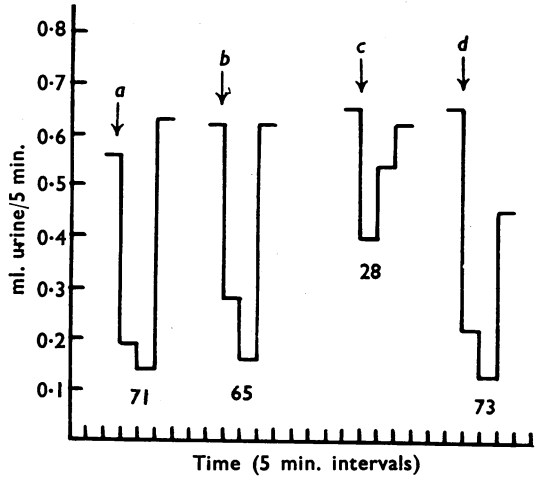


FIG. 4.—Effect of incubating pitressin with the glomerular and the tubular fractions of rat kidney for 30 min. at 38°. Antidiuretic assays. Intravenous injections of 0.4 ml./animal. Rat, 240 g. *a*, 200 μU. pitressin in glomerular fraction after incubation. *b*, 200 μU. pitressin in glomerular fraction before incubation. *c*, 220 μU. pitressin in tubular fraction after incubation. *d*, 220 μU. pitressin in tubular fractions before incubation. The amount of pitressin added to the fractions was proportional to the weight of suspended renal tissue. The numerals below the responses indicate % antidiuresis.

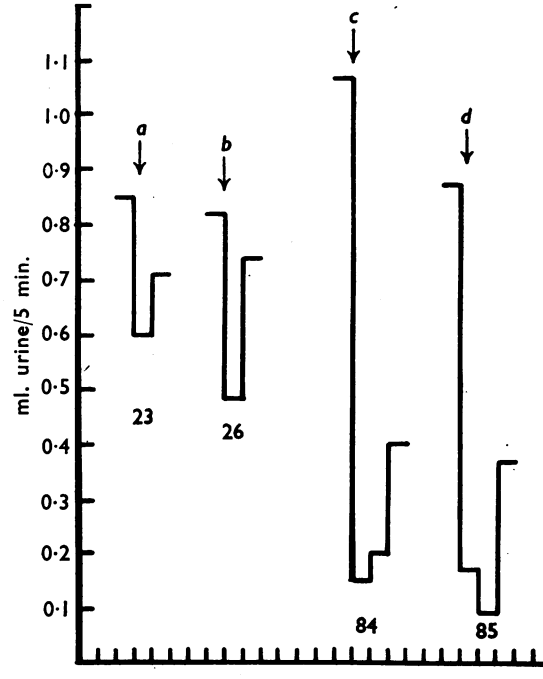


FIG. 5.—Incubation of arterial blood of the rat containing 10 units heparin and 1.0 mU. pitressin/ml. for 30 min. at 38°. Antidiuretic assay, intravenous injections. Rat, 108 g. *a*, 0.1 ml. of non-incubated blood diluted to 0.4 ml. with 0.9% NaCl solution. *b*, 0.1 ml. of incubated blood diluted to 0.4 ml. *c*, 0.3 ml. of non-incubated blood diluted to 0.4 ml. *d*, 0.3 ml. of incubated blood diluted to 0.4 ml. The numerals below the responses indicate % antidiuresis. The antidiuretic activity of incubated blood is 102.3% of that of the non-incubated mixture.

sole and Giere, 1954) and the animals maintained in anaesthesia up to 10 min. No significant antidiuretic effects were obtained with kidney extracts of such animals.

*Inactivation of Vasopressin by Kidney Homogenates.*—Our findings suggested that the kidney not only clears and excretes but also inactivates vasopressin *in vivo*. It seemed interesting therefore to study the rate of inactivation of vasopressin by kidney homogenates. Fig. 3 shows the rapid disappearance of antidiuretic activity in incubated mixtures of rat kidney homogenates and pitressin: 0.75% of the added activity remained after 30 min. of incubation and less than 0.4% after 60 min. These results are in good agreement with previously published results of one of us (Zaidi, 1955) and with those of Dicker and Greenbaum (1956) which were obtained with rat kidney homogenates from which aqueous extracts had been made. In an attempt to establish which renal tissue element was concerned in the inactiva-

tion of vasopressin, the hormone was incubated with homogenates of the glomerular and the tubular fractions of rat kidney. The effect of incubation of a dose of pitressin with the same amounts (in terms of solids) of glomerular and tubular homogenate is compared on Fig. 4. A substantial loss of activity was only obtained with the tubular fraction.

*Inactivation of Vasopressin by Rat Blood.*—It was possible that inactivation of vasopressin by kidney homogenate was partly due to blood trapped in the kidneys. However, Fig. 5 shows that incubation of vasopressin with heparinized rat blood for 30 min. caused no demonstrable loss of antidiuretic activity.

*Antidiuretic Assays of Liver Extracts of Unanaesthetized Rats.*—Since the liver may be another major site of vasopressin clearance in the rat, similar experiments were done with extracts of liver. Only slight and transient antidiuretic effects were seen with extracts of livers of normal animals (5 experiments). Expressed in terms of sensitivity of the assay animals (most of which responded to 3 μU. pitressin/100 g.), the antidiuretic activity was less than 0.44 mU./liver or less than 57 μU./g. liver.

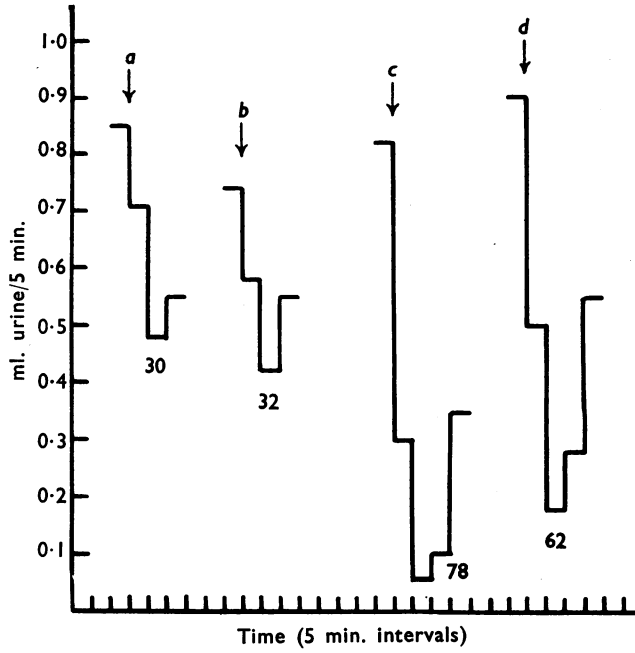


FIG. 7.—Recovery of endogenous antidiuretic activity in rat plasma added to rat liver suspension. Mixture immediately extracted by the acid ethanol method. The plasma concentration of antidiuretic hormone was raised by anaesthetizing the donor rat with ether. Antidiuretic assay. Intravenous injections. Recipient rat, 225 g. *a*, 0.2 ml. plasma. *b*, 0.2 ml. of extract of liver homogenate mixed with the plasma (1 ml. of extract was equivalent to 200 mg. wet weight of liver + 1 ml. plasma). *c*, 0.4 ml. plasma. *d*, 0.4 ml. extract The numerals below the responses indicate % antidiuresis. The recovery of antidiuretic activity from liver-plasma mixture was 88.4%.

*Recovery of Vasopressin Added to Liver Extracts or Ground Liver Tissue.*—Fig. 6 shows that the antidiuretic action of pitressin added to liver extract was neither enhanced nor diminished. When pitressin was added to finely divided rat liver and immediately extracted with acid ethanol, the mean recovery was 86.0 ± 3.4 (9)% of the added activity.

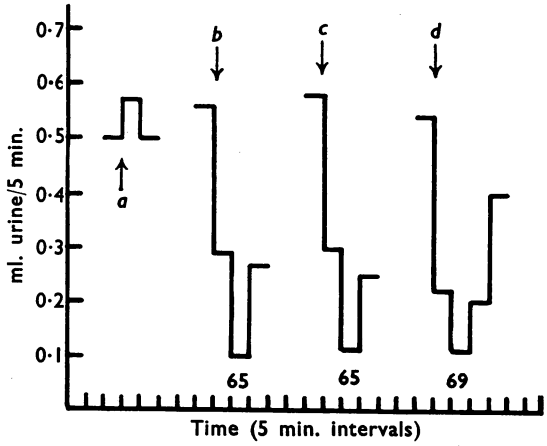


FIG. 6.—The antidiuretic effect of pitressin added to an acid ethanol extract of liver. One ml. of extract was equivalent to 200 mg. wet weight of liver. Rat, 235 g. Intravenous injections. *a*, 0.3 ml. liver extract. *b*, 50 μU. in 0.3 ml. of 0.8% NaCl solution, *c*, 50 μU. in 0.3 ml. liver extract. *d*, 50 μU. in 0.3 ml. of 0.9% NaCl solution. The numerals below the responses indicate % antidiuresis.

*Antidiuretic Activity in Liver Extracts of Rats Anaesthetized with Ether.*—In contrast to the results obtained in unanaesthetized animals, pronounced antidiuresis was produced by extracts of the liver of rats which had been kept under ether anaesthesia for 10 min. In 10 experiments the mean antidiuretic activity/liver was equivalent to 5.8 ± 0.8 mU. pitressin or to 0.74 ± 0.2 mU./g. liver, suggesting the possibility that ether anaesthesia interferes with the inactivation of endogenous vasopressin in the liver.

To ascertain whether the endogenous antidiuretic hormone could be recovered from liver tissue, the following experiment was done: The plasma of jugular blood enriched with endogenous vasopressin by subjecting the rat to ether anaesthesia was mixed with finely divided liver tissue obtained from an unanaesthetized animal; the

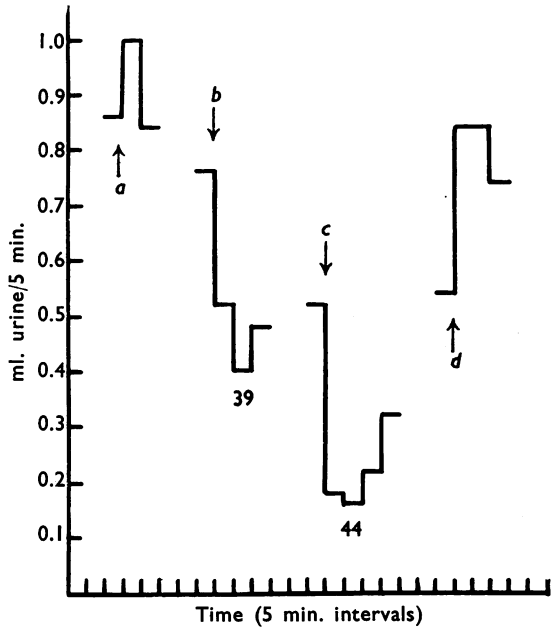


FIG. 8.—Comparison of the antidiuretic effect of liver extracts of hypophysectomized and intact rats. Both animals had been anaesthetized with ether for 10 min. and were then killed by decapitation. Antidiuretic assay. Intravenous injections. Recipient rat, 210 g. *a*, 0.4 ml. of liver extract of hypophysectomized rat. *b*, 100 μU. pitressin in 0.8 ml. of 0.9% NaCl solution. *c*, 0.8 ml. of liver extract of intact rat. *d*, 0.8 ml. of liver extract of hypophysectomized rat. The numerals below the responses indicate % antidiuresis.

mixture was immediately extracted with acid ethanol and the antidiuretic activity of the extract compared with that of the plasma. Fig. 7 shows a recovery of 88.4% of the "endogenous" antidiuretic activity from the liver extract. The recovery in another experiment was 92.2%.

The results are compatible with the possibility that the antidiuretic activity found in the livers of the anaesthetized animals was due to endogenous vasopressin. To test this assumption further, liver extracts were prepared from hypophysectomized rats which again had been anaesthetized with ether for 10 min. Only one of these experiments was entirely successful in that neurohypophysectomy in the donor was satisfactory and the recipient rats responded to sufficiently low doses of pitressin.

Fig. 8 shows that no antidiuretic effect was produced by the liver extract of the hypophysectomized and anaesthetized rat.

Attempts to study the inactivation of the antidiuretic factor in extracts of the livers of the anaesthetized animals by thioglycollic acid had to be abandoned, since intravenous injections of sodium thioglycollate itself occasionally produced an antidiuretic effect. The latter findings are contrary to the experience of Ames and van Dyke (1951) in rats anaesthetized with ethanol.

*Antidiuretic Activity in Liver Extracts of Rats Injected with Pitressin.*—Table IV shows that after the intravenous injection of 100 mU. pitressin/100 g. into unanaesthetized rats, substantial amounts of antidiuretic activity were recovered from the liver of the injected animal in one instance only.

TABLE IV

ANTIDIURETIC ACTIVITY OF ACID ETHANOL EXTRACTS OF LIVER FROM UNANAESTHETIZED RATS INJECTED WITH 100 mU. PITRESSIN/100 G.

| Min. Killed After Injection | % Recovery of Injected Dose |
|---|---|
| 1 | <0·47, <0·46 |
| 3 | 0·61, <0·46, <0·43, <0·12, <0·92 |
| 6 | <1·0, <0·97, <0·43, <0·48 |
| 9 | <0·48 |
| 12 | <1·0, <0·50 |
| 15 | <0·52 |

## DISCUSSION

Vasopressin has been shown to be inactivated by liver and kidney tissue *in vitro* (Heller and Urban, 1935; Jones and Schlapp, 1936; Larson, 1938; Christlieb, 1939; Eversole, Birnie, and Gaunt, 1949; Dicker and Greenbaum, 1954, 1956); but it is difficult to decide to what extent these results apply to the fate of the hormone *in vivo*. First, it seems likely that the rates of permeation and inactivation of the active principle will differ according to whether tissue homogenates or slices are used or whether the hormone circulates through an organ *in situ*. Secondly, cell injury in *in vitro* experiments may cause activation of enzymes or alternatively release enzyme inhibitors. Our results suggest that, *in vivo*, the kidney and liver inactivate vasopressin more rapidly than slices of these organs (Dicker and Greenbaum, 1954) and perhaps as rapidly as homogenates. We found only traces of antidiuretic activity in extracts of kidneys and livers of unanaesthetized rats decapitated 3 min. after the intravenous injection of pitressin, and no activity, demonstrable with our method of assay, was found in the organs of animals killed from 6 to 15 min. after the injection. However, some inactivation probably occurred also in the time taken (approximately 3 min.) to dissect the organs and to arrest enzymatic activity.

No vasopressin-like activity was found in the liver and kidneys of unanaesthetized rats in normal water balance. When rats were kept under

ether anaesthesia, killed by decapitation and bled, no antidiuretic activity could be demonstrated in the kidneys, but the liver contained substantial amounts of an antidiuretic substance. We cannot be certain that the latter was of neurohypophysial origin though the characteristics of the inhibitory effect and some results in hypophysectomized rats suggest it. Ether anaesthesia has been shown to increase the blood concentration of vasopressin (Ginsburg and Heller, 1952, 1953c; Ames and van Dyke, 1952; Eversole and Giere, 1954), but even if this raised blood level is taken into account the amounts of antidiuretic activity found were too large to have been due solely to the hormone content of the blood trapped in the liver. If ether anaesthesia interferes with the inactivation of the antidiuretic principle in the liver but not in the kidney, this would suggest that the mechanism of inactivation in the two organs is different.

In the rat, the kidneys play a threefold role in the metabolism of the antidiuretic hormone: (*a*) they remove a substantial fraction of the hormone from the blood (Ginsburg and Heller, 1953a; Crawford and Pinkham, 1954; Dicker, 1954), (*b*) they inactivate some of the cleared vasopressin, and (*c*) they excrete antidiuretic activity into the urine. So far as the process of inactivation is concerned there seem to be two possibilities: first, that vasopressin is transformed in the kidney to a less active metabolite which is then excreted, and secondly that some of the hormone escapes unchanged into the urine and that the unexcreted portion is completely inactivated. Dicker and Greenbaum (1954) suggested the first as the likely process, but recent experiments of Ginsburg, Heller, and Zaidi (1956) do not support this concept.

We are unable to say to what extent the results reported can be applied to the fate of "physiological" amounts of vasopressin in the body. The amounts injected in the present experiments or released by ether anaesthesia were large as compared with the very small doses needed to produce antidiuresis in the water-loaded rat. They are likely, for example, to have produced vascular changes which are not encountered when physiological amounts of the antidiuretic hormone are released.

It should be remembered, on the other hand, that, under pathological conditions like haemorrhage (Ginsburg and Heller, 1953c; Ginsburg and Brown, 1956), very large amounts of vasopressin may be released in the rat. Apparently such quantities can be inactivated with great rapidity.

The following question may be asked: if, as the present results suggest, a large dose like 100 mU./100 g. can be inactivated within the first few minutes after intravenous injection, why does the antidiuretic effect persist for 18 min. and longer (Ginsburg and Heller, 1953a)? Two answers may be given. First, since the plasma clearance of the antidiuretic hormone follows an exponential course (Ginsburg and Heller, 1953a), much smaller quantities of the hormone will be removed from the circulation (and subsequently inactivated) at low rather than at high plasma concentrations. The delay in reaching the minimum antidiuretic plasma level will therefore be considerable when a dose of the order of 100 mU./100 g. is injected. Secondly, changes in the effector cells may continue after the disappearance of the hormone from the blood and from the kidney.

Some similarity between the fate and metabolism of vasopressin and that of another active polypeptide, insulin, may be noted. The main sites for inactivation of insulin in the body, liver, and kidney (Weisberg, Friedman, and Levine, 1949; Haugaard, Vaughan, Haugaard, and Stadie, 1954; Elgee and Williams, 1954) are the same as those for vasopressin. The renal excretion of insulin, on the other hand, seems to be of less importance than that of antidiuretic hormone. The fate of other polypeptide hormones has not been sufficiently investigated to permit a comparison with that of vasopressin. However, the possibility of a common metabolic pathway of these active principles obviously deserves further study.

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