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THE STATE IN THE BLOOD AND THE EXCRETION BY THE KIDNEY OF THE ANTIDIURETIC PRINCIPLE OF POSTERIOR PITUITARY EXTRACTS

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Extracts of the posterior pituitary (pituitrin), when mixed with blood or certain tissue suspensions and subcutaneously injected into rats, no longer display the antidiuretic activity of pure pituitrin [Heller & Urban, 1934, 1935]. The following evidence led to the conclusion that the inactivation of the hormone is due to adsorption: (1) The process of inactivation is very rapid. (2) The rapidity of the process of inactivation does not depend on the amount of antidiuretic hormone added. (3) The inactivation is reversible. Heating releases the biologically active hormone.

Using body fluids and tissues from several sources it was found that, for each tissue, the adsorption capacity is of specific magnitude. The number of milliunits[1] of pituitrin inactivated by a standard amount of tissue or fluid was accordingly termed its specific adsorbing capacity (s.a.c.). An inactivation of the antidiuretic principle by human serum has also been observed by Dietel [1933]. Theobald [1934] mentions a reversible inactivation of the hormone by both corpuscles and serum *in vitro*.

The s.a.c. of blood is less than that of any other tissue investigated, but it is large enough to inactivate amounts of the antidiuretic principle which are many times larger than the minimum effective dose. The means by which coagulation of the blood is prevented does not seem to make any difference to its adsorbing capacity. It seems therefore unlikely that

[1] The term milliunit is used to express the antidiuretic activity of 10^{-4} c.c. of Pituitary (Posterior Lobe) Extract B.P. The extract employed in the investigation described in this paper was Messrs Parke, Davis and Co.'s "Pituitrin".

the behaviour of circulating blood is qualitatively different from the behaviour of blood *in vitro*. On the contrary, it can be expected that addition of the antidiuretic hormone to circulating, i.e. moving, blood secures a more rapid adsorption. If we then assume that an inactivation of the antidiuretic principle takes place *in vivo* as well as *in vitro*, how are we to explain the fact that small doses of the hormone should be completely adsorbed and nevertheless have an action on the kidney? That such renal action exists has been amply demonstrated by the work of Starling & Verney [1925], Burgess *et al.* [1933] and others.

An explanation can probably be found with the help of the following findings. Heller & Urban [1935] have shown that the mammalian kidney excretes the antidiuretic hormone in an active form. The doses of posterior pituitary extract used in these preliminary experiments were so large that they transcended the S.A.C. of the blood. That is to say a considerable fraction of the injected hormone was sure to have been "free", i.e. not adsorbed by the blood colloids. If it can be shown, however, that the bound as well as the free antidiuretic hormone can be excreted, then the apparently contradictory findings, that a substance which is inactivated by the blood nevertheless has a renal action and is excreted by the kidney, can be explained by the assumption that the hormone is liberated by some unknown process in the kidney.

In this paper an attempt was made to investigate this possibility by obtaining further data about the physiological activity of small amounts of the antidiuretic factor when mixed with blood *in vitro* or when injected intravenously, and by a quantitative study of the elimination of posterior pituitary hormone by the normal kidney.

Methods

In all but one instance male rabbits were used throughout the experiments. They received a standard diet during at least a week before the beginning of the experiments. This diet consisting of oats, bran and water produced an acid urine which seemed desirable in view of the possible inactivation of pituitrin in alkaline media [Gaddum, 1930]. The animals received no food for 16 hours before the beginning of the experiments, water being allowed up to 6 hours before the experiments. For the assay of the antidiuretic potency of urine and blood samples, Burn's [1931] method of subcutaneous injection into rats was used. This method has been found to yield satisfactory results for the estimation of amounts ranging from 2 to 12 milliunits pituitrin per 100 g. rat. Further details of the method are described in a previous paper [Heller & Urban, 1935].

The antidiuretic effect is measured by the time taken to reach the maximum rate of water excretion. This will be referred to as *inhibition time*. The normal inhibition time, i.e. the delay after giving the standard amount of tap water, was found to be 86 ± 11 min. in the present series of 148 rats.

The antidiuretic activity of samples of urine was estimated in terms of doses of pure pituitrin which were found to possess equal antidiuretic action when tested on the same animals. Several groups of four rats each were standardized first with pure pituitrin. The estimations of the rabbit's urine were made two or three days later. A series of dilutions of the urine samples was prepared. 0·5 c.c. of diluted urine per 100 g. rat was injected into each of a group of four rats. Other sets of four rats each were injected with 0·5 c.c. per 100 g. rat of urine which had been diluted to different degrees. A double control is effected by this procedure. The results can first be compared with those observed after the injection of pure pituitrin a few days ago. Secondly, if sample A, for instance, is found to contain 10 milliunits per c.c. urine in the dilution 1:1, then the diuresis of rats injected with a dilution of 1:5 should be inhibited to a degree which corresponds to a concentration of 2 milliunits of pituitrin per c.c. It is thought that by this method gross errors in the assay of the antidiuretic principle were avoided. Controls were performed to make sure that mixtures of acid urine and pituitrin lose none of their activity if kept for 12 hours in the refrigerator. It should be added that an antidiuretic action of pure urine was in no case observed in our experiments.

The urine samples were obtained in the experiments with the rabbit by bimanual expression of the contents of the bladder. As a control a few animals were catheterized after this procedure, but no residual urine was obtained.

The samples of pituitary extract were kept at 0° C. The same batch of "pituitrin" was used throughout any given series of experiments. Urinary chlorides were determined by Volhard's method and urinary proteins by the gravimetric method of Folin & Denis [1914].

Results

The adsorption of the antidiuretic factor of posterior pituitary extracts by rabbit's blood in vitro

The blood was collected from the carotid arteries of rabbits anæsthetized with ethyl urethane. Coagulation was prevented either by defibrina-

tion or by addition of 0·1 g. sodium citrate to 10 c.c. of blood. 0·5 c.c. saline containing the appropriate dose of pituitrin was added to 9·5 c.c. of blood, and 1 c.c. of this mixture per 100 g. rat was then injected subcutaneously into a group of four rats.

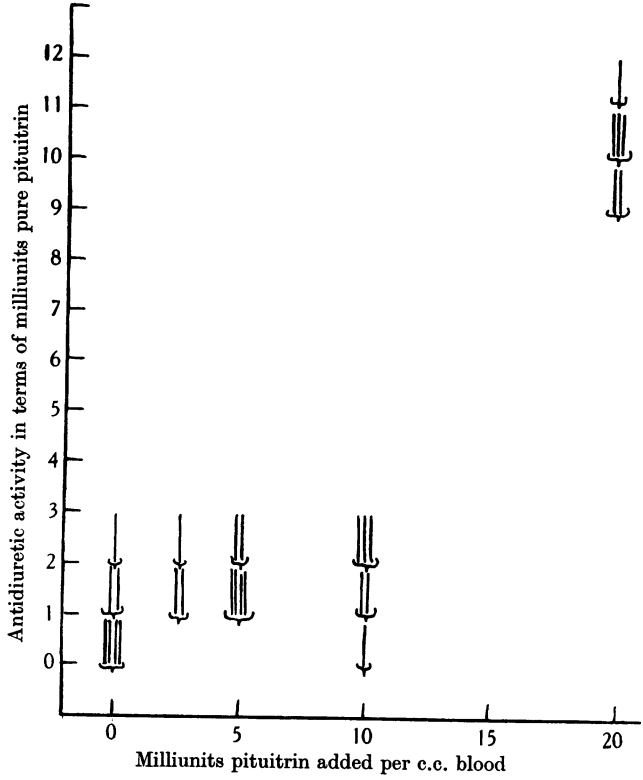

Fig. 1. Specific adsorbing capacity of rabbit's blood for the antidiuretic principle of posterior pituitary extracts. The points of the ordinate were obtained by comparing the inhibition times (see p. 83) of rats injected with blood-pituitrin mixtures with those of rats which were injected with pure pituitrin only. The antidiuretic activity of the control samples (pure blood) is shown under "0 milliunits added". For some unknown reason some of these samples had a slight antidiuretic activity, i.e. the rats had a higher inhibition time than with water alone. Each vertical line represents the equivalent in pure pituitrin of a sample of a pituitrin-blood mixture injected into a group of four rats. The length of the line indicates the probable range of error of the method.

A reference to Fig. 1 shows (1) very little or no difference between the antidiuretic activity of pure blood and mixtures of blood with pituitrin up to the concentration of 10 milliunits per c.c., and (2) an antidiuretic activity equivalent to about 10 milliunits of pure pituitrin of mixtures

of blood with 20 milliunits per c.c. Both these findings indicate approximately 10 milliunits as the specific adsorbing capacity of rabbit's blood.

Amounts of pituitrin below 10 milliunits per c.c., i.e. below the S.A.C. of rabbit's blood, therefore, should rapidly be adsorbed and inactivated after intravenous injection into a normal rabbit. Five experiments were performed. The carotid artery was exposed in ether anæsthesia and the animals were then permitted to recover. Four to six hours later 4 p.c. of the rabbit's body weight of water was given by a stomach tube. After 25–30 min. the first (control) sample of blood was obtained by puncture of the carotid artery, a local anæsthetic being employed. The pituitrin was then injected intravenously. A second and a third blood sample were taken 1–2 and 5–8 min. after the injection. The doses of pituitrin chosen were calculated to produce concentrations ranging from 5 to 10 milliunits per c.c. of circulating blood. The blood volume was assumed to be 8 p.c. of the body weight. The samples were either citrated or defibrinated. The antidiuretic potency of the blood samples was tested on two groups of four rats each.

For example, in one of these experiments subcutaneous injections of 1 c.c. per 100 g. rat of blood removed from a rabbit before the injection of the antidiuretic hormone resulted in inhibition times (see p. 83) of 142 min. in one group of four rats and 113 min. in another group of four rats. 6·5 milliunits of pituitrin per c.c. blood were then injected intravenously into the rabbit. Blood samples were taken from the carotid artery two minutes and five minutes after the animal received the injection of pituitary hormone. Injections of 1 c.c. of these blood samples per 100 g. rat were made into four groups of four rats each. The inhibition times were 97 and 127 min. after injection of the blood samples which were removed after a delay of two minutes, and 105 and 97 min. after the injection of the blood samples removed after five minutes. There is no significant difference between the antidiuretic activity of the controls and of the other blood samples. In other words the hormone was inactivated within two minutes after injection. The other experiments gave similar results in that either all or the bulk of the injected hormone was inactivated within the first two minutes after intravenous injection.

The ultrafiltrability of the antidiuretic hormone

The formation of an adsorption compound with the blood colloids implies a change in the ultrafiltrability of the antidiuretic principle. The published results of ultrafiltration and dialysis experiments as summarized in Table I are in favour of this conclusion. It will be seen that

TABLE I. Dialysis and ultrafiltration of the antidiuretic and the pressor principle of posterior pituitary extracts in solutions containing (*a*) no colloids; (*b*) serum or whole blood. A.P. = antidiuretic principle. P.P. = pressor principle (no difference in "chemical" or "physical" properties has so far been demonstrated between the antidiuretic and the pressor principle. Experiments with the latter were, therefore, included in this table)

Nature of filter used	Nature of colloid-free medium used	Results	Nature of colloid-containing medium used	Results	Author
6 p.c. collodion dissolved in alcohol-ether mixtures of varying concentrations	0·1 p.c. glacial acetic acid	A.P. and P.P. diffuses rapidly and quantitatively	—	—	Smith & McClosky, 1924
Collodion	0·25 acetic acid	P.P. dialyses rapidly	—	—	Kamm, 1928
4 p.c. collodion	Ringer solution	A.P. present in ultrafiltrate	—	—	Dietel, 1933
Collodion	?	A.P. passes freely	Citrated human plasma	Pituitrin demonstrable in ultrafiltrates of plasma containing 2 milliunits pituitrin per c.c.	Byrom & Wilson, 1934
Candles impregnated with 7 p.c. collodion in acetic acid	Acid saline	Quantitative recovery of P.P.	Plasma of *p*H 4·5	Ultrafiltrate completely destitute of pressure effect	De Wesselow & Griffith, 1934
10 p.c. collodion membrane on sintered glass Büchner filter	0·5 c.c. (5000 milliunits) pituitrin added to 5 c.c. saline	Ultrafiltrate contains less than 10 milliunits per c.c.	0·5 c.c. pituitrin and 0·5 acetic acid added to 9 c.c. blood plasma	Ultrafiltrate contains no appreciable amount of A.P.	Theobald, 1934
Ultracella filter "fein" (holding up benzopurpurin molecular weight about 800)	—	Ultrafiltrate contains considerable amounts of P.P. and A.P.	10 c.c. pituitrin were added to 50 c.c. blood of pregnant woman (200 milliunits per c.c.)	Ultrafiltrate contains less than 20 milliunits per c.c.	Theobald, 1934
10 p.c. nitrocellulose	Normal saline	Recovery in direct proportion to the volume filtered	2000 milliunits added to dog's blood (40 milliunits per c.c.)	First traces of pituitrin recoverable	Levitt, 1936

the antidiuretic hormone in a solution free of colloidal matter diffuses and filters freely through comparatively dense membranes, an indication of the low molecular weight of the "free" hormone. All but one series of observations, however, show that either no antidiuretic activity is found in ultrafiltrates of the hormone with blood or serum, or that very large doses of posterior pituitary extract must be added before the first traces of antidiuretic activity appear in the filtrates. These findings suggest strongly that the antidiuretic hormone is rendered non-ultrafiltrable by adsorption to the blood colloids.

Further ultrafiltration experiments were performed with mixtures of pituitrin and rabbit's blood. The filters used were membranes of 7 p.c. collodion in glacial acetic acid made on the surface of sintered glass Büchner filters. The concentrations of pituitary hormone used were 8 and 10 milliunits per c.c. defibrinated rabbit's blood. The antidiuretic activity of the ultrafiltrates was tested by subcutaneous injections into rats. No appreciable amounts of the antidiuretic principle were found in the ultrafiltrates of these mixtures (Table II). Pituitrin dissolved in the same volume of saline was found to pass freely through the collodion ultrafilters. An adsorption of the antidiuretic principle by the filters can, therefore, be excluded.

TABLE II. The difference in ultrafiltrability of the antidiuretic hormone in mixtures with (*a*) saline; (*b*) defibrinated rabbit's blood. Any given series of experiments was performed on the same group of rats at intervals of two days

	Inhibition time in min.
1. Rats receiving 5 p.c. of body weight of water only	68
2. Rats receiving 5 p.c. of body weight of water + 1 c.c. per 100 g. rat of the ultrafiltrate of a mixture containing 8 milliunits pituitrin per 10 c.c. of defibrinated blood	68
3. Rats receiving 5 p.c. of body weight of water + 1 c.c. per 100 g. rat of the ultrafiltrate of a mixture containing 8 milliunits pituitrin per 1 c.c. of saline	195

So far the concentration of the antidiuretic hormone in the pituitrin-blood mixtures was below the S.A.C. of rabbit's blood (see p. 85). However, free, i.e. unadsorbed, antidiuretic hormone should be present in an ultrafiltrate of blood containing an amount of pituitrin above the S.A.C. of rabbit's blood. This we were able to demonstrate in several experiments. Table III shows an example of these results.

The S.A.C. of rabbit's blood for the antidiuretic hormone can, therefore, be estimated as above 10 and below 20 milliunits pituitrin per c.c. blood. These figures are in satisfactory agreement with those obtained by the biological method of the preceding section.

TABLE III. The content of "free" pituitrin, as measured by the antidiuretic effect, of ultrafiltrates of blood samples containing (*a*) an amount of antidiuretic hormone assumed to be near or equal to the S.A.C. of rabbit's blood (8 milliunits per c.c.) and (*b*) an amount assumed to be above the S.A.C. of rabbit's blood (20 milliunits per c.c.). Any given series of experiments was performed on the same group of rats at intervals of two days

	Inhibition time in min.
1. Rats receiving 5 p.c. of body weight of water only	97
2. Rats receiving 5 p.c. of body weight of water + 1 c.c. per 100 g. rat of the ultrafiltrate of a mixture containing 8 milliunits pituitrin per c.c. of saline	225
3. Rats receiving 5 p.c. of body weight of water + 1 c.c. per 100 g. rat of the ultrafiltrate of a mixture containing 8 milliunits per c.c. of blood	113
4. Rats receiving 5 p.c. of body weight of water + 0·5 c.c. per 100 g. rat of the ultrafiltrate of a mixture containing 20 milliunits per c.c. of blood	180

The excretion of the antidiuretic hormone in the urine

Experiments on rats [Heller & Urban, 1935] have shown that a part at least of a large intravenous dose of the antidiuretic hormone is excreted by the kidney in an active state. However, neither the quantity excreted nor the time relations of the excretory process were investigated. These further details are analysed by the experiments described in the following section.

A larger animal, the rabbit, was substituted for the rats of our previous experiments. The animals were placed in metabolism cages. Either 30 or 45 min. before the injection of the antidiuretic hormone 4 p.c. of the animal's body weight of warm tap water was given by stomach tube. The antidiuretic hormone was injected into the ear vein. The urine was collected every 30 and in some experiments every 15 min.

(*a*) *Amounts of antidiuretic principle excreted after intravenous injection of doses ranging from* 1 *to* 10 *milliunits per c.c. of circulating blood.* The quantities of antidiuretic hormone excreted by the same rabbit after repeated injections of identical amounts on different days vary only little. For example: 10 milliunits per c.c. blood were injected intravenously into rabbit No. 5; 294 ± 42 milliunits or 11–15 p.c. of the amount injected were found in the urine. Three days later the animal received the same dose. 320 ± 60 milliunits or 11–18 p.c. of the amount injected were excreted. A series of experiments was then performed with doses rising from 1 to 10 milliunits per c.c. blood (Table IV). It was found that the total amounts of antidiuretic hormone excreted were almost identical. In other words the percentage of antidiuretic hormone excreted in the

TABLE IV. Excretion in the urine of the antidiuretic principle of posterior pituitary extracts by the normal rabbit after intravenous injection of doses ranging from 1 to 10 milliunits pituitrin per c.c. blood. The blood volume was assumed to be 8 p.c. of the body weight

No. of animal	Initial concentration of antidiuretic hormone per c.c. circulating blood (in milliunits pituitrin)	Total amount of antidiuretic principle excreted (in milliunits)	Percentage of antidiuretic principle excreted
2	10	212 ± 42	8–12
	2	220 ± 60	37–64
5	10	294 ± 42	11–15
	10	320 ± 60	11–18
	2	310 ± 18	55–62
6	5	400 ± 80	22–33
	2	252 ± 84	28–57
	1	252 ± 36	72–96
10	10	350 ± 70	16–23
	2	338 ± 8	82–87
11	10	250 ± 10	20–22
	5	244 ± 42	34–48

urine decreases as the amount injected increases. These results apply to five out of a series of six rabbits. No relation was found between the total amounts excreted and the weight of the animals.

It follows that within the concentrations quoted the total amounts of antidiuretic hormone excreted in the urine are to a high degree independent of the hormone concentration in the blood.

(*b*) *The time relations of the excretion of the antidiuretic principle.* The urine was collected at intervals of 15 min. after the injection. Upon estimation of the antidiuretic activity of the samples it was found that, with doses ranging from 10 to 1 milliunits per c.c. blood, the bulk of the excreted amount was eliminated within the first 15 min. The second sample obtained between 15 and 30 min. contained only 10–15 p.c. of the total amount excreted. The experimental procedure used does not permit us to decide whether (1) the antidiuretic activity of the second sample is due to a "washing out" of the residue of the pituitrin excreted during the first 15 min., or (2) the antidiuretic principle is really excreted between the 15th and 30th minute after injection, or (3) the antidiuretic hormone content of the second sample is due to both those processes. In no normal rabbit was any antidiuretic activity observed in urine samples obtained later than 30 min. after the intravenous injection.

The possibility that any significant amount of the excreted antidiuretic principle was destroyed during the storage of the urine in the bladder was excluded by estimations of the antidiuretic activity of

mixtures of pituitrin and urine before and after incubation at 39° C. for 5 hours.

(c) *The excretion of the antidiuretic principle in a rabbit with nephritic changes of the kidneys.* It was mentioned above that divergent results had been obtained with one of the rabbits used in the excretion experiments (see p. 89). Two experiments were performed with this animal (No. 8): 10 and 5 milliunits pituitrin respectively per c.c. blood were injected intravenously and the excretion of the hormone determined in the usual way. The animal differed from the other five of the series in the following points. (1) The total amount of hormone excreted was considerably higher, i.e. 700–1000 milliunits as compared with the 160–480 milliunits of the "normal" animals. (2) The excretion of active hormone extended into the 2nd hour after the injection, whereas the normal animals excreted the antidiuretic principle within 30 min. (3) The water diuresis was not inhibited. Normal animals after the intravenous injection of 425 and 850 milliunits per kg. rabbit excreted 3–10 c.c. urine during the 1st hour. For the same period the figures obtained with animal No. 8 were 35 and 21 c.c. of urine.

In connexion with the last point it will be remembered that a diminished sensitivity to the antidiuretic hormone of the pituitary has been thought to be present in cases of human nephritis [Lebermann, 1929; Hart, 1929]. It was, therefore, investigated whether the abnormal behaviour of rabbit No. 8 was coincident with diseased kidneys.

At the time when the above-mentioned experiments on this rabbit were performed only traces of protein were present in the urine. Such traces, i.e. amounts below 20 mg./100 c.c., are not uncommonly found in the urine of rabbits and are hardly of any pathological significance. Four weeks after the experiments, however, a definite proteinuria was present in rabbit No. 8 (130 mg./100 c.c. in the urine collected during 24 hours). This increase in the amount of protein excreted was thought to be significant as it could not be demonstrated in any of the other animals of the same series, some of which had been injected with large doses of pituitrin even more frequently. Animal No. 8 was finally killed and sections of the kidneys were examined. The stains used were hæmatoxylin and eosin and Weigert's iron hæmatoxylin and van Gieson. Dr G. R. Cameron had the kindness to investigate the sections for pathological changes and gives the following report:

"Scattered through an otherwise normal kidney are glomeruli in various stages of degeneration. Some show distortion of shape, are more cellular and contain an occasional polymorphonuclear leucocyte and

lymphocyte. Others are greatly contracted, being reduced to about one quarter the normal diameter. In these the basement membrane lining the endothelial cells of the tufts are adherent to the capsule. No actual cell crescents however are present. The convoluted tubules appear swollen in places, but there is no obvious pathological alteration of their cells. Blood vessels are normal. The condition suggests a mild degree of subacute local glomerulo-nephritis."

The renal action of "bound" antidiuretic hormone

It has been shown in the first two sections of this paper that the antidiuretic principle up to concentrations of about 10 milliunits per c.c. is adsorbed by some colloidal constituent or constituents of the blood. This adsorption compound when injected subcutaneously is not, or very slowly, absorbed from the subcutis and, therefore, has no appreciable action on the kidney. When brought in contact with the kidney by intravenous injection, however, the adsorption compound seems to have the renal action of the "free" hormone.

Rabbits with a satisfactory diuretic response to a given quantity of water were selected. A few c.c. of blood were obtained from the ear vein of the unanæsthetized animal and either defibrinated or mixed with sodium citrate. An hour later 2 p.c. of the body weight of water were given by stomach tube and the animal was placed in a metabolism cage where it remained for 3 hours. A second dose of water amounting to 4 p.c. of the body weight was then given. Finally, 45 min. after the second administration of water, a mixture of 2–4 c.c. of the rabbit's own blood with pituitrin or the same amount of pituitrin diluted with saline was injected intravenously. The urine was collected every 30 min. The activity of the saline-pituitrin and blood-pituitrin mixtures was assessed by determinations of the urine volume and the characteristic increase in the elimination of chlorides. Blood alone had no influence on either the water diuresis or output of chlorides. The doses of pituitrin injected varied from 8 to 0·7 milliunits per kg. rabbit. The concentration of antidiuretic hormone in the pituitrin-blood mixtures was either 10 or 5 milliunits per c.c. blood. Little or no difference could be found between the action of the pituitrin-saline and the pituitrin-blood mixtures as measured by the degree of inhibition of water diuresis and the increase in chloride elimination (Fig. 2). Similar results were obtained in five other experiments. Diuresis experiments with rabbits do not admit the same accuracy in interpretation as results obtained with dogs or rats. We hesitate, there-

fore, to suggest that the results of the injections with bound and free pituitrin are quantitatively identical.

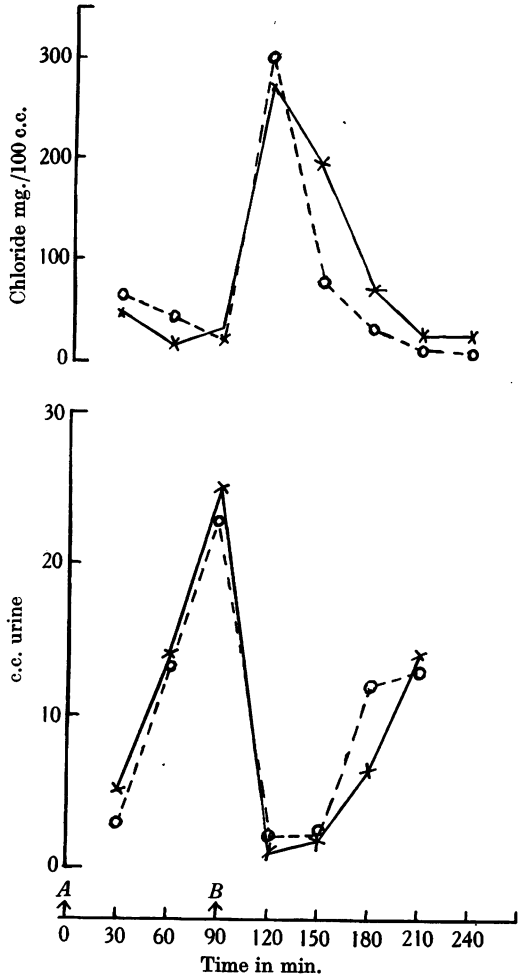

Fig. 2. Rabbit no. 15, ♂, 2300 g. The action of an intravenously injected pituitrin-blood mixture on water diuresis and urinary chlorides as compared with the action of the same amount of pituitrin dissolved in saline. Four c.c. of the rabbit's own defibrinated blood containing 20 milliunits pituitrin, and 4 c.c. normal saline containing the same amount respectively were injected. Continuous line = pituitrin + saline; broken line = pituitrin + blood. *A* = water by stomach tube; *B* = intravenous injection.

There seem to be two possibilities which may explain the renal action of the adsorbed antidiuretic principle. The hormone can be (1) adsorbed

without involvement of its active groups. It is conceivable that in this case the adsorption compound could still have a renal action if brought in contact with the renal cells while its absorption after subcutaneous injection would be prevented by its large molecular weight. The antidiuretic principle could (2) be adsorbed with involvement of its active groups. Under these circumstances a renal action of the intravenously injected hormone could only occur after decomposition of the adsorption compound, i.e. after liberation of the hormone in the kidney. In view of the fact that such a liberation of the antidiuretic principle has actually been demonstrated in the excretion experiments of the preceding section, the second possibility seems the likelier explanation.

Discussion

Mixing of the antidiuretic principle of posterior pituitary extract (pituitrin) with blood *in vitro* results in the formation of an adsorption compound between the hormone and some colloidal constituent or constituents of the blood. This could be demonstrated by the loss of renal activity of blood-pituitrin mixtures after subcutaneous injections into rats, the large molecular weight of the adsorption compound preventing its absorption. The "bound" state of the antidiuretic principle in mixtures with blood could, secondly, be shown by the observation that ultrafilter membranes which are permeable to "free" pituitrin become impermeable to the hormone when it is in the adsorbed state. The adsorbing capacity of the blood for the antidiuretic hormone is limited but constant within the limits of our method for the same species of animal. For the rabbit it was found to be in the neighbourhood of 10 milliunits per c.c. blood. There is strong evidence that the formation of an adsorption compound occurs *in vivo* as well as *in vitro*. An important part of this evidence is furnished by the finding that the activity of suitably small doses of intravenously injected pituitrin, as measured by the rat method, disappears from the blood more rapidly than can be explained by either elimination by the kidney, destruction in the blood or absorption into the tissues.

When small amounts of pituitrin are intravenously injected into rabbits a large proportion is excreted in the urine in a free state. If larger amounts of the hormone are injected the amount excreted in the urine is not proportionately increased. Thus as the amount of hormone injected is increased, the proportion of the hormone which is excreted in the urine decreases (see Table IV). The above observations are based on experiments in which the amounts of antidiuretic hormone injected

have ranged from 1 to 10 milliunits per c.c. blood. They are not likely to apply to amounts of pituitary hormone which are outside this range.

It is evident from the foregoing results that a part of the adsorbed antidiuretic hormone is excreted in the urine in a free state. It is, therefore, concluded that the adsorbed antidiuretic hormone is liberated in the kidney; in other words that the kidney possesses the faculty to elute the adsorbed hormone from its colloidal carrier.

There seems to be no other instance of a similar excretory mechanism for a physiologically active non-foreign substance, but an interesting parallel can be found in the urinary elimination of certain organic dyes, especially phenol red. These seem to be adsorbed by the colloids of the blood and liberated by the kidney in much the same manner as the antidiuretic hormone of the pituitary gland [Marshall & Vickers, 1923; Marshall & Crane, 1924; Marshall, 1931; Grollman, 1925; Bennhold, 1932; Shannon, 1935; Goldring *et al.* 1936].

Since an abundance of evidence indicates [Wearn & Richards, 1924; Richards & Walker, 1930; White, 1932] that the normal glomerulus is only permeable for ultrafiltrable substances, it seems justifiable to assume that non-ultrafiltrable substances like "bound" phenol red or "bound" antidiuretic hormone are excreted by the tubules. In the case of phenol red this is strongly suggested by the work of Marshall [1931], Richards & Walker [1930], Chambers & Kempton [1933], Shannon [1935] and Goldring *et al.* [1936].

The physiological significance of the adsorption of the antidiuretic hormone may be, that small amounts of hormone circulating in the blood are thereby able to avoid elimination by the glomeruli and are automatically guided to the tubules, the probable site of their renal action.

Summary

1. The antidiuretic principle of posterior pituitary extracts (pituitrin) is adsorbed by some colloidal constituent or constituents of the rabbit's blood to the extent of about 10 milliunits per c.c. blood. Evidence is presented which leads to the conclusion that the adsorption of the antidiuretic hormone takes place *in vivo* as well as *in vitro*.

2. The antidiuretic principle dissolved in saline passes rapidly through a collodion ultrafilter, but the ultrafiltrate of a mixture of rabbit's blood with the hormone in concentrations up to 10 milliunits displays no appreciable antidiuretic activity.

3. If antidiuretic hormone is injected intravenously into rabbits a fraction of the injected amount is excreted in the urine. Within a certain

range (1–10 milliunits of pituitrin per c.c. blood) the amount of antidiuretic hormone excreted by the kidney is highly independent of the hormone concentration in the blood. The proportion of antidiuretic hormone excreted in the urine decreases as the amount injected is increased. The urinary excretion of the antidiuretic principle by the rabbit ceases approximately 30 min. after the intravenous injection.

4. These observations applied consistently to five out of a series of six rabbits. The sixth or "abnormal" animal was found to suffer from glomerulo-nephritis.

5. The kidney possesses the faculty to liberate the adsorbed antidiuretic principle.

6. It is suggested that small amounts of the antidiuretic hormone circulating in the blood are thus liberated and eliminated by the tubules.

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