REVIEW ARTICLE

ANTIDIURETIC SUBSTANCES

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AN antidiuretic substance may be defined as one which produces a decrease in urine volume after water has been administered. This definition excludes influences which lead to long lasting depression of kidney function, as for instance the oliguria following adenohypophysectomy or in myxœdema, or again substances which cause a tubular necrosis. It seems likely, however, that in some oliguric states in which the renal response to water is also impaired, the occurrence of antidiuretic substances in the circulation (or of increased amounts of such substances) may be a factor contributing to the abnormalities of water excretion. A discussion of the humoral basis of such conditions may therefore be regarded as being within the scope of this survey.

ESTIMATION OF ANTIDIURETIC ACTIVITY

All methods which involve the use of mammals as test animals have been primarily designed for the estimation of the posterior pituitary antidiuretic principle. The criterion used is uniformly the inhibition of water excretion in a hydrated animal, the methods may therefore be used for the assay of antidiuretic substances generally. For further analysis (see, e.g., Hare, Melville, Chambers and Hare[1]) glomerular filtration rate may be determined by a clearance method simultaneously with changes in urine volume. This permits distinction between a glomerular and a tubular antidiuretic effect. The smaller the size of the species the larger a dose of postpituitary extract has apparently to be given per unit of body weight to obtain a comparable antidiuretic effect: well marked inhibitory effects have been observed in 15 kg. dogs after the intravenous injection of 0·25 to 0·5 milliunits of vasopressin[1,2] while the minimum effective intravenous dose in mice weighing 20 g. is of the order of 0·05 milliunits[3]. The use of small animals is therefore indicated only where very small amounts of antidiuretic material are available or when dogs cannot be used in a particular laboratory. Assays on dogs and rabbits have the advantage that standard and unknown can be compared in the same animal. Intravenous injection is the mode of administration of choice unless the amounts of antidiuretic material are abundant, though activity equivalent to 0·4 mU (=milliunits) of vasopressin per 100 g. can be estimated with accuracy by subcutaneous injection into rats[4]. However, injections other than intravenous are only safe to use for quantitative purposes if the presence of " augmentor " material in the test fluid has been excluded. Numerous substances, inorganic and organic, have been reported by Noble, Rinderknecht and Williams[5] to

enhance the antidiuretic action of posterior pituitary extracts, but recent findings indicate that these augmentors may not be quite as ubiquitous as suggested by the results of these authors. While Noble and his co-workers found, for instance, that antidiuresis in rats was much prolonged when horse blood or serum was added to posterior pituitary extract, Dicker and Ginsburg[6] showed recently that rat plasma and serum did not augment the action of vasopressin in rats. Ideally, assays of post-pituitary antidiuretic hormone should be performed on animals with diabetes insipidus, i.e., on animals in which interference from the endogenous hormone is eliminated. Such animals—dogs after stalk section—have actually been used by Hare, Melville, Chambers and Hare[1] with excellent results. In usual practice, however, it seems sufficient to inhibit neurohypophysial secretion by repeated high water loads.

Classification of Antidiuretic Substances

Considering the multiplicity of mechanisms by which water diuresis can be inhibited, it would seem best to classify antidiuretic substances according to their site of action.

Substances Producing Antidiuresis by Extrarenal Action

Delay of water absorption from the gastrointestinal tract has been observed after the administration of anæsthetics and hypnotics in rats and rabbits[7]. The magnitude of the inhibitory effect varied with the depth of anæsthesia and the anæsthetic used. In rats in which the amount of unabsorbed water was estimated by weighing the alimentary tract, ether or chloroform anæsthesia inhibited the rate of disappearance of water from the gastrointestinal tract to such a degree that this alone explained the absence of water diuresis. Hypnotics such as urethane, paraldehyde and phenobarbitone sodium had a less pronounced effect; 0·01 g. of chloralose per 100 g. rat was hardly inhibitory. Experiments with ether and chloretone on rabbits suggested that anæsthetics may tend to diminish alimentary absorption in this species also. More work is indicated to show whether the effect of a comparable dose of a hypnotic varies from species to species and whether the delay in the disappearance of a gastrointestinal water load increases with the depth of the anæsthesia as seems likely. Further investigation is also needed to analyse the mechanism of this inhibitory effect. Are we dealing with a depression of the absorptive function of the epithelium or is the decrease in the rate of disappearance of the administered water from the alimentary canal due to delay in gastric emptying time? The latter possibility may apply considering that Dicker[8] has shown that the diuretic response to water in rats injected with sodium cyanate is severely impaired for this reason. But whatever the mechanism, delay in the absorption of water into the circulation cannot be neglected as a causal factor—if only a contributory one—in the antidiuretic action of anæsthetics. Other extrarenal mechanisms which could possibly interfere with water diuresis appear to be of lesser importance. It is well known that many steroids induce

retention of water, an effect partly due to increased sodium reabsorption by the renal tubules, but probably also due to extrarenal changes. It was shown by Zuckerman, Palmer and Hanson[9] for example in rats that single injections of certain amounts of α-œstradiol, progesterone and desoxycorticosterone acetate increase the water content of the uterus and the vagina in a matter of hours. Concurrently the water content of other tissues, e.g. skeletal muscle and the skin declined or remained unchanged, which strongly suggests an extrarenal action. The overall change in water balance after desoxycorticosterone is likely to be water loss[10], but the contrary may obtain after œstradiol and progesterone[11,12] and this may possibly interfere with water diuresis. Thyroid extract and thyroxin enhance water diuresis under certain conditions[13] and it can therefore be expected that anti-thyroid drugs will inhibit it. This has actually been shown by Brücke and Lindner[14] in rats after implantation of thiouracil tablets. The mechanism of this effect requires further elucidation.

Substances Producing Antidiuresis by Influencing Renal Blood Flow

Any experimental procedure which lowers systemic arterial pressure below a certain value may be expected to lower glomerular pressure to such a degree that filtration stops and the flow of urine ceases. In the absence of diuretics the minimum arterial pressure compatible with the elaboration of urine has been shown[15,16] to be about 75 mm.Hg. In the intact animal, however, the relation between systemic blood pressure and urine flow is not likely to be a simple one. Even the "minimum value" may be assumed to depend in part on the procedure or agent used to produce the reductions in general pressure. For instance, if a drug is used which has a differential effect on the vasa afferentia and vasa efferentia of the glomerulus, intraglomerular pressure will not bear a direct relationship to general pressure. Furthermore, it has been shown by Rydin and Verney[17] that hæmorrhage in trained dogs causes inhibitions of water diuresis which in duration and degree are quite disproportionate to the fall of systemic blood pressure involved. It is conceivable that depressor drugs invoke the same mechanism. The type of antidiuresis obtained after blood loss resembles strongly that produced by emotional stress which has been shown to be due to stimulation of the neurohypophysis.

Adrenaline. The "type substance" used to demonstrate the possibility of a differential action on the glomerular vessels has been adrenaline. Numerous investigations have shown that the effect of this hormone on urine flow depends on the dose given and on the mode of administration. Intravenous injection of small doses of adrenaline (e.g. 0·02 mg. per animal) into an unanæsthetised dog results in a profound but fleeting inhibition of water diuresis[17,18,19] while large intramuscular or subcutaneous injections (1 to 0·475 mg./kg.) produce an increase in urine flow[20,21]. There was no correlation between systemic blood pressure and rate of urine flow in these experiments suggesting that adrenaline

modified diuresis mainly by influencing intraglomerular pressure. Direct proof of this was obtained by Winton[22] in experiments in heart-lung-kidney preparations of the dog, perfused at constant arterial pressure. He could show that, as in the intact animal, low concentrations of adrenaline induced "diuresis" accompanied by a small reduction in blood flow without the complication of a rise in arterial pressure. Higher concentrations of adrenaline produced a reduction in urine flow together with a more profound reduction in blood flow. The changes in glomerular pressure accompanying adrenaline diuresis were found to involve rises from the normal of 60 per cent. of the arterial pressure to values up to 90 per cent.; the reductions in urine flow due to higher concentrations of adrenaline were accompanied by glomerular pressures ranging down to 30 per cent. of the arterial pressure[22]. Additional light on this mechanism is provided by the work of Richards and Plant[23] who, in 1922, found that in eviscerated cats, rabbits and dogs blood flow decreases and kidney volume and urine volume increase when very small doses of adrenaline are injected. A consistent reduction of renal blood flow after adrenaline was also demonstrated by measurements on unopened blood vessels by means of a thermostromuhr[24,25,26]. It is clear from these findings that the diuretic effect of adrenaline is best interpreted as being due to constriction of the vasa efferentia, accompanied perhaps by dilatation of the afferent vessels[27,28]. When larger doses are given to anæsthetised or unanæsthetised dogs both the afferent and efferent arterioles are constricted and a diminution of urine flow results[29,20,21]. Observations made on man[28,30] suggest that the effects of adrenaline on the human kidney are much the same as those on animals. Ephedrine and pholedrine given intramuscularly to healthy subjects reduced renal plasma flow and increased the filtration fraction[31] without much change in the glomerular filtration rate. The action of these amines thus resembles that of adrenaline.

Tyramine. Motzfeldt[32] observed an antidiuretic effect lasting 2 hours in a rabbit after 10 mg. of tyramine had been given subcutaneously, but Burn, Truelove and Burn[33] using rats failed to get an antidiuretic response with this substance.

Histamine. Histamine would appear to inhibit water diuresis only when large doses are given. Molitor and Pick[34] found that doses of the order of 1 mg. per animal injected subcutaneously or intravenously into unanæsthetised dogs had a pronounced antidiuretic effect. Gilman and Kidd[35] observed antidiuresis in dogs after intravenous injection of 2·5 μg. of histamine per kg., but Theobald and White[36] failed to obtain an inhibitory response after subcutaneous injection of 0·5 mg. per dog. Motzfeldt[32] has reported that 20 mg. of histamine by mouth or 1 mg. subcutaneously inhibits a water diuresis in rabbits; the antidiuretic effect of 0·4 mg. given subcutaneously was very slight. In the writer's experience[37] doses of 1 to 10 μg./kg. injected intravenously into unanæsthetised rabbits have no inhibitory action; 20 μg./kg. given to one animal caused a small and transient decrease in urine flow. Effects on man seem to vary

considerably. A slight antidiuretic effect of 0·2 mg. injected subcutaneously was noted by Gibson and Martin[38] in a subject suffering from diabetes insipidus, but Weir and his co-workers[39] failed to obtain a decrease of urine flow (dose of histamine not stated) in cases of the same disease. Reubi and Futcher[40], who injected 0·15 to 0·7 mg. of histamine subcutaneously into subjects with elevated and with normal blood pressure, say that " significant changes in urine volume were not regularly observed." In a footnote however, they mention an additional patient who after the injection of 0·5 mg. showed a marked antidiuretic effect.

Little is known about the mechanism by which histamine may cause antidiuresis. The plethysmographic studies of Dale and Laidlaw[41] in the cat showed a decrease in renal volume possibly due to arteriolar constriction. Decreases in urea and creatinine clearance observed in man[42] likewise suggested a vascular action. More recent investigations[40] have shown that doses of the order of 0·5 mg. of histamine given subcutaneously to healthy subjects decrease mannitol and *para*-aminohippurate clearance and slightly increase the filtration fraction. The renal extraction percentage of mannitol and para-aminohippurate was not consistently influenced, nor was there much change in the systemic blood pressure, though flushing and headaches was experienced by all the subjects. The authors ascribe the fall in para-aminohippurate clearance (= effective renal plasma flow) to constriction of the efferent arterioles. A wider range of doses and preferably intravenous administration will clearly have to be used in future experiments to clarify the mode of action of histamine on the kidney.

Adenosine Triphosphate. It has been suggested by Green[43] that adenosine triphosphate may be liberated from damaged muscle in sufficient quantities to play a part in the shock syndrome and to account for " traumatic oliguria." When adenosine triphosphate was given with water to healthy subjects[44] the onset of water diuresis was not delayed, but urine volume was reduced. However, as pointed out by Keele and Slome[45] it is most improbable that adenosine triphosphate, a renal vasodilator, is responsible for the reduction in renal blood flow seen in practically every case of shock[46]. Houck, Bing, Craig and Visscher[47] found subsequently that adenosine triphosphate, adenosine and adenylic acid infused intravenously into dogs induced a transient fall in creatinine clearance and urine flow but a well-marked diuresis in the post-infusional period.

The effects of postpituitary extracts on renal blood flow and filtration rate. Injection of small doses of posterior pituitary extract or of vasopressin have either been found to leave glomerular filtration rate and effective renal plasma flow unchanged[48,49,50] or to produce some rise of inulin, creatinine and diodone clearance[51,52]. Only when very large intravenous doses are used (e.g., 300 to 650 mU per dog in the experiments of Wakim, Herrick, Baldes and Mann[53]) have significant decreases of renal blood flow been observed. There is thus little doubt that in mammals, including man, posterior pituitary extracts and vasopressin

given in reasonable doses produce their antidiuretic effect by enhancing tubular water reabsorption. Glomerular antidiuresis may, however, be of importance in lower vertebrates which are unable to concentrate their urine beyond the plasma osmotic level. This is suggested, for example, by the results of Burgess, Harvey and Marshall[54], who found that very small doses of vasopressin (1 mU/kg.) markedly lowered the glomerular filtration rate in alligators without producing a hypertonic urine. Similarly, Sawyer[55] showed in the frog that the oxytocic fraction causes constriction of the afferent arterioles and cessation of glomerular blood. Further discussion of this problem will be found in a recent review by the writer of this article[56].

The action of vasoactive substances on intrarenal blood distribution. It has been postulated by Trueta, Barclay, Daniel, Franklin and Pritchard[57] and before them by Frey[58] and Fuchs and Popper[59] that diversion of blood from the renal cortex through a " medullary by-pass " may be involved in the antidiuretic action of such substances as vasopressin, nicotine, adrenaline and ephedrine. This assumption was based mainly on observations of the intrarenal distribution of injected materials and of the colour of the blood in the renal vein after the drugs had been injected. Quantitative methods such as measurements of renal blood flow and renal oxygen A-V difference, and simultaneous determinations of the inulin and para-aminohippurate extraction ratios have since failed to confirm the diversion of any appreciable quantity of blood through uncleared channels in antidiuresis induced by the release of endogenous posterior pituitary hormone or by administration of vasopressin, adrenaline and histamine in dogs and man[60,61,62,63]. Nor could intrarenal shunt mechanisms be demonstrated in rats. Cortical anæmia could only be produced in this species when intravenous doses of posterior pituitary extract of the order of 3,000 mU per rat (!) were administered[64]. Rabbits anæsthetised with pentobarbitone sodium (nembutal) (the same species and anæsthetic were used by Trueta *et. al.*) were investigated by Kahn, Skeggs and Shumway[65]. They injected adrenaline (0·15 mg./kg.), vasopressin (750 to 2,000 mU/kg.), renin and hypertensin, but were unable to demonstrate the existence of two potential renal circulations or of a renal medullary by-pass by histological methods. Moyer *et al.*[61], on the other hand, did observe a differential distribution of India ink in the kidneys of rats injected intravenously with 0·02 mg. of adrenaline. They then studied renal blood flow and renal oxygen A-V difference after intravenous injection of the same dose. A significant rise in blood pressure was recorded in all the rabits investigated. The renal blood flow decreased by as much as 70 per cent., the mean A-V difference increased by 313 per cent. due to decreased oxygen content of the blood in the renal vein. The picture given by the injection of India ink and adrenaline was thus clearly not associated with arterialisation of the blood in the renal vein. Moyer and his co-workers[61] conclude that their evidence does not support " the hypothesis of an active patho-physiological renal vascular shunt following injection of adrenaline in rabbits."

Summing up, it has been shown in man, the dog, the rabbit and the

rat that changes in intrarenal blood distribution (renal shunts) are of little or no importance in the antidiuretic effect produced by the vasoactive drugs so far tested.

Substances Producing Antidiuresis by Direct Action on the Tubules

Posterior pituitary extracts. The antidiuretic hormone of the neurohypophysis is at present the only substance for which a direct and primary effect on renal tubular water reabsorption has been fully established. Such subjects as the tissue of origin of the hormone, the results of experimentally produced or clinical antidiuretic hormone deficiency and the physiological control of its secretions have been fully discussed in recent review articles[66,67,68,69,70]. The exact site of action of the antidiuretic hormone in the renal tubule and the cellular mechanism of tubular water absorption against the plasma osmotic gradient remain, however, largely a matter for conjecture. The micropuncture studies of Walker, Bott, Oliver and MacDowell[71] suggest that the volume of glomerular filtrate while remaining in osmotic equilibrium with the plasma has been reduced at the end of the proximal tubule by about two-thirds and possibly more. These experiments were performed at moderate rates of urine flow and the osmotic pressures of proximal tubular urine has still to be measured at the extremes of maximum water diuresis and oliguria, but indirect evidence has since been advanced[72,73] which makes it very likely that water absorption in the proximal tubules is carried out iso-osmotically, i.e., without osmotic work being performed directly on water. The action of the antidiuretic hormone must therefore be referred to a more distal site. The scanty evidence available points, on the whole, to the thin segment of the loop of Henle as the tubular site of action of the antidiuretic hormone. The thin segment is typically developed in mammals and birds only and these vertebrate classes are said to be the only ones which respond to the antidiuretic hormone by increasing tubular water reabsorption against the plasma osmotic gradient[54]. It appears further[74] that carnivores which elaborate a more concentrated urine than herbivores, have a relatively longer thin segment and Sperber[74] and Koefoed[75] have shown that the thin segment of certain desert animals which are able to elaborate a more concentrated urine than other mammals[76,77,78], is better developed than that of other rodents which have a better access to water. Moreover, the results of direct cryoscopy in kidney slices from dehydrated rats, reported by Wirz, Kahn and Hargitay[79] in 1950, suggest that the thin segment is an important site of concentration. The difficulty remains, to picture the flat non-secretory epithelium of the thin segment as the site of an active transfer of water against the osmotic gradient[72,63]. It is for this reason that Trueta and his co-workers[57] have suggested that the antidiuretic effect of the neurohypophysial hormone may be due or partly due to an increase in passive reabsorption of water into the blood of the vasa recta resulting from a readjustment in the distribution of the intrarenal blood flow between cortex and medulla (but see the previous section).

Schaumann[80] thinks that the vasopressor principle constricts Henle's tube, whereby the flow of the glomerular filtrate would be slowed so that water reabsorption is increased. However, it is difficult to visualise so complicated a process as the transfer of water against the osmotic gradient as occurring without metabolic changes in the effector cells. There is some tentative evidence that respiratory processes may be concerned in this transfer mechanism[81,82,83], though it must be admitted that proof for a cellular action of posterior pituitary hormones (except that on the frog skin[84]) is not extant.

Substituted cinchoninic acids. These compounds are of particular interest since it has been shown[85] that they may influence enzymatic transfer mechanisms in the proximal renal tubule. They have also been reported by Marshall and his co-workers[86,87] to be antidiuretic in the normal dog and this effect was found to be independent of changes in glomerular filtration. They were active when given by mouth[86]. Marshall[88] found that in two dogs with diabetes insipidus, 3-hydroxy-2-phenylcinchoninic acid retained its inhibitory effect on water diuresis. Clinical studies revealed further that the same compound reduces water output in cases of human diabetes insipidus[89,90]. These findings were strongly suggestive of a direct action on the water reabsorbing elements in the tubules, especially so since it has been shown that the posterior lobe of human cases of diabetes insipidus is very highly depleted of the antidiuretic hormone[91,92,93]. However, considering the minute amounts of antidiuretic hormone needed to inhibit water diuresis in the dog and in men[94] some residual antidiuretic hormone may have been liberated by the cinchoninic acid derivative. The problem has recently been re-investigated in the rat by Maren and Bodian[95] in Marshall's laboratory. The authors found that in animals with diabetes insipidus (but in whom 19 to 63 per cent. of neurohypophysial tissue was still present) 3-hydroxy-2-phenylcinchoninic acid and nicotine (see next section) failed to have an antidiuretic effect. But 7-chloro-3-hydroxy-2-phenylcinchoninic acid, an antidiuretically more active member of the same group, did no longer inhibit water diuresis in 7 out of 10 rats with diabetes inspidus. The authors interpret their results as suggestive of mediation of the hypothalamo-neurohypophysial system in the antidiuretic action of 3-hydroxy-cinchoninic acids in the rat. Further investigation of the site of action of the compounds is planned by them.

Substances Producing Antidiuresis by Liberation of Neurohypophysial Antidiuretic Hormone

It is clear from the foregoing discussion that certain requirements have to be fulfilled before a substance may be assumed to produce an antidiuretic action by liberation of antidiuretic hormone. Some of these are as follows: the unknown substance should retain its antidiuretic action after denervation of the kidneys, it should inhibit water diuresis by increasing tubular water reabsorption and its inhibitory action should disappear after removal of the neurohypophysis. Since it is notoriously difficult or perhaps impossible to remove all neurohypophysial tissue

surgically and since stalk transection may possibly lead to hypertrophy of the neurohypophysial elements cranial to the lesion, the last requirement mentioned is not an easy one to fulfil (see, e.g., the paper of Maren and Bodian[95]) and it may be advisable to attempt the demonstration of an increased antidiuretic activity in body fluids after the suspected stimulant has been injected. A comparison of the form and the time course of the inhibitions of water diuresis obtained with those seen after the injection of small doses of posterior pituitary extracts may help and so may estimations of chloride concentrations in the urine. An increase in the *amounts* of chloride excreted cannot be expected invariably since it has been shown[50,51,96,94] that antidiuretic effects can be obtained with pitressin which are not accompanied by a rise in the amounts of chloride or sodium in the urine.

Increased liberation of antidiuretic hormone by a variety of substances has been postulated, but while this action has been well analysed in some of the instances, the evidence in others is rather fragmentary. In particular, it is not clear whether some of the antidiuretic effects observed were not due to unspecific stimuli.

Acetylcholine, nicotine, atropine, morphine, yohimbine and ergot alkaloids. Molitor and Pick[97] as long ago as 1924 had found that choline and acetylcholine inhibited water diuresis in dogs. Pickford[98,99] confirmed and extended their work. She showed that intravenous doses from 0·2 to 1 mg. of acetylcholine per dog were effective in atropinised animals and that they remained so after denervation of the kidney. Changes of blood pressure or release of excess of adrenaline could be eliminated as causing the decreases of water excretion observed in her experiment. The time course of the inhibition followed closely that produced by injection of small intravenous doses of posterior pituitary extract. Neurohypophysectomy abolished the inhibitory effect of doses of acetylcholine as large as 50 mg. per animal and prevented the rise in urinary chloride output observed in normal dogs. Acetylcholine injected directly into a supraoptic nucleus inhibited water diuresis in dogs with denervated kidneys, eserine added to the injected acetylcholine prolonged the inhibitory effect, eserine by itself had also an inhibitory action, *all* results compatible with the assumption that secretory stimuli are transmitted to the supraoptic cells by acetylcholine.

Since acetylcholine in Pickford's experiments had exhibited its antidiuretic action in the presence of atropine, this effect was possibly due to its nicotine-like action. Nicotine itself can therefore be expected to inhibit water diuresis by release of antidiuretic hormone. It is interesting therefore that Motzfeldt[32] found in 1917 that nicotine has an antidiuretic effect in rabbits. He remarked on the similarity between the action of nicotine and that of posterior pituitary extract. Burn, Truelove and Burn[33] showed more recently that the subcutaneous injection of 0·25 mg. of nicotine was effective in rats, but that it fails to inhibit water diuresis in hypophysectomised rats maintained on anterior pituitary extract. In human beings water diuresis was completely inhibited by 0·5 to 1·0 mg. of nicotine injected intravenously; the smoking of cigarettes

produced a similar effect[33,100,94]. The antidiuretic activity present in the urine of healthy subjects (unused to smoking) after one cigarette was estimated as equivalent to 10 to 20 milliunits of posterior pituitary extract per 100 ml.[101]. Smoking and the injection of nicotine may prove to be valuable diagnostic tests for the differentiation of true diabetes insipidus from psychogenic polydipsia[102,103]. Atropine given intravenously in doses up to 2 mg. per animal did not prevent the development of a water diuresis in Pickford's[98] dogs and unpublished experiments of Ginsburg and Heller on rats failed to show an inhibition of water diuresis when atropine sulphate in subcutaneous doses of 0·5, 5·0 and 50·0 mg. per animal were given. Older reports[104,105] seemed to indicate that water diuresis in dogs is interrupted by this drug. Ssargin and Nussinboim[106], who found an antidiuretic effect in mice after the subcutaneous injection of very large doses of atropine (5 mg./20 g. of mouse) have suggested—on insufficient evidence—that this alkaloid releases antidiuretic hormone. Further investigation is undoubtedly needed, but it is of interest to note that at least some substances which have stimulant effects on the central nervous system are said to inhibit water diuresis by increasing the secretion of the antidiuretic hormone. This mechanism of action has been suggested for yohimbine [107], the antidiuretic action of which, together with that of corynanthine had previously been demonstrated by Zunz and Vesselovsky[108]. In Fugo's[107] experiments yohimbine did not decrease the polyuria of dogs with diabetes insipidus. The antidiuretic action of the central stimulants picrotoxin and tetrahydro-β-naphthylamine reported in rabbits appears to be mainly due to vascular effects, since Koiwa[109] observed marked decreases of creatinine clearance after these drugs had been administered. Certain ergot alkaloids (ergotoxine, ergotamine, ergocristine and ergocristinine have been found to decrease water diuresis[110,111], whereas others (ergotinine, ergotaminine, ergosine and ergosinine) have been reported to enhance water excretion[112]. It seems likely that the inhibiting ergot alkaloids are those which—like tetrahydro-β-naphthylamine—have stimulant action on sympathetic centres. But the mechanism of their inhibitory effect has still to be investigated.

Morphine has been shown to inhibit water diuresis by numerous investigators[113,114,104,115,116,117,118,119,120]. Omnopon (injection of papaveretum), codeine, dihydrocodeinone (dicodid) and pethidine have also been reported[120,121] to have this inhibitory effect, which however is not shown by papaverine. The inhibitory effect of morphine is apparently only obtained when large doses are administered, since Walker[100], who gave a therapeutic dose of morphine (20 mg. of morphine sulphate) subcutaneously to students, failed to observe antidiuretic effects. It is interesting that he did so even when nausea and vomiting were produced in some of his subjects. Species differences in sensitivity can, in addition, obviously not be excluded. Sager[122] found that morphine like posterior pituitary extract increased tubular water reabsorption and decreased chloride reabsorption. De Bodo[123], who gave comparatively large doses of morphine (5 mg./kg. subcutaneously or 2·5 mg./kg. intravenously) to dogs could show that the drug retains its inhibitory

effect after removal of one adrenal and denervation and demedullation of the other, that it fails to act in dogs with permanent diabetes insipidus due to a high hypophysial stalk section, that it does not increase the inhibitory effect of a dose of posterior pituitary extract injected into a diabetes insipidus dog, that it inhibits a saline diuresis to lesser degree than one produced by water and that chloride concentration and output are increased. The excretion of an antidiuretic substance in the urine during morphine diuresis has been reported by Lipschitz and Stokey[124]. Release of antidiuretic hormone by large doses of morphine seems therefore likely. Since morphine has been shown to have anti-cholinesterase properties[125,126,127] the involvement of acetylcholine in the inhibitory effect has been suggested.

Hypnotics and Anæsthetics. Lack of correlation between the hypnotic and the antidiuretic action of a number of substances was first emphasised by E. P. Pick and his school[128,129]. In Bonsmann's experiments[129] on dogs, for instance, only 3 out of 11 dogs slept "deeply" after 0·5 mg. of phenobarbitone sodium per kg. had been administered, but the diuresis was inhibited in all animals to much the same degree. Similar results with the same drug were obtained by Walton[130]. In Theobald's[131] experiments with chloralose, on the other hand, satisfactory hypnosis was obtained with 0·125 mg./kg. but the renal response to water administration was almost normal. The differences between the action of various hypnotics have been explained by Pick as due to a differential depression of cortical and subcortical centres suggested also by the different order in which sensory and motor responses are influenced. The matter requires further investigation. It is complicated by the difficulty to assess depths of hypnosis in objective terms and by the observation that small doses of hypnotics lead to a state of "excitement" in experimental animals (and thus cause an antidiuretic effect) while larger doses of the same substance fail to have such an action[131,132]. Again, it can hardly be doubted that as the dosage of any hypnotic is increased, vascular and cardiac effects will be produced which will lead to impairment of renal function.

The renal mechanism by which hypnotics depress water diuresis has only been sparingly investigated. Corcoran and Page[133] found that dogs given 30 mg. of pentobarbitone per kg. have usually normal values for inulin clearance, diodone clearance and urine volumes. Occasionally, however, and for no clear reason, while systemic blood pressure, for instance, was normal or slightly elevated, "renal failure" as characterised by a marked drop in the clearance values and a decrease in urine output developed. The experiments are open to the criticism that inulin and diodone dissolved in a solution containing 0·9 per cent. of sodium chloride and 2 per cent. of sodium sulphate were intravenously infused in these clearance studies. The hypertonic solution must obviously have altered tubular water reabsorption and may indeed have influenced glomerular filtration rate. That an increase of tubular water reabsorption (mediated by antidiuretic hormone) may be mainly concerned in the antidiuresis produced in dogs by medium doses of phenobarbitone or full anæsthetic doses of pento-

barbitone sodium (amytal sodium), has been suggested by de Bodo and Prescott[132], who found in animals with permanent diabetes inspidus that water diuresis was not inhibited even when full anæsthetic intravenous doses of the three barbiturates had been administered. The antidiuresis produced by chloral hydrate, barbitone sodium and phenobarbitone sodium in rabbits is, according to Koiwa[109], mainly due to a decrease of the rate of glomerular filtration which would not be surprising considering that urine volume in this species is more liable to glomerular control than that of other adult mammals[134].

Studies on the effect of ether and cyclopropane on man and the dog give, on the whole, a uniform picture. Impairment of renal function as manifested by a decrease of glomerular filtration rate (mannitol or creatinine clearance), effective renal plasma flow and urine volume begins to be present in plane 2 of stage 3, the renal effects of cyclopropane being more pronounced than those of ether[135,136,137]. The magnitude of these effects increases with the depth of anæsthesia[136]. Some increase in the mannitol U/P ratio was noted by Burnett and his co-workers[138] and may have been secondary to the decrease of glomerular filtration rate. The depression of renal blood flow is not clearly correlated with the level of the systemic arterial pressure. It would appear to be chiefly due to neurogenic constriction of the renal arterioles since Bayliss and Brown[129] have shown in the dog that the fall in creatinine clearance and urine flow caused by ether is largely prevented by renal denervation.

Dimercaptopropanol. Earl and Berliner[140] found that the intramuscular injection of 1 ml. of 10 per cent. dimercaprol in oil inhibits water diuresis in dogs. The antidiuretic effect was absent in dogs with permanent diabetes insipidus and they suggested therefore that dimercaprol inhibits water diuresis by stimulating the secretion of antidiuretic hormone. Since intramuscular injections of dimercaprol are well known to be painful and the experiments were not controlled by injections of the oily medium in which the dimercaptopropanol was suspended, it cannot be excluded that the investigators dealt with an unspecific effect, viz., with antidiuretic hormone release due to a painful stimulus or emotional stress[17,2,141].

Rate of Inactivation of the Antidiuretic Hormone and Water Diuresis

After injection of posterior pituitary extract or vasopressin, the urine contains a substance which inhibits water diuresis[142,143,144,145,146,147] and increases blood pressure[148,149,150,181]. However, the amounts of antidiuretic and pressor substance found in the urine account only for a fraction of the dose injected. Hence there arise two possibilities, viz., first that the neurohypophysial principle is chemically modified during its passage so that an antidiuretic-vasopressor substance of weaker potency is eliminated or, secondly, that the hormone preparation is partly inactivated in the blood and in the tissues. The latter possibility is more likely to apply since it has been shown by Heller and Urban[142] and later by Jones and Schlapp[149], Larson[150], Christlieb[151] and Eversole, Birnie and

Gaunt[152] that tissue suspensions and simple saline or glycerol extracts inactivate posterior pituitary extracts. Liver, kidney, spleen, skeletal muscle, small intestine and brain were used; per g. of tissue, liver was generally found to be most effective. Defibrinated blood[142], blood rendered incoagulable with chlorazol-fast pink[149] and serum[142] inactivate the antidiuretic and pressor activities slowly; plasma[149,153,154] seems to be ineffective[149,153]. Since inactivation is prevented when the tissue extracts are boiled or heated at 65°C. an enzymatic process of inactivation was suggested[142]. Larson[150], who attempted to characterise the enzyme(s) concerned, found that the pressor factor was not inactivated when mixed with tissue extract at *p*H 9·8 or above, or at *p*H 4·5 or below. He assumed therefore that an ereptase was concerned. He stated further that aminopolypeptidase from dog intestine, kidney or liver inactivates while dipeptidase does not. Birnie[155] has recently investigated the enzyme (or enzyme system) in the liver which inactivates the posterior pituitary antidiuretic principle. He could show that the maximum activity with liver extracts is obtained at *p*H 6·5 and at 37°C., that the inactivating system is non-dialysable and that it can be precipitated by adjustment to *p*H 5·2 or by half-saturation with ammonium sulphate. Copper sulphate or zinc sulphate are effective inhibitors.

It is impossible as yet to say to what extent enzymatic inactivation of antidiuretic hormone takes place *in vivo*, though there is some indication that the phenomenon is of significance in the intact animal. This is suggested by Haynal's[156] observation that the pressor action of posterior pituitary extracts is considerably weaker after intravenous injection into animals with an anastomosis between the inferior vena cava and the portal vein and by results of Eversole, Birnie and Gaunt[152], who found that vasopressin had less of an antidiuretic effect when injected into a site with hepatic portal drainage than when introduced into the general circulation. Similar results have been obtained by Møller-Christensen[157], who compared pressor responses after intrajugular and intrasplenic injections of vasopressin.

It seems thus possible that the loss of the diuretic response to water in certain pathological states is due to decreased inactivation of antidiuretic hormone. Animals or men suffering from adreno-cortical insufficiency, for instance, cannot excrete water at a normal rate. Birnie[155] demonstrated a decrease in the vasopressin-inactivating ability of extracts prepared from livers of adrenalectomised animals, which may not be irrelevant in this connection. A similar decrease in vasopressin inactivation has recently been shown by Birnie and Blackmore[158] in the writer's laboratory to occur in the livers of mice kept on protein deficient diets. Water diuresis in such animals is markedly delayed and reduced[159,160,158].

Antidiuretic Substances in Body Fluids and Tissues

Posterior pituitary gland. Difficulties of identification and estimation of the antidiuretic hormone in body fluids have induced investigators to search for changes in the hormone content of the neurohypophysis in cer-

tain experimental and clinical conditions. The amounts of antidiuretic activity in the gland of "normal" laboratory animals[161,162,163,164,165,166,167], and man[168,169,170] are known, and seem to be of a similar order of magnitude when expressed per mg. of posterior pituitary tissue[56]. The pituitary of a desert animal, the kangaroo rat, contains unusually high amounts of the hormone[167]. Calculated per mg. of dry tissue the neural lobe of newborn infants and newborn rats contains considerably less antidiuretic hormone than that of the adult[165,170]. There may be a causal linkage between this fact and the low sensitivity of the newborn of these species to the antidiuretic principle[171,172], it may, on the other hand, be only an expression of the immaturity of the gland at birth comparable with that of the hypothalamus.

Diminution of hormone content has been reported after section of the pituitary stalk[173,174,145], after the intravenous injection of 5 per cent. sodium chloride solution[175], in subjects with diabetes insipidus[91,92,93], in a case of hæmochromatosis involving the pituitary[93], and in rats with alloxan diabetes[176]. Changes in the glands of animals subjected to experimental dehydration have also been investigated both with histological and with pharmacological methods, but the results are controversial. Some workers[164,174,177] found a decrease in the potency of postpituitary extracts obtained from rats deprived of water for various periods, but lately[176] increases in hormone content have been demonstrated. These results are not necessarily incompatible. It seems possible that a period of glandular exhaustion follows one of increased activity stimulated by a larger demand for the hormone. The significance of Chambers' report[175] that the antidiuretic potency of the rat pituitary decreases when hypertonic sodium chloride solution is substituted for drinking water is difficult to assess in this connection. Marked increases in hormone content have been claimed to occur in cases of liver cirrhosis with ascites, hypertension, cardiac œdema, diabetes mellitus and Addison's disease[92,93].

Blood. The "physiological" rate of release of the antidiuretic hormone by the neurohypophysis has been estimated in the dog by determining the minimum dose of posterior pituitary extract which, when injected intravenously, reduces the urine volume of diabetes insipidus dogs to normal values[178], the figure arrived at was 1 to 5 milliunits per hour. Verney[179] obtained a similar figure (0·06 milliunits/minute) by comparing the antidiuretic response to the intracarotid injection of sodium chloride solution with that of an intravenous injection of the same solution to which graded doses of posterior pituitary extract had been added.

Several investigators have attempted to assay blood for antidiuretic activity. The results are contradictory and confusing. This is perhaps not surprising considering that the species and the site from which the blood was obtained, the condition of the donor animal, the methods of assay and the material used varied widely. Melville[180], for example, who used alcoholic extract of venous (external jugular?) blood obtained from normally hydrated dogs, found antidiuretic activity equivalent

to 10 mU/100 ml., but Gilman and Kidd[35], using the same method of extraction, were unable to confirm his results. Hare, Hickey and Hare[145] collected blood from the external jugular vein of dogs which had been deprived of water for 2 to 4 days and injected it immediately (no coagulant was added) after removal, but failed to detect any antidiuretic action (in terms of the sensitivity of their method of assay = less than 1 mU/100 ml.). Marx[182] transfused large quantities (10 to 15 per cent. of the blood volume) of heparinised blood from the femoral artery of one dog into the jugular vein of another dog and noted an inhibition of water diuresis in the recipient animal if the donor dog had not been previously hydrated; blood from diuretic donor animals had no effect. Walker[183] collected blood by cardiac puncture from rabbits during a water diuresis, but obtained no antidiuretic response. In similar experiments with blood from rabbits which had been without food and water for from 18 to 120 hours, he observed " moderate antidiuresis " in 15 out of 32 cases. Chang and his co-workers[184] tested extracts of external jugular blood prepared according to Melville's method which they obtained from dogs in chloralose anæsthesia during stimulation of the central end of the vagus and found antidiuretic activity to be present. Ames, Moore and van Dyke[185] stimulated the neurohypophysis by intracarotid injection of hypertonic saline into dogs and found antidiuretic activity equivalent to 10 mU/100 ml. in heparinised blood from the external jugular and about 5 times as much in blood from the internal jugular. The serum of male rats injected with anterior pituitary extracts or with chorionic gonadotrophin has been reported[186] to contain increased amounts of antidiuretic activity. However, the investigators were puzzled by the observation that the antidiuretic substance, although demonstrable when injected into recipient animals, apparently did not interfere with the water diuresis of the donor animal itself.

Recent results in rats will probably help to clarify this unsatisfactory picture. In 1949 Birnie, Jenkins, Eversole and Gaunt[187] published a short paper in which they reported that the serum of normal rats in doses of 1 ml./animal, tested by injection into other rats, had an antidiuretic effect, and that this effect was enhanced by adrenalectomy. Dicker and Ginsburg[6] confirmed the presence of an antidiuretic factor in rat serum. But while the antidiuretic activity found by the American workers disappeared rapidly, Dicker and Ginsburg found that the antidiuretic titre remained unchanged in serum kept up to 18 hours. They then repeated and confirmed the finding of Heller and Urban[142] that serum inactivates vasopressin. It seemed unlikely, therefore, that the antidiuretic factor with which they were dealing was of posterior pituitary origin. The suggestion was rather that the stable antidiuretic activity observed by them originated during coagulation, since rat *plasma* was not found to have any antidiuretic action. Discussion of the discrepancies between the findings of the two groups of investigators made it clear that the techniques used differed in several respects. While Dicker and Ginsburg, for instance, collected blood by decapitation, Birnie *et al.* per-

formed heart punctures; the American workers injected the serum within a few minutes after the collection of the blood sample, Dicker and Ginsburg's sample had in most instances been collected at least 30 minutes before injection. Re-investigations of the problem[154] showed that serum prepared from blood obtained from the right ventricle or the external jugular contained an antidiuretic factor which quickly disappeared on standing, and another " stable " antidiuretic substance which was still present after 20 hours. Serum from blood collected by decapitation or from the carotid artery contained the " stable " activity only. Similarly, plasma from carotid blood failed to produce an antidiuretic effect, i.e., when assayed by a method which detects 0·4 mU of vasopressin per 100 g. of rat[4], while plasma from the external jugular gave a marked antidiuretic response. Plasma from the external jugular of sheep and mice was likewise antidiuretic. Since the external jugular vein of the rat and the sheep, but not that of the dog or man, carries the main venous outflow from the head, it seems not unlikely from these results that the labile antidiuretic substance in external jugular and cardiac serum and the antidiuretic factor in external jugular plasma is of neurohypophysial origin. This is also suggested by recent findings of Birnie, Eversole, Boss, Osborn and Gaunt[188]. These authors observed that the antidiuretic action of fresh cardiac serum was due to increased tubular water absorption, that thioglycollic acid inactivates the labile substance and that it was not present in hypophysectomised animals. However, even assuming that the antidiuretic activity in external and jugular plasma is due to the neurohypophysial antidiuretic hormone, there is no certainty that the antidiuretic activity found represents normal values, i.e., that it relates to the release of antidiuretic hormone under basal conditions. Anæsthesia is probably not an important factor[188], but at present it cannot be excluded that the removal of blood as such acts as a stimulus for increased hormone liberation. This objection applies particularly to the rat experiments in which a relatively large fraction of the animal's blood is withdrawn (see the results of Rydin and Verney, p. 611). Thus such factors as the site from which blood is collected, the volume removed, the rapidity with which it is tested, the species of animal used and the presence of other antidiuretic substances, will have to be carefully considered before any deductions concerning the quantity of antidiuretic hormone in the circulating blood can be attempted by direct estimation.

The presence of antidiuretic substances in blood, serum and plasma has been described in a number of clinical conditions: fainting[189], normal pregnancy[190], hypertension[191,192], and acute hepatitis[193]. These reports need confirmation. Marx and Schneider[194] found antidiuretic activity equivalent to 5 to 10 milliunits per 100 ml. in alcohol extracts of normal human plasma and a decrease in antidiuretic potency in the plasma of cases of pituitary obesity, no decrease in a case of diabetes insipidus and an increase in normal pregnancy. Lloyd and Lobotzky[195] reported a relative increase of unidentified antidiuretic activity in serum (relative,

i.e., to the level of diuretic corticosteroids) in Addison's disease, liver cirrhosis and premenstrual water retention, but doubted its pituitary origin. The claim[196,197] that ultrafiltrate of blood plasma obtained from cases of pre-eclamptic and eclamptic toxæmias contains increased amounts of the pituitary antidiuretic hormone has been denied by de Wesselow and Griffiths[198], Byrom and Wilson[199], Theobald[200], Hurwitz and Bullock [201], Melville[202] and Levitt[203].

Urine. Most workers agree that normal urine is devoid of antidiuretic activity. The following species have been investigated: rat[142,204,205,206], rabbit[147], cat[144], dog[145,207], and man[206,208]. These negative findings may only mean that the assay methods used were not sufficiently sensitive or that the active material was not concentrated enough or was not extracted by a suitable method. The presence of antidiuretic material in the urine of normally hydrated men, dogs, cats, rabbits and rats has actually been reported by Arnold[209] and Walker[183], but both authors supply strong evidence against a neurohypophysial origin of their material. Antidiuretic pituitary activity in large amounts seems, however, to be present in the urine of the kangaroo rat, a desert animal whose neurohypophysis is normally under pronounced environmental stress[167].

The excretion of an antidiuretic substance in the urine in a variety of experimental and clinical conditions has been reported. It was likely from previous investigations that such specific stimuli as dehydration[144,145,204,205,207,208,210,211,212,213], intracarotid injection of hypertonic salt solutions[213,214], and electric stimulation of the neurohypophysis[215,216] release amounts of the hormone sufficiently large to lead to its excretion by the kidney, but there are in many instances considerable doubts about the derivation of the antidiuretic substances which have been found in the urine in so many pathological states. Their occurrence has been described in experimental conditions like nephrogenic hypertension[206], treatment with desoxycorticosterone[217], after adrenalectomy[210], in rats on high fat[218] and on protein deficient diets[160], and in the following diseases: toxæmia of pregnancy, pre-eclampsia and eclampsia during the phase of acute water retention[219,220,221,212], acute hæmorrhage nephritis with œdema, and nephrosis[222], Cushing's syndrome[222], hypertension[206,223], congestive heart failure[224], liver cirrhosis with ascites[225,226,227,228], and œdema of starvation[229].

The identification of the antidiuretic factors found in body fluids with the neurohypophysial antidiuretic hormone is difficult not only because the endogenous principle may have different chemical and physical properties when compared with those of commercial postpituitary extracts[211,230,231,232,233,234] but also because of the probability that antidiuretic substances of non-pituitary origin are elaborated in the body. It is conceivable, for instance, that in some of the pathological conditions mentioned increased amounts of steroid hormones are released, which by promoting tubular sodium reabsorption, lead to water retention and the prevention of water diuresis. Furthermore, as shown in the following section, a number of antidiuretic substances have been found in body tissues whose status has still to be elucidated.

Antidiuretic substances in body tissues. Antidiuretic substances of hepatic origin have been described by Lampe[235], Glaubach and Molitor[236], Theobald and White[36], Walker[237], Schaffer *et al.*[221], Ham and Landis[211] and Baez *et al.*[238]. The latter workers have identified the hepatic antidiuretic substance as a definite entity, viz., ferritin (see also Mokotoff *et al.*[239]). Another antidiuretic substance—enteramine—has been extracted from mammalian gastic and duodenal mucosa and from spleen[240] and renal extracts containing renin have been shown[210,241] to inhibit water diuresis under certain circumstances. The placenta from patients with toxæmia of pregnancy contains antidiuretic material[211], extracts from normal placenta were almost inactive. It has also been reported[242] that the hormone-like substance relaxin present in serum and the ovary of pregnant sows, causes water retention in rabbits. Some of the tissue antidiuretic substances may conceivably be identical with the antidiuretic hormone or derivatives of that hormone, but the pharmacological and chemical properties of others have been sufficiently well defined to exclude this possibility.

Conclusion

Antidiuretic substances are of little therapeutic interest, but they are, nevertheless, of considerable concern to the pharmacologist: our knowledge of the chemistry of the neurohypophysial antidiuretic hormone is still very scanty, witness the conclusions reached in a very recent review[243]. After careful sifting of the evidence all the authors consider safe to state is that the specific activities of the neurohypophysis is likely to be due to closely related polypeptides of a molecular weight of about 2000. However, it is not known whether these polypeptides may occur separately in the body or whether they are circulating and are excreted as a single protein molecule. As long as this lack of knowledge of the chemical nature of these principles obtains, only the crudest chemical tests (resistance to acids and alkali, thermolability) help in their identification. The main burden falls on attempts of identification by pharmacological methods, though even careful application of these methods can only reveal probabilities and not certainty. Even so there is no cause for pessimism. Recent work has gone far in showing which chemical, physical and emotional stimuli are likely to release antidiuretic hormone from the pituitary, though stricter criteria (see p. 616) will have to be applied to verify such stimulant action for a number of drugs for which it has been claimed. The search for antidiuretic activities in blood and urine is proceeding so vigorously that the many results reported cannot be summed up concisely; each piece of work has to be judged on its very different merits.

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