# ANTIDIURETIC SUBSTANCES

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In this review the discussion will be limited to a consideration of those antidiuretic substances which are found in animal tissues and body fluids. The qualification antidiuretic, as used here, relates to those substances which cause water retention either by an action in the kidney or on other tissues. For the purpose of discussion, antidiuretic substances have been classified in four groups as shown in the table of contents. For the sake of brevity the letters ADH have been used in some places where "posterior pituitary extract containing vasopressor and antidiuretic fractions" would have been more accurate.

## *Detection and Assay*

Since reliable methods of detection and assay are essential for the identification and proper understanding of the action of antidiuretic substances, a short

account follows of the methods available. So far, owing to our ignorance of the nature of most antidiuretic substances, biological assay is necessary. All the methods depend on testing tissue extracts or body fluids from one animal on a second test animal(s) which is in a state of water diuresis. Most often the method of Burn (23), or a modification of it (80), has been used. In this method observation is made of the time taken for a group of rats to excrete an orally given volume of water with or without the subcutaneous injection of the material to be tested. The unknown substance can also be administered intraperitoneally (12). Instead of rats, mice have been used (166). It seems, however, that mice are not wholly satisfactory, since their control rates of water excretion are less regularly reproducible than those of rats. Bigger animals such as rabbits and dogs have also been used, and have the advantage that both standard and unknown can be compared on the same animal, and that fewer animals are necessary (16, 36, 95, 143, 170, 173, 246). Recently, Jeffers, Livezey and Austin (121) have described a sensitive rat method in which the substances to be tested are given intravenously during ethanol narcosis, and in which standard and unknown can be used on the same animal during the course of a single set of observations. Man, too, has been used as a test animal (18). The sensitivity of the tests varies greatly. The rat method of Burn detects about 20 mU. of antidiuretic activity, that of Jeffers, Livezey and Austin 0.02 mU. In the rabbit 0.2 mU. can be detected (246), and in the dog 0.1 mU. (170). The methods mentioned were specifically designed to measure the activity of the antidiuretic hormone of the posterior lobe of the pituitary (ADH). Occasionally discrepancies have appeared that can be accounted for only by assuming that antidiuretic substances are made in tissues other than the posterior lobe (7, 246), or that the test method was unsuitable. At least in a number of instances the latter was true. Body fluids may contain substances which, on subcutaneous injection, "augment" the action of antidiuretic hormone by stabilising it, or causing it to be more slowly absorbed (167). Sometimes, too, subcutaneously or intraperitoneally injected material causes pain, and both pain and excitement, as will be shown later, may lead to antidiuresis (239). As an example of the confusion that may arise from the use of unsuitable test methods may be cited the finding that rat serum obtained by heart puncture was more actively antidiuretic than serum collected from the carotid artery (81). The sera were tested on rats and administered subcutaneously. Further difficulties of interpretation arose from the finding that carotid plasma was without antidiuretic effect, and that there appeared to be two antidiuretic substances, one stable and the other unstable (81). Ames and van Dyke (4), using the method of Jeffers, Livezey and Austin, *i.e.*, intravenous administration of the test substance, obtained consistent results that were easy to explain. They could find no ADH in rat blood collected by decapitation provided that the animals were tranquil until the moment of death, but could always detect ADH in serum collected by heart puncture or under ether anaesthesia, even if the rats had first been hydrated. The ADH concentration was identical in serum and heparinised plasma. Under no conditions of blood collection could ADH be detected if the rats had first been hypophysectomised. Thus it is evident that

great care must be taken to control the circumstances in which body fluids are collected, and, as far as possible, the material to be tested should be injected intravenously (49) rather than subcutaneously or intraperitoneally, since it is then less likely to evoke secretion from the test rat's own posterior lobe.

### 1. SUBSTANCES ACTING DIRECTLY ON THE CELLS OF THE RENAL TUBULES

There is one tissue substance that is now generally believed to be antidiuretic by virtue of a specific effect on the cells of the renal tubules, namely, the antidiuretic hormone of the posterior lobe of the pituitary. The evidence for the site of origin of ADH, that it may indeed be considered a hormone, and that it is antidiuretic and not diuretic as was thought at first, has been given in previous reviews (67, 97, 169, 178, 240), and therefore will not be repeated.

There have been suggestions that ADH has mainly an extrarenal action. Two of these suggestions can quickly be discarded.

#### *The effect of ADH on the absorption of water from the alimentary canal*

Several workers have shown that posterior pituitary extract does not prolong the emptying time of the stomach (26, 39, 105, 186), nor delay absorption from the intestine. Thus, delayed absorption cannot be considered a factor in the antidiuretic effect of ADH.

#### *The effect of ADH on the ureters*

That the ureters go into spasm after posterior pituitary extract administration has been put forward as an explanation of its antidiuretic effect (148, 150). This may, in part, account for the action if fairly large doses of extract are given, but is certainly not the whole or most important reason. Ross and Stehle (192) could find no spasm, and antidiuresis always occurred in the experiments of Verney (238) on the isolated kidney, and of Klisiecki, Pickford, Rotschild and Verney (126), and of Pickford (176) on the conscious dog, in which a cannula had been inserted whose tip lay in the pelvis of the kidney, thus eliminating all possibility of ureteral obstruction to the flow of urine.

#### *The effect of ADH on other extrarenal sites*

Molitor and Pick are the chief protagonists of the view that ADH acts extrarenally. They and their co-workers published a series of papers in which they believed they had shown that posterior lobe extract acts either on a water control centre in the central nervous system, or on the water-binding capacity of the tissues generally (161–164). Theobald (222) could not repeat, and criticised their work, and Janssen (120) showed by means of spinal cord and nerve sections that the antidiuretic action of posterior lobe extract does not depend on the integrity of the central nervous system. Buschke (26), following a different line of investigation, found that in nephrectomised rabbits the administration of posterior pituitary extract raised the blood chloride concentration. He concluded, therefore, that posterior pituitary hormone acts on the tissues to release chloride, and that this, in turn, affects the excretion of water. There is no evidence that with moder-

ate water loading physiological amounts of ADH cause tissue hydration. On the contrary, v. Konschegg and Schuster (129) were the first amongst many to show that during antidiuresis posterior pituitary extract increased the water content of the blood. This observation has been confirmed in various animals, including man (15, 39, 190, 237). In dogs, for instance, pituitary antidiuresis may give rise to marked salivation (222). Thus, the presentation of insufficient water to the kidneys cannot be the cause of pituitary antidiuresis. There is further evidence of direct renal action in that the polyuria of the isolated perfused kidney is lessened if the kidney is supplied with posterior pituitary extract (210). This point was strikingly shown by Verney (238), who included a head in the perfusion circuit. Provided that the pituitary gland was present in the head the polyuria of the isolated kidney was reduced, but if the gland had been removed the polyuria persisted. The weight of evidence is all in favour of the antidiuretic effect of posterior pituitary extract being a direct renal one. To say this is not to exclude the possibility that extracts may in addition, and under some circumstances, have an action, perhaps nonspecific on the tissues (149, 213, 232, 259).

*Site of action of ADH in the kidney*

Accepting that the antidiuretic action of ADH is renal, it is then necessary to seek for the exact site of action in the kidney. The site certainly varies with the type of animal observed. Below the order Reptilia posterior pituitary extract is not antidiuretic. In reptiles, using alligators, Burgess, Harvey and Marshall (21) found that pituitary antidiuresis was accompanied by a fall in glomerular filtration rate (GFR), with no suggestion of increased water reabsorption in the tubules. In chickens the same observers found that antidiuresis could be induced with only a small change in GFR, though with larger doses of pitressin the filtration rate was markedly depressed. For rabbit, rat, dog and man there is a mass of evidence, both direct and indirect, that antidiuresis occurs without change in the vascular relations in the kidney. Direct measurements of renal blood flow have been made by means of the Rein thermostromuhr, and no change from normal was found as the result of administering ADH to conscious dogs (94, 244). Walker, Schmidt, Elsom and Johnston (248), observing the effect of ADH in both dogs and rabbits, found no parallelism between renal blood flow and urine flow, and concluded that the changes they observed in the latter depended on alterations in the activity of the tubule cells. Indirect evidence has been obtained by means of renal clearance measurements, and has given no cause to believe that pituitary antidiuresis is necessarily associated with changes in GFR or renal plasma flow (RPF) (199, 206). Lamport (138), by calculations based on his own results and those of others, found that in pituitary antidiuresis there was no consistent change in intracapillary pressure or effective arteriolar resistance in the kidney of the dog. A few observers have tried to obtain information about the action of ADH by measuring renal oxygen consumption under various conditions. The earlier work is difficult to interpret as it was done on isolated kidneys or anaesthetized eviscerated animals (65, 127). More recently, Cutting and McCance (42, 43) observed the metabolic rate of kidney slices from adult

and infant rats and noted that posterior pituitary extract increased oxygen consumption of adult kidney, but was almost without action on infant kidney slices. If we allow that ADH has an action on the kidney and causes it to do work, these facts correlate well with the known ineffectiveness of ADH as an antidiuretic in young infants.

It may safely be concluded that in mammals, when ADH is used in physiological amounts, its effect is due to a specific action on the tubule cells of the renal epithelium.

*The role of the renal nerves in the action of ADH*

Motzfeldt (165) believed that the antidiuretic action of posterior pituitary extract was renal in origin, and that the sympathetic nervous system played some part in the reaction. He made his observations on anaesthetized rabbits. Later observers, using unanaesthetized animals, have not been able to show that the peripheral sympathetic nerves are in any way concerned in the action of ADH (64, 126, 194, 223). For example, Samaan (194) made his observations on conscious dogs whose two kidneys had been separately cannulated, and one of which had been denervated by stripping the walls of the artery and vein and separating the kidney from all its attachments except the main blood vessels and ureter. In this well controlled experiment he could find no difference in the response of the two kidneys to ADH.

*The action of ADH on the tubules*

Burgess, Harvey and Marshall (21) pointed out that birds are the lowest of the vertebrate series to respond to ADH by a reduction in the rate of urine flow without the necessary accompaniment of a marked reduction in GFR, and that they were also the first animals to show a thin segment in the loop of Henle. These authors therefore made the interesting suggestion that the thin segment is the site both of water reabsorption and of the action of ADH. As the thin segment looks admirably adapted to the transfer of water these suggestions were accepted and are often quoted as though both hypotheses were proven. Further, it was noticed that in certain desert rodents the thin segment is longer than in related species living near ample water supplies (128, 208). The idea of Burgess *et al.* is attractive, but should not be allowed to close the mind against other possibilities. With regard to the observations on birds, a close examination of the evidence shows in how many ways the kidney of the bird differs from that of dog and man. In the first place, relatively large amounts of ADH have to be used to produce antidiuresis, the GFR is extremely variable, and there is a distinct tendency for it to rise during water diuresis and to fall during hormonal antidiuresis. Further, the onset of diuresis after the administration of water by stomach tube may show the typical mammalian latency (126) between the peak of water load and the peak of diuresis, or there may be no latency at all. Again, ADH reduces the rate of urine flow even in dehydrated chickens excreting an already concentrated urine (130). Finally, the thin segment of Henle's loop is present to a variable extent in different birds and is always short (114). It has to be remembered, too,

that birds and mammals are separately developed branches of the vertebrate tree and that hormones common to both may not have been put to the same use, as has happened with oxytocin in the Amphibia (102, 211). Thus, in birds, the thin segment may well be concerned with the reabsorption of water, but that is not to say that it is also the site of action of ADH. Deductions made from observations on rats can perhaps be more easily transferred to other mammals; but, again, rodents are in many ways highly specialised. Ames and van Dyke (3) found that the kangaroo rat, an inhabitant of desert country, had relatively more ADH in its pituitary gland than the common laboratory rat, and excreted absolutely more ADH into its urine. Thus, desert rats may have an increased supply of ADH and longer thin segments for it to act on (128, 208), or they may have a double and unrelated protection against water loss. That such animals do not rely on their kidneys alone for water conservation was shown by Schmidt-Nielsen and Schmidt-Nielsen (197), who found that they lose only half as much water through the lungs as do most rodents.

Although in the thin segment the cells are flat and have an inactive appearance this does not, of itself, prove that they have no power to do work. It has been shown that the epithelial cells in the gills of eels and some other teleosts are able to transfer water and chloride from blood to seawater against a high osmotic pressure and, moreover, that the chloride and water are conveyed independently of each other (125, 196). However, the working cells appear to be large ones with central nuclei at the base of the leaflets, and are unlike the flat respiratory epithelium (125a). There is here, then, not even an indirect argument for the hypothesis that the thin segment of the loop of Henle does secretory work. In general, the cuboidal cells of the distal tubules appear much more likely to take an active part in the formation of the final urine.

Wirz, Hargitay and Kuhn (256) looked for the site of urine concentration by means of cryoscopic observations on kidney slices from rats. They found that the osmotic pressure of the kidney rose steadily from the outer border of the cortex to the tip of the papilla. They interpreted this as meaning that tubular urine was concentrated in the descending limb, diluted again in the ascending, and finally concentrated in the collecting duct. These findings suggest the importance of two parts of the tubules in the concentration of urine. This work is, however, open to the criticism that diffusion may have occurred from, say, the highly concentrated urine in the collecting ducts to the relatively less concentrated solution in the loops of Henle. This point may be settled by work now in progress on golden hamsters, in which the papilla protrudes into the hilus of the ureter, so that both capillary blood and urine from specific tubules can be collected from that part of the kidney (Wirz, personal communication).

Smith (207) discusses the results of some observations (252) on the response of dogs to osmotic diuresis with and without pitressin infusion, and shows that they could be interpreted as indicating that the function of the thin segment is the passive reabsorption of water so that the distal limb receives urine isosmotic with the plasma, though not of the same composition, many constituents having been absorbed in the proximal tubule (248). The distal tubule is, then, responsible

for the final adjustment of urine composition by the reabsorption of Na and water, its activity being hormonally controlled. In the case of water the hormone concerned would be ADH.

There is yet another possibility; Ljungberg (146) by means of a 3 mm.-diameter punch collected radial cylinders from the kidneys of rabbits and examined them in serial section for their Cl content at various levels from the surface. After making allowance for the fact that all varieties of tissue in any section were included in the final Cl estimate, it was found that there was least Cl at the junction of cortex and medulla, that the epithelium of the proximal tubules contained 99–111 mg./100 g. and that the cells of the collecting ducts contained 2,500–2,700 mg./100 g. The deduction made was that the collecting tubules were actively concerned in Cl reabsorption. However, a different interpretation is possible, namely, that a high Cl concentration in the distal and collecting tubules is linked with the final stage of water reabsorption (207). If this is the case, then possibly, the cells of the collecting ducts are one of the sites of action of ADH.

It is evident that, with the information at present available, it is not possible to make any final statement as to the exact point of action of ADH. Fairly certainly the action lies beyond the proximal tubule. It may influence reabsorption in the thin segment; but, more probably, it acts on the cuboidal cells of the distal tubules. Lastly, there is the possibility that ADH may have some effect on the cells of the collecting ducts.

### *Concentration of posterior lobe extract necessary to produce antidiuresis*

In the alligator, Burgess *et al.* (21) found that 5 mU. pitressin/kg. given *intramuscularly* produced a perceptible diminution in the rate of urine flow. This dose, which in the alligator depends for its action on a decreased GFR, may be compared with the effective *intravenous* dose in other animals, in which its action is on the epithelial cells of the tubules. In a dog of 12–15 kg. a marked inhibition of appreciable duration may follow the injection of 1 mU. posterior lobe extract (171). Transient inhibitions follow the intravenous injection of as little as 0.1–0.2 mU. (170, 176, 222). Thus, in the dog full inhibition could be produced by about 0.08 mU./kg. intravenously. In rats 0.4 mU./100g. is effective, and in mice the minimum effective dose is 0.05 mU./20g. (104). In these rodents, then, relatively more extract must be used than in dog or man. Hart and Verney (98) calculated for man that, of hormone released normally from the posterior lobe, the concentration in the plasma which causes partial inhibition of maximum water diuresis is less than one part in $1.66 \times 10^{-10}$.

### *The rate of ADH release from the posterior lobe*

Attempts to measure directly the normal output of ADH are hampered by the small amount of hormone that need be present in the blood to inhibit urine flow, and also by the difficulty of obtaining blood direct from the gland. Observations have frequently been made on dehydrated animals in which ADH concentration is likely to be high, and therefore easier to estimate. Often, measurement is made of the quantity of ADH in urine collected over a number of hours. This

method has the advantage that samples for assay are easy to collect; also it has been shown that 20–30% of hormone added to the circulating blood was excreted into the urine, where it is fairly stable (122). By such means it has been estimated that dehydrated rats excrete 80 mU. in 24 hr., and that in 6 hr. after NaCl administration rats produce 10–20 mU. of urinary ADH (78). In the urine of severely dehydrated cats 20–400 mU. were found (117). Since none could be found in similar circumstances after hypophysectomy, the pituitary origin of the antidiuretic material was indicated (118). During water diuresis in dogs the amount of ADH liberated by a brief emotional stimulus is about 5–10 mU. (168, 170). Since in dogs 1 mU. is sufficient to cause maximum inhibition of urine flow, it is evident that in the absence of a heavy water load less than this amount in the circulation will be enough to maintain urine flow at the resting rate. Hare, Hickey and Hare (95), using both normal dogs and those with diabetes insipidus, came to the conclusion that usually there was less than 1 mU./100 ml. blood present in the circulation. By giving continuous intravenous infusions of ADH to dogs with diabetes insipidus Shannon (199) found that 1–5 mU./hr. caused the maximal antidiuretic action. Greater amounts than this did not produce a greater antidiuresis. Probably, then, in the normal dog, the release from the gland of 5 mU./hr. would be sufficient to maintain a low rate of urine flow. About the same rate of release seems to be effective in man (139). Normal subjects were kept in a state of full hydration in order to depress endogenous ADH production, and long-continued intravenous infusions were given of varying concentrations of pitressin. For the maximal effect the amount of hormone needed in man was 5 mU./kg./hr.

Measurements have been made of the amount of hormone found in the gland in different animals and man (3, 32, 101–103, 107, 134, 204, 205). These estimates are interesting; for instance, the gland of the newborn human infant contains 20% lessA DH/mg. dry gland than that of the adult. This information, however, tells one nothing of the rate of release of the hormone into the circulation.

### *The disappearance of ADH from the blood*

If, during water diuresis in the dog, 0.5 mU. is injected intravenously its inhibitory effect will have disappeared in 30–40 min. (222). It has also been shown that intravenously injected posterior lobe extract disappears rapidly from the blood and is in part, at least, bound by the plasma proteins and the tissues (106, 122, 221). The bound hormone is, however, antidiuretic on intravenous administration into a hydrated animal, so that the binding does not render it inactive. Possibly, too, there is enzymatic destruction of ADH, especially in the liver (11, 106).

### *Form and composition of ADH as released from the posterior lobe*

As yet it is uncertain in what form ADH is released from the posterior lobe; it may be as a separate hormone, or as part of a large protein molecule whose other portion contains the oxytocic hormone (1, 236). The antidiuretic and oxytocic principles have been prepared separately in highly active form although

each of the final fractions was contaminated with the other to the extent of about 1% (123). Against this, there is evidence that by using the gentlest possible methods of chemical concentration and by ultracentrifugation all the active substances concentrate to the same extent (93, 191, 236), suggesting the existence of a single large molecule from which active fractions break off. In man and rat Simon and Nagy (205) and Simon (203) found that the oxytocin and vasopressin content of the glands generally ran parallel. Vaichulis (235) noticed that hormones from autolysed glands showed a greater solubility than those from fresh ones. Both the observations favour the idea of a mother substance. As Stehle (212) points out, even if the gland elaborates a single hormone with multiple activities it does not necessarily mean that that is the form to which the physiological actions are due. If a parent molecule exists it is not known how it is broken into the separate parts. A difficulty in accepting the unitary idea is the report that the various hormones do not occur in the same proportion in all species (74). Recently, too, Cross (41a) has shown in diuretic rabbits that 0.4–1.0 mU. posterior pituitary extract causes an antidiuresis resembling that seen on suckling, whilst the "let-down" of milk, which most workers agree is due to the oxytoxic factor, requires the injections of 50–200 mU. These facts strongly suggest that the two hormones can be independently released from the gland.

It is generally believed that the pressor fraction and ADH are one and the same. Certainly they seem to behave in much the same way towards chemical reagents. Heller (100), however, found that ADH was more stable than the pressor fraction over a wide range of pH, and he prepared solutions with considerable antidiuretic potency, but little pressor action.

The pressor-antidiuretic fraction of posterior lobe extract appears to contain the following amino acids; cystine, tyrosine and perhaps arginine (119, 183, 212, 242).

### 2. SUBSTANCES WHOSE SITE OF ACTION IS IN THE CENTRAL NERVOUS SYSTEM

Water comes within the definition of a substance found in living tissues, and its concentration in the body is obviously of first importance in regulating the output of ADH. The subject is a vast one and is discussed in a number of books and articles (67, 169, 178, 207, 213). In this place no more than a brief summary of recent information will be given. The quantity of ADH liberated from the posterior lobe depends on the water load of the body (176). The electrolyte concentration of the blood also depends on the water load. Changes in osmolarity of the blood stimulate or inhibit as yet unidentified receptors in the hypothalamus (239, 240). The stimulus, on reaching the supraoptic nuclei, is transmitted down the pituitary stalk by the nerve fibres arising from the cells of these nuclei, and causes the release of more or less ADH by the posterior lobe of the pituitary (58, 67, 178, 179).

However, it is evident that control of ADH production depends also on factors other than water concentration. Urine flow may be reduced at times when it would seem physiological for it to increase or remain high. For instance, an emotional stimulus occurring during the course of a water diuresis results in a

diminished rate of urine flow despite the still high water load (168, 170, 171). Certain sensory stimuli also inhibit urine flow during water diuresis (222). Brief exercise, in so far as it has an emotional content, is also inhibitory (193, 239, 240). Finally, the onset of water diuresis or the inhibition of water excretion may be initiated by conditioned reflexes or hypnosis (27, 28, 59, 86, 110, 140, 155, 156). Thus, processes occurring within the central nervous system, either in the cerebral cortex or in lower centres, may influence ADH release. It is, therefore, interesting to examine by what means these internal stimuli are transmitted to the posterior lobe.

### *Acetylcholine*

Two substances are known that are chemical transmitters for at least some peripheral nerve endings, namely, acetylcholine (ACh) and noradrenaline. As regards noradrenaline, there is so far no evidence that it is a central transmitter at synapses on the path to the posterior lobe, though sympathin is found in the hypothalamus in higher concentration than elsewhere in the central nervous system (243). There is growing evidence that ACh is a central transmitter, particularly to the supraoptic nerve cells (58, 66). In 1924 Molitor and Pick (162) drew attention to the fact that choline HCl administered intravenously, delayed the onset of water diuresis. They also remarked that its ester, ACh, was antidiuretic on intravenous, but not on subcutaneous, injection. They ascribed the effect of choline to a change in water distribution. Dikshit (52, 53) found that the injection of ACh into the cerebral ventricle of cats produced effects similar to electrical stimulation of those parts. This latter central action of ACh suggested that perhaps this substance might play a part in the liberation of ADH, and experiments were made on the effect of ACh on urine flow. It was shown that in conscious normal dogs the intravenous injection of ACh during the course of water diuresis resulted in a temporary reduction in the rate of urine flow, but that no inhibition occurred if the posterior lobe of the pituitary had previously been removed (177). An intact pituitary gland, then, was necessary for the antidiuretic action of ACh. In later experiments on dogs under light chloralose anaesthesia, an attempt was made to locate the site of action of ACh by injecting it in a small volume of 0.9 % NaCl solution direct into one or other supraoptic nucleus (179). As a result of this proceeding an established water diuresis could be inhibited, provided the posterior lobe was intact. Injections made into other parts of the hypothalamus were without effect on the rate of urine flow. From these observations it appeared possible that ACh was a transmitter to the supraoptic cells. This explanation became highly probable after prolonged observation of dogs into whose supraoptic nuclei diisopropylfluorophosphonate (DFP) had been injected (58). In these animals after several days of polyuria the daily level of fluid exchange returned to normal, but intravenously administered ACh was without effect on the rate of urine flow for as long as 89–139 days after DFP injection. Towards the end of this number of days there was a period when ACh was sometimes effective and sometimes not. After this length of time ACh resumed its normal inhibiton of the rate of urine flow, as always without altera-

tion in either GFR or RPF (58). In confirmation of the central action of ACh was the observation that if it was injected into the carotid artery rather than into the malleolar vein, far smaller doses were effective in reducing a diuretic rate of urine flow (181). Lastly, nicotine is antidiuretic in normal animals and man, but not in those with diabetes insipidus (22, 25, 33, 144, 219). Thus, there is good reason to believe that ACh is a substance concerned in controlling the output of ADH owing to its transmitter action on the cells of the supraoptic nuclei.

*Adrenaline*

The part played by adrenaline (or noradrenaline) in the central control of ADH release is complex and its physiological function uncertain. Observations on the emotional inhibition of water diuresis in conscious dogs (171, 239, 240) made it clear that endogenously liberated adrenaline, or adrenaline injected intravenously one half minute before the occurrence of the emotional stimulus, prevented the appearance of the expected antidiuresis. The lack of an antidiuretic response after adrenaline was not due to renal insensitivity to ADH. The action seemed to be a central one and, moreover, independent of changes in systemic blood pressure. Since it has been shown that in the periphery, under certain conditions, ACh and adrenaline are antagonistic (19, 20, 147, 153) there was the possibility that centrally, also, adrenaline interfered with the action of ACh. It was found that if 10–15 $\mu$g. adrenaline was injected intravenously just before, or with, a dose of ACh sufficient to decrease a diuretic rate of urine flow, then, on about half of such occasions the antidiuresis failed to appear (57). As ACh alone is rarely ineffective this again suggested a central action of adrenaline, perhaps even on the supraoptic nerve cells. If, instead of giving the drugs intravenously, they were given by intracarotid injection, 2 $\mu$g. adrenaline given 11–38 sec. before 0.2 mg. ACh regularly prevented the ADH-releasing action of the latter (Pickford and Watt, unpublished work). Thus, both ACh and adrenaline are concerned with the central control of ADH release. In all probability ACh acts by transmitting impulses to the supraoptic nuclei. The mode of action of adrenaline has yet to be determined. It may directly interfere with the physicochemical reactions of ACh, or there may be local capillary sensitivity to adrenaline, so that the vessels surrounding the supraoptic cells constrict and ACh is prevented from reaching its site of action, or lastly, adrenaline may stimulate other neurones having an influence on the supraoptic cells.

*Oxygen lack and carbon dioxide excess*

It has been shown that both anoxia and hypercapnia may, by an action on the central nervous system, alter vascular conditions in the kidney and hence the rate of urine flow. In conscious men and animals anoxia generally causes polyuria (202), but in anaesthetized animals oliguria results from severe anoxia, though polyuria may be seen if the anoxia is mild (10, 215). Although it is possible in anaesthetized animals to simulate renal anoxia by the administration of large amounts of adrenaline (230), nevertheless, the adrenal blood vessels can be ligatured and the anoxic response induced as before. The adrenals, therefore, are

not necessary for the appearance of the reaction. Intact renal nerves are, however, essential if the antidiuretic effect of anoxia is to be seen. After denervation of the kidneys the rate of urine flow passively follows the systemic blood pressure, but with intact renal nerves anoxia causes a reduction in renal blood flow and the kidney becomes pale and shrunken (230). Possibly there are chemoreceptors in the spinal cord. Franklin, McGee and Ullmann (69) found that the anoxic response of the kidney occurred as usual both after decerebration and, in one instance, after transection of the spinal cord at D4. They did not suggest that the response is always initiated at this level, only that it can occur when these centres alone are intact. There is some evidence that there are cerebral cortical centres which may influence renal blood vessels. Hoff, Kell, Hastings, Gray and Sholes (108) found that prolonged stimulation of the proreus and sigmoideus gyri and orbital surfaces of the cerebral cortex induced a marked renal ischaemia, but they could not suggest under what circumstances these centres would go into action. The last observations were made on cats.

Hypercapnia causes anuria in both dogs and rabbits, even after generous hydration, but if the kidneys are denervated urine is still excreted slowly. In rabbits the surface of the kidney can be seen to pale (55, 69). Draper and Whitehead (55) tried to determine whether the anuria could be accounted for by diversion of the renal blood supply from cortex to medulla. They examined frozen sections of the kidneys of rabbits, into whose blood stream mercuric sulphide had been injected. They found that after denervation the cortical glomeruli of the hypercapnic kidney were clearly visible and must have had a circulation of blood. In the innervated kidney the cortex was pale and, judging by the photograph published, so also were the medulla and the juxtamedullary region. There must, then, have been a closing down of the circulation through the whole kidney. In dogs they were not able to obtain a contrasting picture between innervated and denervated kidney. The analysis of the mode of action of central anoxia and hypercapnia is complicated by the number of centres that may be thrown into activity or inhibited, such as adrenal medullary centres, centres linked with the adrenal cortex or pituitary, and the vasomotor centre as a whole.

### 3. SUBSTANCES AFFECTING THE CIRCULATION OF BLOOD THROUGH THE KIDNEY

Under this heading are included substances acting directly on the blood vessels, or on the nerve endings supplying the vessels.

Richards and Plant (188) and Dreyer and Verney (56) were amongst the first to point out that intrarenal blood pressure is largely independent of systemic blood pressure. It cannot, therefore, be expected that systemic blood pressure changes, unless considerable, will have much effect on the production of urine. Far more important from this point of view, is what happens in the kidney itself. The production of urine depends in the first place on filtration from the glomeruli into the capsular spaces, and for this purpose the minimum pressure necessary seems to be about 75 mm. Hg (255). Any differential reactivity of the vasa efferentia and afferentia of the glomeruli may cause a change in the rate of urine

flow owing to a change in the effective filtration pressure. Further, if the blood supply to some glomeruli is cut off whilst that to others supplying a different or similar type of nephron opens, then again the rate of production and the composition of the final urine may alter.

The substances to be considered in this section are adrenaline, (and noradrenaline), histamine, adenylic acid derivatives, renin and hypertensin, and vasopressin.

### *Adrenaline and noradrenaline*

The injection of adrenaline may lead to either polyuria or oliguria according to the dose and route of administration. In intact man, dog, rabbit, rat and guinea pig the intramuscular or subcutaneous injection of adrenaline results in polyuria. There is also polyuria if the drug is given slowly or in small doses by the intravenous route (41, 73, 111, 145, 229). Thus, polyuria follows if the blood concentration of administered adrenaline is low. With high blood concentrations the rate of urine flow is reduced. The oliguric action is easily seen following a single intravenous injection, when the inhibition is fleeting, or continuous intravenous infusions (57, 177, 193, 222, 223). Unless the kidneys have been denervated there is no correlation between systemic blood pressure and urine flow. Therefore, if the rate of urine flow is seen to change, it is probably because the adrenaline has had an intrarenal effect. There is no evidence that it has any specific action on the cells of the tubules. Richards and Plant (188), observing that in the perfused rabbit kidney adrenaline increased both the resistance to perfusion and the volume of the kidney, suggested that the polyuric effect was due to increased filtration brought about by constriction of the efferent arterioles of the glomeruli, accompanied by dilatation, or no change, in the afferent arterioles. Winton's (254) observations on the isolated kidney of the dog seem to bear out this suggestion. He found that low concentrations of adrenaline caused an increase in the rate of urine flow accompanied by only a slight reduction in the blood flow, and an increase in filtration pressure. Greater concentrations of adrenaline decreased the rate of urine flow with a fairly marked reduction in blood flow and glomerular pressure. The reduction in renal blood flow has also been observed in intact animals by the use of the thermostromuhr (99, 209, 234). Smith (207), citing unpublished work of Gomez, thinks that adrenaline diuresis cannot be explained by a preferential efferent vasoconstriction. On the basis of clearance observations made in man he believes that adrenaline constricts the venules, and so prevents water reabsorption. Also the venular constriction, by altering the interstitial pressure and water content, would explain the increase in volume noticed by Richards and Plant. Adrenaline antidiuresis is easily explained by a general vasoconstriction of the smaller renal arterioles, and venules according to Smith (35, 89, 229, 254).

Clearance measurements show that with doses of adrenaline sufficient to produce oliguria (about 2$\mu$g./min.) both GFR and RPF are reduced in dogs (182). Some workers believe that denervation of the kidneys sensitises them to the action of large doses of adrenaline (133, 152). Others have not observed this phenomenon (182).

Eränkö and Karvonen (60) find that if large (0.5 mg.) doses of adrenaline are given intravenously to conscious dogs a biphasic antidiuretic effect is seen, the second part of which is long-lasting and takes 20 min. to reach its maximum. Urine collected during the antidiuretic phases contains an antidiuretic substance, as judged by its effect when injected intravenously into the dog from which the urine was collected. The authors think that large doses of adrenaline cause the release of ADH from the posterior lobe. The work is still in its early stages. Possibly, the renal ischaemia that must follow the use of such large doses of adrenaline is the cause of the prolonged antidiuresis. Verney and Vogt (241) noticed that prolonged antidiuresis could result from a 5–120 sec. compression of the renal artery in the conscious dog, and that the antidiuresis was associated with hypertension in unilaterally nephrectomized animals.

From the information available it seems that noradrenaline acts on the kidney more powerfully than adrenaline, but in the same way, at any rate as far as the excretion of water is concerned (24, 182). In rats noradrenaline increases absolutely the excretion of Cl, a phenomenon not seen after the administration of adrenaline (61, 111).

### *Histamine*

A consideration of the action of histamine on the kidney is important since it has repeatedly been shown that histamine is released into the circulation from all injured tissues, whether the injury is mechanical or due to allergy, and that the liberated histamine can cause profound disturbances at many sites.

Most observations on the kidneys have been made after subcutaneous administration of histamine, and the results vary. In man, any antidiuretic effect of 0.15–0.7 mg. is generally slight or absent. The subjects examined were either normal, hypertensive or suffering from diabetes insipidus (75, 187, 250). In dogs Molitor and Pick (162) found that antidiuresis followed a total dose of 1 mg., whereas Theobald and White (223) observed no renal action after a total dose of 0.5 mg. Gilman and Kidd (79) gave 2.5 μg./kg. intravenously to dogs and did observe antidiuresis. The writer has noticed slight or sometimes marked antidiuresis follow the intravenous injection of 20 μg. into a dog of 20 kg. weight. The same variability in response has been noticed in rabbits (104, 162). Most workers agree that the changes in renal function are not directly dependent on the fall in systemic blood pressure that occurs. Reubi and Futcher (187) measured the PAH and mannitol clearances in man after subcutaneous histamine and found that in normal subjects both GFR and RPF fell. This, they believe, was due to afferent arteriolar constriction. The renal volume changes seen by Dale and Laidlaw (44) could also be interpreted as due to arteriolar constriction. Bjering (14) found in man that creatinine and urea clearances fell, and thought that both glomerular and tubular vessels constricted. It is evident that the exact mode of action of histamine is not settled. In man histamine induces general arteriolar constriction, unlike adrenaline the response to which shows regional differences. This fact, with the evidence mentioned, makes it reasonable to suppose that histamine induces arteriolar constriction in the kidney. The injection of histamine also causes the release of adrenaline from the adrenal medulla

and this may influence the final result, either by adding to the constrictor effect or, in smaller amounts, by partially counteracting the histamine antidiuresis.

There are some data on the effects of certain types of shock on the kidneys of dogs. After acute haemorrhagic and traumatic shock PAH clearances are normal until the RPF falls very low (37, 174). At first sight this might suggest that histamine is not a factor in causing the oliguria of shock, but these observations were acute ones made on anaesthetized dogs, whereas in man death tends to occur a few days after injury. Chronic experiments show that the kidney of the dog is capable of recovery if its circulation has not been cut off for too long, or too frequently in a short space of time (37, 174). Dog's kidneys seem to be more resistant than those of man, in whom a relatively short-lived ischaemia may be so damaging to the kidney that its functional recovery is too slow to permit survival of the rest of the organism. Cort and Barron (38) found that in cats the anuria of trauma could be relieved by the injection of local anaesthetics into the splanchnic nerve or the spinal roots supplying the kidney. It is possible, then, that ischaemia and anuria following injury may be partly reflex in origin and partly due to excessive amounts of histamine and other vasoactive substances present in the blood.

*Adenylic acid derivatives*

These are substances which, like histamine, are known to be released into the circulation from injured tissues. Tested on normal men adenosinetriphosphate reduces the volume output of the kidney (87). However, all these substances are powerful vasodilators for the kidney (124) and their antidiuretic action has been found to run parallel with their effect on the general blood pressure and to be rapidly reversible (113). Any antidiuresis occurring as a result of the presence of these substances is probably due to the general vasodilatation and the fall in blood pressure that they cause.

*Renin and hypertensin*

In conscious hydrated rabbits renin first causes a brief antidiuresis which is followed by diuresis (115). Pickering and Prinzmetal (175) attribute the antidiuretic effect to an action on the glomerular vessels and a reduction in the rate of filtration. Results on dogs vary to some extent in the hands of different workers. Pickering and Prinzmetal observed a well-maintained antidiuresis in the conscious dog, whilst Merrill, Williams and Harrison (160) generally found a slight or well-marked diuresis as the result of the intravenous administration of renin, whether or not the dogs had been hydrated. The latter workers noticed that the kidney volume increased at the same time that the renal blood flow decreased, an effect similar to that seen after adrenaline (188).

In both dog and rabbit hypertensin has the same effect on the kidneys as renin. This similarity of action is not surprising since in all probability renin is merely the enzyme which, with the hypertensinogen of the plasma, forms hypertensin (17, 172). In the main, the action of these two substances appear to be diuretic rather than antidiuretic. What function, if any, they have in the normal organism is not known.

*Vasopressin, other vasoactive substances, and the intrarenal redistribution of blood*

Since it is probably impossible to separate the ADH of the posterior pituitary from the pressor fraction, and since the former is effective in far smaller doses than the latter, the examination of the unmixed action of vasopressin on the kidney is not possible. Given in doses at least 7 times larger than those necessary to produce antidiuresis, vasopressin decreases the renal blood flow in dogs and frogs (244). In mammals generally the vasopressor action seems to be of pharmacological rather than physiological interest to the kidney.

The suggestion has been made by Trueta, Barclay, Daniel, Franklin and Prichard (231) that posterior pituitary substances and adrenaline alter renal function by causing a redistribution of blood in the kidneys, and that the antidiuretic effect of the former is due to constriction of cortical arterioles with a transference of blood to medullary nephrons, where the long thin segment reabsorbs a large part of the tubular fluid. A somewhat similar suggestion was originally made by Frey (70, 71). Certainly the juxtamedullary nephrons are arranged differently from the cortical. As Huber (114) showed, the former have a much longer thin segment and the distal tubule (in rabbits) is about half as long as that in the cortical nephrons. Also the pattern of the surrounding capillaries is different, and the efferent arteriole of the juxtamedullary glomerulus is larger than the afferent (231). The work of Trueta *et al.* (231) was done mainly on rabbits, rats and cats, and they observed the renal circulation radiologically using thorotrast as the contrast medium. Recently, Daniel, Peabody and Prichard (45) have extended the observations to include dogs, sheep and monkeys. In some, but not all, individuals of all these species, under the conditions of their experiments, a closing down of the cortical blood supply was observed. The effective conditions were direct or reflex stimulation of the renal nerves, tissue trauma by the application of a tourniquet to one or both hind limbs, intravenous pituitrin 0.1–0.17 units/kg. rabbit, intravenous pitressin 0.2–20 units/kg. rabbit, intravenous adrenaline 0.1–0.17 mg./kg. rabbit, and equivalent doses for other animals. Daniel *et al.* also gave 5 $\mu$g. adrenaline into the aorta. As far as antidiuresis is concerned the doses used were monumental and might well cause a blood diversion which would not be seen with smaller, but effectively antidiuretic, doses. Given intravenously 5 $\mu$g. adrenaline is enough to cause maximal antidiuresis. Given intra-aortically, as was done by Daniel *et al.*, this dose would mean a very high concentration in the renal blood. Having seen that large doses had a renal vascular action, it is a pity that Trueta *et al.* made no attempt to test the response to physiological amounts of the extracts. There is a further criticism of their suggestion that hormone antidiuresis is vascular and not specifically tubular in origin, namely, that after denervation of the kidneys they found no change in blood distribution in the kidneys. It has frequently been shown that ADH is an antidiuretic in the denervated as in the normal kidney (170, 171, 194, 223) Thus, it is unlikely that a hormonal mechanism causes the vascular changes, or that the vascular changes have anything to do with antidiuresis. Other workers using both similar methods to and different methods from those of Trueta *et al.* have not always come to the same conclusions. It is

easy to cause ischaemia of the whole kidney, less easy to cause cortical ischaemia only. However, the latter has been induced fairly often in rabbits, more rarely in rats and dogs, and so far, not at all in healthy men, or in men suffering from essential hypertension or congestive cardiac failure (6, 83, 112, 157, 216). In man it is obviously impossible to use enormous doses of pitressin, and information regarding renal blood flow and function has to be obtained indirectly by means of clearance measurements. There is, in fact, no evidence that *normal* renal excretory function in man, dog or a number of other mammals in any way depends on intrarenal redistribution of blood.

This is not the place to discuss general renal physiology, but the question of blood redistribution in the organ cannot be left here. Three facts, amongst others, have to be taken into consideration; there is no reason to invoke, nor evidence for, intrarenal blood redistribution as a cause of pituitary or adrenaline antidiuresis when small but adequate doses are used; the kidney is richly supplied with nerves, but it has yet to be proved that these are concerned with renal excretory activity; under certain experimental and clinical conditions the blood supply to the cortical nephrons is diminished. On the last point the work of Trueta *et al.* has already been mentioned. Goodwin, Sloan and Scott (83) could at times, and particularly by direct stimulation of the renal nerves, induce cortical ischaemia in the kidneys of rabbits, cats, dogs and monkeys. Hoff, Kell, Hastings, Gray and Sholes (108) and Hoff, Kell, Hastings, Sholes and Gray (109) found that prolonged intermittent stimulation of certain gyri of the cerebral cortex resulted in renal cortical, but not juxtamedullary, ischaemia. To the writer there are only two possible ways of accounting for the conflicting evidence as to the importance of intrarenal blood redistribution for renal function. Either cortical ischaemia with medullary engorgement is a wholly abnormal phenomenon, occurring only in response to extreme provocation, or it is normal and connected with an endocrine activity of the kidney, not with its excretory function. In favour of the first possibility is the fact that extreme methods have to be applied to cause renal cortical ischaemia. In favour of the second are the following points. We know that a hypertensive substance, renin, is found in the kidney, and that a vasoexcitor material (VEM) (201) can be collected from it. We know, too, that in some ill-understood way, there is a connexion between the kidney and certain hypertensive states, both clinical and experimental. Renal nerve supply and occasional intrarenal blood redistribution may be concerned with the production of these materials. Under what circumstances this system is activated, why it is more reactive in some species and some individuals than in others, and what place it holds in the normal, as against the pathological, economy are quite unknown.

Recently, a highly vasoactive and antidiuretic substance has been extracted from the posterior salivary glands of certain octopi, and from some viscera of mammals, and named enteramine (60b). Its identity with 5-hydroxy-tryptamine has been established (60a). By means of thiosulphate and *p*-aminohippuric acid clearance measurements on rats and dogs it was found that its antidiuretic action was due to powerful constriction of the glomerular afferent arterioles, and that

it was effective in doses equivalent to 2 mg. fresh salivary tissue per 100 g. rat. The minimal effective dose for antidiuresis in dogs was not determined (60c, 60d).

### 4. MISCELLANEOUS ANTIDIURETIC SUBSTANCES AND CAUSES OF ANTIDIURESIS

#### *Ferritin*

It has for a long time been recognised that the liver stands in a special relationship with the kidney (76). Hepatic disease is accompanied by disturbance in water balance and either oliguria or, as in dogs with an Eck fistula (40), polyuria may result. The liver, too, seems to be involved in the first stages of water distribution after water ingestion (2). Finally, a large number of workers have extracted antidiuretic substances from the liver (9, 82, 137, 159, 195, 224, 245). One of these antidiuretic substances, originally called VDM (vasodepressor material), has now been identified as ferritin. It can be formed in the spleen and muscles as well as in the liver, and its production seems to depend on hypoxia of the organs concerned. Ferritin is found in the circulation in renal hypertension in dogs, and in man in essential hypertension, congestive heart failure and the hyporeactive phase of traumatic or haemorrhagic shock, but not in healthy men (201). The activity of ferritin runs parallel with its nitrogen content, that is, with its protein and not its iron-containing moiety (158). The suggestion is that ferritin appears in the blood in shock as the result of hepatic ischaemia (201). The normal liver can inactivate ferritin, but an anoxic liver loses this power, so that increasing amounts accumulate in the blood. The antidiuretic action of ferritin occurs without alteration in blood pressure, GFR or RPF, and is not seen in dogs with diabetes insipidus (207). Presumably, therefore, its action is mediated through the posterior lobe of the pituitary. It is unknown what function ferritin has in the normal animal.

#### *Hepato-renal syndrome*

In contrast to the polyuria that occurs in some hepatic diseases, in others oliguria and ascites predominate, but there is no evidence that the water retention is due to the production of ferritin. In hepatic cirrhosis the presence of low plasma albumin and ascites do not run parallel. In some cases treatment with liver extract lessened the ascites without raising the plasma albumin, and albumin could be administered without benefit to the oliguria (185, 249). Thus, water retention cannot be due simply to the osmotic effect of low plasma protein. Ralli (185) and her colleagues found that urine from patients with hepatic cirrhosis often had an abnormally high content of antidiuretic substance as judged by the water excretion rate of rats after subcutaneous administration of the urine. This antidiuretic substance appeared to have some of the characteristics of commercial pitressin (217). A similar substance could also be found in the urine in cases of infective hepatitis, and diminished in amount with the approach of convalescence (136). Ralli suggested that water was retained in these cases because liver damage had reduced the power of that organ to inactivate ADH. In favour of this idea was the finding that ADH was effectively inactivated by an enzyme that could

be extracted in more abundance from liver than from spleen, whole blood or muscle (11, 62). Also, in rats whose livers were damaged by a high fat-low protein diet the power of excreting water was markedly depressed and delayed, and moreover, could be improved by supplementing the diet with liver extract and NaCl (142). These animals, like patients with cirrhosis, excreted large amounts of antidiuretic substance in the urine. Gopalan (85) states that he found antidiuretic material in the urine of men suffering from nutritional oedema. The above results could all be interpreted as supporting Ralli's suggestion that excess ADH is the cause of hepatic ascites and oedema. However, she and her co-workers themselves found certain facts that do not agree with this hypothesis. First, they found that in hepatic cirrhosis all renal functions are depressed (141), a phenomenon which is only seen if very large amounts of ADH are administered, and secondly and more important, they are quoted by Smith (207) as stating that the antidiuretic substance obtained from patients with cirrhosis will not inhibit water diuresis in the dog when given intravenously, though it is antidiuretic on intraperitoneal administration to the rat. This at once suggests that the problem should be reinvestigated using the rat intravenous method, and bearing in mind the errors that may arise when other routes of administration are used (49). In other words, it is an interesting suggestion that water retention in hepatic disease is due to inadequate ADH destruction, but it is very far from proven.

The abnormal water metabolism in liver disease can more simply be explained as due to altered electrolyte balance. The retention of water and the ascites are not necessarily due to renal disease, since with improvement in the hepatic condition water excretion may return to normal (141). Farnsworth (63) believes that in congestive heart failure and hepatic cirrhosis some disturbance in Na and Cl excretion precedes oedema formation, and not the reverse. Goodyer, Relman, Lawrason and Epstein (84) came to the same conclusion as the result of observations on the rate at which infused NaCl was excreted by normal subjects, and by patients with and without ascites. They believe that there is evidence for specific impairment of the renal mechanism for the excretion of Na in patients with cirrhosis accompanied by ascites and oedema. They made no suggestion as to the cause of the increased Na reabsorption.

### *Adrenal cortical disorders*

In Addison's disease ingested water is only slowly excreted and there is a tendency for water intoxication to appear. Birnie, Jenkins, Eversole and Gaunt (13) suggested that the liver may, in part, be the cause of the oliguria. At first they thought that there was a sensitisation to ADH. Later Birnie (11) found that rat liver extracts made after adrenalectomy of the donors were less able to inactivate ADH than extracts of normal livers, so that the apparent sensitisation was, perhaps, due to a diminished destruction of ADH. It was also found that after adrenalectomy the antidiuretic titre of the serum was raised, but could be reduced again by the administration of adrenal cortical extract, deoxycorticosterone or NaCl (12). The tests were made on rats and the serum injected

intraperitoneally. It was thought that the increased antidiuretic activity of the serum was due to the already mentioned deficiency in the system which usually destroys ADH. However, it should be noted that although treatment reduced the antidiuretic titre of the serum, water excretion did not return to normal. Possibly therefore, there was at no time a real change in the ADH content of the serum, but only of some substance which had been acting as an augmentor to the endogenously formed hormone.

The changes of water excretion in Addison's disease are most readily explained as due to the inevitable marked electrolyte disturbance and low blood pressure. The subject of the adrenal cortex and water metabolism is too vast to be further considered here, and references may be found in Gaunt, Birnie and Eversole (72a) and in Smith (207).

*Antidiuretic substances found in pregnancy*

For the greater part of normal pregnancy there seems to be neither specific water retention nor measurable amounts of antidiuretic substances in serum or urine (50, 54, 131, 151, 251), but late in apparently normal pregnancies some women show a lessened capacity for water excretion, and the same thing has been observed during parturition (50, 151). Some observers claim that pregnant serum contains a substance antagonistic to ADH and melanophore hormone, and particularly is this antihormone found in the first part of normal pregnancy. Very little of this substance can be found in the serum of women who are not pregnant (135, 198).

There is a considerable divergence of opinion as to what occurs in eclampsia and pre-eclampsia. Anselmino, Hoffman and Kennedy (5) thought that they had demonstrated the presence of an antidiuretic substance in the blood of eclamptic women, and that this substance was of posterior pituitary origin. Theobald (222) showed that their methods were not reliable, and that their claims could not be substantiated, particularly with regard to the origin of the antidiuretic material. A number of other workers agreed with Theobald (30, 116, 131, 143). On the other hand, Teel and Ried (220) found that there was an antidiuretic substance in the urine of eclamptic women, especially during the phase of acute water retention. The quantity of antidiuretic material found was correlated with the degree of water retention and not with the hypertension or albuminuria. Others (91, 92, 195) too, have found an antidiuretic substance in the urine in pre-eclampsia, but think that it differs from true ADH. Still others believe that they have shown the existence of an increased sensitivity to vasopressin in pre-eclampsia (29, 50) and that in this condition patients react to injections of vasopressin by a rise in blood pressure and fall in urine volume which is far greater than that seen in normal women. Kustner (135) believes that the antihormone present in normal pregnancy is lacking in eclampsia.

In the general confusion it seems clear that excessive production of ADH with primary water retention is not the *cause* of eclampsia or hypertension in pregnancy. It remains to be decided whether antidiuretic substances of unknown origin are formed in considerable amounts in eclampsia and pre-eclampsia, or

whether their apparent discovery is due to the use of unsuitable test methods. The latter seems the more probable.

*Various steroids*

There is general appreciation of the fact that in certain phases of the sexual cycle of females some alteration or disturbance of water metabolism often occurs. In some species an obvious alteration is physiological and is accompanied by water retention (132); in others water retention is not apparent, and, if it does occur, may be abnormal. The question arises whether the known changes in steroid balance are directly or indirectly the cause of altered water metabolism.

Zuckerman, Palmer and Hanson (260) made observations on the water content of some of the tissues of rats after the administration of a single dose of oestrogens. The measurements were made at 6-hourly intervals for 3 days. A biphasic change in water content was found, with an inverse relationship between uterus, vagina and skin and other tissues. The uterus returned to its normal weight in 72 hr. and all other organs in 48 hr. As no figures were given for the total water content of the animals or their body weight changes, it is not possible to say whether there was an actual water retention or only a redistribution of water in the body. Guthkelch and Zuckerman (90) compared the red blood cell counts during phases of the sexual cycle in pig-tailed macaques and rhesus macaques. The pig-tailed species displays sexual skin swelling, and at such a time the red blood cell count increased from 4.5 to 5.75 million with subsidence to normal at the next mid-cycle. There was also a diminished urine output during the phase of sexual skin swelling. The rhesus displays colour changes but no skin swelling; nevertheless, a similar concentration of red blood cells occurred. There was no clear evidence of body weight change and true water retention. When the rhesus was ovariectomised and given oestrone a rise in the red blood cell count was seen during the early follicular phase. No generalisation was possible on the basis of these results. Witten and Bradbury (258) made blood counts and blood volume estimates on women during the intermenstrual period in order to avoid the complication of blood loss. After injections of oestrone or oestradiol propionate they found that the red blood cell count fell to an extent that could be accounted for by the simultaneous increase in blood volume. Friedlander, Laskey and Silbert (72) state that, in women, ovariectomy results in a marked decrease in blood volume, and that administration of oestrogenic preparations increases it again. These results are in contrast to those of Zuckerman, but this may be due to species differences. The difference between pig-tailed and rhesus macaque has already been mentioned, and Friedlander, Laskey and Silbert themselves noted that in humans when the blood volume rose the plasma volume sometimes fell, whereas in cats both blood and plasma volume rose.

In observations made on dogs, Thorn (226, 227, 228) and his colleagues found that whether or not the dogs were adrenalectomised the administration of oestrone, progesterone, pregnanediol or testosterone led to the retention of Cl, Na and water. The same workers made balance studies on women and found that Cl, Na and water were retained at two periods of the menstrual cycle, during

the premenstrual phase and in mid-cycle (228). With the onset of menstruation the retained water and electrolytes were excreted. Similar changes were seen in women after hysterectomy, but not after both hysterectomy and ovariectomy. The changes in water and electrolyte metabolism were, then, probably related to the ovary and oestrogens. There were, however, indications that the weight gain depended on a cyclical increase in hunger and thirst, since by controlling the diet the weight gain could be avoided. Some women show a well-recognised state of premenstrual tension. This is related to an increase in weight with water retention and, sometimes, obvious general oedema. The basal metabolic rate may be low. The fall in BMR, but not the oedema, can be prevented by thyroid administration (8). It has also been noticed that the administration of progesterone can give rise to all the signs of premenstrual tension in those liable to the condition (77). Progesterone has also been shown to prevent the usual postpartum loss of water in mice (46, 47). Frank (68) states that irradiation of the ovaries led to a 3-year cure in patients suffering from premenstrual tension. These observations all point to changes in water metabolism being dependent on the balance of steroids in the body, but do not show how it is brought about. Thomas (225) thought that the tissues were made hydrophilic by a rise in oestrogen concentration, but suggested no cause for the hydrophilia. A number of workers believe that Na retention is the first event, and water retention is secondary (34, 88, 189, 218). From the observations of Thorn and Engels (226) that changes in Na and Cl excretion due to administration of sex hormones were seen in both normal and adrenalectomised dogs, it appears that adrenal hormones are not necessary for the changes that occur. In all the conditions mentioned water retention is best explained as due to a steroid induced alteration in electrolyte balance.

Two other possibilities may be briefly considered, first, the action of oestrogens on the anterior lobe of the pituitary, and second, their action on the posterior lobe. Oestrogens are known to depress certain activities of the anterior lobe, and so may result in depression of renal function (180, 253). Shapiro (200) found that following large daily injections of oestradiol benzoate to 5 patients there was marked decrease in their urinary output, and that the same reduction was seen in a patient with diabetes insipidus. He suggested that anterior lobe activity had been sufficiently depressed to disturb renal function.

Cavallero, Corbetta and Malandra (31) found that in ovariectomised rats there was a reduction in the ADH content of the pituitary that was not seen if the operated animals had been treated with oestrogens. They suggested that with a changed steroid balance more ADH was produced. Martin, Herrlich and Fazekas (154) postulate that, following adrenalectomy, an initial stimulation of the posterior lobe is due to early dehydration, with a secondary augmentation due to electrolyte imbalance.

## 5. SUMMARY

Urine output may be reduced and water retained in the body for a number of reasons, many of which affect water only secondarily. For instance, any process or substance which alters the electrolyte balance will almost certainly also affect

water excretion. As examples may be mentioned hepatic and adrenal disorders, and the changes in steroid balance that occur during the sexual cycle. More directly, water excretion may be diminished when the blood pressure in a part or the whole of the kidney is reduced, or where the renal vessels undergo general constriction. Such renal vascular changes may be caused by substances in the circulating blood, or by activity of the renal nerves. Finally, water excretion may be reduced by the specific action of the antidiuretic hormone of the posterior lobe of the pituitary, and also, therefore, by all substances and conditions that increase the rate of release of the hormone into the circulation. A study of the literature makes it evident that ADH is the only known tissue substance whose normal function it is to control water excretion *per se*, and that it does so by an action on the cells of the renal tubules.

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