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THE INHIBITION OF WATER DIURESIS BY AFFERENT NERVE STIMULI AFTER COMPLETE DENERVATION OF THE KIDNEY.

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In a recent paper [Theobald, 1934] it has been shown that water diuresis may be inhibited in the dog by afferent nerve stimuli associated with acupuncture in the lumbar area. We propose first, through the description of three related experiments, to offer proof that the renal nerves play no essential part in such inhibition, and second, through a consideration of the results of these experiments with others of cognate interest, to suggest its underlying cause.

Experimental work.

A bitch, on which a plastic operation to expose the urethral orifice had been performed some weeks previously, was anæsthetized with a mixture of chloroform and ether, the anæsthesia being continued with ether alone. After the abdomen had been opened with full surgical precautions the left renal artery and vein were defined and freed completely from the surrounding tissues. The numerous nerve fibres lying on the artery were then cut and the vessel itself cleaned with gauze. After the vein and ureter had been similarly cleaned the kidney was lifted forward and separated from all fascial connection with the body. At this stage the renal artery was painted for a distance of about a half an inch with phenol. After the kidney had been sutured to its bed, the right kidney was treated in a similar manner. The abdomen was then closed and the animal returned to its kennel.

Three weeks later it was placed in a Pavlov stand, and the observations recorded in Fig. 1 were made. At *C* 250 c.c. of warm tap water were given by stomach tube, and when the diuresis was becoming estab-

lished a needle was introduced (the skin and subcutaneous tissues having been previously anæsthetized by infiltration with a 2 p.c. solution of novocain) and moved about in the tissues between two lumbar spines for 10 min. (*A*, Fig. 1). During this time the change in rate of urine flow was reversed, the ebb continuing after removal of the stimulus until the original rate of flow was reached, and at this level it remained for some 10–20 min. The rate subsequently increased and rose uninterruptedly to its peak value of 30 c.c. in 10 min. At *B* the needle was again introduced between the vertebral spines and moved about for 3 min., a procedure which was followed by a fall in the rate of urine flow from 28 to 4·5 c.c.

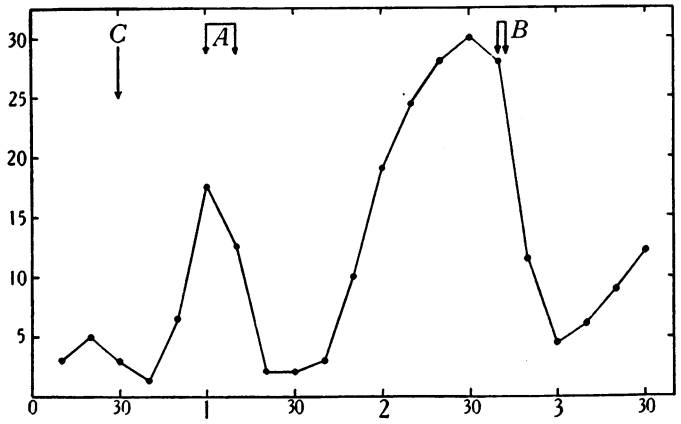

Fig. 1. Each point on the curve represents the volume of urine secreted in the previous 10 min. At *C* 250 c.c. warm tap water were given by mouth. *A* and *B* = periods during which hypodermic needle remained in region of fourth lumbar interspace. Ordinate = c.c. urine. Abscissa = time in minutes and hours.

per 10 min. That this sequence is causal is shown by the fact that the rate of secretion thereafter increased. Such denervation of the kidneys, then, as has been described above, does not prevent the occurrence of the inhibition we are considering. Indeed its grossly parallel nature in the innervated and denervated organs is shown by a second experiment in which denervation of one kidney only was effected, and the response of each separately recorded. In this experiment a bitch was anæsthetized, and the left kidney denervated in the manner already given. Both ureters were then extended to the vulva by the technique described by Klisiecki, Pickford, Rothschild and Verney [1933]. The right kidney was not disturbed by this operation, and the only nerves affected were those running along the ureter.

Results obtained from this animal three days after the operation just described are shown in Fig. 2, and it is evident that there is no essential difference in the responses of innervated and denervated kidneys to the inhibitory influence of afferent nerve stimulation on an established diuresis. Indeed the close correspondence of these effects makes it reasonable to assume that the inhibition of secretion is produced in the two kidneys by the same physiological means.

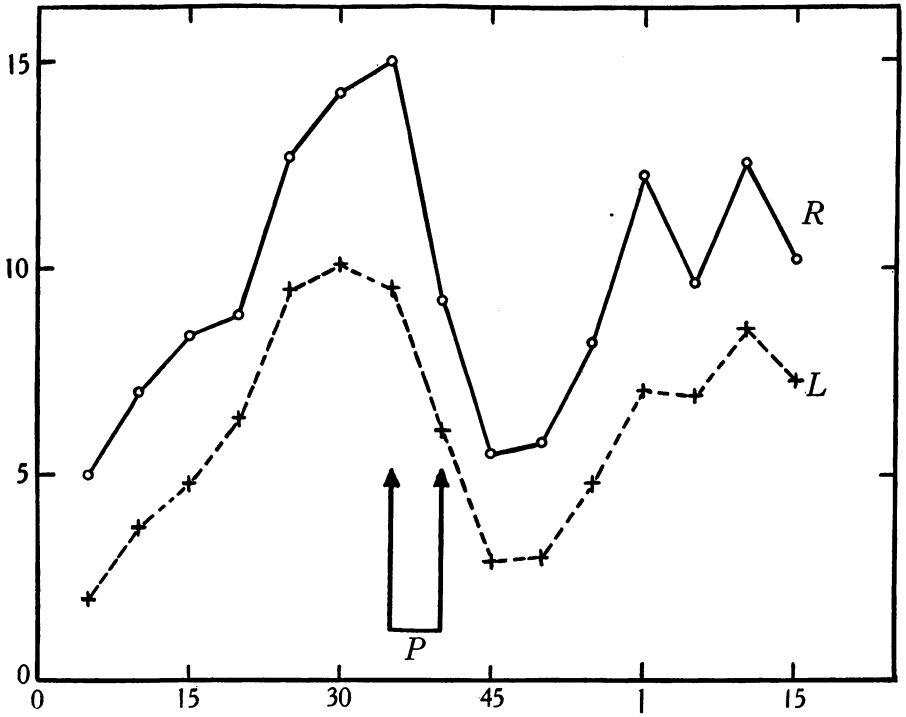

Fig. 2. Each point on the curve represents the volume of urine secreted in the previous 10 min. At zero time 250 c.c. warm tap water were given by mouth. R = urine flow from right kidney and L = that from left. P = period during which hypodermic needle remained in region of fourth lumbar interspace. Ordinate and abscissa as in Fig. 2, *q.v.*

While it might be legitimately assumed that the experiments already described had proved that the renal nerves played no essential part in the inhibition of water diuresis by afferent nerve stimuli, it was decided to place the issue beyond cavil.

The operation designed for this purpose consists essentially in the introduction and fixation of cannulæ in the abdominal aorta and inferior vena cava above and below the points of entry of the renal vessels,

followed by complete section of the aortic and caval walls at the levels of the cannulæ, and isolation of the intervening segments.

Three dogs were used, two in the preparation of the requisite cannulæ, the third as the experimental animal. The abdomen of the first dog was opened under chloroform-ether anæsthesia and with full surgical precautions. The segment of inferior vena cava below the entrance of the renal veins was defined and freed from adherent fat and fascia, and the lumbar tributaries divided between ligatures of fine silk. Strong ligatures were then tied around the upper and lower ends of the caval segment, and the intervening portion was excised and placed in warm sterile Ringer's solution. The animal was then killed by deepening the anæsthesia, the chest opened and the intrathoracic portion of the vena cava excised and placed in Ringer's solution. The segments of vein, having been washed in the Ringer's fluid, were used immediately to line glass cannulæ, of selected length and bore, which had been previously sterilized.

The cannulæ consist of sections of glass tubing of various sizes. The ends have edges which are slightly everted, and two shallow grooves are etched on the external face. These pass round the periphery of the cannula at a distance of 2 mm. from the central transverse plane (see *W*, Fig. 3). The sizes we have found particularly useful are, for the vein, 15 mm. long, with an internal diameter of 8 mm., and for the artery, 12 mm. long with an internal diameter of 6·5 mm.

A suitable length of vein was threaded through the cannula, its ends everted over the edges and then drawn to meet at the centre of the cannula. It was then fixed in position by fine silk ligatures tied in the positions of the etched grooves (*x*, Fig. 3) In the preparation of such a cannula it was inevitable that air and liquid should be retained between the interior of the glass segment and the wall of the vein which lined it. These fluids were withdrawn by means of a syringe and fine hypodermic needle, and as they were aspirated the vein expanded to continuous contact with the inner wall of the glass tube. The segments of vein removed from the first dog proved sufficient to line three cannulæ which were placed in a closed sterile vessel containing Ringer's solution. The second dog was then anæsthetized, and three further cannulæ were prepared in exactly the same manner.

Anæsthesia of the third dog was then induced with a mixture of chloroform and ether and continued with ether alone. The abdomen was fully opened in the midline and the edges of the wound were held apart by a self-retaining retractor. The aorta and inferior vena cava were then defined from their bifurcations below to the superior mesenteric artery and first lumbar vein above. After this definition the right renal artery,

vein and ureter were divided between ligatures, and the kidney removed. The lumbar tributaries of the vena cava were then divided between ligatures, and the corresponding part of the vein freed in this way from

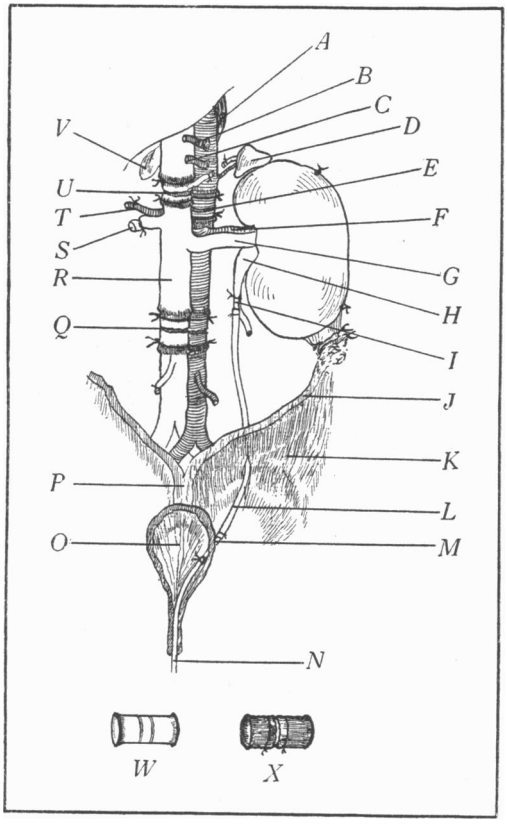

Fig. 3. *A* = left crus of diaphragm. *B* = cœliac axis. *C* = superior mesenteric artery. *D* = left suprarenal gland. *E* = upper aortic cannula. *F* = left renal artery. *G* = left renal vein. *H* = renal pelvis. *I* = silver ureteric cannula. *J* = left fallopian tube. *K* = left broad ligament. *L* = rubber tube connecting the upper ureteric cannula *I* with the glass cannula *M* which passes through the intravesical part of the ureter. *N* = rubber tube connecting *M* with the exterior. *O* = bladder. *P* = uterus. *Q* = lower caval cannula. *R* = inferior vena cava. *S* = right venal vein. *T* = right renal artery. *U* = upper caval cannula. *V* = right suprarenal gland. *W* = glass cannula. *X* = glass cannula covered with a segment of vena cava and ready for insertion.

all tributaries other than the left renal vein. The corresponding aortic segment was isolated by a similar procedure. The next stage consisted in the introduction and fixation of the two caval cannulæ. For this purpose the aorta was temporally occluded by a clip just proximal to its bifur-

cation, and the vena cava by two others, the one just caudal to the left renal vein, the other some 4–5 cm. lower. An opening was made in the cava just above the lower clip, and a few cubic centimetres of a sterile solution of heparin were allowed to flow over and into the opening. A cannula of appropriate size was then introduced and manœuvred towards the upper clip. This was temporarily removed while the cannula was rapidly passed to a position just above the right renal vein, where it was fixed by two ligatures which had been previously placed in position, and which were tied as near the everted ends of the cannula as possible. While the cannula was being manœuvred upwards, the caval wound was compressed with a finger until such time as the upper clip could be replaced. A second cannula was passed into the cava, and tied in such a position that the caval opening was closed. The vena cava was then completely divided circumferentially at the level of the central transverse plane of both upper and lower cannulæ. After the clips had been removed, attention was directed to the aorta, and here again, by a technique which followed closely that described for the cava, cannulæ were introduced into corresponding positions, and the aortic wall completely transected above and below. It will be observed that the renal circulation was at no time interrupted except during the short period of passage of the upper cannulæ across the openings of the artery and vein, a period which amounted to no more than a fraction of a second. The ureter was then divided at the level of the lower pole of the kidney and extended to the vulval orifice by the method of Klisiecki, Pickford, Rothschild and Verney [1933]. Simple division of the peritoneal attachments of the kidney now allowed the organ to be raised from its lumbar bed, its vessels to be cleaned, and its complete organic isolation certified. A few sutures brought the edges of the divided peritoneum on the posterior wall into apposition, and maintained the kidney in its normal position (see Fig. 3). About 100 c.c. of physiological saline were poured into the peritoneal cavity, and the abdominal wall was closed by two tiers of interrupted sutures. After 100 c.c. of a 10 p.c. solution of glucose in physiological saline had been given intravenously, the animal was returned to its kennel, which was maintained at a temperature of 25° C. for the next 24 hours. The animal made a good recovery, and 2 days after the operation apparently normal responses to the ingestion of water were obtained. One such response is shown in Fig. 4. As will be seen from this figure, the rate of urine flow suffered profound inhibition as the result of passing a hypodermic needle into the fourth intervertebral space and moving it occasionally over a period of 10 min. No attempt was made to

enter the thecal canal and the animal showed little emotional response to the procedure to which it was subjected. The facts that water diuresis can occur in a dog whose one remaining kidney has been completely denervated, and that such diuresis readily suffers inhibition from afferent nerve stimulation, are therefore beyond controversy.

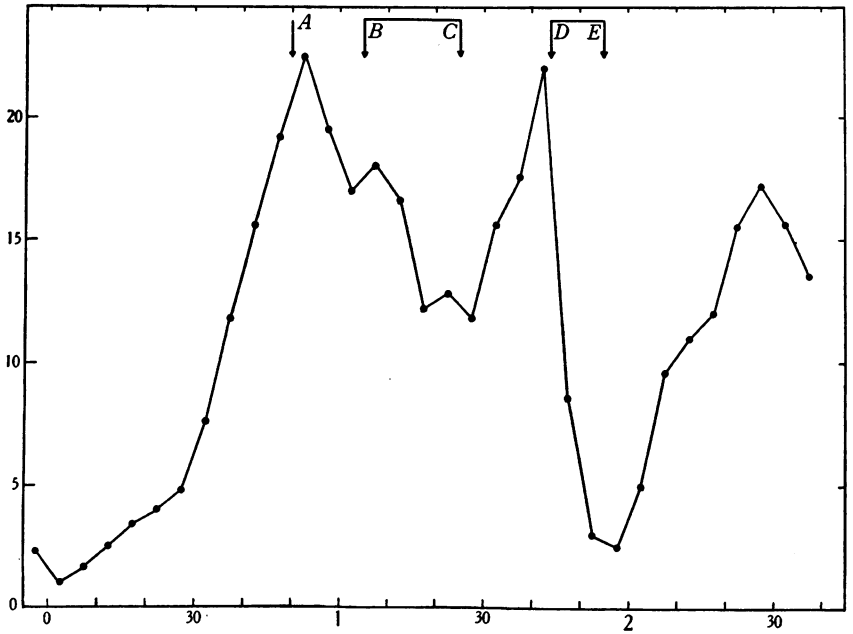

Fig. 4. Rate of urine secretion from left kidney which had been completely denervated. Each point on the curve represents double the total volume of urine secreted in the preceding 5-min. period. At zero time 250 c.c. warm tap water were given by stomach tube. The hair of the mid-lumbar region was cut short with scissors. Lathering with soap and water commenced at *A*. *BC* = period of shaving. A 2 p.c. solution of novocaine was injected into the skin and subcutaneous tissues immediately preceding the period *DE* during which a hypodermic needle was moved about in the region of the fourth lumbar interspace. Ordinate and abscissa as in Fig. 2, *q.v.*

Discussion.

The inhibition of normal urine flow in the mammal, induced by such emotional states as anger and fear and by muscular exercise [Dobreff, 1926], as also the inhibition of water diuresis by similar causes [MacKeith, Pembrey, Spurrell, Warner and Westlake, 1923; Klisiecki, Pickford, Rothschild and Verney, 1933], have been attributed commonly to a redistribution of blood through the intervention of

the vaso-motor system. The hypothesis, however, that the renal nerves themselves are involved in the chain of events leading to inhibition of water diuresis is made unlikely, in so far as the inhibition by muscular exercise is concerned, by the demonstration of the persistence of inhibition after section of those nerves in as complete a manner as has been found possible [Klisiecki, Pickford, Rothschild and Verney, 1933]. In this paper, moreover, we have presented an extension of such evidence to include inhibition of water diuresis by afferent nerve stimuli [Theobald, 1934], and finally brought unequivocal proof to the contention that the renal nerves play no essential part in such inhibition, by the elicitation of the phenomenon in a dog in which organic connection between kidney and animal had been completely severed. Further work alone can decide whether or no we are justified in ascribing the inhibition of water diuresis by emotional states, by muscular exercise and by afferent nerve stimuli to the causal participation of a common underlying factor, but it is clear that the factor or factors concerned operate independently of the renal nerves.

The mildness of the effective stimuli causing the inhibition described in this paper, along with the experimental findings of MacKeith, Pembrey, Spurrell, Warner and Westlake [1923], of Dobreff [1926], and of Klisiecki, Pickford, Rothschild and Verney [1933], make the ascription of this inhibition to a fall in arterial blood-pressure unreasonable. We are therefore driven to conclude that the inhibition is effected by some change in the composition of the blood reaching the kidney, a change, moreover, which may be either humoral or hormonal in nature. In the former case we may imagine the resultant inhibition to be mediated by the tissues, these retaining the water they were previously liberating to the blood stream. Such a change in the tissues could conceivably be effected through efferent channels of a nervous nature. While on the one hand this possibility is not excluded, on the other no evidence exists, so far as we are aware, in its support. The fact, however, that an occasional accompaniment of the inhibition due to afferent nerve stimuli is proteinuria, supports belief in the alternative hypothesis that the kidneys are responding to some specific change in the composition of the blood, rather than to a temporary reduction in the amount of water brought to them. The two hormonal agencies which might theoretically be concerned here are adrenaline and post-pituitary antidiuretic substance. Now it has been shown that inhibition of water diuresis by exercise occurs after bilateral section of the splanchnic nerves [Klisiecki, Pickford, Rothschild and Verney, 1933, see Fig. 2, p. 524]; but in

view of the somewhat conflicting statements concerning the effects of adrenaline on urinary secretion, we decided to determine whether or no inhibition of water diuresis could be attributed to its agency. We were unable to detect any effect on water diuresis in man from the subcutaneous injection of 0·5 c.c. of a 1 : 1000 solution of adrenaline hydrochloride. Moreover, the intravenous injection of adrenaline in such

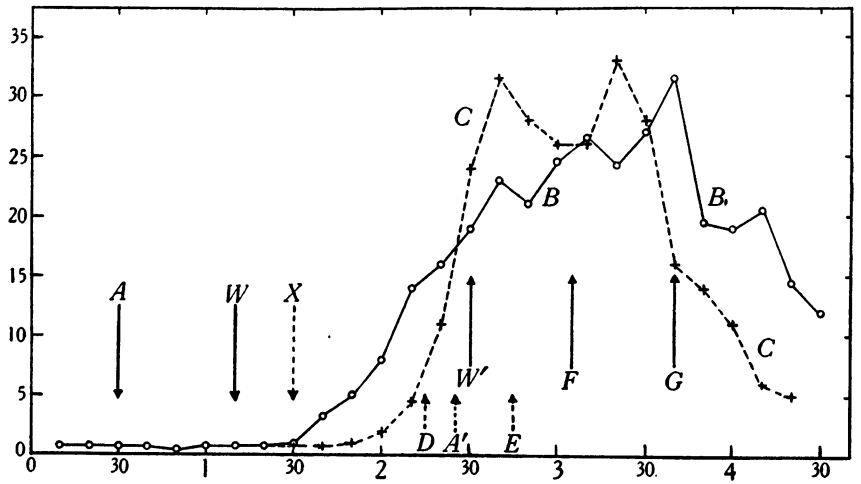

Fig. 5. Curve of urine flow from two dogs to show the effect of the intravenous injection of adrenaline on water diuresis. Each point on the curves represents the volume of urine secreted in the previous 10 min. At *A* the continuous injection of a 1 : 5000 solution of adrenaline hydrochloride was begun and continued in the case of the first dog (curve *B*) for 2 hours, during which time 4 c.c. were injected. 250 c.c. warm tap water were given at *W* and again at *W′*. At *F* 0·5 c.c. and at *G* 1 c.c. of the 1 : 5000 solution were injected intravenously. Curve *C*, obtained from data from the second dog, follows *B* closely for 1½ hours. At *A* the continuous injection of a 1 : 10,000 solution of adrenaline hydrochloride was begun and discontinued at *X* at which time 250 c.c. warm tap water were given by stomach tube. At *D* 0·5 c.c. and at *E* 1 c.c. of the 1 : 10,000 solution were injected intravenously. At *A′* the continuous injection of the 1 : 10,000 solution of adrenaline was recommenced and was discontinued at *E*.

amounts as to cause profound bradycardia and polypnœa in dogs gave but little change in the rate of urinary secretion. The evidence for this latter statement is contained in Fig. 5. Under novocaine anæsthesia a cannula was tied into the external metatarsal vein of a bitch which had been perineotomized some weeks earlier. The animal stood quietly in a Pavlov stand, and the cannula was connected by means of fine rubber tubing with the nozzle of a 2 c.c. syringe, the plunger of which could be slowly advanced by a micrometer screw turned by a small electric motor. As

will be seen, the continuous injection at a rate of 2 c.c. per hour of neither a 1 : 10,000 nor a 1 : 5000 solution of adrenaline hydrochloride affected the normal resting rate of urinary secretion. Dog *B* (curve *B*) was given 250 c.c. warm water by stomach tube at *W*, the slow injection of the adrenaline solution being continued. Water diuresis occurred and was apparently unaffected by the adrenaline. At *F* 0·5 c.c. and at *G* 1 c.c. of a 1 : 5000 solution of adrenaline hydrochloride were rapidly injected intravenously. These doses caused the respiration to become rapid and shallow, and the pulse rate to fall from 130 to 54 beats per min. The rate of urinary excretion fell very steeply for about a minute, and then returned quickly to its previous value. The conclusion may therefore be drawn that the inhibition of water diuresis produced by afferent nerve stimuli is not due to the physiological liberation of adrenaline, seeing that amounts of that substance which cause obvious symptoms of distress exercise so transient an effect on the rate of urinary secretion.

With respect to the possibility of the pituitary body being involved in the processes leading to inhibition of water diuresis by afferent nerve stimuli, the following three facts concerning the action of post-pituitary extract seem to us significant. First, minimal inhibition in the dog may be caused in some animals by the intravenous injection of as little anti-diuretic substance as is associated with 0·0005 oxytocic unit of post-pituitary extract [Theobald, 1934]. Second, the effect caused by the intravenous injection of such an amount of post-pituitary extract does not reach its maximum until nearly 10 min. have elapsed from the time of the injection [Theobald, unpublished observations], a time lag comparable with that observed in the inhibition of water diuresis by afferent nerve stimuli. Third, albumin is sometimes found in the urine after water diuresis has been inhibited whether by minimal doses of post-pituitary extract or by afferent nerve stimuli. We are therefore driven to conclude that the inhibition of water diuresis encountered in the experiments described in this paper, can most reasonably be ascribed to the liberation of anti-diuretic substance from the pituitary body into the blood stream. Proof or disproof of the involvement of the pituitary body in the phenomenon of inhibition of water diuresis by afferent nerve stimuli, by emotional states, and by exercise, nevertheless demands and depends upon experiments of more direct a nature. But whatever the factor immediately responsible for the inhibitory effect on water excretion may prove to be, the experiments described in this paper show, as we think, conclusively, that the effect is transmitted to the kidney through the blood stream and that the agent so transmitted is not adrenaline.

SUMMARY.

1. A method is described by which the kidney of the dog may be completely denervated, and the response of this kidney to the ingestion of water, measured.

2. Water diuresis is shown to occur in such an animal, and to run an apparently normal course.

3. After complete denervation of the kidney, water diuresis may be temporarily inhibited by afferent nerve stimuli. Such inhibition is in all probability directly due to an agent which is transmitted through the blood stream, and whose liberation may be effected by the central nervous system.

4. The rate of urine flow may continue to fall after removal of the original stimulus, and recovery only begin after the flow has persisted at a low rate for some 5–20 min.

5. Reasons are advanced for the view that the humorally transmitted agent is not adrenaline.

6. The facts described find interpretation in, and in turn lend support to, the hypothesis which ascribes the control of water secretion by the kidney to physiological variations in the activity of the pituitary body.

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