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THE REPETITION OF CERTAIN EXPERIMENTS ON WHICH MOLITOR AND PICK BASE THEIR WATER-CENTRE HYPOTHESIS, AND THE EFFECT OF AFFERENT NERVE STIMULI ON WATER DIURESIS.

By G. W. THEOBALD.

(*From the Department of Pharmacology, University College, London.*)

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The complexity and the fundamental nature of the issues involved in the regulation of the water content of the body and its partition have convinced most workers of the need for postulating some central control, but a wide divergence of opinion obtains as to its nature. Molitor and Pick have advanced the hypothesis that the control is vested in a centre situated in the neighbourhood of other vegetative centres in the mid-brain, which when stimulated by a fall in the water content of the blood, causes the tissues to liberate water into the blood. On the other hand, an excess of water in the blood is dealt with by the kidneys. They are not, however, decided as to whether the water centre effects its control by nervous or hormonal means. Like the other vegetative centres the water centre is under the inhibiting control of the cerebral cortex, and if this restraining influence is removed either surgically or by optimum doses of such narcotics as paraldehyde or chloralose, the centre becomes over-active and water diuresis is no longer inhibited by posterior pituitary extract. This extract, in their view, has no direct action on the kidneys or the renal circulation, but acts, either directly or through the water centre, on the tissues, enabling them to hold more water.

This hypothesis is based almost entirely on experiments devised to investigate the anti-diuretic action of posterior pituitary extract. Molitor and Pick [1924] found that the inhibition of water diuresis caused by this extract could not be overcome either by vaso-dilator substances such as amyl nitrite, sodium nitrite, nitroglycerine or papaverine or by diuretics such as caffeine, theobromine or theocin. On the

other hand, it could be overcome by the administration of such diuretics as sodium chloride, glucose and, most powerful of all, urea, which act in virtue of the osmotic pressure they exert. In 1925 these authors stated that posterior pituitary extract did not inhibit water diuresis either in rabbits under paraldehyde narcosis or in those which had been decerebrated, the tests being carried out two or three days after the animals had recovered from the operation. In 1926 they further affirmed that: (*a*) the injection of posterior pituitary extract into dogs under a suitable degree of chloralose narcosis has no influence on the course of water diuresis; (*b*) if the extract be injected intrathecally it inhibits water diuresis even when amounts are used which have no effect when introduced intravenously; (*c*) the inhibition of water diuresis persists for a longer time when the extract is injected intrathecally than when injected subcutaneously.

These conclusions, if they can be substantiated, offer strong evidence in favour of the water-centre hypothesis and indisputable evidence (notwithstanding the fact that Starling and Verney [1925] reported that posterior pituitary extract lowered the rate of secretion of urine in the heart-lung-kidney preparation) that posterior pituitary extract does not exert its anti-diuretic activity directly on the kidneys or the renal circulation. For these reasons Prof. E. B. Verney suggested that it was desirable that the experiments reported by Molitor and Pick in 1926 should be repeated. Before these experiments are described reference will be made to a paper published by Janssen in 1928. Working with rabbits he found: (1) that division of the spinal cord at the level of the fifth cervical vertebra, together with section of both vagi, did not interfere either with the normal concentration of urine or with water diuresis, which was inhibited in the ordinary manner by the injection of posterior pituitary extract; (2) that a given amount of posterior pituitary extract inhibited water diuresis as effectually when injected subcutaneously as when injected intrathecally; (3) that posterior pituitary extract inhibited water diuresis in decerebrate animals, although the tuber cinereum, infundibulum, thalamus, and the brain stem down to the corpora quadragemina had been removed. These experiments, if confirmed, would prove that the water centre could not exert its control by nervous impulses and that posterior pituitary extract could not affect the tissues through the mediation of the water centre. They would not, however, invalidate the hypothesis advanced by Molitor and Pick, which affirmed the possibility that the water centre effected its control by hormonal means and that the posterior pituitary extract acted directly on the tissues.

Experimental work.

The experiments were performed on young bitches on which a plastic operation exposing the urethra [see Klisiecki, Pickford, Rothschild and Verney, 1933] had been performed. A preliminary hydrating dose of 250 c.c. of tepid water was given through a stomach tube and the animal returned to its cage. Some two hours later it was placed in a Pavlov's stand and a female rubber catheter was passed and fixed in position. The urine was collected in a graduated glass cylinder and the amounts were read at 10 min. intervals. After the urine flow had returned to its resting rate a further 250 c.c. of water were introduced into the stomach in the same manner. The time occupied in administering the water was $1\frac{1}{2}$ min.

The effect of the intravenous injection of posterior pituitary extract on water diuresis in dogs under chloralose narcosis.

It is postulated by Molitor and Pick [1925*b*] that the water centre normally receives inhibitory impulses from the cerebrum and that these may be inhibited by a suitable dose of chloralose which selectively narcotizes this area of the brain. It follows that a dog under chloralose narcosis should excrete more urine in a given period after water has been administered than when normal.

Chloralose dissolved in 250 c.c. of water was administered through the stomach tube to three different bitches on thirteen occasions. The optimum dose of this drug was found to be 0·125 g. per kg. of body weight. When this amount was given the animal became sleepy, sagged in the middle, and without passing through any stage of excitement fell fast asleep, usually in about 30 min. As the drug became effective the animal had to be supported by bands passed round the chest and abdomen and fixed to the beam of the stand. The head had also to be supported in a similar manner. While "asleep" it did not mind the prick of a needle or being pinched, but exhibited movements in response to a sharp noise or to its ears being flicked. The renal response to the ingestion of water was usually good, but except in the case of one bitch the amount of urine secreted in a given period and the maximum rate of secretion were lower than normal. If a smaller dose of chloralose was given the animal passed through a stage of intense excitement and water diuresis did not occur. Moreover, possibly because of the violent movements, hæmaturia occurred and blood was noticed in the fæces, which were somewhat violently expelled (Fig. 1).

The preparation of posterior pituitary extract used was "Infundin" (Messrs Burroughs and Wellcome) the oxytocic content of which is standardized in international units. All the ampoules used were taken from one batch which was kept in cold storage. Five units (0·5 c.c.) were diluted with physiological saline to make 10 c.c., and of this 1 c.c. was taken and added to 9 c.c. of physiological saline. Successive dilutions were made in like manner until the required dose was obtained. The standard amount of fluid injected was 1 c.c., this was introduced into the lateral metatarsal vein while the animal stood in the stand, and with the minimum of disturbance. The smallest amount of "Infundin" which

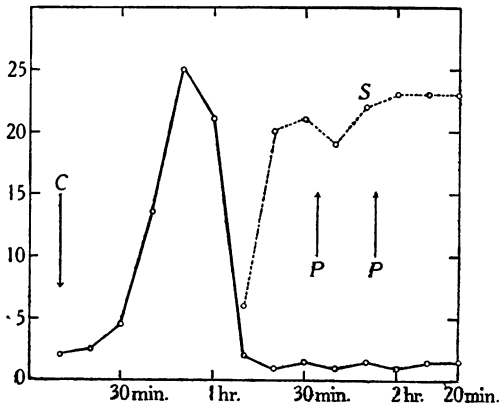

Fig. 1. Curve showing the effect of an insufficient dose of chloralose on water diuresis. At *C* 0·075 g. per kg. of chloralose, dissolved in 250 c.c. of water, was given through a stomach tube. Water diuresis commenced in the normal manner, but at the end of 40 min. the animal became very excited and remained in an excited state during the rest of the experiment. As the rate of urinary secretion fell excessive salivation occurred, the amounts collected being recorded in curve *S*. "Infundin" 0·05 unit was injected subcutaneously on two occasions at points *P* and was without effect on the rate of salivary secretion. The urine secreted during this period contained albumin and blood, blood being also present in the fæces. In this and in the subsequent figures water was given at the points the curves commence; the ordinates represent the amounts of urine or saliva secreted in the preceding 10 minute interval and recorded in c.c.; and the abscissæ represent the times in 30 minute intervals.

when injected intravenously caused "minimal inhibition" of water diuresis was determined for each of the three bitches used, and the amount required was remarkably constant for each animal. By "minimal inhibition" is meant the falling of the rate of urinary secretion during water diuresis from near the peak to approximately the resting rate for about 10 min., after which time it again rises to reach a maximum some

40 min. later. No determinations were made, either in the normal or the narcotized animal, without a previous administration of 250 c.c. of water to hydrate the tissues.

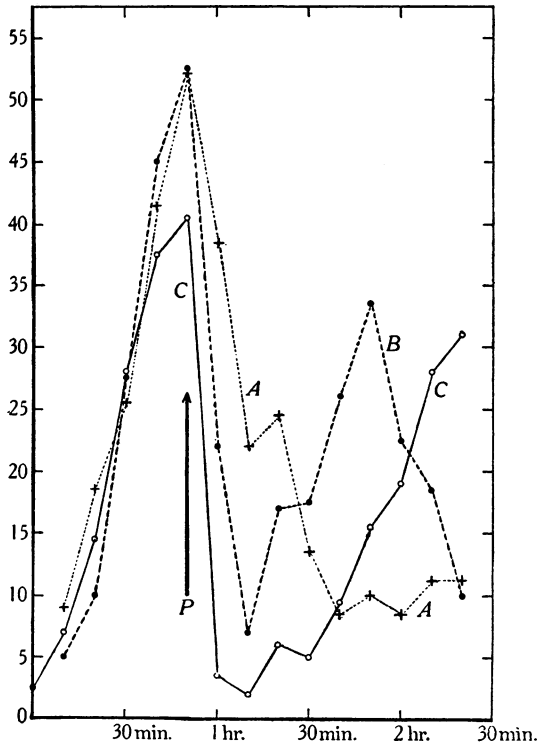

Fig. 2. These three curves were obtained with the same bitch. Curves *A* and *C* represent the effects on water diuresis caused by the intravenous injection of 0·0005 unit of "Infundin" at *P*, the animal being normal in curve *A* and under chloralose narcosis in curve *C*. It will be observed from curve *A* that the animal secreted 85 c.c. of urine during the 30 min. following the injection, while in curve *C*, during the corresponding period of time only 11·5 c.c. were secreted. It is therefore evident that the same amount of "Infundin" inhibited water diuresis more effectively when the animal was under chloralose narcosis than when normal. Curve *B* shows the effect on water diuresis of the intrathecal injection of 0·005 unit of "Infundin," the animal being normal. It is clear that an amount of "Infundin," injected intrathecally into the normal animal inhibited water diuresis less effectively than one-tenth of the amount injected intravenously into the animal under chloralose narcosis.

So soon as the animal was fast asleep and the peak of water diuresis had occurred, the smallest amount of "Infundin" which caused minimal inhibition of water diuresis in the normal state was injected intra-

venously. On each and every occasion the same amount of "Infundin" inhibited water diuresis just as effectually as when the animal was normal. Indeed in one bitch the intravenous injection of 0·0005 unit of "Infundin" was more effective when the animal was narcotized than when normal (Fig. 2, see also Fig. 3).

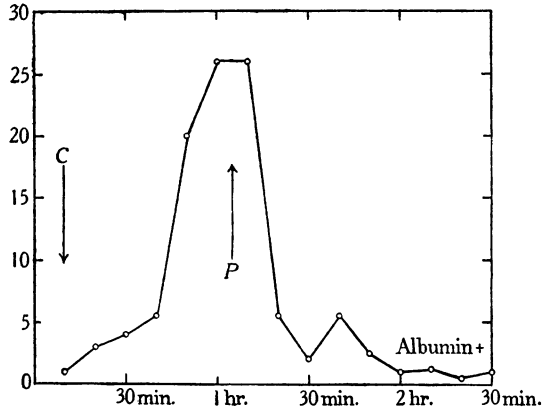

Fig. 3. At *C* 1·5 g. of chloralose dissolved in 250 c.c. of water, were given through a stomach tube. At *P*, 55 min. later, 0·02 unit of "Infundin" was injected subcutaneously and caused maximal inhibition of water diuresis and albuminuria.

The intrathecal injection of "Infundin."

"Infundin," 0·1 unit, was injected intrathecally into a bitch which had been given chloralose in the manner described. The diuresis, which had well commenced, was completely inhibited for 2½ hours, at the end of which time the experiment was discontinued. Two days later, however, it was discovered that the intrathecal injection of 1 c.c. of physiological saline was equally effective in inhibiting water diuresis (Fig. 4). It is not easy to perform lumbar puncture in the dog, and it soon became evident that water diuresis was inhibited even though the needle did not enter the thecal canal. Subsequent experiments on the normal animal proved that attempts at lumbar puncture, if prolonged for from 5 to 10 min., invariably inhibited water diuresis in spite of the fact that the skin and subcutaneous tissues of the area pricked were previously infiltrated with a 2 p.c. solution of novocaine. Diuresis was usually completely inhibited for about 40 min. This result was obtained on many occasions and with five different bitches (Fig. 5).

On two occasions, the one animal being normal and the other under chloralose narcosis, the mere shaving of the back over the area of the

lumbar vertebræ was sufficient to inhibit water diuresis. It was thought that if the splanchnic nerves were blocked evidence might be obtained as to whether both the afferent and the efferent impulses were nervous. The attempt to block these nerves with 12 c.c. of a 1 p.c. solution of novocaine on each side was, however, in itself sufficient to inhibit water diuresis completely. It also became evident that this inhibition of water diuresis by afferent nerve stimuli was more readily caused in animals on which the

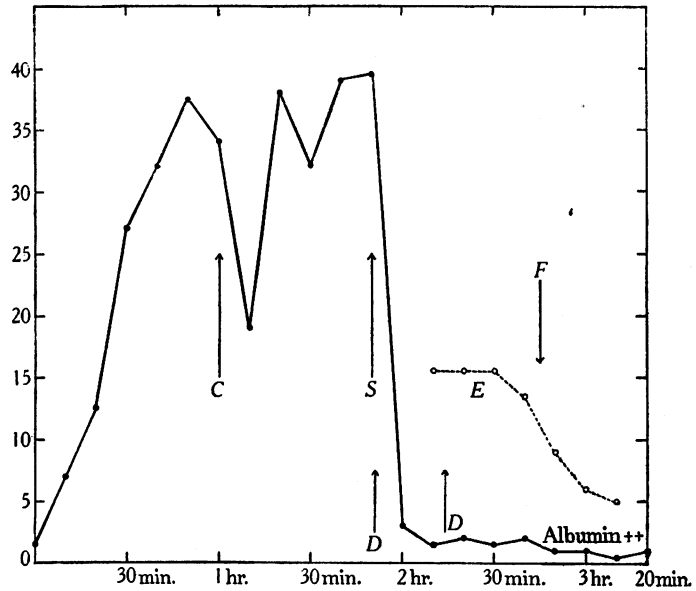

Fig. 4. Curve showing the effects caused by the intrathecal injection of 1 c.c. of physiological saline solution into a bitch under chloralose narcosis. Chloralose, dissolved in 250 c.c. of water, was administered through a stomach tube at *C*. The physiological saline solution was injected intrathecally at *S* and caused immediate and maximal inhibition of water diuresis. The animal defæcated at points *D*. Curve *E* represents the amounts of saliva collected, the secretion of which was inhibited by atropine injected subcutaneously at *F*. After diuresis had been inhibited the urine contained albumin.

"operation" had been performed more than once. In one animal it was found possible to cause a diminution of the rate of urinary secretion during diuresis by making ostentatious preparations for performing the puncture. These observations would suggest the possibility of setting up a conditioned reflex which would inhibit water diuresis.

It may therefore be stated that stimuli ranging in severity from shaving the skin to lumbar puncture inhibit water diuresis for varying periods of time, while the interval from the initiation of the afferent

nerve stimuli to the onset of the inhibition of the diuresis and the period of time for which the inhibition persists vary with the severity and the duration of the stimuli. Whether stimulation of the skin of the lumbar region is more likely to cause this effect than stimulation of any other area must for the present be left unanswered, but shaving the hair from the leg, which has been frequently performed, has on no occasion interfered in the slightest degree with water diuresis. The question as to

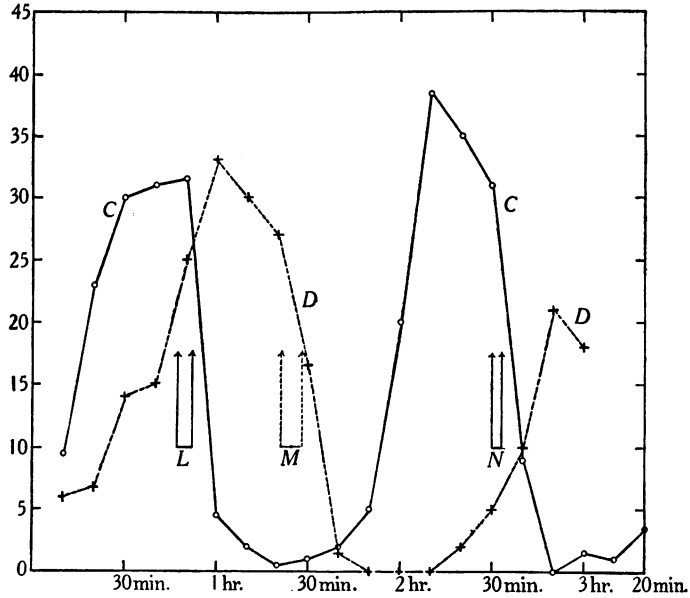

Fig. 5. Curves from two different animals showing the effects of afferent nerve stimuli on water diuresis. In curve *C* the arrows at *L* and *N* indicate respectively the points at which a needle was introduced into and withdrawn from the tissues between two lumbar vertebral spines. At *L* the duration of the stimuli was 5 min. and at *N* 3 min. The arrows at *M* have the same significance for curve *D*, the duration of the stimuli being 7 min. During the inhibition of diuresis which followed these stimuli, albumin was present in the urine secreted by both animals.

whether the inhibition of water diuresis caused in this manner is to be attributed entirely to nervous impulses will be considered in a subsequent paper. The only further point to be mentioned is that the urine, which at the height of diuresis resembled water, became thick and contained albumin after the stimuli had been applied.

It is evident that the phenomenon just described made it extremely difficult to disprove the assertion of Molitor and Pick that amounts of posterior pituitary extract which had no effect when introduced intra-

venously inhibited water diuresis when injected intrathecally. It was, however, possible on one occasion to show that ten times the amount of "Infundin" which definitely inhibited diuresis when given intravenously had no such effect when injected intrathecally (Fig. 6). On three other

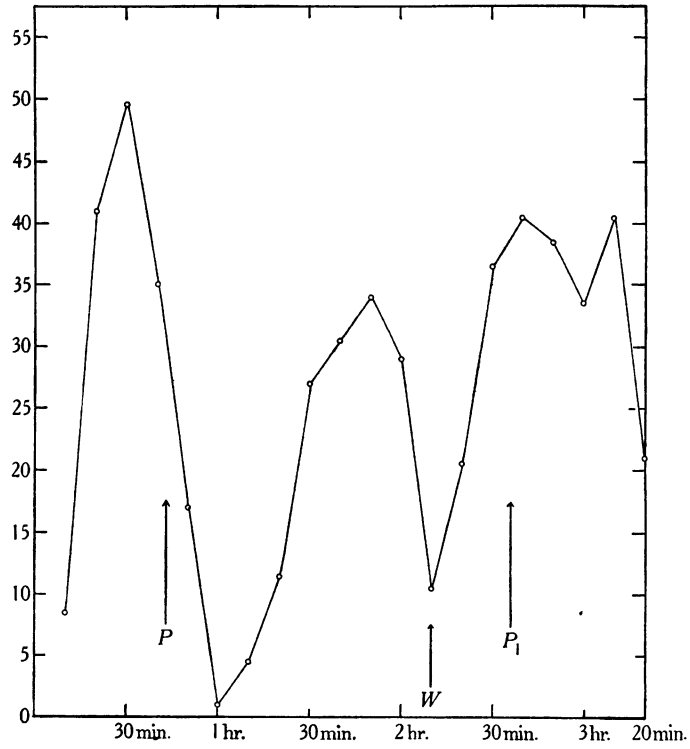

Fig. 6. Curves showing that the intrathecal injection of 0·05 unit of "Infundin" was without effect on water diuresis which was inhibited by the intravenous injection of one-tenth of that dose of "Infundin." At *P* 0·005 unit of "Infundin" was injected intravenously and caused minimal inhibition of water diuresis. The injection would appear to have been made when the rate of urinary secretion was falling, but the fact that the rate subsequently rose to 34 c.c. in 10 min. makes it clear that diuresis was effectually inhibited. At *W* a further dose of 250 c.c. of water was given. At P_1 0·05 unit of "Infundin" was injected intrathecally and was without effect on the rate of urinary secretion.

occasions it was found that a given amount of "Infundin" injected intrathecally inhibited water diuresis less effectually than when introduced intravenously (Fig. 2). Moreover, when 3 units of "Infundin" were injected intrathecally water diuresis was inhibited for 3 hours, whereas when the same amount was injected subcutaneously the inhibi-

tion lasted just over 4 hours. It may be concluded from these results that an amount of "Infundin" which when injected intrathecally has no effect on water diuresis may nevertheless be effective when injected intravenously.

Excessive salivation.

This symptom was observed on several occasions and in all three bitches while under chloralose narcosis. If the animal had been given an excess of water and diuresis was inhibited, excessive salivation invariably occurred. One animal secreted 35 c.c. of saliva in 15 min. and another 151 c.c. in 70 min. (Fig. 1). The symptom could be caused in three separate ways: (*a*) By an insufficient dose of chloralose (Fig. 1). (*b*) By the injection of "Infundin." This fact is of interest in view of statements by other authors that posterior pituitary extract inhibits the secretion of saliva [Gruber, 1929]. (*c*) By afferent nerve stimuli, *e.g.* attempts at lumbar puncture (Fig. 4). The onset of excessive salivation was often very sudden and could be inhibited by an injection of atropine. This vicarious excretion of water is hardly in accord with the view that the tissues are unable to liberate water to the circulation to be excreted by the kidneys.

Albuminuria.

If water diuresis was inhibited either by "Infundin" or by pathic stimuli, the urine subsequently excreted in small amounts almost invariably contained albumin and sometimes blood. It was interesting to observe the clear limpid urine excreted at the rate of 40–50 c.c. in 10 min. suddenly cease and the glass tube connecting the end of the catheter with the cylinder slowly becoming filled with opaque highly pigmented urine sometimes containing a considerable amount of albumin. So soon as diuresis recommenced the urine again became clear and contained no protein. These observations would suggest that the inhibition of water diuresis was effected by a direct action either on the kidneys or on the renal circulation.

Discussion of results.

It is clear that the experiments recorded do not support any of the conclusions reached by Molitor and Pick and indeed that the results obtained are directly contrary to theirs. The reasons for this divergence are:

(1) their experimental methods are open to serious objections;

(2) their conclusions are not altogether supported by the figures they published.

In the first place they made no effort to hydrate the tissues of the animals before obtaining their curves. In their early experiments food and fluid were withheld from the dog for 12 hours before the commencement of the experiment, while the practice observed in the experiments under discussion is not stated. The curves for water diuresis which they have published in their papers bear little resemblance to each other and still less to the normal curves obtained in this laboratory. It may be fairly claimed that they represent the measure of the dehydration of the tissues rather than that of water diuresis. Then, too, although they refer to the effects of posterior pituitary extract when injected intravenously, in practice all their injections were made subcutaneously. The rate of absorption of "Infundin" from the subcutaneous tissues is most variable and even such a large dose as 3 units may take 30 min. before it inhibits diuresis, if it be injected by this route. Further, Molitor and Pick injected the posterior pituitary extract at the same time that they administered the water. They therefore had no guarantee that water diuresis would have occurred even if the extract had not been injected.

A study of their own figures, moreover, makes it evident that they were not justified in drawing the conclusions they did. This study is made difficult because: (1) they did not adopt a standard dose of water, three separate amounts being given to the same dog on different occasions; (2) they used two different commercial preparations of the extract. It is stated that dog "Struppl," while under chloralose narcosis, excreted 54 c.c. of urine in 2 hours, after having been given 100 c.c. of water and 0·05 c.c. of "Physormon" subcutaneously, and that this amount was 72 p.c. of the normal (Protocol 2, p. 114). Elsewhere, however, it is stated that the same dog excreted 93 c.c. of urine in the same time after having been given the same amount of water (Protocol 6, col. 2, p. 119). Clearly 54 c.c. are not 72 p.c. of 93 c.c. Again it is recorded that "Jack" excreted considerably more urine than normal after being given 0·05 c.c. of "Physormon" subcutaneously (Protocol 5, p. 118), and yet just double this amount of the same extract injected by the same route reduced the amount of urine secreted in a given time to 25 p.c. of the normal (p. 115). That exactly half the amount of posterior pituitary extract that has such a marked and prolonged inhibitory effect on water diuresis should have no effect, other than to increase the output of urine, is contrary to our experience. Only one further example will be given. It is recorded that "Struppl" in normal circumstances excreted 106 c.c. of urine in three hours after having been given 100 c.c. of water (Protocol 6, p. 119). It is also shown that 41 c.c. of urine were excreted in the same time after

0·025 c.c. of "Physormon" had been injected intrathecally and the same amount of water had been administered. 41 c.c. thus represent 38·7 p.c. of the normal amount. At the foot of p. 114 it is stated that this dog only excreted 30 p.c. of the normal amount of urine after the same amount of "Physormon" had been injected subcutaneously. From their own figures, therefore, it is clear that the same amount of "Physormon" injected subcutaneously inhibited water diuresis as effectually as when injected intrathecally.

Summary.

1. Water diuresis which occurs while the dog is under chloralose narcosis is inhibited as effectually by the same amount of "Infundin" as when the animal is normal.

2. An amount of "Infundin" which when injected intrathecally has no effect on water diuresis may nevertheless be effective when injected intravenously.

3. An effective dose of "Infundin" when injected intrathecally does not inhibit diuresis for a longer time than when injected subcutaneously.

4. Water diuresis occurring in the dog, whether the animal be normal or under chloralose narcosis, may be inhibited by afferent nerve stimuli, varying in intensity from shaving the skin to lumbar puncture.

5. If water diuresis be inhibited, whether by the injection of "Infundin" or by afferent nerve stimuli, the urine subsequently passed in small amounts usually contains albumin.

6. The hypothesis of Molitor and Pick is not supported by these results, which are directly contrary to those they obtained and on which they based their hypothesis.

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