anaerobic glycolysis of the brain of hypophysectomized rats was reduced 20% below that of normal rats but this was restored to normal by the administration of ACTH.

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Adrenal Medullary Hormones in Water Diuresis.* (18099)

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Recent studies have established the existence of nor-epinephrine both in the adrenal medulla and in natural U. S. P. epinephrine and it is now generally believed that this substance is normally secreted by the adrenal glands(1-4). Many workers(*e.g.* 5-8) have found nor-epinephrine to have biological and pharmacological properties different from those of epinephrine and it has become of general interest and importance to determine which of the many actions ascribed to adrenal medullary secretions are due to epinephrine, nor-epinephrine or both. The diuretic response to water offers an easy and convenient testing procedure for the investigation of one possible difference in the action of these compounds since it has been established that large doses of adrenalin (nor-epinephrine and epinephrine) greatly stimulate the flow of urine in water diuresis(9). The purpose of the experiments reported here, therefore, was to determine whether one or both of the adrenal medullary hormones was responsible for the augmentation of urine flow such as that obtained by Gaunt *et al.*(9). The results indicate that nor-epinephrine rather than epinephrine was the causative agent involved.

Methods. Four types of adrenal medullary hormone preparations† were employed. The adrenalin (Parke-Davis) was predominately l-epinephrine but contained small amounts of nor-epinephrine. The pure compounds used were dl-nor-epinephrine hydrochloride, l-epinephrine bitartrate and l-nor-epinephrine bitartrate monohydrate. Each of these was suspended in peanut oil and administered so that the volume of oil was kept constant (0.05 ml per 100 g body weight) although the amount of hormone varied from 3 to 30 μg per 100 g body weight. Female rats, weighing approximately 200 g were fasted 18 hours but allowed water *ad libitum*. They were placed in individual metabolism cages, injected subcutaneously with hormone, and given orally one dose of water (3 ml per 100 sq cm of body surface)(11) warmed to body temperature. Urine output was recorded at half-hour intervals for a period of 3 hours.

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TABLE I. Showing Mean Effect on Water Diuresis Produced by Various Types of Adrenal Medullary Hormones in Intact Rats.

Series	Type of preparation	No. of animals	% water excreted at indicated min. 30	% water excreted at indicated min. 60 ± S.E.*
1	Controls	19	3.3	30.5 ± 3.7
2	Adrenalin, 30 γ	11	9.5	43.8 ± 5.9
3	DL-nor-epinephrine, 30 γ	12	29.3	79.7 ± 7.6
4	L-nor-epinephrine, 30 γ	12	26.2	84.5 ± 8.2
5	L-epinephrine, 30 γ	12	5.2	34.1 ± 5.1
6	Adrenalin, 3 γ	12	15.1	45.6 ± 5.8
7	DL-nor-epinephrine, 3 γ	11	31.3	61.0 ± 6.2
8	L-nor-epinephrine, 3 γ	14	8.8	49.9 ± 6.9
9	L-epinephrine, 3 γ	15	6.0	27.9 ± 4.9

$$S.E. = \pm \sqrt{\frac{\Sigma d^2}{n(n-1)}}$$

The rate of excretion of the administered water was the major criterion of response. Control rats were injected subcutaneously with 0.05 ml peanut oil per 100 g body weight, but otherwise they were handled in the same manner as those receiving hormones.

Since N, N-dibenzyl-beta-chlorethylamine (Dibenamine) is known to block the action of administered epinephrine(12), it was decided to use this drug in a series of experiments. A 5% solution of commercially prepared dibenamine hydrochloride was diluted with 0.9% saline so that 1 ml contained 10 mg. This was injected subcutaneously in the amount of 0.5 mg (0.05 ml) per 100 g body weight, 14 hours prior to water or hormone administration.

In order to study the effects of hormones and dibenamine in animals without adrenal medullary secretion another group of animals were medullectomized 3 to 4 weeks prior to testing in order to allow regeneration of the cortex. The operations were carried out under ether anesthesia and the glands enucleated by pressure after their capsules had been nicked.

Results. As seen in Table I, the injection of adrenalin was less effective in stimulating diuresis than was nor-epinephrine. Estimations taken from the increment in urine flow over the control value at the 60 minute interval indicated that 30 μg of dl-nor-epinephrine was about 3 times more effective than the same quantity of adrenalin, and at the 3 μg level the dl-nor-epinephrine was 2 times more effective than adrenalin. Such results pointed toward the possibility that the diuretic action of the adrenalin used was due to its nor-epinephrine content; this was further borne out by the fact that l-epinephrine failed to augment urine flow whereas l-nor-epinephrine was highly effective in this respect.

Although it is not shown in the tables, the diuretic response obtained with adrenalin and the nor-epinephrine compounds lasted about 2 hours. At the end of 3 hours, the controls had excreted over 80% of the administered water and had approached, but not equalled, the excretion level of those animals that had been treated.

Obvious signs of overdosage, such as gasping, stretching, twisting, etc., lasted for about 90 minutes after the injection of the higher doses of all preparations. At the 3 μg level, however, dl-nor-epinephrine produced no grossly observable overdosage effects, while l-nor-epinephrine produced detectible responses; adrenalin and l-epinephrine elicited marked but less severe reactions of the types noted above with larger doses. We do not know whether this low dosage is within a physiological range because estimations on the rate of secretion of nor-epinephrine have

11. Benedict, F. G., Vital Energetics, Carnegie Institution of Washington, Washington, D. C., 1938, p. 174.

12. Nickerson, M., *J. Pharmacol. and Exp. Therap.*, 1949, v95, 27.

TABLE II. Showing Mean Effect on Water Diuresis Produced by Adrenalin and DL-nor-epinephrine in Rats Treated with Dibenamine.

Series	Type of preparation	No. of animals	% water excreted at indicated min.	
			30	60 ± S.E.
1	Controls	19	3.3	30.5 ± 3.7
2	Dibenamine, .5 mg	11	1.5	21.4 ± 4.7
3	Dibenamine, .5 mg + adrenalin, 30 γ	12	1.4	5.5
4	Dibenamine, .5 mg + DL-nor-epinephrine, 30 γ	11	4.4	43.5 ± 5.2

TABLE III. Showing Mean Effect on Water Diuresis Produced by DL-nor-epinephrine and Dibenamine in Adrenal Medullectomized Rats.

Series	Treatment	No. of animals	% water excreted at indicated min.	
			30	60
1	Medullectomized	11	9.0	33.7 ± 3.9
2	Medullectomized + DL-nor-epinephrine, 3 γ	11	25.8	68.9 ± 4.3
3	Medullectomized + .5 mg dibenamine	11	18.5	40.8 ± 4.3
4	Medullectomized + .5 mg dibenamine + DL-nor-epinephrine, 30 γ	9	24.1	76.4 ± 6.6

not as yet been made.

Effects of treatment with dibenamine. As seen in Table II, a small amount (0.5 mg) of dibenamine caused a reduction in urine flow. Higher doses of this drug were used but the results are not tabulated here; 2½ mg per 100 g of body weight, the dosage employed by Tepperman and Bogardus(13) in other experiments, was antidiuretic to such an extent that urine flow was almost completely inhibited for 2 hours. The dibenamine blocked and reversed the otherwise diuretic action of adrenalin but only partially blocked the diuresis caused by nor-epinephrine.

Effects of demedullation (Table III). In confirmation of Gaunt *et al.*(9) animals lacking an adrenal medulla responded to diuresis tests in a normal fashion, and when such animals were treated with 3 μg of nor-epinephrine they responded as well as did intact controls. The injection of dibenamine into medullectomized rats did not cause a reduction in the diuretic response to water as it did in intact animals. Furthermore, when 30 μg of dl-nor-epinephrine was administered to demedullated dibenamized rats the nor-epinephrine was just as effective as when given to intact controls. In other words, under these conditions, dibenamine did not alter the diuretic effect of nor-epinephrine.

Discussion. The results reported here show clearly that nor-epinephrine exhibits a diuretic influence when injected subcutaneously in intact rats which is approximately three times greater than that seen with adrenalin. That the diuretic action of the adrenalin used is due to its nor-epinephrine content seems plausible in view of the fact that pure l-epinephrine failed to augment urine flow. Such findings may be of help in reconciling conflicting reports in the literature concerning urine flow after the administration of adrenal medullary hormone preparations(9, 10,14,15) although the mode of administration and the species employed may have to be taken into consideration in arriving at any generalized conclusions. The finding that adrenal demedullated animals respond to a water diuresis test in no way different from intact animals confirms the work of Gaunt *et al.*(9) but is at variance with the results of Stein and Wertheimer(10). The basis for the discrepancy between these two

13. Tepperman, J., and Bogardus, J. B., *Endocrinology*, 1948, v43, 448.

14. Houck, C. R., *Fed. Proc.*, 1950, v9, 63.

15. Hayes, H. W., and Mathieson, D. R., *Endocrinology*, 1945, v37, 156.

groups of workers is still obscure. The problem of the part played by the adrenal medulla in water metabolism under normal conditions is one of considerable importance and the finding reported here that small doses of dl-nor-epinephrine stimulate water diuresis without giving other gross overdosage effects makes it feasible to seek further for evidence of a physiological role for the adrenal medulla in such processes. We intend to explore such possibilities in extension of this work by attempting an analysis of these actions of nor-epinephrine in terms of circulatory and renal mechanisms. It is pertinent to point out that the lack of gross overdosage effects with dl-nor-epinephrine may be due to the lesser toxicity of nor-epinephrine as compared with epinephrine(8).

It is well established that dibenamine will block and often reverse some of the actions of epinephrine(12). This proved to be the case in our experiments for adrenalin but a reversal effect was not seen with nor-epinephrine. In other words, in the intact rat, dibenamine more effectively blocks the action of injected adrenalin than it does nor-epinephrine. However, in the absence of the adrenal medulla dibenamine does not have any blocking action on nor-epinephrine and its characteristic water retaining properties are absent. It is possible that the blocking action of dibenamine on water diuresis is dependent upon the presence of either endogenous or administered medullary epinephrine. It would be of interest to see if this holds true for other types of dibenamine blockage.

Summary. The injection of non-epinephrine in the rat is more effective in causing an augmentation of water diuresis than is adrenalin. Pure l-epinephrine has no stimulating action on diuresis.

Dibenamine causes a water retention in intact rats but fails to do so in adrenal medullectomized rats.

Dibenamine blocks and reverses the diuretic activity of adrenalin in intact rats but it less effectively blocks such activity of nor-epinephrine. Dibenamine, in the dosage used, was incapable of blocking the action of nor-epinephrine in medullectomized animals.

These results indicate that the diuretic action of adrenal medullary hormone is not due to epinephrine but to the presence of nor-epinephrine.

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Influence of Blood Incompatibilities on Measurement of Blood Volume by Cell-Tagging Methods.* (18100)

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During a recent series of direct comparisons in dogs of blood volume determined with radio iron (Fe^{55}), CO and dye (T-1824)(1), we sometimes encountered reactions to the injections of donor blood. In one dog the reactions were so severe that the tests were discontinued. In a second animal with very severe reactions we decided to complete the test and then found that both the CO volume, which normally agrees with the dye-volume, and the radio-iron volume were extremely low as compared with the results of the dye determination. Subsequently, we noted that if the blood for CO saturation and injection was drawn from the recipient dog itself in-

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