Although the number of animals used is not large enough to detect a slight change in response it is evident that the administration of Vitamin C in the manner and amounts here employed has no marked effect either upon the phenomena of sensitization or the subsequent anaphylactic reaction in dogs.

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An Antidiuretic Substance Present in the Urine of Dehydrated Rats.

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Gilman and Goodman[1, 2] have reported the discovery of an antidiuretic substance, which they identify as pituitrin, in the urine of animals dehydrated for varying periods of time. These authors have suggested that the effective concentration of a hormone responsible in any part for the reabsorption of water in the renal tubules must increase as the demand for reabsorption becomes greater, as in states of dehydration. It has, further, been reported by Heller[3, 4] that a portion of pituitrin injected into the animal body appears in the urine.

We have repeated a part of the work of Gilman and Goodman, and are able to confirm their statement that an antidiuretic substance can be recovered from the urine of dehydrated rats.

Healthy, adult male rats were deprived of water for periods of 24, 48, and 72 hours. Their urine was collected during 24-hour periods in sufficient 1% acetic acid to keep the samples weakly acid. An attempt was made to keep the samples free from contamination with fecal matter by lining the collecting funnels with glass wool. In most cases, this was successful. Urine so collected was filtered and dialyzed from 5 to 8 hours against running water, through a "viscose" membrane with a wall thickness of 0.0008 inch. It was then concentrated *in vacuo* to approximately 10 cc. When it was

1 Gilman, A., and Goodman, L. S., *Science*, 1936, **84**, 24.

2 Gilman, A., and Goodman, L. S., *J. Physiol.*, 1937, **90**, 113.

3 Heller, H., and Urban, F. F., *J. Physiol.*, 1935, **85**, 502.

4 Heller, H., *J. Physiol.*, 1937, **89**, 81.

necessary to hold the urine for any length of time between steps in the processing, it was kept in an ice box.

The assay for antidiuretic activity was performed according to the method of Burn[5] with the exception that the time required for the excretion of one-half the fluid given by gavage was used as an index of diuresis, rather than the time necessary to reach the point of maximum rate of excretion. These 2 points correspond closely. Healthy male rats weighing between 200 and 300 g were used. Each rat was given tap water to the extent of 10% of its body weight by stomach tube. Each urine concentrate was assayed collectively on 4 rats. Each rat was injected with 2.5 cc of the urine concentrate. Urinary output was measured at 15-minute intervals. Interpolation within 15-minute periods was performed when necessary to determine the "mid-excretion point."

Results. (A) Control Group. Fifty control determinations were made. Instead of urine concentrate, 2.5 cc tap water was injected into each rat of an assay group. The time required to reach the "mid-excretion point" was 87 ± 2.8 minutes. This figure corresponds closely with the figure 86 ± 11 minutes reported by Heller[4] as the result of 36 similar control determinations.

(B) Non-dehydrated Group. Six determinations were made on the urine of groups of 12 rats collected during a 24-hour period in which the animals had free access to water. The time required to reach the "mid-excretion point" was 100 ± 5.7 minutes.

(C) Rats Dehydrated 24 Hours. Six determinations were made on the urine of groups of 14 rats collected during a 24-hour period in which the rats received no water. The time required to reach the "mid-excretion point" in this series of tests was 147 ± 19 minutes.

(D) Rats Dehydrated 48 Hours. Nine determinations were made on the urine of groups of 14 rats collected during the last 24 hours of a 48-hour period during which the animals were allowed no water. The "mid-excretion point" in this series was reached after 134 ± 13 minutes.

(E) Rats Dehydrated 72 Hours. Ten determinations were made on the urine of groups of 14 rats collected during the last 24 hours of a 72-hour period of water deprivation. The time required to reach the "mid-excretion point" in this series was 137 ± 9.2 minutes.

The foregoing results are summarized in Table I.

[5] Burn, J. H., *Quart. J. Pharm.*, 1931, **4**, 517.

TABLE I.

	No. of assays	Mean "mid-excretion time"	Probable error
A. Control group	50	87	± 2.8
B. Non-dehydrated rats	6	100	± 5.7
C. Rats dehydrated 24 hours	6	147	±19.0
D. " " 48 "	9	134	±13.1
E. " " 72 "	10	137	± 9.2

(F) Pitressin Controls. Forty cc human urine from a non-dehydrated subject was added to 20 cc of 1% acetic acid. The solution was exposed on our collecting stand for 48 hours, then filtered, dialyzed and concentrated. Upon assay it produced a "mid-excretion point" at 94 minutes. To a similar quantity of the same urine 50 mille units of pitressin were added, and the same process repeated. This produced a "mid-excretion point" at 181 minutes. Urine to which 200 mille units of pitressin were added, and similarly treated produced a "mid-excretion point" at 280 minutes.

In our series there is no significant difference between the antidiuretic effect of tap water and the urine concentrate of well hydrated rats, for the difference between the means of these groups is 13, a figure less than 3 times the probable error in the assays. There is, however, a significant difference between the control mean and that obtained by the injection of the urine concentrate of dehydrated animals.

It will be noted that in our series the amount of antidiuretic substance excreted reached a fairly uniform level after 24 hours of dehydration, and remained at about this level for the duration of the dehydration period. In this respect, our results differ from those of Gilman and Goodman, who found that the excretion of antidiuretic substance reached a maximum level at the end of 48 hours' dehydration and remained at about this level during periods of dehydration ranging up to 96 hours. The fact that a level of excretion of antidiuretic substance is reached and maintained is in accord with the observation of Heller[4] that within certain ranges, the amount of antidiuretic substance excreted by the kidney during experiments in which the hormone (pituitrin) is added to the blood, is definitely independent of the blood hormone concentration.

Ingram, Ladd and Benbow[6] have recently reported the detection of antidiuretic substance in the urine of dehydrated but not normal cats. Results somewhat contradictory to ours and those of

[6] Ingram, W. R., Ladd, L., and Benbow, J. T., *Proc. Am. Physiol. Soc.*, April, 1938, p. 107.

Gilman and Goodman have recently been reported in abstract by Walker.[7]

Conclusion. An antidiuretic substance can be obtained from the urine of dehydrated rats. This substance is not present in significant quantities in the urine of non-dehydrated rats. Its action upon water diuresis is similar to that of pitressin, though we cannot say that it is pitressin.

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Effect of Choline on Atherosclerosis in the Rabbit Aorta.*

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The cholesterol content of rat liver is increased by the feeding of cholesterol,[1, 2] and its accumulation is accompanied by the deposition of large amounts of neutral fat. The increased cholesterol is chiefly in ester form.[3] The fatty livers produced by feeding 1% of cholesterol can be prevented by feeding large amounts of choline.[2] In the rabbit, cholesterol feeding results in the deposition of cholesterol in the aorta in the form of atheromatous plaques.[4-8] Atherosclerosis in rabbits has been prevented by feeding a pancreas extract.[9] The antagonism exhibited between choline and cholesterol deposition in the rat led us to investigate the effect of choline on the formation of plaques in the rabbit aorta.

Twelve female rabbits, 1.3 to 3.0 kg in weight, averaging 2.14 kg were divided into 3 groups. The first group received Purina

[7] Walker, A. M., *Proc. Am. Physiol. Soc.*, April, 1938, p. 210.

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[1] Chanutin, A., and Ludewig, S., *J. Biol. Chem.*, 1932, **102**, 57.

[2] Best, C. H., Channon, H. J., and Ridout, J. H., *J. Physiol.*, 1934, **81**, 409.

[3] Okey, R., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1003.

[4] Cowdry, E. V., *Arteriosclerosis*, Macmillan & Co., 1933.

[5] Rosenthal, S. R., *Arch. Path.*, 1934, **18**, 473.

[6] Menne, F. R., Beeman, J. A. P., and Labby, D. H., *Arch. Path.*, 1937, **24**, 612.

[7] Turner, K. B., and Bidwell, E. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 656; 1935, **62**, 721.

[8] Aylward, F. X., and Stott, Wm., *Biochem. J.*, 1937, **31**, 2055.

[9] Huber, M. J., Brown, G. O., and Casey, A. E., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 441.