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Antidiuretic Substance Levels in Rat Plasma

By FREDERIC A. GIERE

In recent years it has been our desire to evaluate the presence of antidiuretic substance (ADS) in the blood of animals under various experimental conditions (Giere, 1951; Eversole, Giere and Rock, 1952; Giere and Eversole, 1954; Eversole and Giere, 1954). For these determinations we have used the method of Birnie, *et al.* (1950) and also our modification of the technic of Ames and van Dyke (1952). The results herein reported constitute a continuation of the above work to include other physiological and experimental conditions including such controversial subjects as the concentration of ADS in the adrenalectomized rat (Birnie, *et al.*, 1950; Ames and van Dyke, 1952; Robinson and McFarlane, 1956).

MATERIAL AND METHODS

The method employed for the assay of plasma is our modification (Giere, 1957) of the technic of Ames and van Dyke (1952). Female rats in the 'normal state' (*i.e.* non-fasted and without thermal shock for the previous five days) are hydrated by gavage with 5 ml/100 gm body weight of a 0.05% NaCl solution. This solution serves to place a water load on the animal and also to provide a reserve of sodium and chloride ions. About 30 minutes later, the rat is further hydrated with 5 ml/100 gm body weight of an 11% ethanol solution. The ethanol serves to hydrate the rat, to anesthetize it for surgical processes and to shut off the endogenous anti-diuretic principle by blocking the posterior pituitary. If the rat is handled roughly, or is injured during the stomach tubing process, it is not likely that the animal will be suitable for assay purposes. About 30 minutes after the administration of the ethanol the rat should be anesthetized sufficiently to be insensible to pinching the paws. The urethral catheter is then placed in position, and an indwelling polyethylene cannula placed in the jugular vein. Within an hour the urine flow should be equal to or greater than one ml per ten minutes. The urine volume is recorded and a sample saved if electrolyte concentrations are to be determined. When the urine flow is plateaued for two or three readings, a known quantity of antidiuretic principle is injected *via* the jugular vein. The ADS is diluted daily from refrigerated stock solution of vasopressin. The urine flow after each sample is collected for three minutes, recorded and discarded. The sample from three to thirteen minutes is the

urine sample regarded to be influenced by the injections, since this has given time for the hormone to act on the nephra. When the rate of urine flow has returned to the base level the other test substances may be injected for assay of ADS. The blood from such a rat is collected by draining the trunk after swift decapitation over a funnel and centrifuge tube filmed with 0.2 ml heparin. The blood is centrifuged and injected within ten minutes of decapitation. The urine samples may be analyzed by flame photometry for the concentration of sodium and potassium cations.

The animals which underwent adrenal medullectomy were permitted to recover sufficiently long to permit regeneration of the cortex. Those placed in the cold were in cages in a walk-in refrigerator (4°C) for periods of 18 hours to 1 week.

The stages of the estrous cycle were determined by examination of vaginal smears.

RESULTS AND DISCUSSION

Adrenal medullectomized male rats with regenerated cortices were placed in the cold for periods of 18 hours, 72 hours, and 1 week. The plasma from these rats was assayed for ADS in the manner described above and there was no detectable increase in the concentration of ADS, but rather a diuresis was apparent.

Bilaterally adrenalectomized male rats (maintained with tap water and Purina Chow *ad libitum*) were sacrificed at 3 days, 7 days, 10 days and 24 days and their plasma assayed for ADS. No ADS was detected in samples from 3-10 days, but the increase in ADS at 24 days was statistically significant ($t = 2.1$). The mean concentration was 19 $\mu\text{U}/\text{ml}$ plasma.

No difference in the concentration of plasma ADS was detected at the several stages of the estrous cycle (anestrus, metestrus, estrus), nor were differences noted between young males (less than 150 gm body weight) and old males (greater than 250 gm body weight).

In an earlier paper we had reported that the diuretic action of the adrenal medullary hormones in the rat may, in part, be due to their blocking of the release of posterior pituitary hormones. It would appear from our data reported here that the 'cold diuresis' in the assay rat after the injection of plasma from an adrenal medullectomized rat could not be effected by medullary hormones blocking the posterior pituitary. Gordon (1950) has also reported that the adrenal medulla is not essential for the activation of the anterior pituitary gland by stressing agents as judged by the concentration of the adrenal ascorbic acid.

Previous investigators have reported increased concentration of

ADS in adrenalectomized rats (Birnie, *et al.*, 1950) and others have reported no change in ADS concentration (Robinson and MacFarlane, 1956). As nearly as can be determined from the published results, the plasma or serum samples were taken from rats about 1 to 2 weeks after adrenalectomy. Our results would indicate there to be no increase in ADS at that time, however the increased ADS in rats adrenalectomized a longer period of time was quite obvious and significant.

Although Heller (1957) has stated that there are significant differences in the pressor activity of rat pituitaries associated with the estrous cycle, we have not observed any differences in the concentration of the ADS in the plasma of female rats associated with the estrous cycle.

SUMMARY

We interpret these data as indicating that (a) cold diuresis is independent of the adrenal medulla, (b) there appears to be an increase in plasma ADS of long term adrenalectomized rats, (c) there is no apparent change in plasma ADS with the age of the rat, nor (d) is there a change in plasma ADS associated with the estrous cycle.

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