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An Intravenous Assay for Antidiuretic Substance

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In recent years it has been our desire to evaluate the presence of antidiuretic substance (ADS) in the bloods 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 Ames and van Dyke's (1952) modification of the intravenous method of Jeffers, Livezey and Austin (1942). Although these technics are qualitatively accurate it has been our experience that they are oftentimes difficult to duplicate. The following technic is offered as a semi-quantitative procedure which can be duplicated by a practiced individual.

Female rats are taken in a 'normal state' (i.e. a non-fasted condition, with water *ad libitum* and without exposure to thermal shock in the animal room for the previous five days). It is desirable that they be in the weight range of 175-225 grams, as smaller rats are sometimes difficult to catheterize, the urine flow is not great and the larger animals show great variation in response to administered ADS.

The rat is 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. 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. The ethanol serves to hydrate the rat, to anesthetize it for surgical processes and to shut off the endogenous antidiuretic principle by blocking the posterior pituitary gland (Ames and van Dyke, 1952).

About 30 minutes after the administration of ethanol the rat should be anesthetized sufficiently to be insensible to pinching the paws. When this level is reached, the rat is placed in a supine position and secured to an operating board. The urethral catheter is then placed in position.

The catheter is fashioned from a 100 mm length of Clay Adams 0.038 in. OD, 0.023 in. ID polyethylene tubing. A mariah may be made about 20 mm from one end. It is desirable to 'round' the end of the tubing so that it will follow the lumen of the urethra.

One lip of the urinary papilla is grasped with a mouse tooth forceps and the catheter picked up with the working hand. It is desirable to

wet the catheter with physiological saline. The greatest success with the insertion of the catheter is enjoyed when it is directed somewhat *caudad* and dorsal along the ventral side of the urethral lumen. The tubing should then be advanced and directed anteriorly by rolling the tube back and forth between the thumb and forefinger. When the catheter is in position urine will drain freely. The catheter is held in position by passing a thread through the urinary papilla and then tying the papilla around the tube in the manner of a purse-string.

The ventral neck is shaved of hair and a 30 mm incision made parallel to the longitudinal axis. The musculature is parted with forceps to expose the jugular vein. When the vein is free of connective tissue, two lengths of nylon ligature are passed under the vein. One is placed *craniad* and one *caudad* on the free section of the vein. The anterior ligature is secured. While grasping the vein with forceps, a nick or hole is made with a saline lubricated 23 gauge hypodermic needle. The infusion tube is directed into the hole a distance of more than 15 mm until blood can be aspirated by an attached syringe, and then is held fast by the posterior ligature. The infusion tube is fashioned from a 30 cm length of 0.024 in. OD, 0.011 in. ID Clay Adams polyethylene tubing one end of which should be beveled to afford easy entrance into the vein, and the other end fitted with a Clay Adams Plastic Tubing Adapter. A one ml syringe filled with physiological saline is always attached except during the injection of test materials. The incision is closed by suture.

The rat is placed on a board perforated by a hole through which the urethral catheter passes into a suspended syringe (graduated to 0.01 ml and fitted with a stopcock) and is retained in a hardware cloth cage attached to the board. A polyethylene tube about 1 mm OD is inserted into the stomach *via* the mouth and esophagus. A 10 ml syringe attached thereto is filled with 1% ethanol in a 0.025% NaCl solution and a relatively constant water load is maintained on the rat by adding to the stomach a volume of the alcohol-saline solution equal to the volume of urine produced in a ten minute test period.

There may be a short period of antidiuresis during the adjustment period. 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 is 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 a 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

Table 1
Sample Data Sheet from Assay Procedure

Date 8-14-56			
Assay Rat		Long-Evans 240 gm	5% = 12 ml
Urine Sample		Urine/10 min	% Change
		1.16	
		0.45	
		2.27	
		1.82	
	A	2.00	
30 μU Pitressin			
	3 min.	1.02	
	B	1.42	—29
		1.72	
		2.17	
	C	2.15	
1 ml plasma #314 (normal)			
	3 min.	0.77	
	D	2.08	— 3
		1.42	
		2.48	
		2.58	
	E	2.32	
1 ml plasma #315 (in cold box 18 hrs + 4°C)			
	3 min.	0.82	
	F	1.62	—30
		1.70	
		1.98	
		2.31	
	G	2.35	
20 μU Pitressin			
	3 min.	0.98	
	H	1.93	—18

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. The injection of vasopressin reduces the urine flow (c.f. Table 1). When the rate of urine flow has returned to the base level (the old base level or a new level) other substances or other quantities of the same substance may be injected *via* the jugular vein. Trunk blood from a tranquil rat swiftly decapitated shows no ADS, i.e. there is no alteration in the rate of urine flow following the administration of heparinized plasma from a rat so treated. The blood from such a rat is collected by draining the trunk 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 the sodium and postassium cations.

It is our belief that this is a reliable semi-quantitative procedure for the assay of antidiuretic substance because the responses due to certain unknown substances may be bracketed by the responses from two known substances, and a comparison thereby may be made.

Literature Cited

Ames, R. G., and H. B. van Dyke, 1952. Antidiuretic hormone in the serum or plasma of rats. *Endocrinol.* 50:350-60.

Birnie, J. H., W. J. Eversole, W. R. Boss, C. M. Osborn, and R. Gaunt, 1950. An antidiuretic substance in the blood of normal and adrenalectomized rats. *Endocrinol.* 47:1-12.

Eversole, W. J., F. A. Giere, and M. H. Rock, 1952. Effects of adrenal medullary hormones on renal excretion of water and electrolytes. *Am. J. Physiol.* 170:24-30.

Eversole, W. J., and F. A. Giere, 1954. Effects of adrenal medullary hormones on antidiuretic substance (ADS) in blood. *Fed. Proc.* 13:137.

Giere, F. A., 1951. A study of the possible sites of action of the adrenal medullary hormones in water diuresis. *Anat. Rec.* 111:142.

Giere, Frederic A., and W. J. Eversole, 1954. Effects of adrenal medullary hormones on antidiuretic substance in blood serum. *Science* 120:395-6.

Jeffers, W. A., M. M. Livezey, and J. H. Austin, 1942. A method for demonstrating an antidiuretic action of minute amounts of Pitressin. *Proc. Soc. Exp. Biol. & Med.* 50:184-8.

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