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Production of Diabetes Insipidus in the Rat.* (24275)

CARL S. ALEXANDER† (Introduced by Edmund B. Flink)
Veterans Admin. Hospital, and Department of Medicine, University of Minnesota Medical School, Minneapolis

Reports dealing with diabetes insipidus in partially hypophysectomized rats have seldom described operative technics(1-6). This report sets forth in detail the surgical technic of neurohypophysectomy as performed in over 500 animals during the past 5 years, and also more recent experience with hypothalamic lesions produced with a stereotaxic instrument. The water exchange in acute and chronic DI as well as other conditions resulting from such lesions will also be discussed.

Methods. Sprague-Dawley rats of either sex, weighing approximately 200 g, were subjected to neurohypophysectomy by the parapharyngeal approach. Clean but not sterile technic was used. The procedure as described by Smith(7) and Thompson(8) has been modified in 2 important respects: 1) ether is used as the anesthetic because it avoids respiratory depression often seen with barbiturates, and 2) after making a midline neck incision extending from lower jaw to sternal notch, the trachea is incised just below the thyroid cartilage parallel and between 2 rings of tracheal cartilage. A glass tube 4 mm in diameter, 5-6 cm long and tapered to 2 mm at the end serves as a tracheal cannula thru which ether may be administered. Except during actual administration of the anesthetic, it is important to remove ether bottle to avoid CO_2 accumulation and hypoxia. A bloodless plane of dissection between deep strap muscles on the right side of neck can be found by gently retracting with 2 pairs of curved forceps. The digastric muscle is not cut as by Thompson(8) because retraction of the trachea permits adequate exposure of the basisphenoid area of skull. Two curved paper clip retractors are now placed in the plane separating the deep neck muscles dissected on the right side. The exposed basisphenoid area, which can be recognized by the V-shaped muscles inserting along the midline, is freed of its muscle attachment. An important landmark can now be seen—intersection of a longitudinal ridge (crista occipitalis) with the blue transverse cartilage (occipito-sphenoid synchondrosis). Approximately 1 mm rostral to this intersection a hole is drilled and enlarged with a No. 9 dental burr (a smaller size burr may be used to start the hole). Two precautions should be observed during drilling; occasionally a blood sinus in the bone located in rostral portion of hole may be entered, resulting in a brisk oozing of blood which can be stopped only by sustained pressure best applied using #3 round dental pel-

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† Veterans Admin. Clinical Investigator.

TABLE I. Results of Various Procedures Designed to Produce Diabetes Insipidus in the Rat.

Procedure*	No. operated	% mortality	% of survivors developing Acute DI	Chronic DI
DI	300	20	60	20
C	20	20	30	13
C + DI	200	22	70	25
DI + KS or C + DI + KS	30	50	30	20

* DI, neurohypophysectomy; C, cautery of stalk; KS, hypothalamic lesions.

lets serving as tampons. To some extent this injury in bone sinus can be avoided by centering the hole *at the intersection* in older animals. The second precaution is to avoid drilling completely through the inner table of skull, but to leave a thin shelf of bone which is then carefully removed by a curved, sharp, pointed pick and fine curved forceps. Removal of bone in this manner prevents damage to the gland itself by the burr. The procedure should then be completed with the aid of a binocular dissecting microscope with magnification of 7 to 10X or a loupe. After the bone is completely removed, the underlying tough dural membrane enclosing the pituitary gland is slit down the middle with aid of the pick. The pink anterior lobe immediately bulges into the opening. Complete exposure of the anterior lobe may be afforded by hooking the pick under the free margin of the membrane and pulling each half of the membrane toward the outer margins of the hole as far as possible. Gentle retraction, using 2 picks, one on either side of the anterior lobe, or one pick and a capillary suction pipette, results in a plane of separation permitting division of anterior lobe into 2 halves between which lies the white, fluffy, vermis-like intermediate lobe. The gland of the female is relatively larger and more friable than that of the male. By gentle manipulation of the dissecting needles and curved capillary suction tube, the dark purple-colored posterior lobe can be mobilized, and while restraining the 2 halves of the anterior lobe with the fine curved forceps, suction is abruptly applied to the intermediate and posterior lobes. After tracheal secretions have been aspirated, a single suture consisting of 6-0 silk joined to an atraumatic needle ("eye suture") is placed on either side of the tracheal ring bordering the tracheal opening. Trial closure of the tracheostomy is carried out before the tie is secured. Before suturing, skin margins are freed from underlying connective tissue to allow more space for accumulation of any serous fluid. Ballooning of wound after closure indicates an air leak either from trachea or from fistula in the hypopharynx. The former can easily be repaired but the fistula is difficult or impossible to repair; such animals are best discarded since death will most certainly occur early in the postoperative period. Final step in the procedure is aspiration of blood and mucus from nares and oropharynx. With skill entire procedure takes 15-20 minutes. Requirements for postoperative care are simple: Room temperature should be 78-80° F, animal in individual cage for at least 10 days, and regular stock diet. *Use of cautery.* The technic is the same as described above with certain modifications. After separation of the anterior lobe from the neurohypophysis, the exposed end of a No. 26 varnished copper wire electrode is applied to the proximal end of stalk. The indifferent electrode is placed in the rectum. A current of 3 to 5 milliamperes‡ for 15 to 18 seconds is applied to the stalk without damaging the neighboring anterior lobe. Usually this results in appearance of small bubbles in the fluid bathing the electrode and sometimes even an eschar. The neurohypophysis is then gently dissected free from the coagulum and aspirated as described above. *Use of stereotaxic instrument.*§ The technic employed has been similar to that described by McCann(9) with minor modifications. The aim is to destroy the supraoptic and paraventricular nuclei producing ADH.

Results. A summary of results covering operative mortality and the development of acute and chronic DI in 550 rats is given in

‡ May be furnished by a D.C. Heathkit regulated power supply with a milliammeter and voltmeter in the circuit.

§ The instrument and atlas of cross-sectional anatomy of the rat brain are available from Johnson Instrument Co., Berwyn, Ill.

Table I. In this study DI is considered present when urine output is 3 or more times that of normal rats of comparable weight on a Fox-chow, water *ad lib.* diet. Approximately 6% of animals operated excreted 100 ml or more of urine/day at some time during postoperative period (Table II). In ½ the group with transient DI a residual defect in urine concentration persisted despite urine output within the range of normal to 3 times normal. The remainder, ½ of the group, returned to a normal condition. Chronic DI developed more frequently after hypothalamic lesions than with other technics, and the highest urine outputs occurred in these animals. But this advantage was more than offset by higher mortality and the work involved in performing an extra procedure (hypothalamic lesions in normal rats are less satisfactory). Three such animals had sustained polyuria, excreting 150-240 ml of urine for several weeks and in 2 of the 3 obesity due to hyperphagia was also present.

Early postoperative course. Urine output may be quite variable for the first few days. Passage of dilute urine within hours after surgery occurred even before animals began to drink water. Despite the extreme degree of fluid exchange seen early in some of these animals, there was a sudden or gradual decline in rate of water exchange to a level only 2 to 3 times normal. However, in about 50% of such animals 2 permanent abnormalities remained: 1) urine specific gravity persisted at lower than normal value and did not reach that of controls even after large isolated doses of pitressin, and 2) when the animal was offered 1% NaCl in place of water as sole drinking fluid there was a sharp increase in level of fluid exchange far exceeding the response of a normal rat to this maneuver (Fig. 1). An atypical course in one animal charactreized by unexplained extreme fluctuations in fluid exchange is illustrated in Fig. 2.

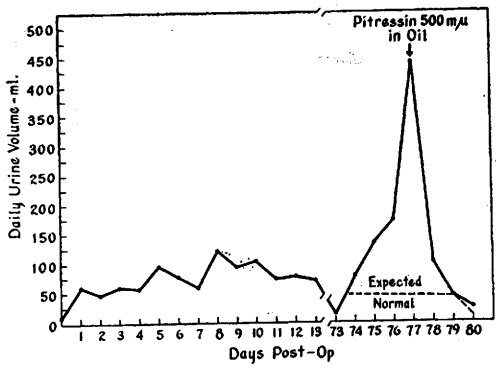
Chronic diabetes insipidus. One-third of

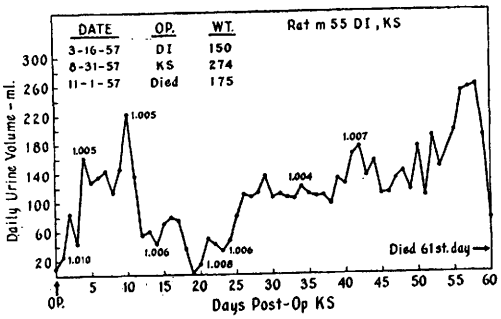
TABLE II. Excretion of 100 ml or More of Urine per Day (1 Day or Longer) in Survivors of Various Operative Technics.

Procedure*	No.	Urine output (ml/day) Avg	Range	Sp. gravity Avg	Range
DI	18	148	105-170	1.007	1.005-1.008
C	1	182		1.009	
C + DI	11	150	113-175	1.007	1.003-1.012
C+DI+KS	2	222	186-258	1.004	1.003-1.005
	2†	178	161-166	1.004	1.012-1.025

* See footnote, Table I.

† Polyuria was associated with hyperphagia and obesity.





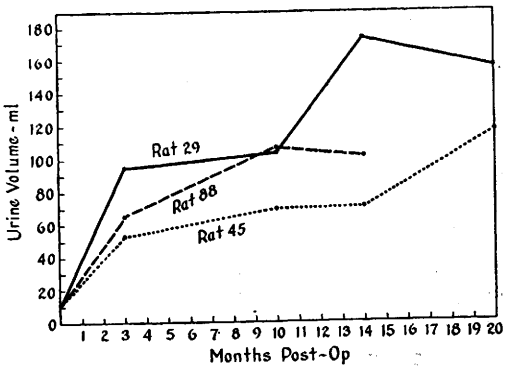


FIG. 1 (top). Pattern of fluid exchange in neurohypophysectomized rat. Exaggerated response to drinking 1% NaCl substituted for water on 73rd postoperative day.

FIG. 2 (center). Unusual course in fluid exchange in rat with neurohypophysectomy and electrolytic lesions of the hypothalamus.

FIG. 3 (bottom). Avg daily urine output on stock diet in 3 neurohypophysectomized rats.

all animals with acute DI developed chronic DI. That diabetes insipidus can be both severe and permanent is illustrated in Fig. 3 which shows average daily urine output over a long period of 3 animals subjected to neurohypophysectomy. Diabetes insipidus animals with severe polyuria generally do not show signs of anterior pituitary deficiency such as inactivity, weakness, fur changes or testicular atrophy.

Other postoperative syndrome. Electrolytic lesions made with the stereotaxic instruments produced a variety of other sequelae deserving mention. In contrast to the syndrome characterized by viciousness, hyperphagia, polydipsia, polyuria and eventual obesity, other animals with brain lesions made in the same manner refused to eat, drink or move about the cage and died within a few days. Life could not be prolonged beyond an extra few days by administration of DCA, cortisone or parenteral glucose and saline. In some rats arching of spine and ataxia on forced movement was noted. Others were extremely agitated as soon as they recovered from anesthesia and circled the cage incessantly as if trying to escape. They, too, ate and drank little or nothing and within a few days died in a state of apparent exhaustion. It is important to note that many of these animals excreted dilute urine early postoperatively even in complete absence of food or water intake. Pitressin administration produced an increase in urine concentration without appreciably affecting the volume.

Discussion. The existence of permanent DI in the rat has been questioned by some workers(10,12), but others have observed DI of long duration(1,4,5). Diabetes insipidus itself has been arbitrarily defined and therefore the reported duration and severity may differ from one report to another. Compared to values reported by others(1,4,5,6), it is evident that excretion of urine exceeding 100 ml/day by animals weighing 250-300 g reflects severe ADH deficiency. Assuming glomerular filtration rate (GFR) of 0.6 ml/min/100 g body weight for the rat(13), excretion of 100 ml of urine/day by a 250 g rat amounts to 4.6% of total plasma filtered. Assuming for man, 187 liters of plasma is filtered/day, the same fraction excreted would produce 8.6 liters/day which approximates the output of the human with DI of average severity. The highest daily urine output ever recorded in these DI rats (258 ml, corresponding to 11.9% of the total plasma filtered) would be comparable to a urine output in the human of over 21 liters, a figure rarely reported even in most extreme cases of human DI. Inasmuch as maximum concentrating ability of rat kidney is greater than the human kidney (2000 mosm/l *vs.* 1500 mosm/l(14), respectively), urine output in these rats with severe DI is even more impressive.

It is clear from these postoperative results and from the work of others that amount of urine excreted depends not only on ADH, but also on rate of water and solute excretion, which in turn depends on the integrity of the appetite(15-17) and drinking centers(18). Damage to these centers even in absence of ADH leads to oliguria and hyposthenuria and may explain the unexpected appetite for salt (Fig. 1) and fluctuating thirst (Fig. 2). It is well known that diuresis induced in DI subjects by isotonic saline can be interrupted by pitressin. Degree of response is a function of pitressin dosage and rate of solute excretion (21), and Jacobson and Kellogg(22) have even shown that pitressin can inhibit diuresis induced in normal rats with hypertonic saline (0.23 M NaCl). The prompt antidiuresis caused by a relatively large dose of pitressin (Fig. 1) may also be explained in this way.

Summary. Experience with several technics for producing experimental diabetes insipidus (DI) in 550 rats has been described. These include neurohypophysectomy, cautery of stalk, and electrolytic lesions of the hypothalamus produced with aid of a stereotaxic instrument.

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Lipemia in the Rabbit Following Injection of Pituitary Extract.* (24276)

DANIEL RUDMAN† AND FLOYD SEIDMAN (Introduced by David Seegal)
Columbia Univ. Research Divvision, Goldwater Memorial Hospital and Dept. of Medicine, Columbia University, N. Y. City

It has been recognized that the pituitary gland plays a role in regulation of lipid metabolism. Injection of pituitary substances into experimental animals has been reported to produce the following changes: (A) Decrease in fat content of carcass and adipose tissue, and increase in fat content of liver(1, 2); (B) Increase of ketone bodies in blood and urine(2,3,4); (C) Depression of respiratory quotient(5); (D) Increase in plasma content of unesterified fatty acids(6). These effects appear to result from mobilization of lipid from fat depots, with subsequent deposition of fat in the liver, and increased oxidation of fatty acids associated with accumulation of ketone bodies(2,7,8). Increase in liver fat content has been observed following injection of anterior pituitary extract, and of various anterior pituitary fractions, including adrenotropic, growth, and thyrotropic preparations (9,10). Increase in plasma unesterified fatty acids has been observed after injection of growth hormone(6). A further effect of pituitary material upon circulating lipids has been described. Evans(11), Munoz(12), Baumann(13), and Campbell(14) observed lipemia in dogs and rabbits made diabetic by repeated injections of anterior pituitary extract or of purified growth hormone. Greenbaum(15) described a transitory increase in plasma triglyceride of rats receiving daily injections of growth hormone. Seifter(16) reported that a dialyzable material obtained from posterior lobe of hog pituitary is inhibitor of post-heparin plasma clearing factor and causes an increase in plasma lipids of animals with liver damage, or of animals fasted for 20 hours. The present study was undertaken to determine the effect of injections of crude pituitary extract and of various purified pituitary fractions, upon serum lipid pattern of the rabbit.

Methods. Male rabbits weighing 2.5 to 4 kg were used. They were fed Purina rabbit chow with the exception that in experiments where blood sugar determinations were made

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† Senior Research Fellow, N. Y. Heart Assn.