

## Natriuresis and Kaliuresis in Saline-Expanded, Long-Term Hypophysectomized Rats (41595)

ROBERT O. BANKS

*Department of Physiology (ML No. 576), University of Cincinnati College of Medicine, Cincinnati, Ohio 45267*

---

**Abstract.** The ability of unanesthetized, long-term hypophysectomized (HPX) rats to excrete a saline challenge was evaluated. HPX rats were hypophysectomized 1-2 months prior to experiments. Saline was administered either by intragastric gavage or by intravenous infusion. For 3 hr following gastric saline loading, HPX rats excreted significantly less sodium and potassium than did intact rats. When saline was administered intravenously, both intact and HPX rats excreted similar amounts of sodium during 3 hr of continued volume expansion. On the other hand, potassium excretion was again markedly lower in saline expanded HPX rats than in intact rats. Saline expanded HPX rats in both series of studies had lower excretion rates of creatinine and had lower plasma aldosterone and corticosterone concentrations than expanded intact rats. Urine osmolalities were not significantly different between intact and HPX rats, either before or after volume expansion, indicating that there was at least partial recovery of posterior pituitary function in the HPX animals. These results suggest that adequate anterior pituitary function is required for supporting the kaliuresis but not the natriuresis associated with saline expansion.

---

Although it has been recognized since 1901 that extracts of the pituitary gland possess natriuretic as well as antidiuretic properties (1), the ability of hypophysectomized animals to regulate sodium excretion under conditions of sodium loading has not been widely studied. Moreover, information that is available is conflicting in nature. Lichardus and Ponc have reported that an attenuated natriuretic response ensues when rats are volume expanded with either whole blood (2) or saline (3) shortly after hypophysectomy (within 1 to 2 hr of surgery). On the other hand, Kaloyanides *et al.* (4) did not find a compromised volume-induced natriuresis by isolated kidneys perfused with blood of hypophysectomized dogs. In the latter study hypophysectomized dogs were treated with antidiuretic hormone and the natriuretic response to volume expansion was evaluated 24 hr following surgery.

No studies have been reported on the ability of long-term hypophysectomized animals to adjust the excretion rate of sodium in response to a sodium challenge. Consequently, in the current study the competency of rats to excrete a saline load was evaluated 1 to 2 months following hypophysectomy. Since long-term hypophysectomized animals do not tolerate anesthesia and surgery well, the studies reported in this paper were performed on unanesthetized rats.

**Methods.** Female Sprague-Dawley rats weighing 200-250 g were used. Hypophysectomies were performed by the supplier (Charles River Breeding Laboratories). Hypophysectomized (HPX) rats were housed in departmental small animal quarters for at least 1 month prior to the initiation of renal function studies. Standard rat chow (Purina) and tap water were provided *ad libitum*. Intact (I) rats were housed for at least 1 week after arrival.

Two experimental protocols were employed: in one series, rats were volume-expanded by the administration of saline intragastrically while in the second series, saline was infused intravenously.

**Intragastric (IG) protocol (Series I).** Nine intact and nine HPX rats were evaluated. At the beginning of each experiment, the animals were exposed to ether for 3-5 sec to induce a reflex emptying of the bladder (5). The initial urine sample was discarded and each rat was then placed in a metabolic cage. Spontaneously voided urine was collected during the next 3 hr (urine was always aspirated into a syringe immediately after voiding). At the end of this 3-hr control period the animals were again exposed to ether for 3-5 sec. A volume of saline equal to 5% of the rat's body weight was then administered to each animal via a stomach tube (18-gauge gavage needle). Rats were returned to the metabolic cages,

spontaneously voided urine was collected, and, after 3 hr, ether again was used to stimulate emptying of the bladder. Immediately following collection of the urine sample, rats were decapitated and blood was collected into heparinized tubes.

**Intravenous (IV) protocol. Series IIa—saline expansion.** Six intact and six HPX rats were used in this series. Rats were placed into wire-mesh restraining cages and sterile saline was infused ( $0.1 \text{ ml kg}^{-1} \text{ min}^{-1}$ ) via a 25- or 27-gauge needle positioned in a tail vein. The needle was attached, via PE50 tubing, to a syringe infusion pump (Harvard Apparatus Model 925). Urine was collected in a petri dish placed beneath the animal. Prior to and after each urine collection period a voiding reflex was initiated with ether inhalation, as described above. Following a 2-hr control urine collection period, the rats were volume expanded with saline, administered at a rate of  $2 \text{ ml kg}^{-1} \text{ min}^{-1}$  for 15 min and then  $0.5 \text{ ml kg}^{-1} \text{ min}^{-1}$  for 2.75 hr. During saline expansion three consecutive 60-min urine samples were obtained.

The intravenous protocol was repeated three times on control rats and two to three times on HPX rats (values for repeated determinations on each rat were averaged so that the mean of both the HPX and the intact group reflect  $n = 6$ ). One week was allowed to elapse between studies on individual rats. After the final experiment was performed on each rat and immediately following the third hour of saline expansion, the animal was decapitated and blood was collected into heparinized tubes.

**Series IIb—nonexpanded.** Six intact and six hypophysectomized rats were placed into wire cages and control urine samples collected as described in Series IIa. The rats were then decapitated and blood collected into heparinized tubes.

**Analytical procedures.** Sodium and potassium concentrations in urine and plasma ( $P$ ) were determined by flame photometry (Advanced Instruments Corp.). Endogenous creatinine concentrations in urine and plasma were measured using the picric acid method of Folin and Wu (6). It should be noted that endogenous plasma creatinine estimations using a picric acid determination include "non-creatinine chromogens" (7). Plasma concen-

trations of aldosterone (aldo) and corticosterone (Cmpd B) were measured using the radioimmunoassay of Gomez-Sanchez *et al.* (8) and Ray *et al.* (9), respectively. Urine osmolalities were measured by freezing point depression (Precision Inst).

Statistical analyses were based on Student's  $t$  test for pooled data. Values were accepted as significantly different when the probability of no difference was less than 5%. Data are reported as means  $\pm 1$  SEM.

**Results. Intra gastric series.** Selected physiological variables obtained from unanesthetized, intact and hypophysectomized rats in this series of experiments are summarized in Table I. Prior to saline expansion the rate of sodium excretion ( $U_{\text{Na}}V$  where  $V$  = urine flow rate and  $U$  = urine concentration) was significantly lower in the HPX rats compared with intact rats. During saline expansion there were significant increases in the excretion rate of Na by both groups of rats. However, HPX rats excreted only  $44.0 \pm 5.9\%$  of the administered sodium load compared to  $72.9 \pm 4.8\%$  by intact rats ( $p < 0.005$ ). In addition, the change in Na excretion rate ( $\Delta U_{\text{Na}}V$ , in  $\mu\text{eq } 3 \text{ hr}^{-1} \text{ g body wt}^{-1}$ ) induced by saline was

TABLE I. EFFECTS OF SALINE ON SELECTED PHYSIOLOGICAL VARIABLES OF ANIMALS IN SERIES I

	Pre-expansion	Saline expansion
Intact rats		
$U_{\text{Na}}V$ , $\mu\text{eq hr}^{-1} \text{ g BW}^{-1}$	$0.70 \pm 0.03$	$2.6 \pm 0.1^*$
$U_{\text{K}}V$ , $\mu\text{eq hr}^{-1} \text{ g BW}^{-1}$	$0.87 \pm 0.10$	$1.07 \pm 0.07$
$U_{\text{Cr}}V$ , $\mu\text{g hr}^{-1} \text{ g BW}^{-1}$	$1.7 \pm 0.1$	$2.1 \pm 0.07^*$
$U_{\text{os}}$ , mOs/kg $\text{H}_2\text{O}$	$735 \pm 60$	$543 \pm 21$
$P_{\text{aldo}}$ , ng/dl	—	$25 \pm 5$
$P_{\text{Cr}}$ , $\mu\text{g/ml}\S$	—	$15 \pm 1$
$P_{\text{Na}}$ , $\mu\text{eq/ml}$	—	$145 \pm 3$
HPX rats		
$U_{\text{Na}}V$ , $\mu\text{eq hr}^{-1} \text{ g BW}^{-1}$	$0.33 \pm 0.07\ddagger$	$1.4 \pm 0.1^*\ddagger$
$U_{\text{K}}V$ , $\mu\text{eq hr}^{-1} \text{ g BW}^{-1}$	$0.67 \pm 0.07$	$0.37 \pm 0.03^*\ddagger$
$U_{\text{Cr}}V$ , $\mu\text{eq hr}^{-1} \text{ g BW}^{-1}$	$1.6 \pm 0.1$	$1.5 \pm 0.1$
$U_{\text{os}}$ , mOs/kg $\text{H}_2\text{O}$	$634 \pm 50$	$602 \pm 80$
$P_{\text{aldo}}$ , ng/dl	—	$5 \pm 1\ddagger$
$P_{\text{Cr}}$ , $\mu\text{g/ml}\S$	—	$13 \pm 1$
$P_{\text{Na}}$ , $\mu\text{eq/ml}$	—	$143 \pm 3$

Note. Values are means  $\pm 1$  SEM.  $P_{\text{K}}$  values were not measured in Series I.

\*  $p < 0.05$  compared to preceding period.

†  $p < 0.05$  compared to same period for intact rats.

§ Value represents creatinine and chromagen.

only  $3.2 \pm 0.4$  in HPX rats but  $5.6 \pm 0.4$  ( $p < 0.005$ ) in intact rats.

The rate of  $K^+$  excretion ( $U_KV$ ) was similar in both groups of rats prior to saline expansion. However, during the 3 hr following saline administration, the rate of potassium excretion decreased significantly in hypophysectomized rats but did not change in the intact groups.

Saline expansion resulted in changes in the excretion rate of creatinine ( $U_{Cr}V$ ) that were similar to volume-induced changes in  $K^+$  excretion. That is, prior to saline expansion both groups of rats excreted similar amounts of creatinine whereas following expansion intact rats excreted significantly more creatinine than HPX rats.

There were no significant differences in the concentration of creatinine + chromogens in the plasma of volume expanded intact vs HPX rats. Similarly, urine osmolalities were not significantly different in the two groups, either before or after saline expansion. On the other hand, the concentration of aldosterone in sa-

line expanded HPX rats was 1/5 that found in saline expanded intact animals.

*Intravenous studies.* Table II summarizes selected physiological variables obtained from unanesthetized, intact, and hypophysectomized rats prior to and during 3 hr of continued intravenous saline expansion (Series II). During each collection period the rate of  $Na^+$  excretion by HPX rats was either equal to or greater than that found in intact rats.

Intact rats were characterized by a marked kaliuresis during saline expansion, a phenomenon which was, however, significantly attenuated in the hypophysectomized group. During the second and third hours of expansion the excretion rates of  $K^+$  HPX rats were not significantly different from preexpansion values.

Creatinine excretion rates prior to saline expansion were similar in both groups of rats. However, differences in  $U_{Cr}V$  between the two groups of rats were observed when the animals were volume expanded. Specifically, during the second and third hours of expan-

TABLE II. EFFECTS OF SALINE ON SELETED PHYSIOLOGICAL VARIABLES OF ANIMALS IN SERIES II

	Pre-expansion	Saline expansion		
		First hour	Second hour	Third hour
<b>Intact rats</b>				
$U_{Na}V$ , $\mu\text{eq hr}^{-1} \text{g BW}^{-1}$	$0.44 \pm 0.05$	$4.0 \pm 0.3^*$	$5.1 \pm 0.3^*$	$4.7 \pm 0.3$
$U_KV$ , $\mu\text{eq hr}^{-1} \text{g BW}^{-1}$	$0.45 \pm 0.05$	$1.30 \pm 0.07^*$	$1.10 \pm 0.08^*$	$0.83 \pm 0.08^*$
$U_{Cr}V$ , $\mu\text{g hr}^{-1} \text{g BW}^{-1}$	$1.3 \pm 0.1$	$2.3 \pm 0.1^*$	$2.0 \pm 0.1$	$1.8 \pm 0.1$
$U_{os}$ , $\text{mOs/Kg H}_2\text{O}$	$570 \pm 56$	$410 \pm 9^*$	$397 \pm 8$	$389 \pm 10$
$P_{aldo}$ , $\text{ng/dl}$	$51 \pm 10^*$	—	—	$18 \pm 3$
$P_{Cr}$ , $\mu\text{g/ml}\S$	$19 \pm 1^*$	—	—	$12 \pm 2$
$P_{cmpdB}$ , $\text{ng/ml}$	—	—	—	$391 \pm 98$
$P_{Na}$ , $\mu\text{eq/ml}$	$143 \pm 1^*$	—	—	$149 \pm 5$
$P_K$ , $\mu\text{eq/ml}$	$6.3 \pm 0.2^*$	—	—	$5.2 \pm 0.3$
<b>HPX rats</b>				
$U_{Na}V$ , $\mu\text{eq hr}^{-1} \text{g BW}^{-1}$	$0.89 \pm 0.12\ddagger$	$5.2 \pm 0.4^*$	$4.6 \pm 0.3$	$5.2 \pm 0.4$
$U_KV$ , $\mu\text{eq hr}^{-1} \text{g BW}^{-1}$	$0.37 \pm 0.05$	$0.76 \pm 0.11^{*\ddagger}$	$0.44 \pm 0.03^{*\ddagger}$	$0.41 \pm 0.05\ddagger$
$U_{Cr}V$ , $\mu\text{g hr}^{-1} \text{g BW}^{-1}$	$1.2 \pm 0.1$	$2.4 \pm 0.3^*$	$1.4 \pm 0.03^{*\ddagger}$	$1.5 \pm 0.1\ddagger$
$U_{os}$ , $\text{mOs/kg H}_2\text{O}$	$465 \pm 17$	$369 \pm 7$	$415 \pm 9$	$476 \pm 24$
$P_{aldo}$ , $\text{ng/dl}$	$13 \pm 6^{*\ddagger}$	—	—	$4 \pm 2\ddagger$
$P_{Cr}$ , $\mu\text{g/ml}\S$	$20 \pm 1^*$	—	—	$16 \pm 2$
$P_{cmpdB}$ , $\text{ng/ml}$	—	—	—	ND $\ddagger$
$P_{Na}$ , $\mu\text{eq/ml}$	$145 \pm 2^*$	—	—	$154 \pm 6$
$P_K$ , $\mu\text{eq/ml}$	$6.7 \pm 0.5^*$	—	—	$5.0 \pm 0.2$

Note. Values are means  $\pm 1$  SEM. ND = nondetectable.

\*  $p < 0.05$  compared to preceding period.

$\ddagger p < 0.05$  compared to same period for intact rats.

$\S$  Values represents creatinine + chromogen.

$^*$  Values are from rats in Series IIb, all other values are from rats in Series IIa.

sion there was a lower excretion rate of creatinine by HPX rats whereas both groups excreted similar amounts during the first hour of expansion.

The concentrations of creatinine + chromogens, corticosterone and aldosterone in plasma obtained following the third hour of expansion are also given in Table II. The concentration of creatinine + chromogens was similar in both groups of rats whereas aldosterone and corticosterone concentrations were significantly lower in HPX compared to intact rats. Corticosterone values in HPX rats were nondetectable, i.e., values were less than 2 ng/ml, clearly demonstrating that the pituitary gland had been completely excised.

Finally, urine osmolalities were not significantly different between intact and HPX rats prior to or during the 3 hr of saline expansion.

*Series IIB.* The plasma concentration of creatinine + chromogens in nonexpanded HPX ( $n = 6$ ) and intact rats ( $n = 6$ ) was not significantly different, averaging  $20 \pm 1$  and  $19 \pm 1$   $\mu\text{g/ml}$ , respectively. By contrast, the concentration of aldosterone was significantly higher ( $p < 0.05$ ) in the nonexpanded intact rats ( $51 \pm 10$  ng/dl) than in the nonexpanded HPX rats ( $13 \pm 6$  ng/dl).

**Discussion.** The present study demonstrates both similarities and differences in the renal handling of solute during saline expansion between intact and long-term hypophysectomized rats. It is of particular interest to note that hypophysectomized rats did increase the excretion rate of sodium to the same extent as did intact rats, a fact clearly demonstrated by the results of the intravenous series of experiments. Moreover, the fact that intact and HPX rats had comparable changes in  $\text{Na}^+$  excretion during intravenous but not during intragastric expansion, strongly suggests that the compromised natriuresis of HPX rats in the Series I was due to a reduced absorption rate of saline from the gastrointestinal tract. Hypophysectomy has been found to delay gastric emptying (10) and to reduce intestinal absorption rate of carbohydrate (11), fat (12), and sugar (13).

The results of the current study are in agreement with those of Kaloyanides and co-workers (14). They also reported that pituitary function is not a factor in determining the ability of kidney to modulate sodium ex-

cretion during volume expansion. By contrast, Lichardus and Ponc (2, 3) observed that hypophysectomized rats are characterized by an attenuated natriuresis during volume expansion when experiments were performed shortly following surgery (within 1–2 hr). It is possible that surgical trauma, not duplicated in sham-operated controls, accounts for the compromised natriuretic response to volume expansion observed in short-term hypophysectomized rats. Alternatively, since it has been well established that elements of posterior pituitary function recover following hypophysectomy (14–16), a fact attested to by the lack of a water diuresis in the HPX rats used in the current study, it is also possible that a natriuretic function of the posterior pituitary gland recovers with time. Clearly, however, anterior pituitary function is not reestablished following hypophysectomy (plasma corticosterone concentrations were nondetectable in the current study) and is not necessary for the natriuresis associated with intravenous saline expansion.

The renal handling of  $\text{K}^+$  during volume expansion was affected by hypophysectomy. Excretion of  $\text{K}^+$  by HPX rats in both the intragastric and intravenous experiments was markedly reduced during saline expansion as compared to intact rats. In the intragastric series  $\text{K}^+$  excretion rate by HPX rats actually decreased during expansion while in the IV series only a small and transient kaliuresis was observed.

Many factors including the glomerular filtration rate (GFR) are known to affect the rate of  $\text{K}^+$  excretion (17). In the current study there appeared to be differences in the GFR between saline expanded intact and HPX rats. Although creatinine clearances could not be obtained with the protocol used in the current study, the fact that endogenous plasma creatinine + chromogen values were not significantly different between nonexpanded intact and HPX rats, or between intact and HPX rats during the third hour of expansion suggests that differences in creatinine excretion rate between the two groups were due to differences in GFR (creatinine clearance in the female rat is equal to the GFR (18)). Along these lines, it should be noted that the profile of  $\text{K}^+$  excretion in both series of experiments was qualitatively similar to the profile of cre-

atinine excretion. Specifically, in the IG series both creatinine and  $K^+$  excretion decreased in HPX rats during expansion while the same two variables tended to increase in expanded intact rats. Similarly, in the IV series HPX rats were characterized by an increase in creatinine and  $K^+$  excretion only during the first hour of expansion. Both variables returned to basal values during the second and third hours of expansion. Intact rats were characterized by higher excretion rates of creatinine and  $K^+$  during each hour of expansion (compared to preexpansion values). These facts are further illustrated in Fig. 1 which is a plot of  $K^+$  excretion rates ( $\mu\text{Eq hr}^{-1} \text{g BW}^{-1}$ ) vs the rate of creatinine excretion ( $\mu\text{g hr}^{-1} \text{g BW}^{-1}$ ) during each hour of IV saline expansion. There was a significant correlation between these two variables in both groups of rats. Linear regression analysis of the data shown in Fig. 1 provides the following least squares equations:  $y = 0.522X - 0.02$ ,  $r = .621$  ( $p < 0.001$ ) for intact rats and  $y = 0.298X + 0.0064$ ,  $r = .724$  ( $p < 0.001$ ) for hypophysectomized rats. Nonetheless, the slopes of these regression equations are significantly different ( $p < 0.05$ ). Therefore, it is likely that other physiological variables also contribute to the observed differences in  $K^+$  excretion rate between volume expanded intact and HPX rats.

Willis and Bauer (5) have concluded that during saline expansion, differences in the plasma concentration of aldosterone between

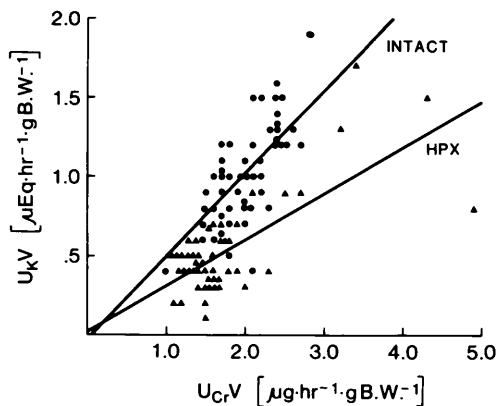


FIG. 1. Amount of potassium excreted by intact (●) and HPX (▲) rats during each hour of intravenous saline expansion is plotted vs the amount of creatinine excreted during the same time interval.

groups of rats will result in differences in sodium excretion rate. Since plasma aldosterone concentrations were markedly different between saline expanded intact and HPX rats the data from the current study also permit an evaluation of the potential role of the mineralocorticoid in regulating  $\text{Na}^+$  excretion during volume expansion. Thus, the plasma concentrations of aldosterone were four- to fivefold greater in intact compared to HPX rats in both series of studies. Nonetheless, excretion rates of sodium during expansion were not significantly different in the Series II animals and were actually lower (not higher as would be predicted based on plasma concentrations of aldosterone) in the HPX rats compared with intact rats in the intragastric studies. These results do not support the contention that differences in plasma concentrations of aldosterone during saline expansion markedly affect the rate of sodium excretion.

In summary, the results of this study demonstrate that long-term hypophysectomized rats have a compromised natriuretic response to intragastric but not to intravenous saline loading. These results do not provide evidence for a functional role of the anterior pituitary gland in modulating changes in the renal handling of a sodium load. By contrast, long-term hypophysectomized rats are characterized by an attenuated kaliuretic response to both modes of saline loading, clearly demonstrating the necessity of adequate anterior pituitary function for supporting the kaliuresis associated with saline expansion. Evidence suggests that the reduced  $K^+$  excretion rate by saline expanded hypophysectomized animals is related to both attenuated glomerular and tubular functions.

The author is grateful to Dr. Laxmi Srivastava for the aldosterone and corticosterone radioimmunoassays and to Mrs. Reiko Tanaka for her technical assistance. This research was supported by a grant from the National Institutes of Health (HD 12160).

1. Magnus R, Schaffer EA. The action of pituitary extracts upon the kidney. *J Physiol* 27:IX, 1901.
2. Lichardus B, Ponc J. Effects of hypophysectomy on sodium excretion in rats without blood dilution during blood volume expansion. *Experientia* 28:471-472, 1973.
3. Lichardus B, Ponc J. On the role of the hypophysis

- in the renal mechanism of body fluid volume regulation. *Endocrinologie* 61:403-412, 1973.
4. Kaloyanides GJ, Cohen L, DiBona GF. Failure of selected endocrine organ ablation to modify the natriuresis of blood volume expansion in the dog. *Clin Sci Molec Med* 52:351-356, 1977.
  5. Willis LR, Bauer JH. Aldosterone in the exaggerated natriuresis of spontaneously hypertensive rats. *Amer J Physiol* 234:F29-F35, 1978.
  6. Folin O, Wu H. A system of blood analysis. *J Biol Chem* 38:81-110, 1919.
  7. Relman AS, Levinsky NG. In: Strauss MB, Welt G, eds. *Disease of the Kidney*. Boston, Little, Brown, pp80, 1963.
  8. Gomez-Sanchez C, Kerm DC, Kaplan MM. A radioimmunoassay for plasma aldosterone by immunologic purification. *J Clin Endocrinol* 36:795-798, 1973.
  9. Ray SK Jr, Garza R, Maroulis G, Abraham GE. Radioimmunoassay of plasma corticosterone. *Anal Lett* 7:109-118, 1974.
  10. Enriquez de Salamanca F Jr, Garcia-Morato Castano V, Lopez-Porrúa JM, Castro-Rial Canosa M. Efectos de la hipofisectomia en el funcionalism. *Arch Med Exp* 16:379-412, 1953.
  11. Russell JA. The effects of hypophysectomy and of anterior pituitary extracts on the disposition of fed carbohydrate in rats. *Amer J Physiol* 121:755-764, 1938.
  12. Verzár F, McDougall EJ. In: *Absorption from the Intestine*. London, Longmans, Green pp147, 1936.
  13. Jacobson ED, Magnani TJ. Some effects of hypophysectomy on gastrointestinal function and structure. *Gut* 5:473-479, 1964.
  14. Billenstien DC, Leveque TF. The reorganization of the neurohypophysial stalk following hypophysectomy in the rat. *Endocrinology* 56:704-717, 1955.
  15. Moll J, De Wied D. Observations on the hypothalamo-posthypophysial system of the posterior lobectomized rat. *Gen Comp Endocrinol* 2:215-228, 1962.
  16. Raisman G. Electron microscopic studies of the development of new neurohaemal contacts in the median eminence of the rat after hypophysectomy. *Brain Res* 55:245-261, 1973.
  17. Harvey AM, Malvin RL. Comparison of creatinine and inulin clearances in male and female rats. *Amer J Physiol* 209:849-852, 1965.
  18. Brenner BM, Berliner RW. The transport of potassium. In: Orloff J, Berliner RW, eds. *Handbook of Physiology, Sect 8, Renal Physiology*, Washington D.C. Amer Physiol Soc, pp497-519, 1973.
- 

Received July 6, 1982. P.S.E.B.M. 1983, Vol. 172.