

Table II. Sodium balance in 9 rats before and 6 weeks after bilateral resection of superficial kidney cortex

Day of experiment	Sodium intake	Before resection		After resection	
		Sodium excretion	Sodium rejection (%)	Sodium excretion	Sodium rejection (%)
4	60	47 ± 21	0.06 ± 0.03	62 ± 19	0.1 ± 0.04
5	1500	1947 ± 295	2.4 ± 0.67	753 ± 205	1.1 ± 0.6
6	1500	2265 ± 302		1150 ± 198	2.9 ± 1.0
7	1500	1349 ± 450		1666 ± 330	3.3 ± 0.98
9-11	1500	1991 ± 771		1444 ± 219	
12	60	152 ± 47		50 ± 11	
13	60	48 ± 13	0.08 ± 0.01	40 ± 17	0.16 ± 0.02

Means and SD. values are expressed in $\mu\text{Eq. Na}/100 \text{ g body weight}/24 \text{ h}$.

Discussion. Even when kidney mass is considerably reduced, normal sodium balance can be maintained^{7,8}. In the present study sodium balances were examined in rats subjected to a special type of partial nephrectomy: bilateral surgical ablation of the whole superficial kidney cortex. In such rats (group IV) a delay in sodium excretion was observed when daily sodium intake was suddenly increased. This seemed to be due to a slower increase in fractional sodium rejection. However, even these rats can maintain their sodium balance at a high intake. They need only about 2 days longer for adaptation. The critical question is: whether the slower adaptation to sodium loading is an intrinsic quality of these kidneys containing almost only juxtamedullary nephrons, or whether this might be due to extrarenal factors. Since serum electrolyte and -protein concentration, hematocrit, PV and EZV were not different in the rats of all groups, the first explanation should be favoured.

Reduction of kidney mass and number of glomeruli were comparable in the rats of groups III and IV. Therefore, this reduction alone does not seem to be responsible for the delayed sodium adaptation in group IV. Since this phenomenon was observed in the same way 1 week or 12 weeks after surgery, the rate of hypertrophy obviously does not influence the modification in sodium balance.

With respect to the question of the heterogeneity of nephron populations and their possible relations to sodium excretion^{1,9,10}, our results may be interpreted as follows: the data presented here may be compatible with the view that superficial nephrons are of some importance – at least for the rapidness of sodium excretion when daily sodium intake is suddenly increased.

Summary. Bilateral resection of the whole superficial kidney cortex (approximately 75% of glomeruli) was carried out in rats. These animals needed some 2 days longer to restore their sodium balance when placed from low to high sodium intake in comparison with rats subjected to other types of partial nephrectomy.

M. HOHENEGGER, A. SONNTAG and
I. SZEMEREDI¹¹

*Institute for General and Experimental Pathology,
University of Vienna, Währingerstrasse 13,
A-1090 Wien (Austria), 17 March 1975.*

Table III. Kidney weight and GFR after partial nephrectomy

		Time after surgery	
		10 days	12 weeks
Kidney weight (% of controls)	I ^b	100	100
	II ^c	60	84
	III ^c	62	82
	IV ^b	72	88
GFR (ml/min/g kidney wt.)	I	1.01	0.98
	II	1.05	0.90
	III	0.85	0.90
	IV	0.68 ^a	0.63 ^a

I, controls; II, unilateral nephrectomy; III, 4/6 nephrectomy; IV, bilateral resection of superficial cortex. Means of 3-5 rats in each group.

^a $p < 0.05$ to controls (I). ^b2 kidneys. ^c1 kidney.

⁷ J. P. HAYSLETT, M. KASHGARIAN and F. H. EPSTEIN, *J. clin. Invest.* 48, 1002 (1969).

⁸ R. G. SCHULTZE, H. S. SHAPIRO and N. S. BRICKER, *J. clin. Invest.* 48, 869 (1969).

⁹ M. WALSER, in *The Kidney* (Eds. C. ROUILLER and A. F. MÜLLER; Academic Press, New York and London 1971), vol. 3, p. 182.

¹⁰ F. WRIGHT and G. GIEBISCH, *Kidney int.* 1, 201 (1972).

¹¹ Acknowledgment. The authors thank Dr. A. Sabanas, Chicago, for his help in preparing the manuscript.

Stimulation of Autonomic Nerves to the Urinary Bladder of the Rat

Electrical stimulation of the pelvic nerves^{1,2} or hypogastric nerves³ causes contraction of the detrusor muscle in the rat. The contractile response of the rat bladder to stimulation of the pelvic nerves at physiological frequencies is probably caused via cholinergic fibres^{1,3-6} and this seems also to be the case at stimulation of the hypogastric nerves in the guinea-pig⁷ and in the rat^{2,6}.

The contraction of the detrusor muscle caused by stimulation of one pelvic nerve has by a number of investigators been described to be confined to the ipsilateral half of the bladder in the dog, cat and rabbit⁸⁻¹¹. Others suggest that the entire detrusor can be made to contract by stimulation of one pelvic nerve, although the response of the contralateral side is weaker than that of the ipsilateral in the cat and dog^{12,13}. In the rat post-

ganglionic axons from either pelvic nerve appear morphologically to distribute bilaterally, while the functional overlap between the right and left pelvic nerve at electrical stimulation has been found not to exceed 20%¹⁴.

In the present investigation, the interaction between cholinergic fibres from the different autonomic nerves to the bladder was studied by stimulation of the pelvic nerves unilaterally and bilaterally and by simultaneous stimulation of the pelvic and hypogastric nerves.

Material and methods. 20 male albino rats of the Wistar strain weighing about 350 g were used. The rats were anaesthetized with chloralose (100 mg/kg), given through a cannula in a femoral vein after induction with ether. The bladder was exposed and the ureters were ligated. A glass cannula was inserted into the bladder through an incision in the urethra. The bladder was filled with 0.25 ml of physiological saline and the pressure developed by the detrusor muscle was recorded by means of a transducer and a polygraph.

The hypogastric nerves were cut distal to the hypogastric ganglia and the distal ends were stimulated jointly, using a bipolar electrode. Each pelvic nerve was stimulated after section proximal to the pelvic plexus, which in the male rat forms a distinct ganglion located on the lateral surface of the prostate gland¹⁵. 3 Grass stimulators supplied with stimulus isolation units, giving rectangular pulses with a duration of 2 msec a frequency of 0.1–30 Hz and of supramaximal intensity (10 V) were used.

Eserine sulphate, 0.2–0.4 mg/kg, was injected through the cannula in the femoral vein. Higher doses caused fasciculations of the skeletal muscles and deterioration of the preparation. For statistical evaluation of the data Student's *t*-test was used. The 0.05 level of probability was accepted as significant.

Results. The response to electrical stimulation of one pelvic nerve for 15 sec was a contraction of the detrusor muscle. Maximal pressure response was obtained at stimulation frequencies of 15–20 Hz increasing the intravesical pressure from a resting pressure of about 10 mm Hg (see Table). Similar responses were obtained whether the opposite pelvic nerve was intact or cut in advance. The size of the contraction caused by unilateral pelvic stimulation amounted to about 60% of the response to bilateral stimulation. As can be seen in the Table, there was a functional overlap between the right and the left pelvic nerve; the sum of the responses produced by the nerves stimulated separately (Dx + Sin) was larger than the response produced when both nerves were stimulated together (DxSin). The degree of convergence by fibres from the right and left pelvic nerve was expressed as % overlap = [(Dx + Sin) - DxSin] × 100/Dx Sin. After the injection of eserine the response to stimulation of one pelvic nerve was increased by about 40%, while the response to simultaneous stimulation of both pelvic nerves was not significantly changed. After eserine the response to bilateral stimulation did not differ significantly from the response to unilateral stimulation.

In another series of experiments the hypogastric nerves were stimulated which also caused a contraction of the detrusor muscle. The optimal stimulation frequency was 15–20 Hz. Section of the pelvic nerves did not change the response to hypogastric nerve stimulation or vice versa. In these experiments the pressure response to stimulation of the pelvic nerves bilaterally at 20 Hz was 53.1 ± 6.6 mm Hg (mean ± S.E., *n* = 7), the hypogastric nerves 2.6 ± 1.0 mm Hg and all nerves simultaneously 56.1 ± 7.1 mm Hg. There was no functional overlap between the pelvic and hypogastric nerves in any of the rats. In 4 rats the response to simultaneous stimulation of the nerves even exceeded the sum of the responses to stimulation of each pair of nerves separately. After eserine the response to stimulation of the hypogastric nerves was increased to 7.8 ± 2.7 mm Hg (*p* < 0.05, *n* = 9), while the responses to bilateral pelvic nerve stimulation or to stimulation of all nerves simultaneously were not significantly changed.

In a third series of experiments the response to unilateral stimulation of the pelvic nerves was 38.0 ± 5.3 mm Hg (*n* = 6), the hypogastric nerves bilaterally 2.6 ± 0.9 mm Hg and the 3 nerves simultaneously 42.3 ± 5.8 mm Hg. There was no functional overlap between one of the pelvic nerves and the hypogastric nerves. In 3 rats simultaneous stimulation of the nerves instead caused a contraction which was larger than the sum of the responses to separate stimulation of the nerves.

Discussion. The functional overlap between the right and left pelvic nerve found in this study was also found by CARPENTER and RUBIN¹⁴. The bilateral distribution of axons from each pelvic nerve in the rat bladder described by these authors probably constitutes the morphological basis for the increase of the response to unilateral pelvic nerve stimulation after eserine shown in the present study.

¹ S. VANOV, Br. J. Pharmac. 24, 591 (1965).

² P. ALM and M. ELMÉR, Acta physiol. scand., in press (1975).

³ F. G. CARPENTER and S. A. RAND, J. Physiol., Lond. 180, 371 (1965).

⁴ G. B. CHESHER, J. Pharm. Pharmac. 19, 445 (1967).

⁵ M. ELMÉR, Acta physiol. scand. 87, 223 (1973).

⁶ M. ELMÉR, Acta physiol. scand. 93, 202 (1975).

⁷ P. MANTEGAZZA and K. M. NAIMZADA, Eur. J. Pharmac. 1, 402 (1967).

⁸ M. J. GIANNUZZI, J. Physiol., Paris 6, 22 (1863).

⁹ G. DEBAISIEUX, Névralg. 13, 121 (1912).

¹⁰ K. TORBEY and W. F. LEADBETTER, J. Urol. 90, 395 (1963).

¹¹ A. C. DIKNO, R. DAVIS and J. LAPIDES, Invest. Urol. 11, 178 (1973).

¹² E. H. INGERSOLL, L. L. JONES and E. S. HEGRE, Proc. Soc. exp. Biol. Med. 88, 46 (1955).

¹³ E. H. INGERSOLL, L. L. JONES and E. S. HEGRE, Am. J. Physiol. 189, 167 (1957).

¹⁴ F. G. CARPENTER and R. M. RUBIN, J. Physiol., Lond. 192, 609 (1967).

¹⁵ O. R. LANGWORTHY, Invest. Urol. 2, 491 (1965).

	Dx	Sin	DxSin	<i>p</i>	Overlap (%)	<i>p</i>	<i>n</i>
Before eserine	39.9 ± 3.1	37.1 ± 3.1	62.4 ± 4.1	< 0.001	23 ± 3	< 0.001	14
After eserine	55.5 ± 4.9	53.0 ± 5.3	69.7 ± 6.5	> 0.1	53 ± 3	< 0.001	6
<i>p</i>	< 0.02	< 0.05	> 0.1		< 0.001		

Pressure responses in mm Hg of the rat urinary bladder to electrical stimulation at 20 Hz of the right pelvic nerve (Dx), the left pelvic nerve (Sin) and both nerves simultaneously (DxSin) before and after the injection of eserine 0.2–0.4 mg/kg. Values are mean ± S.E.; *n* = number of rats; *p* = significance.

According to GRIFFITHS¹⁶ and MACDONALD and M'CREA¹⁷ unilateral bladder responses in dogs and cats are obtained only when the distal ends of cut pelvic nerves are stimulated, while stimulation of intact nerves leads to bilateral contractions more pronounced on the stimulated side. The response in the opposite half was suggested to be dependent on a sacral reflex initiated by stimulation of afferent fibres in the ipsilateral pelvic nerve, the efferent impulses passing through the intact nerve of the contralateral side. The present results in the rat, however, are in agreement with those of INGERSOLL et al.^{12,13} in the cat and dog showing bilateral responses to unilateral stimulation of the distal ends of cut pelvic nerves, with or without section of the opposite pelvic nerve.

The responses to pelvic nerve stimulation found in this study were not changed by section of the hypogastric nerves or vice versa, which was also found by INGERSOLL et al.¹³ in the dog and by EDVARDSEN¹⁸ in the cat. The contractile response to hypogastric nerve stimulation in the cat is caused via adrenergic fibres¹⁹. EDVARDSEN¹⁸ found no synergistic effects of concomitant pelvic and hypogastric nerve stimulation in the cat but only reduction of the detrusor contraction as compared to the response to stimulation of the pelvic or hypogastric nerves separately. Sympathetic inhibition of intramural parasympathetic ganglia was suggested, since adrenergic nerve terminals forming synaptic structures around non-adrenergic cells have been demonstrated histochemically in the bladder wall of the cat²⁰. The urinary bladder of the rat in contrast to other species contains no intramural ganglion cells². In the present investigation in the rat the pelvic and hypogastric responses were instead added at simultaneous stimulation and in some rats this response

was even bigger than the sum of the responses produced by the nerves stimulated separately. This might indicate a spatial summation of effector cells innervated by cholinergic fibres from the pelvic and hypogastric nerves, suggesting that some muscle fibres are dependent on impulses from both nerves for contraction. This postulates a convergence by some of the fibres from the pelvic and hypogastric nerves. In the rat bladder cholinergic neuro-terminals are in contact with every muscle cell²¹.

Zusammenfassung. In der Harnblase von Ratten wurde der Druck während einseitiger und zweiseitiger Pelvicusreizung gemessen. Nach Injektion von Eserin wurde bereits bei einseitiger Reizung eine maximale Blasenkontraktion erhalten. Zusätzliche Reizung der Nn. hypogastrici führt – ohne funktionelle Überlappung – zu weiterer Kontraktionssteigerung.

M. ELMÉR²²

*Institute of Physiology, University of Lund,
Sölvegatan 19, S-223 62 Lund (Sweden),
24 February 1975.*

¹⁶ J. GRIFFITHS, *J. anat. Physiol.* 29, 61 (1895).

¹⁷ A. D. MACDONALD and E. D. M'CREA, *Q. Jl. exp. Physiol.* 20, 379 (1930).

¹⁸ P. EDVARDSEN, *Acta physiol. scand.* 72, 234 (1968).

¹⁹ P. EDVARDSEN and J. SETEKLEIV, *Acta pharmac. toxic.* 26, 437 (1968).

²⁰ B. HAMBERGER and K.-A. NORBERG, *Int. J. Neuropharmac.* 4, 41 (1965).

²¹ A. EL-BADAWI and E. A. SCHENK, *Am. J. Anat.* 179, 405 (1966).

²² This work was supported by grants from the Medical Faculty, University of Lund.

Digestive Enzymes in the Excreta of the Larvae of *Sesamia inferens* Walker and *Chilotraea infuscatellus* Snell. (Lepidoptera: Insecta)

The fate of the digestive enzymes is unknown¹ but they are generally thought to be inactivated or resorbed². HOBSON³ detected trypsin, and glycyl-glycine dipeptidase in the excreta of *Lucilia sericata*, ENGELMAN⁴ casually observed the activity of trypsin in the excreta of *Leucophaea maderae*, and YANG and DAVIS⁵ found chymotrypsin, trypsin and amylase in the excreta of *Aedes aegypti*. During the course of studies of digestive physiology, various digestive enzymes were detected in the hindgut contents of the larvae of *Sesamia inferens*⁶ and *Chilotraea infuscatellus*⁷. To find out the fate of these enzymes, a study of the excreta was undertaken.

Materials and methods. 20 mg (wet weight) of fresh excreta of the last larval instar of each insect was collected after feeding sugarcane slices. It was homogenized in 0.5 ml of 0.1 M phosphate buffer (pH 8.2, the pH of the midgut contents), centrifuged at 3000 g for 15 min and the supernatant was used as the enzyme source. The enzymes were tested as described elsewhere⁷.

Results and conclusion. The excreta of the larva of *S. inferens* showed appreciable activity of aminotripeptidase, leucine aminopeptidase and glycyl-L-leucine dipeptidase, and a very weak activity of trypsin, prolinase and glycyl-glycine dipeptidase. All these enzymes were present in the hindgut contents of the larva.

In the excreta of the larva of *C. infuscatellus* appreciable activity of aminotripeptidase, leucine aminopeptidase and prolinase was seen, and a very weak activity of trypsin and glycyl-glycine dipeptidase. All these enzymes

were detected in the hindgut contents of the larva; and besides these, the contents also contained chymotrypsin and glycyl-L-leucine dipeptidase.

The activity of amylase, cellulase, α -glucosidases (maltase, melezitase, sucrase and trehalase), β -glucosidase, α -galactosidase, β -galactosidase, β -fructosidase, carboxypeptidase and prolidase was absent in the excreta; although sucrase, trehalase and β -glucosidase were present in the hindgut contents, while in the hindgut contents of *C. infuscatellus* a very weak activity of α -galactosidase and β -fructosidase was also detected.

The presence of the enzymes in the hindgut contents but their absence in its tissue and excreta suggests that they are either denatured, self-hydrolyzed or digested. The absence of the enzymes in the excreta but their presence in the hindgut tissue and its contents reflects the possibility of their resorption in the hindgut. EVANS and PAYNE² have also suggested the absorption of the enzymes in the posterior gut regions.

¹ M. F. DAY and P. F. WATERHOUSE, in *Insect Physiology* (Ed. K. D. ROEDER; John Wiley & Sons, New York 1953), p. 318.

² W. A. L. EVANS and D. W. PAYNE, *J. Insect Physiol.* 10, 657 (1964).

³ R. P. HOBSON, *J. exp. Biol.* 8, 109 (1931).

⁴ F. ENGELMAN, *J. Insect Physiol.* 15, 217 (1969).

⁵ Y. J. YANG and D. M. DAVIES, *J. Insect Physiol.* 17, 2119 (1971).

⁶ A. K. AGARWAL, submitted for publication (1974).

⁷ A. K. AGARWAL, in preparation.