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The Implantation of Puromycin into the Neurosecretory Nuclei of the Rat

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Summary. Following the bilateral implantation of puromycin into the paraventricular nuclei of rats, the neurosecretory cells became atrophic and the amount of aldehyde-fuchsin (AF) positive material in the neural lobe decreased. In these rats, urine excretion and water intake increased remarkably. The supraoptic nuclei of the rats were not affected by this treatment. After the unilateral implantation of puromycin in the paraventricular nucleus, the neurosecretory cells of the implanted side became atrophic, while those of the unimplanted side hypertrophied. The neural lobe contained similar amounts of AF-positive material to those of the control rats with unilateral cholesterol implants. In the rats implanted bilaterally with puromycin immediately above the supraoptic nucleus, the neurosecretory cells of this nucleus contained little or no AF-positive material, and urine excretion and water intake increased greatly. The cells of the paraventricular nucleus remained unchanged in these rats.

Key words: Puromycin — Paraventricular nucleus — Supraoptic nucleus — Neurosecretory material — Urine excretion — Water intake.

Introduction

Sachs and Takabatake (1964) showed with pharmacological methods that puromycin injected intraventricularly inhibited synthesis of vasopressin in the rat. Zambrano and de Robertis (1967) observed by electron microscopy that puromycin injected subarachnoidally inhibited the synthesis of neurosecretory material in the cells of the supraoptic nucleus of the rat. In these experiments, there is a possibility that the puromycin affected first non-neurosecretory neurons and that, in turn, these neurons transferred inhibitory information to the neurosecretory cells. In the present study, a cannula containing puromycin was implanted into the paraventricular nucleus or the supraoptic nucleus of the rat so that puromycin could act directly on the neurosecretory cells.

Materials and Methods

1. Paraventricular Implantation. Female rats of the Wistar strain were obtained from a commercial source when they were about one month old and from that time on their body weights were measured weekly. They were used for the experiments when they weighed between 280 and 350 g. Each rat was transferred to "a metabolic cage" with a funnel at the bottom for urine collection, after the rat had gone through at least two regular estrous cycles. After transferring them to the metabolic cages, a vaginal smear was taken daily and examined together with their urine volumes and water intake. Their body weights were measured weekly during the experimental periods. For the implantation of puromycin, it was mixed with cholesterol in the ratio of 0.05% by weight, and then the mixture was tamped into stainless steel cannulae with inside diameters of 0.7 mm. The implantations into the paraventricular nuclei of the rats were performed 10 to 15 days after their transfer to the

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cages. By then they were adapted to the experimental conditions and showed regular estrous cycles, normal urine volume and normal water intake. With the aid of roentgenography the tip of the cannula was placed exactly in the paraventricular nucleus as described by Uemura and Kobayashi (1971). Bilateral or unilateral implantations were performed. In a preliminary experiment, it was found histologically that the maximum diffusion of puromycin from the tip of the cannula was about 700 μ.

The rats were divided into four groups: (1) 19 rats with bilateral puromycin implants (experimental), (2) 15 rats with bilateral cholesterol implants (control), (3) 8 rats with unilateral puromycin implants (experimental), and (4) 8 rats with unilateral cholesterol implants (control). In Groups 1 and 2, rats were killed in groups of three or four 12, 17, 25 and 40 days after the implantation. In four of Group 1 rats with bilateral puromycin implant, the implantation was so far anterior to the paraventricular nuclei that the data of these rats were excluded. In Groups 3 and 4, rats were killed in groups of four 20 and 40 days after the implantation.

2. Supraoptic Implantation. Puromycin was mixed with cholesterol in the ratio of 2% by weight and then the mixture was tamped into stainless steel cannulae with inside diameters of 0.7 mm. The cannulae containing the mixture were implanted bilaterally into the supraoptic nuclei of three Sprague-Dawley female rats (200–250 g) after they showed normal urine volume and water intake in metabolic cages. The rats were killed 12 days after the implantation. The implantation and other procedures were the same as those of the paraventricular implantation.

After killing the rats, the whole brain including the pituitary was fixed in Bouin's solution for 48 hours, and then a small block containing the pituitary and hypothalamus was trimmed and fixed for additional 24 hours. Sections were made at 10 μ from the paraffin-embedded tissues and double-stained with Gomori's paraldehyde-fuchsin and toluidine blue (Asai *et al.*, 1969) or paraldehyde-fuchsin only. The diameters of the nuclei of the cells of the supraoptic and paraventricular nuclei were measured. In each rat a minimum of 30 nuclei were measured. The presence of neurosecretory material in the neural lobe was noted.

Observations

I. Effects of Puromycin on Paraventricular and Supraoptic Nuclei and Neural Lobe (Pars nervosa) of the Hypophysis

In the group with bilateral implantation of puromycin in the paraventricular nucleus, the diameters of the cell nuclei in the paraventricular nuclei were signifi-

Table 1. Nuclear diameters of the cells of the paraventricular nucleus and amount of neurosecretory material in the neural lobes of rats implanted bilaterally with puromycin or cholesterol

Days after implantation	Bilateral cholesterol		Bilateral puromycin		P
	Nuclear diameter (μ)	Neurosecretory material in neural lobe	Nuclear diameter (μ)	Neurosecretory material in neural lobe	
12	9.66 ± 0.03[a] (4)[b]	+++[c]	8.41 ± 0.15(4)	++	$p < 0.01$
17	9.48 ± 0.17(3)	+++	7.22 ± 0.04(3)	+	$p < 0.01$
25	9.83 ± 0.08(4)	+++	9.04 ± 0.09(4)	++	$p < 0.01$
40	9.57 ± 0.16(4)	+++	9.70 ± 0.08(4)	+++	not significant

[a] Mean and standard error.
[b] Numbers of rats are indicated in parentheses.
[c] +++ large amount, ++ moderate amount, + trace or absence.

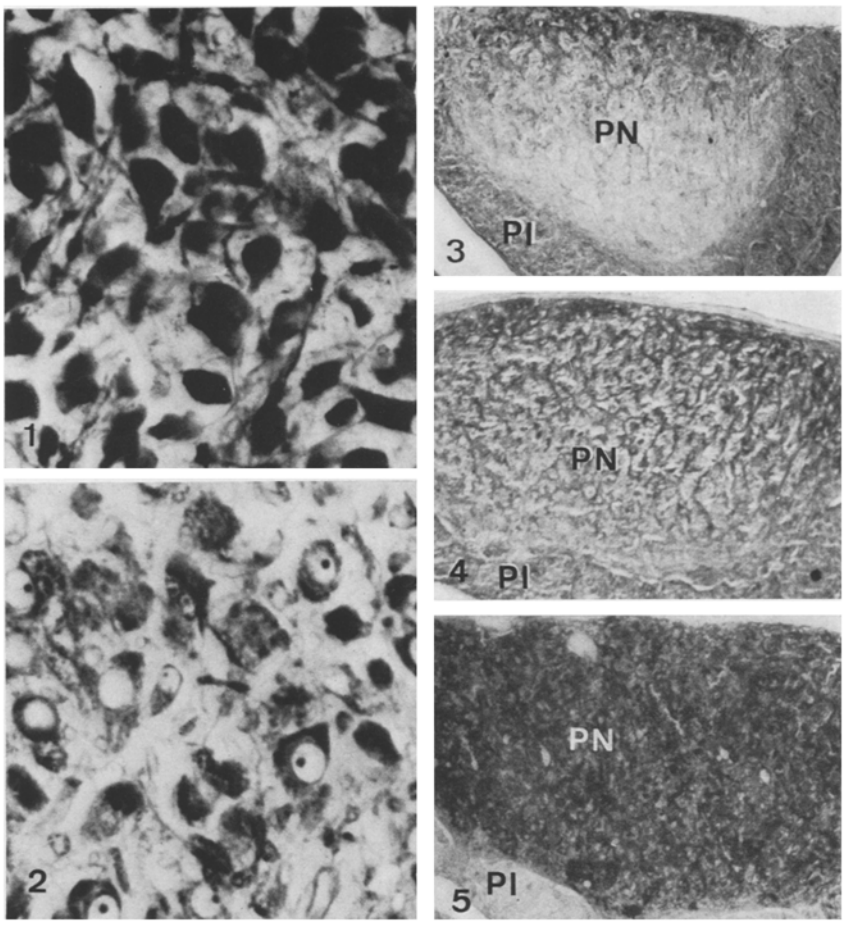


Fig. 1. Atrophic cells of the paraventricular nucleus 17 days after the implantation of puromycin. AF-toluidine blue staining. × 480

Fig. 2. Cells of the paraventricular nucleus 25 days after the implantation of puromycin. Note the restoration of cell activity. AF-toluidine blue staining. × 480

Fig. 3. Depletion of neurosecretory material in the ventral portion of the neural lobe 17 days after the bilateral implantation of puromycin. *PI* pars intermedia; *PN* neural lobe. AF-toluidine blue staining. × 84

Fig. 4. Reaccumulation of neurosecretory material in the ventral portion of the neural lobe 25 days after the implantation of puromycin. For abbreviations see Fig. 3. AF-toluidine blue staining. × 84

Fig. 5. Neural lobe of a control rat. For abbreviations see Fig. 3. AF-toluidine blue staining. × 84

cantly smaller than those of the control (cholesterol-implanted) groups killed 12, 17 and 25 days after the implantation (Table 1). The rats killed 17 days after the implantation showed maximal damage as shown by the loss of a defined nuclear structure; very few cells had measurable nuclei (Fig. 1). The diameters

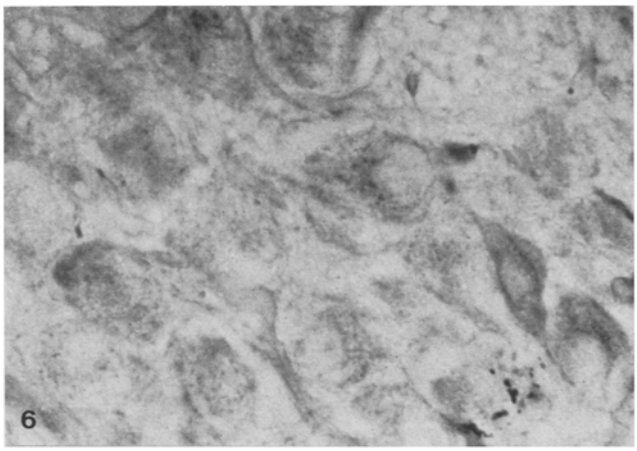
of the cell nuclei began to increase sometime after the 20th day after implantation (Table 1 and Fig. 2). Since the nuclei were more prominent and better defined in the rats killed 25 days after the implantation than those killed after 12 and 17 days. However, the cell nuclei were still significantly smaller than those of the control group. The cells of the paraventricular nuclei in the experimental groups became completely normal 40 days after the implantation and their nuclei were almost the same size as those of the control rats (Table 1).

The neural lobes of the rats of the control (cholesterol-implanted) group showed no change in the amounts of neurosecretory material at any stage. However, in the experimental groups, as well as changes in the cellular structure of the paraventricular neurons, there were also changes in their neurosecretory activity as shown by the amount of neurosecretory material in the neural lobe. In the neural lobes of those rats that were killed 12 days after implantation of puromycin, there was less neurosecretory material and this was scantly distributed compared to the control groups (Table 1). Seventeen days after implantation, when the cellular damage was maximal, neurosecretory material was almost absent in the ventral part of the neural lobe (Table 1 and Fig. 3). Twenty-five days after implantation, when the cells in the paraventricular nucleus had begun to recover, neurosecretory material reappeared in the ventral portion of the neural lobe (Table 1 and Fig. 4). After 40 days, the amount of neurosecretory material equalled that of the control rats (Table 1 and Fig. 5).

In the group with unilateral implantation of puromycin, the cells of the paraventricular nucleus of the implanted side showed damage when observed after 20 days, but they became normal 40 days after the implantation (Table 2). The cells of the unimplanted side seemed to be hyperactive in those rats which were killed 20 days after the implantation (Table 2). Their nuclei were significantly larger than those of the cholesterol implanted rats (Table 2). Fourty days after implantation, they had lost most of their hyperactivity, but were still slightly more active than the cells of the other side. There was no change in the amount of neurosecretory material in the neural lobe of these rats (Table 2). In the group with unilateral implantation of cholesterol there was no change in the paraventricular nuclei or in the neural lobes (Table 2).

Table 2. Nuclear diameters of the cells of the paraventricular nucleus and amount of neurosecretory material in the neural lobes of rats implanted unilaterally with puromycin or cholesterol

Days after implantation	Number of rats	Puromycin implanted side (μ)	Puromycin unimplanted side (μ)	p	Neurosecretory material in neural lobe
20	4	7.91 ± 0.08	11.62 ± 0.03	$p < 0.01$	+++
40	4	9.55 ± 0.04	9.92 ± 0.10	$p < 0.05$	+++
		Cholesterol-implanted side (μ)	Cholesterol-unimplanted side (μ)		
20	4	9.73 ± 0.13	9.51 ± 0.09	$p > 0.05$	+++
40	4	9.82 ± 0.09	9.35 ± 0.14	$p > 0.05$	+++



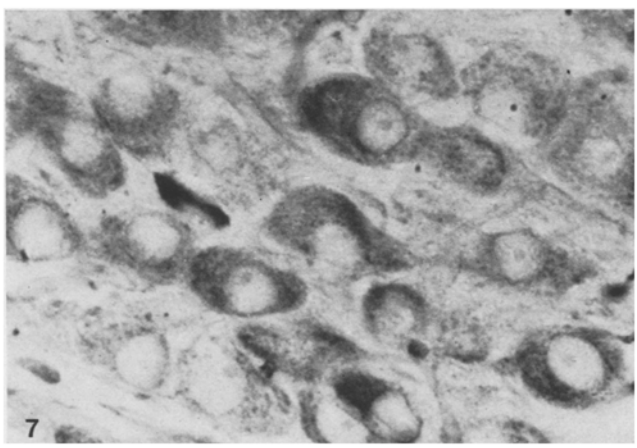


Fig. 6. Neurons of the supraoptic nucleus of the rat (see Fig. 9a) 12 days after bilateral implantation of puromycin. AF staining. × 800

Fig. 7. Neurons of the supraoptic nucleus of the rat (see Fig. 9b) bearing the tips of puromycin cannulae in the rostro-lateral region of the nucleus. AF staining. × 800

Table 3. Nuclear diameters of the cells of the supraoptic nucleus of rats killed 17 days after the bilateral implantation of puromycin or cholesterol into the paraventricular nucleus

Treatment	Puromycin	Cholesterol
Nuclear diameter (μ)	$10.3 \pm 0.08(3)$[a]	$10.2 \pm 0.03(3)$

[a] Number of rats is indicated in parentheses.

The nuclei of the neurosecretory cells of the supraoptic nucleus did not show any change in diameter following the implantation of puromycin or cholesterol into the paraventricular nucleus (Table 3).

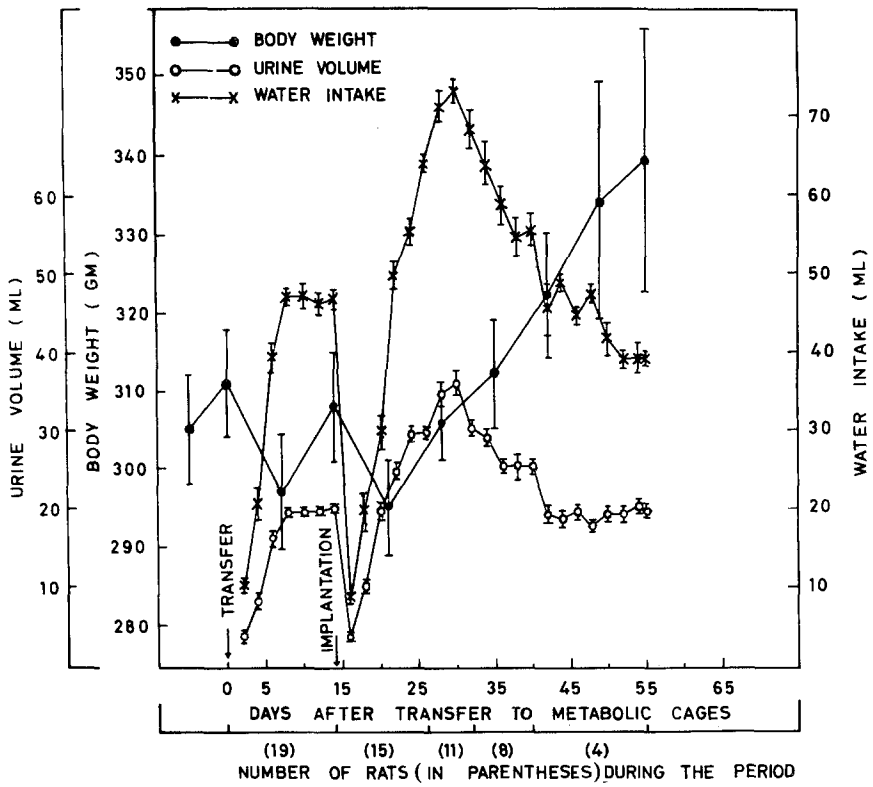


Fig. 8. Body weight, urine volume and water intake of rats bearing puromycin in the paraventricular nucleus

In one rat with bilateral supraoptic implants, the tips of the cannulae were placed 300 to 450 μ above the supraoptic nucleus, covering almost entire dorsal area (Fig. 9a). In this rat, AF-positive material disappeared completely from the neurosecretory cells, and the nucleolus was not prominent (Fig. 6). In the second rat, the tips were placed immediately rostro-lateral to the nucleus (Fig. 9b). AF-positive material decreased slightly only in the cells near the tips, but not in other cells (Fig. 7). In the remaining rat, the tip of one cannula was placed just above the right supraoptic nucleus and the tip of the other cannula was placed immediately lateral to the left nucleus. In the right nucleus, half of the cells showed a decrease of AF-positive material and in the left nucleus no change was observed in the cells. In the neural lobes of all three rats, no significant decrease in AF-positive material was detected. No change in the amount of AF-positive material was induced in the cells of the paraventricular nuclei by the supraoptic implantation.

II. Urine Volume and Water Intake

For a few days after the rats had been transferred to the metabolic cages, the urine volume and water intake per day were remarkably reduced. They were

Table 4. Urine volumes and water intake of rats implanted bilaterally with puromycin or cholesterol

Days before implantation	Number of rats	Urine excretion (ml)		Water intake (ml)	
4	19	19.5 ± 0.92[a]		46.8 ± 1.60	
0	19	18.7 ± 1.67		42.6 ± 2.30	
Days after implantation		Puromycin	Cholesterol	Puromycin	Cholesterol
12	15	29.5 ± 0.66[b]	19.4 ± 0.78	64.3 ± 1.42[b]	42.7 ± 1.00
17	11	36.3 ± 1.27[b]	19.6 ± 0.41	73.3 ± 1.27[b]	49.4 ± 1.39
25	8	25.0 ± 0.75[b]	18.3 ± 0.45	54.8 ± 2.50[b]	46.3 ± 1.57
40	4	19.5 ± 0.50	19.8 ± 0.47	48.5 ± 1.70	51.0 ± 1.29

[a] Mean ± standard error.
[b] Significantly different ($p < 0.01$) from the 0-day group.

around 3 to 5 ml and 10 to 15 ml, respectively (Fig. 8). After 3 to 5 days, they began to increase, and attained normal levels (Fig. 8 and Table 4) 8 days after the transfer. Following bilateral implantation into the paraventricular nucleus, the quantity of urine excreted and water intake decreased for a few days perhaps due to stress; thereafter both increased and reached normal pre-operation values about one week after the operation in all rats (Fig. 8). After this, there were continuous increases, and maximal urine volume and water intake levels were recorded about 15 days after the operation (Fig. 8 and Table 4). These values were significantly higher than those of the control rats. Subsequently, their levels slowly decreased, returning to normal 30 to 40 days after the operation (Fig. 8 and Table 4).

Soon after the puromycin implantation just above the supraoptic nucleus, urine volume and water intake decreased (Fig. 9a), as in the case of the paraventricular implantation. Thereafter, they increased gradually and reached maximal values on the sixth day of the implantation. Thereafter there were large fluctuations until autopsy (Fig. 9a). In the rat in which the tips of the cannulae were placed immediately rostro-laterally to the supraoptic nucleus, the urine volume and water intake showed slight increases (Fig. 9b). In the rat, bearing the tip of one cannula just above the right nucleus and that of the other cannula lateral to the left nucleus, both urine volume and water intake wer unchanged.

III. Body Weight and Estrous Cycle

Whenever rats were transferred to metabolic cages and implanted; thereafter there were decreases in the body weights (10 to 20 g) during the first week, and there were gradual increases (Figs. 8 and 9).

The estrous cycle was almost regular in all rats, except for a few days after the operation when one or two cycles were irregular because of prolonged diestrous phases.

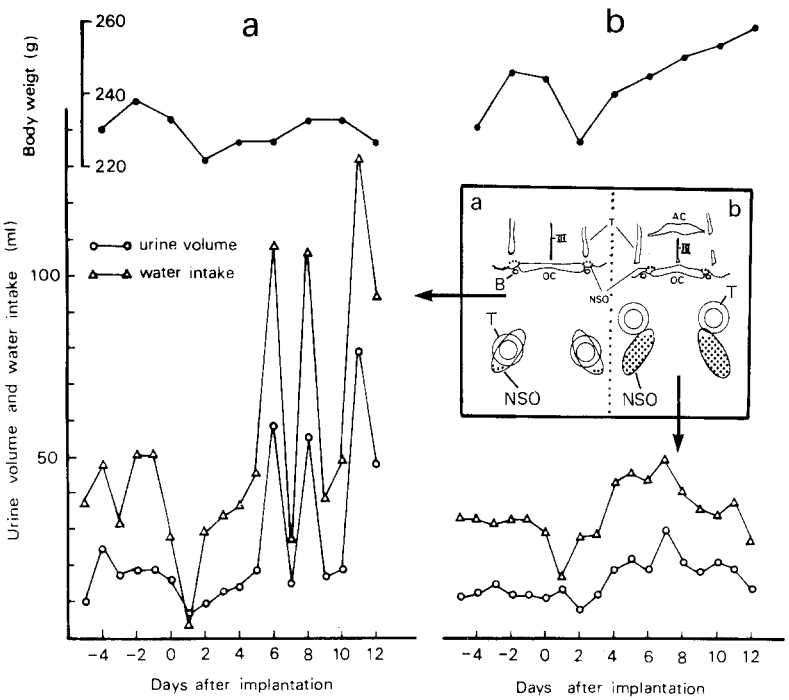


Fig. 9a and b. Body weight, urine volume and water intake of the rat bearing puromycin just above the supraoptic nuclei (a), and those of the rat bearing it immediately rostrolaterally to the supraoptic nuclei (b). Sites of the tips of cannulae containing puromycin are indicated in a rectangle. The two upper figures in the rectangle are cross sections of the hypothalamus through the traces of the implanted cannulae. The two lower figures are dorsal views of the tips (*T*) of the cannulae and regions distributed with neurosecretory cells (*NSO*). Neurosecretory material in the nucleus is indicated by dots. In the rat (a) the neurosecretory material disappeared from almost all the neurosecretory cells, while in the rat (b) the neurosecretory material disappeared only in the cells of restricted regions near the tips of the cannulae. *AC* anterior commissure; *B* blood vessel; *OC* optic chiasma; *T* trace of a cannulae, *III* third ventricle

Discussion

Puromycin implanted into the paraventricular nucleus seems to directly inhibit the biosynthesis of the neurohypophysial hormones. One of these is probably vasopressin, because the urine volume and water intake of rats with puromycin implants showed remarkable increases. This suggests that the paraventricular nucleus can synthesize vasopressin, and that vasopressin produced in this nucleus is to some extent physiologically responsible for water retention in rats. There seems to have been also a direct effect of puromycin on the supraoptic nucleus that inhibited the biosynthesis of vasopressin, since the quantities of urine volume and water intake increased in the implanted rat. Thus it is probable that both the paraventricular and supraoptic nuclei synthesize vasopressin and they are physiologically responsible for water retention in rats,

although it has been reported that the paraventricular nucleus produces oxytocin and the supraoptic nucleus secretes vasopressin (Olivecrona, 1957).

In the rats bearing puromycin in the paraventricular nuclei and showing increased urine volumes, AF-positive material was absent in the ventral portion of the neural lobe. This suggests that the neurosecretory cells producing vasopressin in the paraventricular nucleus send their axons to the ventral portion of the neural lobe. The lack of change in the amount of AF-positive material in the neural lobe of the rats bearing puromycin just above or near the supraoptic nucleus may be due to the fact that the neurosecretory activity was almost at the normal level when they were killed, judging from the urine volume and the water intake level.

In the rats with a unilateral puromycin implant in the paraventricular nucleus, the neurosecretory cells of the implanted side showed atrophy, whereas those of the unimplanted side showed hypertrophy. The neural lobe showed no change in the amount of neurosecretory material. This may be due to the compensatory neurosecretory activity of the hyptertrophied cells of the unimplanted side and may not be due to the hypertrophic activity of the neurosecretory cells of the supraoptic nucleus. This is because the supraoptic nucleus did not show any histological hypertrophic change following bilateral puromycin implantation into the paraventricular nucleus. The paraventricular nucleus did not show any change in amount of AF-positive material following the supraoptic puromycin implantation. These findings suggest that the supraoptic nucleus and the paraventricular nucleus are mutually independent in their function, at least in secretion of vasopressin. The neurosecretory cell groups of both sides of the supraoptic or paraventricular nucleus have some compensatory function with respect to the contralateral side.

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