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HYPOTHALAMO-HYPOPHYSEAL RELATIONS OF EXPERIMENTALLY INDUCED CHANGES IN SALT AND WATER METABOLISM

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In a previous study [14] it has been shown that in hyperfunction of the anterior hypothalamus (magnocellular groups of the supraoptic and paraventricular nuclei) and the neurohypophysis induced by dehydration the adrenocorticotrophic and gonadotrophic functions of the anterior pituitary are increased. In the light of this evidence it became necessary to elucidate whether there exists a causal relationship between the functional state of the anterior hypothalamo-neurohypophyseal system and the changes in the morphological appearance of the anterior pituitary.

In the studies to be reported in the present paper mesencephalic-neurohypophyseal hyperfunction was induced by chronic administration of hypertonic saline while functional balance of the system was achieved by prolonged administration of distilled water. Administration of hypertonic NaCl solution has namely been shown to activate the anterior hypothalamo-neurohypophyseal system by *Hillarp, Ortmann*, as well as in a previous work by the present authors [17] .On the other hand, it could be assumed that chronic administration of distilled water would bring about a state of equilibrium in the above system, due to an inhibition of the antidiuretic hormone mechanism.

Methods

The histomorphological peculiarities of the functional states of the supraoptic and paraventricular nuclei have been described in a previous paper [1, 14]. Of these, the volumes of cytoplasm, nucleus and nucleolus were thought to be the most reliable indicators of functional conditions and these were determined in the experiments. In addition, studies were made of the Gömöri-positive substance content of the cytoplasm of ganglion cells. The functional phase of the nerve lobe was determined on the basis of the morphological syndrome described by *Ortmann, Bargmann* et al., and confirmed by us [14], which includes pituicyte swelling and mitoses, oedema, decrease in the amount of Gömöri-positive substance, and other signs. The morphological appearance of the anterior lobe was evaluated by quantitative and qualitative cytological methods. Adrenocorticotrophic hyperfunction was demonstrated by weighing the adrenal glands and examining them microscopically, weighing the thymus, determining the absolute eosinophil count (4 hours after intraperitoneal injection of 1 to 3 ml of a 5 per cent solution of NaCl, or of 5 to 10 ml of distilled water.) Gonadotrophic function was assessed by determining the number of periodic acid-Schiff positive cells in the adenohypophysis, as well as on the basis of the histological appearance of the ovaries.

Sixty white rats of both sexes, with body weights varying between 100 g and 120 g, were used. They were kept on a well balanced diet. Twenty animals received daily 1 to 3 ml of a 5 per cent NaCl solution, another 20 animals 5 to 10 ml of distilled water, intraperitoneally. 20 rats served as controls. The saline-treated animals were given hypertonic (1,7 per cent) saline to drink, while the water-treated ones received tap water *ad libitum*. The experiment lasted 50 days; then the animals were killed by destroying the medulla oblongata. The pituitary gland was fixed in Susa's solution, the brain in 4 per cent formaldehyde. The specimens were embedded in paraffin. Serial sections made in the horizontal plane of the pituitary were stained by Mallory and Farkas' trichrome method and by Gömöri's chrome-alum haematoxylin method *(Bargmann)* and the periodic acid-leucofuchsin reaction was also carried out. The frontal-plane serial sections of the anterior hypothalamus were stained with haematoxylin and eosin, and by Gömöri's method. The internal organs were stained with haematoxylin and eosin.

The methods used for estimating the volumes of the ganglion cell components of the supraoptic and paraventricular nuclei, for determining the quantitative distribution of cells in the adenohypophysis, and for weighing the organs, have been described along with the method of statistical evaluation [14, 16].

Finally, the mesencephalon of animals treated with water or saline, and of untreated ones, was titrated for antidiuretic hormone according to the method of *Birnie* et al., slightly modified by us. Each of these groups comprised 3 animals.

Results

The animals tolerated the treatment rather well and only one saline-treated and 2 water-treated rats were lost during the experiment. In general, the animals showed a constant increase in weight. The rats treated with hypertonic saline drank in excess unusually great quantities, while those given distilled water hardly touched their drinking water.

TABLE I

Ganglion cell volumes in cubic micron in supraoptic and paraventricular nuclei of rats treated with hypertonic saline and with distilled water

Group	Number of cells counted	Supraoptic nucleus			Paraventricular nucleus			
		Cytoplasm	Nucleus	Nucleolus	Cytoplasm	Nucleus	Nucleolus	Ependyma
Control	700	1513,9 ± 65,9*	230,5 ± 7,0	13,2 ± 0,7	1642,0 ± 72,2	256,3 ± 12,8	11,7 ± 0,6	77,2 ± 2,7
Hypertonic saline	700	2687,9 ± 123,4	340,2 ± 12,5	22,5 ± 0,9	3211,2 ± 266,1	424,8 ± 29,8	23,1 ± 1,1	79,7 ± 3,8
Water	700	1296,4 ± 60,1	223,2 ± 10,2	9,5 ± 0,5	1563,9 ± 55,5	278,1 ± 5,2	9,5 ± 0,3	79,8 ± 4,4

*Standard error

In Table I are shown the volumes of the supraoptic and paraventricular nuclei from animals treated with hypertonic saline, respectively distilled water. It can be seen that in saline-treated animals the mean volume (expressed in

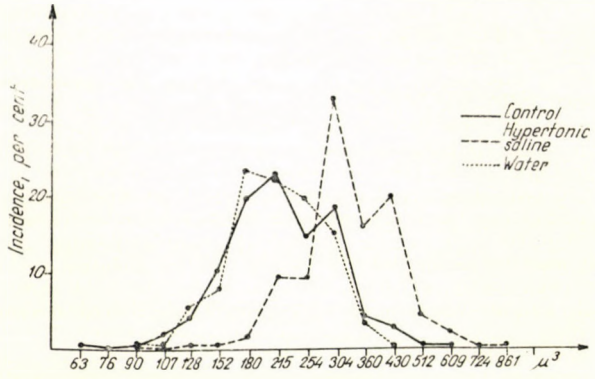

Fig. 1. Nucleus variation curve for supraoptic nucleus

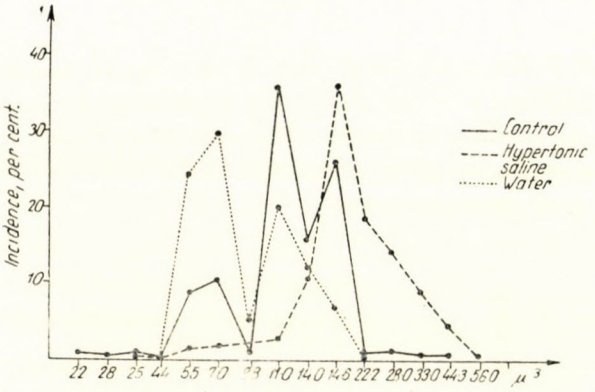

Fig. 2. Nucleolus variation curve for supraoptic nucleus

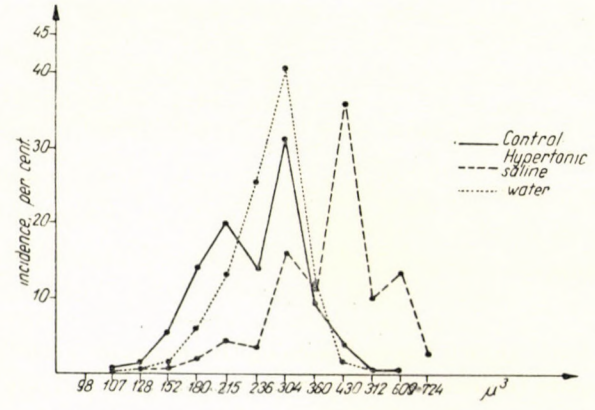

Fig. 3. Nucleus variation curve for paraventricular nucleus

cubic microns) of the cytoplasm, nucleus and nucleolus, respectively, was significantly increased in both the supraoptic and paraventricular nuclei. In rats treated with distilled water the mean volume values did not differ from those of the controls, except for the nucleoli, which were markedly reduced in size in

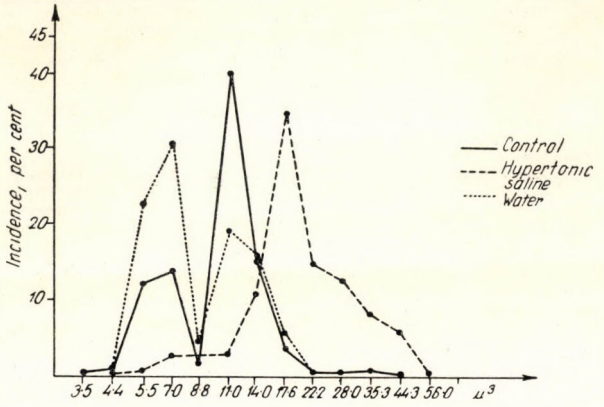

Fig. 4. Nucleolus variation curve for paraventricular nucleus

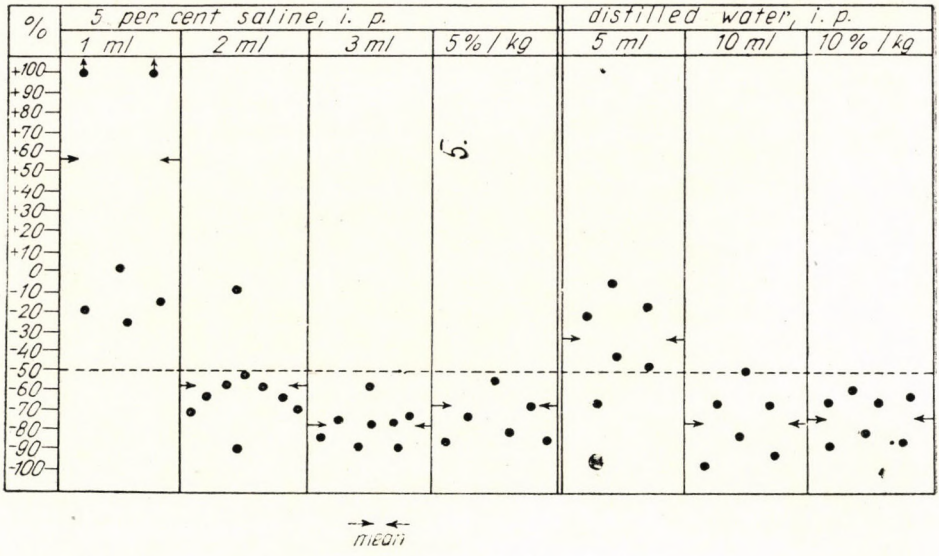

Fig. 5. Changes in eosinophil count, in per cents

both nuclei. There were no changes in the ependyma cell volume in either experimental groups. This is thought to indicate that quantitative ganglion cell changes were isolated and were not due to inadequacies in the technique of embedding. In Figs. 1 to 4 are shown the variation curves for the size of nucleoli and of cell nuclei. The abscisse shows the volumes in cubic micron, in base-2 logarithmic line (with nucleoli, K = 7 ; 2 sub-groups were interpolated between the members

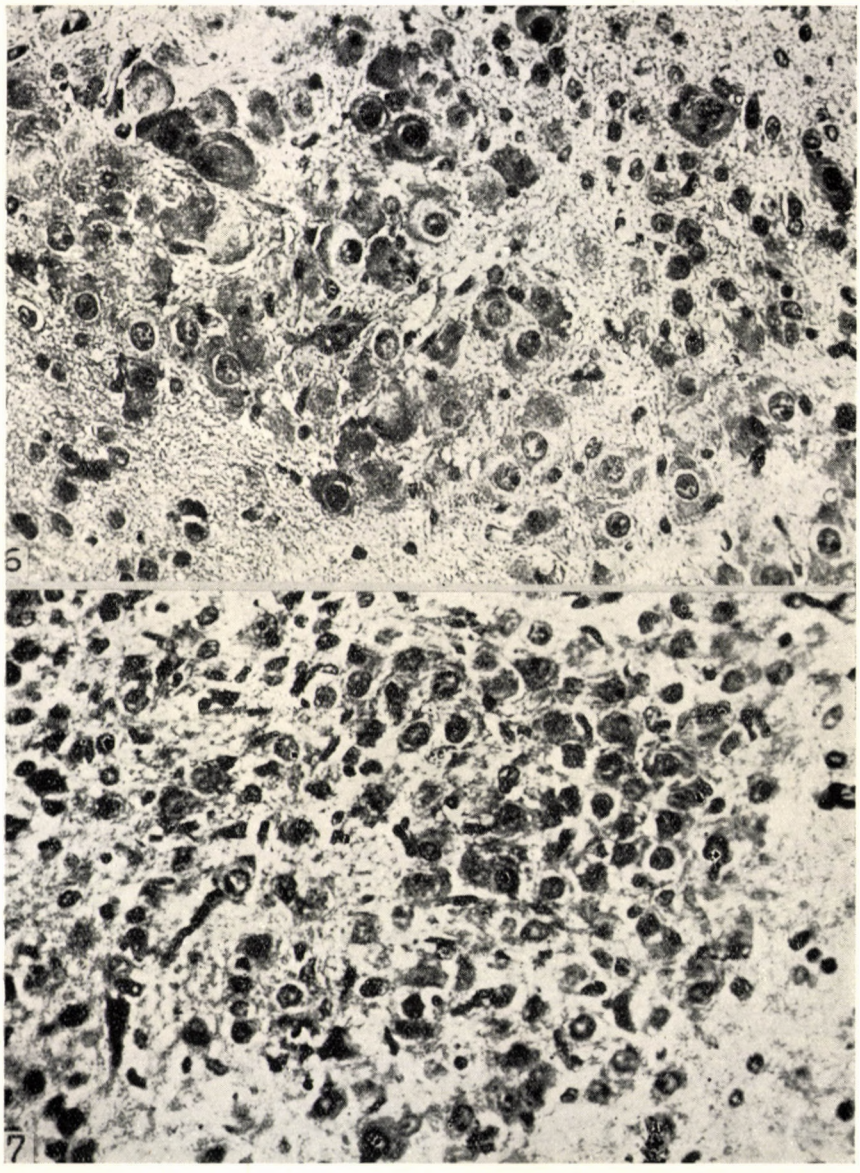

Fig. 6. Paraventricular nucleus from rat after chronic saline treatment. H. E. stain. Magnification, ×400

Fig. 7. Paraventricular nucleus from rat after chronic water treatment. H. E. stain. Magnification, ×400

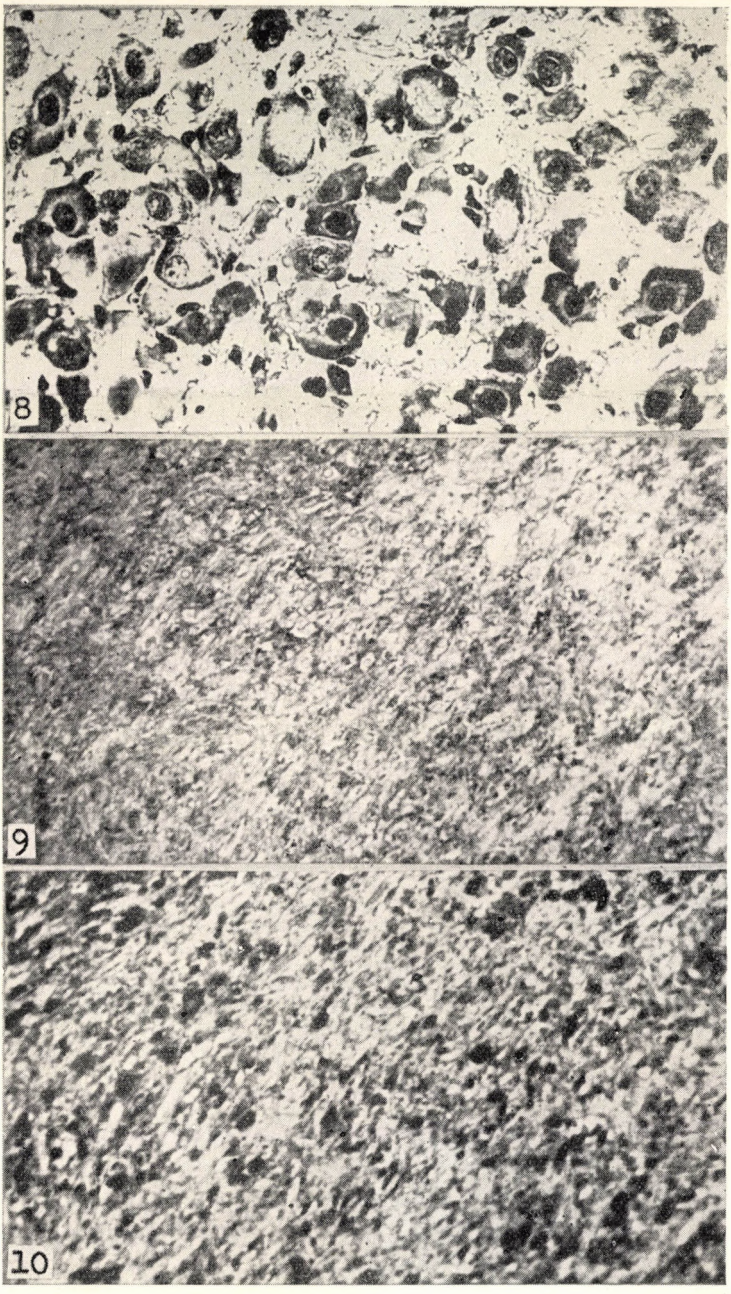

Fig. 8. Supraoptic nucleus from rat after chronic saline treatment. H. E. stain. Magnification, ×400

Fig. 9. Neurohypophysis from rat after saline treatment. Gömöri's stain. Magnification, ×200

Fig. 10. Neurohypophysis from rat after water treatment. Gömöri's stain. Magnification, ×200

of the line. With nuclei, K = 180 ; with 3 sub-groups interpolated), while the ordinate shows the percentual incidence of the values found. According to the figures, the difference found for mean volumes may be established on the basis of the variation curves, too, inasmuch as on hypertonic saline the volume values shifted to the right, toward higher values, while on chronic water treatment the peak of the curves for nucleoli shifted to the left, towards smaller values. (See also the microphotographs in Figs. 6 to 8.)

The hypertrophy of the magnocellular nuclei of the anterior hypothalamus, developing in the course of treatment with saline, is accompanied by a reduction in the amount of Gömöri-positive substance in the cytoplasm of ganglion cells as well as by morphological signs of neurohypophyseal hyperfunction [4, 20, 14], such as the decrease in the amount of Gömöri-positive substance, hyperaemia, oedema, swelling and division of pituicytes, while in chronic water treatment the neurohypophysis of rats is similar in appearance to that of the controls (Figs. 9 and 10). In the latter group some animals were found in which increased amounts of Gömöri-positive substance occurred in the neurohypophysis. It is, however, rather difficult to evaluate such an accumulation in the rat, since considerable amounts of Gömöri-positive substance may be present in the normal animal, too. In the haematoxylin-eosin-stained sections from animals treated with hypertonic saline, perinuclear lightening (Aufhellung) and, at sites, degenerative changes could be detected in the hypothalamic nuclei. The supraoptic and paraventricular nuclei of animals treated with distilled water did not significantly differ from the normal, either in sections stained with haematoxylin and eosin, or in specimens treated according to Gömöri's chrome-alum haematoxylin method.

TABLE II

Antidiuretic activity of the hypothalamus of the rat after treatment with hypertonic saline, respectively water

Group Treatment		2 ml of 1 per cent hypothalamus extract per 100 g body weigh		
		Control	Hypertonic saline	Water
Number of animals		12	12	12
Average body weight ..		213,0	184,8	175,0
Water tolerance test :		2×5 per cent per body weight		
30 min. excretions, in per cent of tolerance dose (ml)	..... 30'	2,0	0,2	0,2
	..... 60'	7,8	6,9	3,8
	..... 90'	16,6	22,4	13,3
	..... 120'	28,7	42,8	28,5
	..... 150'	39,3	54,7	39,9
	180'	44,4	56,1	47,6

The mobilization of adiuretin is confirmed by the fact that in the anterior part of the mesencephalon of rats with chronic saline treatment the antidiuretic activity seemed to be less than in the controls or in water-treated animals (Table II).

Although the treatments employed brought about in the magnocellular nuclei of the hypothalamus and in the neurohypophysis morphological changes indicative of antagonistic functional phases (hyperfunction on administration of saline ; hypofunction on water-treatment), the same quantitative cytological changes developed in the adenohypophysis (Table III). There was a mathematically significant increase in the number of basophil (trichrome staining) and of PAS-positive cells ; the number of acidophil cells was not appreciably increased, while basophil hyperplasia occurred at the expense of chromophob cells. In addition to the quantitative results, the qualitative appearance of the cells of the anterior lobe was also in agreement with the results of experiments in which basophilia had been induced by other means, such as formaldehyde treatment or thirsting. It must, however, be noted that the qualitative changes developing in the adenohypophysis on chronic saline or water treatment appear to be less marked. In the present experiments, too, the number of basophil cells changed parallel with the changes in the number of PAS positive cells. The accumulation of PAS positive cells on saline or water treatment suggests the possibility of a gonadotrophic hyperfunction. The histological appearance of the ovaries seems also to point in this direction : the corpora lutea were unusually large and occurred in greater numbers than in the controls.

TABLE III

Quantitative cytology of adenohypophysis

Group	Number of animals	Quantitative distribution of cells, per cent				
		Basophils	Eosinophils	Chromophobes	PAS +	PAS —
Control	8	5,8± 0,5*	44,4± 1,1	49,9± 1,1	6,0± 0,5	94,0± 0,5
		5,3— 6,3	42,9—46,4	48,2—54,4	5,6— 6,5	93,0—94,5
Water	8	12,1± 0,7	40,2± 1,1	47,7± 1,1	11,9± 0,7	88,1± 0,7
		10,9—13,5	38,2—42,4	46,5—49,2	10,3—13,1	86,9—89,7
Hypertonic saline ...	8	11,0± 0,7	40,3± 1,1	48,7± 1,1	10,7± 0,7	89,3± 0,7
		9,8—12,3	38,6—41,3	46,9—50,1	9,6—12,5	87,6—90,5

*Standard error

The increase in the weight of the adrenal glands and the reduction in the weight of the thymus (Table IV) are adequate indicators of adrenocorticotrophic hyperfunction. Equally relevant are the cell hypertrophy and hyper-

plasia visible in the fasciculate zone of the adrenal cortex. In Fig. 5 are shown the changes in the absolute eosinophil count on treatment with saline, respectively water. The resulting eosinopenia is another evidence of adrenocorticotrophic hyperfunction.

TABLE IV

Weight relations

Group	Number of animals	Initial body weight, gr	Final body weight, gr	Adrenals mg	Thymus mg	Spleen mg
Control	20	—	139,5 ± 3,1*	25,2 ± 1,2	230,2 ± 11,6	474,1 ± 24,5
Water	20	107,2 ± 3,1	149,6 ± 5,4	37,0 ± 2,1	184,4 ± 12,9	649,5 ± 66,7
Hypertonic saline ...	20	107,8 ± 2,4	148,7 ± 6,3	32,5 ± 1,2	165,2 ± 12,7	447,4 ± 30,3

*Standard error

Discussion

Bykov and his school, evolving the doctrines of *Setchenov* and *Pavlov*, have accumulated a great wealth of experimental data during the past decades on whose basis it became possible to work out a new theory for corticovisceral reflexes. Using the Pavlovian method of conditioned reflexes, *Bykov* and his school stated in the light of numerous experimental evidence that a functional correlation exists between the cerebral cortex and the internal organs. According to their investigations, the pathway of corticovisceral reflex runs through the subcortical ganglia of the hypothalamic region and through the endocrine glands (pituitary, thyroid gland, etc.), which, in this way, become included in the realm of cortical impulses (*Balakshina* 1936, *Olnianskaia* 1946, *Krasnogorsky* 1947). In 1939 *Tsareva* showed that the function of subcortical centres depends on cortical impulses.

The pathways and transmission mechanisms of the neural regulative impulses for adenohypophyseal function have not been elucidated. A number of authors [13, 11] emphasize the role of the anterior hypothalamus, while others [10, 8] attribute a significance to the posterior hypothalamic nuclei. *Simms* et al. have shown that a close correlation exists between the functions of the neurohypophysis and the adenohypophysis, while we ourselves have succeeded in demonstrating adrenocorticotrophic and gonadotrophic hyperfunction of the

anterior pituitary in mesencephalic-neurohypophyseal hyperfunction induced by dehydration [14].

The results presented do not support the assumption of a causal correlation between the adrenocorticotrophic function of adenohypophysis and the morphologically traceable functional state of the anterior hypothalamo-neurohypophyseal system. The overproduction of this trophic hormone will namely take place just as well in the presence of a hyperfunction of the two magnocellular nuclear groups, as it will when these systems are brought into a state of equilibrium. On the basis of our investigations it seems that the neural regulation of adrenocorticotrophic function takes place not through the supraoptic and paraventricular nuclei, but through some other pathways.

In the experiments evidence was obtained to show that a very close functional correlation exists between the two magnocellular nuclei of the anterior hypothalamus and the neurohypophysis. It follows that it is justified to speak of an anterior hypothalamo-neurohypophyseal system, one of whose main tasks is to regulate antidiuresis in the capacity of a neurohumoral mediator of integrative actions of the higher planes of the central nervous system.

Summary

Chronic administration of hypertonic saline brought about in the rat a hyperfunction of the supraoptic and paraventricular nuclei and of the neurohypophysis, while chronic water treatment resulted in a state of equilibrium. Although these two types of treatment brought about opposite functional conditions in the anterior hypothalamo-neurohypophyseal system, in both cases an adrenocorticotrophic hyperfunction could be demonstrated, accompanied by basophilic hyperplasia and a parallel increase of PAS positive cells in the anterior pituitary.

On the basis of this experimental evidence we are of the opinion that the adrenocorticotrophic and gonadotrophic functions of the adenohypophysis are not mediated *via* the supraoptic and paraventricular nuclei.

REFERENCES

1. **Bachrach, D., Kovács K.. Traub, A., Horváth É., Korpássy B.;** (1954) Histomorphological signs of hyperfunction in the magnocellular nuclei of the anterior hypothalamus of the rat. Acta Morph. Hung. *4*, 179. 2. **Balakshina :** quoted by Bykov and Kurtsin. 3. **Bargmann W.:** (1950) Die elektive Darstellung einer marklosen diencephalen Bahn. Mikroskopie *5*, 289. 4. **Bargmann, W., Hild W., Ortmann, R., Schieb'er, Th. T. :** (1950) Morphologische und experimentelle Untersuchungen über das hypothalamisch-hypophysäre System Acta Neuroveget. *1*, 233. 5. **Bykov, K. M., Kurtsin, I. T. :** (1949) Кортико-висцеральная теория патогенеза язвенной болезни. Издательство академии медицинских наук СССР. Москва (Hungarian translation). 6. **Birnie, J. H., Jenkins, R., Eversole, W. J., Gaunt, R. :** (1949) An antidiuretic substance in the blood of normal and adrenalectomized rats. Proc. Soc. exper. Biol. Med. *70*, 83. 7. **Tsareva, T. V:** quoted by Bykov and Kurtsin. 8. **Porter, R. W.:** (1952) Alterations in electrical activity of the hypothalamus induced by stress stimuli. Am. J. Physiol. *169*, 629. 9. **Gömöri, G. :** (1939) A differential stain for cell types in the pancreatic islets. Am. J Path. *15*, 497. 10. **Colfer, H. F., de Groot, J., Harris, G. W. :** (1950) Pituitary gland and blood lymphocytes. J. Physiol. *111*, 328. 11. **Heinbecker, P., Pfeiffenberger, M.:** (1950) Further clinical and experimental studies on the pathogenesis of Cushing's syndrome. Am. J. Med. *9*, 3. 12. **Hillarp, N. A.:** (1951) Cell reactions in the hypothalamus following overloading of the antidiuretic function. Acta Endocrinol. *2*, 33. 13. **Hume, D. M.:** (1949) The role of the hypothalamus in the pituitary-adrenal corticalresponse to stress. J. Clin.

Invest. *28*, 790. 14. **Kovács, K., Bachrach, D., Jakabovits, A., Horváth É., Korpássy B.** (1954) Hypothalamo-hypophyseale Beziehungen der Flüssigkeitsentziehung bei Ratten. Endokrinologie *31*, 17. 15. **Kovács K., Bachrach, D., Horváth, É.:** (1953) Nem fajlagos károsodás (stress) és az adenohypophysis gonadotroph működése. Kíséri. Orvostud. *5*, 52. (Hungarian only). 16. **Kovács, K., Korpássy, B.:** (1952) Effect of dietary protein contents on the hypophyseal adrenocortical system and on the lymphatic organs of normal rats and of rats in alarm reaction. Acta Physiol. Hung. *3*, 243. 17. **Kovács, K., Bachrach, D.:** (1951) Hypothalamus and water metabolism. Studies on the antidiuretic substance of the hypothalamus and hypophysis. Acta Med. Scand. *141*, 137. 18. **Krasnogorsky:** quoted by Bykov and Kurtsin. 19. **Olnianskaia:** quoted by Bykov and Kurtsin. 20. **Ortmann, R.:** (1951) Über experimentelle Veränderungen der Morphologie des Hypophysen-Zwischenhirnsystems und die Beziehung der sog. »Gömöri-substanz« zum Adiuretin. Ztschr. f. Zellforschg. *36*, 92. 21. **Simms, E.,, Pfeiffenberger, M., Heinbecker, P.:** (1951) Neuro-endocrine and endocrine influences on the circulating blood elements. Endocrinology *49*, 45.

ГИПОТАЛАМО-ГИПОФИЗЕАЛЬНЫЕ ОТНОШЕНИЯ ЭКСПЕРИМЕНТАЛЬНОГО ВОЗДЕЙСТВИЯ НА СОЛЯНОЙ И ВОДНЫЙ ОБМЕН

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Р е з ю м е

Авторы вызывали дозировкой, в течение продолжительного времени, гипертонического раствора поваренной соли гиперфункцию nucleus supraopticus и paraventricularis, а также и гиперфункцию неврогипофиза. С другой стороны, хронической водной нагрузкой образовалось состояние покоя. Авторы установили, что хотя функциональное состояние системы передний гипоталамус — неврогипофиз в случае двух примененных воздействий имеет противоположный характер, то все-же в обоих случаях происходила адренокортикотропная гиперфункция, а в аденогипофизе возникли базофильная гиперплазия и параллельное размножение ПАСК-положительных клеток.

На основании результатов своих экспериментов авторы того мнения, что управление адренокортикотропной и гонадотропной функции аденогипофиза не состоится при посредстве nucleus supraopticus и paraventricularis.