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Effect of Ethanol on Cytological Changes Induced by Salt Load in Nucleus Supraopticus of Rat.* (25530)

NIELS RAIHA (Introduced by Irving L. Schwartz)
Research Lab. of State Alcohol Monopoly, Helsinki, Finland

It is generally agreed that posterior pituitary hormones are formed in the hypothalamus and transported *via* supraoptico-hypophyseal tract to the posterior pituitary lobe bound to a carrier, neurosecretory material (1). Although this hypothesis of neurosecretion is widely accepted no agreement has been reached as to site of formation of the peptide hormones or to the mechanism of their release. According to Olivecrona(2), formation of oxytocin is primarily located in the paraventricular nucleus and that of antidiuretic hormone (ADH) in the supraoptic nucleus. Conspicuous changes in ganglion cells of the supraoptic nucleus after procedures which increase blood tonicity were first noted by Ortmann(3), and later confirmed by other investigators(4). According to Ortmann(3) cytological changes in cells of nucleus supraopticus after osmotic stress with hypertonic saline are comparable with those found by Hydén(5) in nerve cells activated by other stimuli. It was further suggested that these changes reflect increase of cell activity and hence production of antidiuretic hormone. The diuretic action of ethanol has been explained as due to inhibition of the supraoptico-hypophyseal system(6,7). Kleeman *et al.*(8) in studies on ethanol diuresis in human subjects found that ethanol prevented or minimized the fall of urine flow and free water clearance that characteristically follows administration of hypertonic solutions of sodium chloride. In the present investigation the cytological changes of the supraoptic nucleus of rats induced by hypertonic salt solution were compared with those of rats receiving ethanol in addition to hypertonic salt solution.

Material and methods. White male rats weighing 150 to 170 g were used. The animals were divided into 4 groups of 7 rats. Twelve hours after withdrawal of food, rats in Group I received an intraperitoneal injection of 6 ml of water, Group II, 6 ml of 10% (w/v) solution of ethanol, Group III, 6 ml of 5% NaCl solution and Group IV, 6 ml of 5% NaCl solution containing 10% ethanol. Each rat was placed in metabolic cage and urine collected 24 hours. Rats used for histological studies were divided into 3 groups of 4 rats. 5 ml of 10% ethanol solution was administered by stomach tube twice a day during 5 days to the control group. The second group received an equal amount of 2.5% NaCl solution and the third group a solution containing 10% ethanol in 2.5% NaCl. On 6th day animals were killed by decapitation. The hypothalamic region was fixed for 24 hours in Bouin's fluid, treated with alcohol and embedded in paraffin. Transverse sections of 3 to 5 μ were cut through the supraoptic nucleus and stained according to Gomori's chromhematoxylin method.

Results. Table I shows total urine excre-

TABLE I. Total Urine Excreted in ml during 24 Hours in 4 Experimental Groups of Rats.

	Group I	Group II	Group III	Group IV
	Water	Ethanol	NaCl	NaCl + ethanol
	7.5	9.5	4.5	9.0
	7.0	9.0	5.0	8.0
	6.5	10.0	6.8	8.5
	4.5	7.5	3.5	7.0
	8.0	9.0	5.6	6.5
	6.0	8.0	5.5	7.5
	7.0	11.0	6.5	7.0
Mean	6.64	9.21	5.34	7.64

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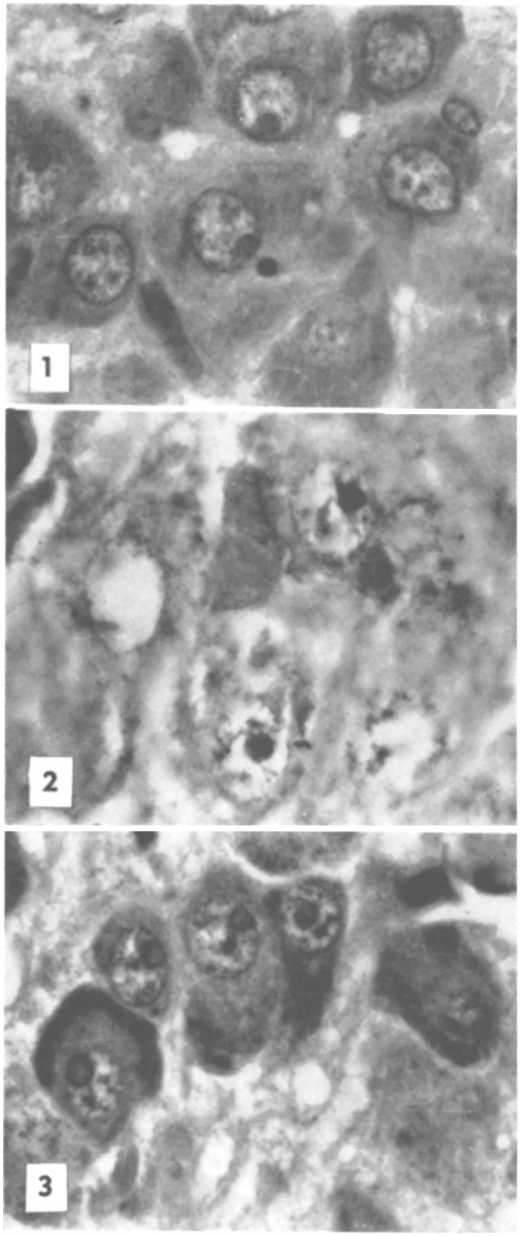

FIG. 1. Cells of supraoptic nucleus of a control rat after administration of 5 ml of a 10% ethanol solution by stomach tube twice daily during 5 days. Cells are at rest, with Nissl substance evenly distributed throughout the cytoplasm, nucleus fairly deeply stained and nucleolus small. Magnification 800 ×.

FIG. 2. Cells of supraoptic nucleus of rat after administration of 5 ml of a 2.5% NaCl solution by stomach tube twice daily for 5 days. Cells show signs of degeneration with vacuolized cytoplasm, disappearance of nuclear membrane and disintegration of nucleus. Magnification 800 ×.

FIG. 3. Cells of supraoptic nucleus of a rat after administration of 5 ml of a solution containing 10% ethanol in 2.5% NaCl by stomach tube twice daily for 5 days. Among cells in a stage of rest other cells are seen with deeply stained periphery and enlarged nucleolus. Magnification 800 ×.

tion during 24 hours in the 4 groups. When groups were statistically compared, a difference of high significance ($p<0.005$) was found between the group receiving water and the group receiving ethanol. The difference between the group receiving NaCl solution and the group receiving NaCl solution containing ethanol was also statistically highly significant ($p<0.005$).

The histological pictures of the supraoptic nucleus in the 3 groups of rats receiving ethanol, hypertonic salt solution and hypertonic salt with ethanol are seen in Fig. 1, 2, and 3. In rats of control group, ganglion cells are in a typical state of rest with the Nissl substance evenly distributed throughout the cytoplasm, the nucleus fairly deeply stained and mostly located centrally and the nucleolus small (Fig. 1).

Ganglion cells of animals receiving hypertonic salt (Fig. 2) show signs of necrobiosis and cell degeneration: vacuoles in the cytoplasm, disappearance of nuclear membrane and disintegration of the nucleus. Cells in paraventricular nuclei did not show such marked degenerative changes, and elsewhere in the central nervous system, cells appeared normal.

In rats receiving the same amount of salt as those in the previous group but in a 10% ethanol solution, degenerative changes of ganglion cells in the supraoptic nuclei were not observed (Fig. 3). Among cells in a stage of rest, other cells can be seen with a deeply stained band at the periphery of cytoplasm and with an usually enlarged and intensely stained nucleolus. According to Hydén's classification these cells appear to be in a moderate state of excitation.

Discussion. It has been shown by Eggelton (9) and Strauss *et al.*(10) that following administration of ethanol, renal tubules are capable of responding to exogenous ADH, and concluded that ethanol acts directly on the supraoptico-hypophyseal system and not upon the kidneys. Thus ethanol might affect the

osmoreceptors(11), ganglion cells of supraoptic nucleus or mechanism for ADH release. Since ethanol is distributed very rapidly over the entire water phase of the body, especially in areas of rich blood supply as the brain, and no effective osmotic pressure difference is produced between extracellular and intracellular fluid, it is unlikely that the osmoreceptors can be affected.

Whether ethanol acts directly on the ADH release mechanism, on production of ADH, or on both is difficult to conclude. Results of our study suggest that ethanol can retard development of cytological changes characteristic for exhaustion of ganglion cells in the supraoptic nuclei. Further studies are, however, needed to correlate these cytological changes with production of ADH. The action of ethanol on diuresis occurs relatively rapidly according to Kleeman *et al.*(8), this observation favoring an effect directly on the ADH releasing mechanism. According to recent theories ADH is released into blood at nerve terminals in the posterior pituitary, but whether ADH can be released into blood directly from site of its production following a stimulus is not known. Nervous impulses must also play a role in release of ADH(12) and a depression of nerve conduction with ethanol can be postulated.

Summary. Administration of ethanol to rats loaded with hypertonic salt solution increased urine flow and prevented degenerative changes in ganglion cells of the supraoptic nucleus.

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Influence of Tetrazolium Salts on Human Pseudocholinesterase.* (25531)

GEORGE H. FRIED AND WILLIAM ANTOPOL
Joseph and Helen Yeamans Levy Fn., Beth Israel Hospital, N. Y. City

Tetrazolium salts at present are used as histochemical tools primarily for measuring oxidative enzyme systems underlying cellular metabolism. Tetrazolium compounds also have a pharmacological action when administered to the intact animal. Neurotoxic effects with respiratory embarrassment, convulsions, paralysis, and death ensuing from intravenous administration of a simple monotetrazolium have been described(1). *In vivo* administration of neotetrazolium (NT) exerts a hypotensive action on renal hypertensive rats. Since blood pressure can be reduced in hypertensive states by interference with neural transmission through the sympathetic ganglia, it was postulated that NT might be acting as a ganglionic blocking agent(2). The observation that subcutaneous injection of NT results in marked deposition of formazan in the satellite and other cells of the sympathetic ganglia (3,4) lends credence to this view. It is also of interest that subcutaneous administration of various tetrazolium salts results in varying patterns of formazan deposition(5), and that INT, which is not reduced in the ganglia of the rat does not have an antihypertensive effect (unpublished data). In an effort to delineate the biochemical mechanisms underlying the neurological effects of tetrazolium

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