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)

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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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compounds, determinations were made of the effects of 6 tetrazolium salts on human pseudocholinesterase (ChE). Since the acetylcholine-cholinesterases system is known to play a major role in transmission of nerve impulses, alteration of involved enzyme systems could conceivably account for the neurotoxic effects of tetrazolium. Koelle(6) has offered histochemical evidence for the presence of the pseudoesterase in cytoplasm of the satellite cells of the dorsal root, trigeminal, superior cervical, and stellate ganglia of the cat. In addition, pseudoesterase is more sensitive than true cholinesterase (AChE) to the inhibiting effects of various agents known to depress cellular activity(7) and is more readily influenced by a variety of tranquilizers and hallucinogens which are presumed to act within the central nervous system(8). The presence of a minimum of 3 tertiary amine groups in every tetrazolium salt also suggested a possible effect upon ChE, because this enzyme is specifically inhibited by tertiary amine groups (9).

Materials and methods. The 6 compounds tested for anticholinesterase activity were triphenyltetrazolium chloride (TTC), 2-p-iodo-3 - p - nitro-5-phenyl tetrazolium chloride (INT), neotetrazolium (NT), tetrazolium blue (BT), nitro-neotetrazolium (NNT), and the nitro derivative of blue tetrazolium (NBT). The first 2 are monotetrazoliums, the last 4 ditetrazoliums. ChE activity was determined manometrically(10) with human serum as a source of ChE. Tetrazolium salts (0.5 ml) and human serum diluted 1-50 (0.5 ml) were pipetted directly into the main compartment of the flasks, while ACh (1.0 ml) was added to the sidearm to give a final substrate concentration of 0.02 M. All flasks were adjusted to a final fluid volume of 2 ml with buffer and then flushed with 95% N_2 - 5% CO_2 for 10 minutes. The initial pH was about 7.6, and this value did not appear to change more than several tenths in either direction during the course of the reaction. Substrate was tipped into the main compartment and the flasks equilibrated for 10 minutes at a bath temperature of 30°C. Readings were taken every 10 minutes. Calculations of the velocity constants of cholinesterase activities were based on microliters of CO_2 liberated during the 1st hour. Several experiments were also carried out with benzoylcholine (0.002M) as substrate. Corrections were made for spontaneous hydrolysis. A minimum of 6 experiments was carried out with each concentration of tetrazolium salt tested.

TABLE I. Influence of Tetrazolium Salts on Cholinesterase.

Tetrazolium compound	No. of tertiary amines	Concentration	% inhibition
TTC	3	4.2×10^{-5}M	50 ± 8.2*
INT	3	2.0×10^{-5}M	48 ± 9.2
NT	6	4.2×10^{-6}M	47 ± 9.6
BT	6	1.0×10^{-7}M	42 ±10.3
NBT	6	5.0×10^{-7}M	52 ± 5.3
NNT	6	1.3×10^{-6}M	54 ± 3.6

* S.D. $= \sqrt{\frac{\Sigma d^2}{n-1}}$.

Results. All of the tetrazolium compounds studied inhibited ChE, with BT exerting maximum effects and TTC and INT exhibiting the least inhibition (Table I). TTC and INT produced 50% inhibition at levels of the order of 10^{-5}M. Both NT and NNT produced 50% inhibition at levels of approximately 10^{-6}M, while the corresponding 50% inhibition levels for BT and NBT were of the order of 10^{-7}M. A marked decrease in magnitude of inhibition was encountered with decreasing concentrations of tetrazolium. There was a variable potentiation of ChE activity with 10^{-8}M NT which averaged 25%. Potentiation was not observed with similar low levels of the other tetrazolium salts. Addition of excess glutathione in several instances did not reverse the inhibitory effects of tetrazolium. In selected instances excess substrate (ACh) was found to reverse the inhibitory effects.

Discussion. As might be expected, the ditetrazoliums, possessing greater numbers of tertiary amine groups, exert stronger inhibitory effects than the monotetrazoliums, although each of the tetrazolium compounds tested demonstrated appreciable anticholinesterase activity. The reversal of inhibitory effects by excess substrate indicates that inhibitions are of a competitive nature. Tetrazolium compounds can effect oxidation of sulfhydryl

groups at higher pH ranges. However, since reversal of inhibition did not occur with added glutathione, the inhibition is probably not due to oxidation of sulfhydryl groups.

It has been reported that BT, which exerted maximal inhibitory effects in the present investigations, was most toxic of a series of tetrazolium salts administered to mice *in vivo* (11); this tends to support the thesis that interference with neural enzyme mechanisms underlies the neurotoxic effects. It is not likely that the toxic effects of the tetrazolium salts are due to the intracellular blocking effects of deposited formazan *per se* since this formazan remains in the ganglia long after blood pressure returns to preinjection levels in hypertensive animals treated with tetrazolium(2).

The histochemical advantages obtained by addition of the nitro groups to the tetrazolium nucleus, particularly in terms of greater sensitivity to reducing substances within tissues, are not reflected in changes in anticholinesterase activity of either monotetrazolium or ditetrazolium salts. It would appear that the redox properties of these compounds are totally unrelated to the inhibition effects observed here. Also, while some reports indicate that striking qualitative differences in activities of certain cholinesterase inhibitors exist with different choline ester substrates(12), no indication of this phenomenon was found with tetrazolium salts in the present study. Degree of inhibition and order of inhibitory effectiveness among 4 of the tetrazolium salts studied (BT, NT, INT, TTC) was essentially the same with substitution of benzoylcholine for ACh in several experiments. The exact physiological role of ChE has not been established, but the demonstrated sensitivity of this enzyme to psychotomimetic substances(13, 14) and correlation of neurotoxic effects and anticholinesterase activity in the case of the tetrazolium salts strongly suggest a fundamental significance within the central nervous system.

Summary. Neurotoxic effects of tetrazolium salts may be due to interference with enzyme systems in nerve tissue. Six tetrazolium salts were found to inhibit pseudocholinesterase, with the ditetrazoliums generally exhibiting more marked inhibitory activity than the monotetrazoliums. This may be related to the greater number of tertiary amine groups in the ditetrazoliums.

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