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Ethanol Inhibition of Vitamin A Metabolism in the Testes: Possible Mechanism for Sterility in Alcoholics

Abstract. *Vitamin A (retinol) is essential for spermatogenesis. Alcohol dehydrogenase, the enzyme responsible for ethanol metabolism, is also required for the conversion of retinol to bioactive retinal at the end organ site. Ethanol inhibits the oxidation of retinol by testicular homogenates containing alcohol dehydrogenase. Thus, a possible biochemical mechanism for the sterility of chronic alcoholics is identified.*

Testicular atrophy and aspermatogenesis are found in 50 to 75 percent of male chronic alcoholics with Laennec's cirrhosis (*1*). Histologic study of testicular biopsy and autopsy material has demonstrated marked seminiferous tubular atrophy with loss of germinal elements and, in more advanced cases, total germinal cell aplasia (*2*). The pathogenesis of this lesion is poorly defined, as are its rate of development and reversibility. In one autopsy review, the degree of testicular histologic damage appeared to correlate with liver disease activity, suggesting that testicular and liver injury were coincident and that both were partially reversible (*3*). Our own recent results establish that a high incidence of azoospermia is found not only in patients with Laennec's cirrhosis, but also in chronic alcoholics with relatively mild liver disease (*4*). Whatever the disease mechanism, it is estimated that 9 million adult Americans are alcoholics or alcohol abusers and that 10 percent of these may ultimately develop cirrhosis (*5*). These findings taken together make it almost certain that alcoholism is a common nonfunctional cause of male sterility in the United States, and suggest the possibility that alcohol per se (rather than or as well as liver disease) plays a significant role in the pathogenesis of azoospermia in male alcoholics.

It is well established that vitamin A is essential for spermatogenesis (*6*). Rats develop germinal cell aplasia within 6 to 8 weeks of the initiation of a vitamin A–deficient diet, and full spermatogenesis is restored by replacement therapy. Similarly, vitamin A supplementation is required for the initiation of spermatogenesis in organ culture systems (*7*). Vitamin A is ingested as inactive retinol and is oxidized to active retinal by alcohol dehydrogenase (ADH), the enzyme which metabolizes ethanol to acetaldehyde. Recent studies have demonstrated ADH activity in the retina, and have shown that alcoholics experience night blindness because of the competitive inhibition of retinal formation by ethanol (*8, 9*). We now propose that ADH activity is demonstrable in testicular tissue, that retinol is converted to retinal by testicular ADH, that ethanol inhibits testicular retinal formation, and that "relative vitamin A deficiency" may be a factor in the pathogenesis of sterility in chronic alcoholics.

Testicular and hepatic tissue were obtained from 20 Wistar rats at each of the following ages: 20, 43, 50, and 61 days. Homogenates of both types of tissue were prepared in a 1:9 dilution with 0.32*M* sucrose in 10 m*M* tris(hydroxymethyl)aminomethane (tris) buffer, at *p*H 7.5. These homogenates were then made successively 35 and 60 percent saturated with $(NH_4)_2SO_4$. Essentially all the ADH activity was located in the fraction precipitated at 35 to 60 percent $(NH_4)_2SO_4$. This precipitate was redissolved in 40 m*M* tris buffer,

Table 1. Alcohol dehydrogenase (ADH) activity of rat testes. Forty testes (20 animals) were used for each age. The results are expressed as mean ± standard error of the mean. The protein concentration in the reaction vessel varied for each homogenate but ranged between 30 and 200 μg/ml. The ADH activity is expressed as nanomoles of NADH per minute, retinol oxidation activity as nanomoles of retinal per minute per gram of tissue.

Age of rats	ADH activity			
	Total	Per gram of tissue	Per mg of protein	Retinol oxidation activity
20	966 ± 30	350	59	
43	4585 ± 96	170	30	34.1 ± 2.4
50	3944 ± 82	89	20	22.6 ± 2.6
61	5861 ± 90	120	23	15.0 ± 1.3

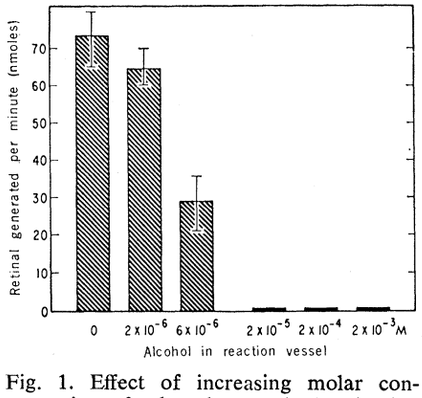

Fig. 1. Effect of increasing molar concentration of ethanol on retinol oxidation by testicular homogenates containing alcohol dehydrogenase activity. The retinol concentration and protein content in the incubation mixture were $6 \times 10^{-6}M$ and 100 μg, respectively. Results are expressed as mean ± standard error of the mean. Note complete inhibition of retinol oxidation at molar concentrations of ethanol equal to or greater than 2×10^{-5}.

*p*H 7.6, and dialyzed in Visking tubing against 8 liters of the same buffer, with two changes over a 48-hour period. The ADH activity was measured according to the method of Bonnishsen and Brink (*10*), which utilizes the generation of reduced nicotinamide adenine dinucleotide (NADH) as ethanol is oxidized. The enzymatic activity for each testicular homogenate at each age of rats studied is shown in Table 1. The overall total testicular ADH activity expressed per gonad pair is lowest in the least mature animals. In contrast, specific activity (ADH activity per milligram of testicular protein) is highest in the 20-day-old animals and declines progressively with increasing maturity.

The ability of these same preparations to metabolize retinol to retinal was assayed according to the method of Mezey and Holt (*9*), where the retinal generated is determined spectrophotometrically by using its known absorption maximum at 410 nm. The enzymatic activity for retinal generation by 35 to 60 percent $(NH_4)_2SO_4$ fractions of rat testicular homogenates is also shown in Table 1. The specific activity of ADH for retinal formation was also highest in the least mature preparation and declined with increasing age, which suggests that the enzyme is present in either the Leydig cells or the Sertoli cells. Finally, as shown in Fig. 1, it was demonstrated that ethanol, in concentrations as low as 1/100 of the concentration of retinol substrate and 1/10,000 of the maximum concentration found in the blood of legally nonintoxicated drinkers, inhibited retinal formation.

The results show that testicular tissue is capable of retinal formation and that, at least in vitro, testicular retinal production is inhibited by coincident ethanol oxidation. While the concentrations of retinol substrate available in testicular tissue in vivo are not at present known, the amounts of ethanol necessary for the inhibition of retinal formation appear to be well below the range of concentrations customarily found in alcoholic individuals. Specifically, it is probable that an average male alcoholic ingesting 33 ounces (~ 1 liter) of whiskey or its equivalent per day (*4*) and maintaining plasma concentrations of 50 to 150 mg per 100 ml for prolonged periods, might suppress testicular retinal production and ultimately interrupt normal spermatogenesis. Preliminary experiments dealing with this specific question have shown that chronic ethanol feeding to pair-fed rats does produce germinal cell injury in the alcohol-fed animal but not in the isocaloric control. Whether this injury is directly related to decreased retinal formation within the testes, however, has not yet been determined. Indeed, the consequences of ethanol ingestion on normal testicular function may be even more far-reaching. The presence of ADH within testicular tissue raises the possibility that ethanol ingestion may alter not only spermatogenesis but also testicular steroidogenesis as a consequence of cofactor utilization and the resultant change in the redox state of the Leydig cells.

DAVID H. VAN THIEL
JUDITH GAVALER, ROGER LESTER
Division of Gastroenterology, Department of Medicine, University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania 15261

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Guanosine 3′,5′-Monophosphate in Sympathetic Ganglia: Increase Associated with Synaptic Transmission

Abstract. *Brief stimulation of cholinergic preganglionic nerve fibers resulted in an increase in guanosine 3′,5′-monophosphate (cyclic GMP) in the bullfrog sympathetic ganglion. When the release of synaptic transmitter was prevented by a high-magnesium, low-calcium Ringer solution, stimulation of preganglionic nerve fibers did not increase cyclic GMP in the ganglion. The increase in cyclic GMP caused by preganglionic stimulation was also blocked by the muscarinic antagonist, atropine. The data indicate that the increase in cyclic GMP is associated with synaptic transmission and support the possibility that cyclic GMP may mediate the postsynaptic action of acetylcholine at muscarinic cholinergic synapses.*

Guanosine 3′,5′-monophosphate (cyclic GMP) is distributed in a wide variety of tissues, but little is known about its functional role (*1*). Cyclic GMP can be elevated in brain by the systemic administration of oxotremorine (*2*), an agent known to affect cholinergic mechanisms in the central nervous system. Moreover, cyclic GMP concentrations in brain slices can be increased by acetylcholine (ACh) or other muscarinic cholinergic agonists (*3*, *4*). These elevations of cyclic GMP can be antagonized by atropine (*2*, *4*), which suggests that the effect may be related to the activation of muscarinic receptors. However, the functional significance of cyclic GMP in the nervous

Table 1. Effect of stimulation of preganglionic nerve fibers on the cyclic GMP content (picomoles per milligram of protein) of bullfrog sympathetic ganglia in normal and high-Mg (20 m*M*), low-Ca (0.4 m*M*) Ringer solution; and in normal Ringer solution and Ringer solution containing atropine alkaloid (free base, 10 μg/ml; from Nutritional Biochemical Co.). Stimulus frequency was 10 hertz. The number of control and of stimulated samples analyzed is *N*; each sample contained 12 ganglia pooled from six bullfrogs. The data are given as mean ± standard error.

Ringer solution	Duration of stimulation (sec)	*N*	Cyclic GMP			
			Control (pmole/mg)	Stimulated (pmole/mg)	Absolute (pmole/mg)	Percentage increase
Normal	120	5	0.85 ± 0.18	1.78 ± 0.40	0.93 ± 0.24	105 ± 12
High-Mg, low-Ca	120	5	0.45 ± 0.08	0.49 ± 0.08	0.04 ± 0.04	13 ± 9
Normal	15	8	0.71 ± 0.10	1.22 ± 0.20	0.50 ± 0.16	76 ± 22
Atropine	15	6	0.46 ± 0.06	0.45 ± 0.08	−0.01 ± 0.05	−4 ± 11