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Action of Dinitrophenol on Rate of Oxidation of Ethyl Alcohol in Vitro.*

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It has recently been shown by Widmark[1] that the injection of dinitrophenol in a dose of 10 mg. per kilo in dogs is effective in increasing the rate of disappearance of a previously administered dose of alcohol from the blood stream. He was not certain whether this was due to an increase in the rate of alcohol-metabolism from the drug itself, or to an increased rate of metabolism due to the hyperthermia, or to an increased elimination by the lungs from hyperventilation. Similar results have been reported by Harger and Hulpieu.[2] That alcohol may be oxidized in the presence of tissues *in vitro* has long been known.[3] We therefore determined whether dinitrophenol could increase this rate of alcohol oxidation *in vitro* by tissues, since this fact was indispensable in deciding as to the mechanism of the increased rate of fall of the blood alcohol concentration. Preliminary control experiments showed that dinitrophenol did not oxidize alcohol directly in the absence of tissues in concentrations used in the present study.

The echnic of Le Breton[4] was used, modified to adapt it to the alcohol method of Cannan and Sulzer.[5] Male white rats of 250 to 300 gm. were killed by a blow on the head and decapitated. The liver was removed, and sections 0.5 mm. in thickness prepared immediately; 300 mg. of the liver slices was placed in a 25 cc. Ehrlenmeyer flask, 3 cc. of 0.1% ethyl alcohol in phosphate buffer of pH 7.4, and 0.3 cc. of the appropriate dilution of dinitrophenol added, and the air displaced by oxygen. Each determination was run in duplicate, with duplicate controls from the same animal, which differed only in the absence of dinitrophenol. The 4 flasks were incubated at 37°C. for 2 hours, being shaken mechanically 120

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1 Widmark, E. M. P., *Biochem. Z.*, 1935, **276**, 268.

2 Harger, R. N., and Hulpieu, H. R., *Science*, 1935, **81**, 6.

3 Battelli, F., and Stern, L., *Compt. Rend. Soc. Biol.*, 1909, **67**, 419; Hirsch, J., *Biochem. Z.*, 1916, **77**, 129.

4 Le Breton, E., *Compt. Rend. Soc. Biol.*, 1935, **118**, 64.

5 Cannan, R. K., and Sulzer, R., *Heart*, 1924, **11**, 148.

times per minute during this period. They were then placed in ice water to stop the oxidation, and the alcohol content determined.

Table I summarizes the results obtained, the rate of alcohol-metabolism being expressed in gm. per kilo of liver per hour. The last column gives the results for change in oxygen consumption as determined by McCord,[6] for comparison. Each value is the average of the duplicate determinations.

TABLE I.

End Concentration Dinitrophenol	Rate of Alcohol Combustion D.N.P.	Control	% Change	Change in Oxygen Consumption (McCord) %
1:100,000				—20
1:200,000	1.198	1.222	—2	
	1.629	1.748	—7	
	1.749	1.844	—5	
1:1,000,000	1.797	1.869	—4	—5.7
1:5,000,000	1.318	1.222	+8	—7.9
1:20,000,000	.814	.767	+6	+18.4
	1.461	1.318	+11	
	1.461	1.437	+2	
	1.293	1.221	+6	

It will be seen that the changes, though small, were consistent. The dinitrophenol increased the oxidation of the alcohol in concentrations of 1:5,000,000 and 1:20,000,000. This stimulating concentration range was of the same order of magnitude as that found by McCord for stimulation of the oxygen consumption, in a medium of the same pH.

Conclusion. At a pH of 7.4, concentrations of dinitrophenol from 1:5,000,000 to 1:20,000,000 slightly increased the rate of oxidation of alcohol by rat liver *in vitro,* while higher concentrations slightly diminished it. This indicated that under some conditions an increase in tissue metabolism produced by dinitrophenol is accompanied by an increased rate of oxidation of alcohol. From this it may be deduced that the increased rate of fall of blood alcohol concentration caused by dinitrophenol in animals may be accounted for, in part at least, by an increased rate of its oxidation in the tissues. Further work, both *in vitro* and *in vivo,* is in progress to investigate this problem more fully.

[6] McCord, W. M., *Am. J. Physiol.,* 1934, **109**, 232.