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Inhibition of Brain Respiration by Ethyl Alcohol at Varied Temperature Levels.

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Jowett[1] has reported that given concentrations of ethyl alcohol produce less inhibition of rat brain respiration at 34°C than at 39°C. The experiments reported here were carried out in order to study the inhibitory effect of ethyl alcohol over a wider range of temperature and concentration than had hitherto been investigated.

Methods. The oxygen consumption of cerebral cortex slices from adult albino rats was measured *in vitro* by the direct method of Warburg at 37.7°C, 30°C and 20°C. The tissue slices were weighed on a micro torsion balance and transferred to respirometer vessels containing Ringer's solution[2] buffered to pH 7.25 with 0.01 M phosphate and containing 0.011 M glucose. The gas phase was oxygen. Ethyl alcohol in appropriate concentration, in one-tenth the total fluid volume, was added from the side arm of the vessels at the end of a 10 minute period of thermoequilibration. Rates of oxygen consumption are expressed as ml of oxygen consumed per mg initial dry weight (determined on aliquots) per hour (QO_2). Complete details of the experimental procedure have been given elsewhere.[3]

Results and discussion. The rates of oxygen consumption of rat brain slices in the presence of various concentrations of ethyl alcohol at 37.7°C, 30°C and 20°C are given in Table I. The values of QO_2 at 37.7°C are the means of 4 determinations while those at other temperatures are single determinations. Additional values of QO_2 (not given in the table) at 37.7°C for the period 120-180 minutes after addition of alcohol are 11.10 at 0.95% alcohol, 10.43 at 2.85%, 6.91 at 4.75% and 2.16 at 6.65%.

The inhibition of brain respiration produced by ethyl alcohol has been reported by Jowett[1] to become progressively greater with time. It may be seen from the table that the progressive inhibition is more marked at 37.7°C than at 30°C. At 20°C this decrease in rate of oxygen consumption with time does not occur within 3 hours. At all of these temperatures the QO_2 of control brain slices, without addition of alcohol, remains constant for at least 5 hours.

In order to minimize the effect of the progressive inhibition in the presence of alcohol, the QO_2 for the period 120-180 minutes after addition of the inhibitor has been selected for purposes of comparison. It is apparent that the minimum effective inhibitory concentration of alcohol increases with decrease in temperature. It is approximately 2.5% at 37.7°C, 6% at 30°C and 7% at 20°C. The percentage inhibition produced by a given concentration of alcohol is greater at 37.7°C than at the lower temperatures. With 9.5% alcohol, for example, the inhibition is 91% at 37.7°C., 64% at 30°C and 21% at 20°C. The absolute level of oxygen consumption in the presence of 9.5% alcohol is remarkably similar at the 3 different temperatures.

At 37.7°C the inhibitory effect of higher concentrations of alcohol was studied. With 23.73% alcohol the QO_2 was 0.93 and further decrease did not occur over a period of 3 hours. The oxygen consumption was completely abolished by 47.5% alcohol. There is thus a large range of alcohol concentration, from about 8% to well over 25%, within which the rate of oxygen consumption is approximately constant. This fraction of oxygen consumption of brain, amounting to about 10% of the control rate at 37.7°C, has been

1 Jowett, M., *J. Physiol.*, 1938, **92**, 322.

2 Dickens, F., and Greville, G. D., *Biochem. J.*, 1935, **29**, 1468.

3 Fuhrman, F. A., and Field, J., 2nd., *J. Pharm. Exp. Therap.*, 1943, **77**, 229.

TABLE I.

Effect of Time and Temperature on Inhibition of Rat Brain Oxygen Consumption by Ethyl Alcohol. See text from experimental details. Oxygen consumption expressed as Q_{O_2} for time periods given.

Temp. °C	Time after addition of alcohol, min.	Conc. of ethyl alcohol—% by volume					
		0	1.90	3.80	5.70	7.60	9.50
37.7	30- 60	11.02	11.74	10.82	9.89	8.24	3.70
	60-120	11.02	11.74	9.68	7.82	3.61	1.39
	120-180	11.02	11.74	8.14	4.58	1.03	1.03
30.0	30- 60	5.25	6.59	5.30	5.46	4.74	4.02
	60-120	5.25	6.08	5.30	5.46	4.17	2.68
	120-180	5.25	5.87	5.30	5.46	3.45	1.91
20.0	30-180 (Constant)	1.96	2.27	1.96	1.85	1.55	1.55

termed the "inhibitor stable" respiration. It has been found to be of approximately equal magnitude in brain slices maintained for over 3 hours without added substrate[4] and in the presence of a number of other inhibitors.[3,5-7]

It may be seen from Table I that low concentrations of alcohol appeared to produce an increase in the rate of oxygen consumption at all 3 temperatures. The effective concentration for this augmentation was about 2%. A similar increase in brain QO_2 with alcohol concentrations of from 1.6 to 3.2% has been reported by Levy and Olszycka.[8] It is possible that this increased rate of oxygen consumption is due to alcohol metabolism by brain tissue.[9]

Summary. The inhibition of oxygen consumption of rat cerebral cortex slices by ethyl alcohol was studied at 37.7°C, 30°C and 20° C. The inhibition becomes progressively greater with time at 37.7°C; such progressive inhibition is less marked at 30°C and is absent at 20°C. The minimum effective inhibitory concentration increases with decrease in temperature. The percentage inhibition with a given concentration of alcohol decreases with decrease in temperature.

[4] Fuhrman, F. A., and Field, J., 2nd., unpublished results.

[5] Fuhrman, F. A., and Field, J., 2nd., *J. Cell. and Comp. Physiol.*, 1942, **19**, 351.

[6] Fuhrman, F. A., and Field, J., 2nd., *J. Pharm. Exp. Therap.*, 1943, **77**, 392.

[7] Fuhrman, F. A., *Abst. of Dissertations, Stanford University*, 1943, **19**, 3.

[8] Levy, J., and Olszycka, L., *Compt. rend. Soc. Biol.*, 1940, **133**, 370.

[9] Himwich, H. E., Nahum, L. H., Rakieten, N., Fazekas, J. F., DuBois, D., and Gildea, E. F., *J.A.M.A.*, 1933, **100**, 651.

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Hemagglutination by Botulinal Toxin.

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In the course of studies of the properties of type A botulinal toxin we have observed that the addition of toxin to red cell suspensions results in agglutination. Crystalline and amorphous preparations of toxin have proven to be equally capable of causing hemagglutination. Observations for agglutination have been positive with red cells from all animal species tested (chicken, guinea pig, rabbit, sheep, man.)