THE INFLUENCE OF MINIMAL NARCOTIC DOSES ON THE RESPIRATION OF ERYTHROCYTES

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The investigations of Warburg in 1921 on the action of narcotics on cell respiration led to the formulation of his general theory of narcosis, according to which the action of a narcotic is attributed to its specific ability to occupy the vital surfaces of the cell structures, thereby displacing the adsorbed nutritive substances and important oxidative enzymes (1). The occasional observations of some investigators, describing a stimulating action of narcotics on the metabolic functions of the cell, seem hardly compatible with Warburg's theory; for, "adsorption displacement," as Warburg calls the phenomenon, might bring about a depression, but not a stimulation of cellular metabolism.

In his experiments on the respiration of the isolated spinal cord of the frog under the influence of ethyl alcohol, Winterstein (2) describes a series of cases in which low concentrations of this narcotic produced acceleration, higher concentrations, on the other hand, produced metabolic depression. Similar results were obtained by Garrey (3) in his experiments on the stimulating effect of ethyl alcohol on the CO_2 production of the heart ganglia of *Limulus polyphemus*. Warburg, who made similar investigations on liver cells, avian erythrocytes, and the central nervous system, could find no such acceleration. Narcotic concentrations lower than those which caused metabolic depression were without effect. In explanation of this discrepancy between his results and those of other investigators, Warburg suggested (9) that metabolic acceleration would result upon administering a narcotic to starving cells. The narcotic would then play the rôle of a nutritive substance and as such might be replaced by glucose or peptone with the same stimulating effect. Of course, in the case of cells such as sea urchin eggs, where lipoid-soluble substances serve as a stimulus to further development, as was first demonstrated by Jacques Loeb, an

increase in the metabolism of the organism was expected and actually found by him to be the case.

In view of these divergent observations it appeared of special interest to investigate systematically and with modern methods the influence of low concentrations of narcotics on cellular respiration, and to limit ourselves to two narcotics: ethyl alcohol, which can be oxidized and utilized by the cell for nutritive purposes, and ethyl urethan, which is hardly oxidized and certainly not useful as a cellular nutrient. Convenient cells for such investigations were mammalian erythrocytes. It seemed further expedient to extend our investigations to starving erythrocytes as well as to blood cells well supplied with nutritive material.

Method

Human blood was obtained by puncture of the cubital vein; rabbit blood from the auricular vein; horse blood directly from the jugular vein at the slaughter house. Sodium citrate was used to prevent clotting. For the experiments on starving blood cells the citrate blood was centrifuged, the supernatant plasma removed, the erythrocytes suspended in the fivefold volume of physiological saline solution, well stirred, centrifuged, and the supernatant fluid again removed. This process was repeated five times to assure complete removal of all the diffusible nutrients.

In the experiments on nourished erythrocytes the citrate blood was centrifuged but once and the supernatant citrate plasma removed. The sedimented cells were thus still saturated with nutritive material.

5 cc. of the thick erythrocyte suspension were then pipetted into small respiration vessels of the type described by Warburg (4), and 5 cc. of the narcotic to be examined, dissolved in physiological saline solution, added. A vessel containing the erythrocyte suspension plus physiological saline solution, and one with the dissolved narcotic alone, served as controls. The vessels, attached to their manometers, were placed in a constant temperature water bath at 37°C. Air served as the gas medium. The experiments lasted no longer than 2 hours to avoid possible interferences due to bacterial growth.

EXPERIMENTAL

In our search for a concentration of alcohol which would stimulate respiration in erythrocytes it was necessary to start off with inhibiting concentrations. Thus, we found that 4 per cent alcohol caused a depression in O_2 consumption of about 35 per cent. As the concentration of alcohol was gradually lowered this depression became smaller,

finally changing into an indifferent behavior when a concentration of 0.5 per cent was reached. Upon lowering the concentration still fur-

TABLE I

Blood	No. of experiments	Concentration of alcohol	Average O_2 consumption in 2 hrs.		Increase or decrease in O_2 consumption
			Without alcohol	With alcohol	
		vol. per cent	*c.mm.*	*c.mm.*	*per cent*
Rabbit	2	4.0	26.9	17.3	−35.7
"	2	2.0	26.9	22.9	−14.9
"	2	1.5	40.1	36.3	−9.5
"	2	0.5	40.1	40.5	+0.1
"	2	0.3	16.8	18.2	+8.3
"	8	0.25	32.5	39.5	+21.5
"	3	0.125	22.7	30.5	+34.3
"	12	0.10	31.3	45.2	+44.5
Human	1	0.5	44.4	40.2	−9.4
"	2	0.25	44.4	57.2	+29.0
"	2	0.10	32.4	46.8	+44.4
Horse	3	0.25	81.5	127.5	+56.4
"	4	0.10	82.4	106.8	+30.0

TABLE II

Blood	No. of experiments	Concentration of urethan in	Average O_2 consumption in 1½ hrs.		Increase or decrease in O_2 consumption
			Without urethan	With urethan	
		per cent	*c.mm.*	*c.mm.*	*per cent*
Human	2	3.0	89.9	52.8	−41.2
"	1	0.02	67.9	72.0	+6.0
"	9	0.015	127.2	118.1	−7.1
"	3	0.005	105.4	94.9	−9.9
Rabbit	1	0.25	28.1	27.4	−2.5
"	1	0.125	27.8	27.0	−2.9
"	2	0.10	28.0	25.0	−10.7
"	1	0.04	84.4	77.9	−7.7
"	2	0.025	84.4	88.0	+4.2
"	3	0.015	39.3	40.5	+3.0
"	1	0.005	26.5	24.6	−7.1

ther we observed a definite increase in O_2 consumption in the range between 0.1 per cent and 0.3 per cent alcohol. To make sure that

this observation was not based on mere chance some twenty-five experiments were made within this range, both on nourished and starving rabbit erythrocytes. All the results indicated a decided respiratory stimulation, also in the cases of human and horse blood cells. In all three instances starving (washed) erythrocytes reacted the same as nourished blood cells, so that no differentiation of these two categories is made in Table I.

In the instance of ethyl urethan, however, we could find no corroboration of the results obtained with ethyl alcohol, either with starving or with well nourished cells. The values given in Table II fluctuate around the zero mark, indicating an indifference on the part of the erythrocytes towards this narcotic. The general trend, however, points to a slight inhibitory effect. The few positive results shown in the table fall within the limits of error of the manometric method and are therefore hardly of any significance. Occasional controls made with the Barcroft-Warburg differential manometer (4) confirmed the results given in Tables I and II.

DISCUSSION

Our experiments indicate that low concentrations of an easily oxidizable narcotic such as ethyl alcohol increase the O_2 consumption of starving as well as nourished erythrocytes, whereas subinhibitory doses of ethyl urethan remain without effect very likely because this narcotic cannot be utilized by the cell. The fact that even well nourished erythrocytes respond towards low concentrations of alcohol with an increased O_2 consumption can be explained by the observations of Durig and coworkers (5), who showed that upon simultaneous supply of alcohol and carbohydrate in the human body the narcotic is given preference over the carbohydrate and is oxidized first. This also seems plausible for the cell, especially since ethyl alcohol readily penetrates the cell wall of the erythrocyte, and as Fleischmann and Trevani (6) showed, is then oxidatively decomposed into acetaldehyde. While Durig could find no general increase in oxidation in the human organism upon alcohol consumption, recent investigations by Bickel and Kanai (7) show that small quantities of this narcotic stimulate, large quantities on the other hand inhibit oxidative processes in the intermediary metabolism of the rabbit. Similar results were recently

obtained by Robertson and Stewart (8), who demonstrated an increased O_2 consumption in brain sections of alcoholized rabbits.

Higher concentrations of either oxidizable or non-oxidizable narcotics bring about respiratory inhibition in cells by more completely displacing from the surfaces of the cell structures not only the adsorbed nutrients, but also the oxidases, so that in the instance of alcohol, this narcotic cannot be burned. Our results can also be fitted into Warburg's "adsorption displacement" theory of narcosis by assuming that low narcotic concentrations do not sufficiently displace the adsorbed nutrients and enzymes, thus making an easily oxidizable narcotic readily accessible to the cell, whereas non-oxidizable narcotics remain indifferent.

SUMMARY

Low concentrations of ethyl alcohol stimulate the respiration of mammalian erythrocytes *in vitro*.

Low concentrations of ethyl urethan remain without effect on, or tend slightly towards depressing the respiration of mammalian erythrocytes *in vitro*.

It is suggested that this may be due to the oxidizable nature of alcohol, and the non-oxidizable nature of urethan, properties which come into evidence only when these narcotics are present in such low concentrations that the threshold of inhibition (narcosis) has not been reached.

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