Effect of Cytochrome C Therapy on Resistance to Decompression and Drug Depression. (17753)

HERBERT S. SALZBERG AND HERBERT P. JACOBI.

From the Department of Biochemistry, College of Medicine, University of Nebraska, Omaha.

Proger and coworkers(1) have reported that cytochrome C is effective in alleviating tissue anoxia. In view of these reports a study was undertaken to determine whether parenterally administered cytochrome C would alter the critical pressure below which an animal could not survive. A considerable species difference in this critical pressure has been observed(2). The determination would, therefore, appear to be sensitive enough to reflect variations in the efficiency of tissue oxygen utilization. Since the action of depressant drugs has been thought to be due to a production of anoxia(3), it was also deemed of interest to ascertain whether cytochrome C treatment would exert an antidotal effect in animals intoxicated with drugs of this type.

Experimental. The decompression chamber used in this investigation was a vacuum desiccator communicating with a large ballast jar which was connected to a vacuum pump. The pressure in the chamber, which contained soda lime to absorb carbon dioxide and water, was measured with a Hg manometer. A needle-valve,* by means of which the rate of decompression could be regulated, was interposed between the chamber and ballast jar. All experiments were conducted at room temperature which remained essentially constant. Cytochrome C was prepared by the method of Keilin and Hartree(4) and dialyzed against distilled water. The final concentration, determined by a method described by Potter(5), was 5 mg of cytochrome C per ml of solution. The preparation proved to be active in a reconstituted succinoxidase system(6). Black mice were used in all experiments except those involving sodium barbital, for which white mice were used. The mice were of either sex and approximately the same age, having an average weight of 25 g. The rate at which the mice were decompressed is shown in Table I.

Two series of mice were used in the decompression experiments. In the first series, the pressure at which the last extensor thrust of the hind legs occurred was taken as the critical pressure, since this endpoint was very close to death(2). The experimental mice were injected intraperitoneally with one ml of the dialyzed cytochrome C solution. The control mice were similarly injected with an equal volume of distilled water. Each mouse was injected one hour prior to decompression. In the second series, each mouse was made to serve as its own control. This was accomplished by using a slightly earlier endpoint in the course of decompression so that subsequent restoration to normal atmospheric pressure would revive the mouse almost immediately. The endpoint chosen, which proved to be very definite, was the first extensor thrust of the legs following the onset of gasping. In this way the critical pressure for each mouse was determined following intraperitoneal injection of one ml of distilled water and again, 24 hours later, following intraperitoneal injection of one ml of the dialyzed cytochrome C solution. Each mouse was injected 2 hours prior to decompression.

To investigate the possibility of antagonism

1. Proger, S., and Dekaneas, D., *Science*, 1946, v104, 389.

2. Stullken, D. E., and Hiestand, W. A., PROC. SOC. EXP. BIOL. AND MED., 1943, v54, 260.

3. Quastel, J. H., in Perspectives in Biochemistry, edited by Needham, J., and Green, D. E., Cambridge University Press, London, 1938, 296.

* We are indebted to Dr. A. L. Bennett, Department of Physiology and Pharmacology, for the construction of this needle valve.

4. Keilin, D., and Hartree, E. F., *Biochem. J.*, 1945, v39, 289.

5. Potter, V. R., in Manometric Techniques and Related Methods for the Study of Tissue Metabolism, edited by Umbreit, W. W., Burris, R. H., and Stauffer, J. F., Burgess Publishing Co., Minneapolis, 1945, 191.

6. Schneider, W. C., and Potter, V. R., *J. Biol. Chem.*, 1943, v149, 217.

TABLE I.
Summary of Results Obtained with Cytochrome C.

A. Rate of decompression							
Time in min.:		0	3	6	9	12	15	18
Barom. press. mm Hg:		atm. press.	398	266	195	156	130	112

B. Effect of Cytochrome C therapy on critical barometric pressure					
Group		No. of mice	Avg pressure (mm Hg)	Range	S.D.
First series	Control	7	177	160-198	14.7
	Cyt. C treated	10	175	145-202	22.8
Second series	Control	21	184	160-228	16.8
	Cyt. C treated	21	183	140-225	18.6

C. Effect of Cytochrome C therapy on time required for loss of righting reflex					
Group		No. of mice	Avg time (min.)	Range	S.D.
20% alcohol (0.2 ml/10 g)	Control	10	2.2	0.9-6.2	1.5
	Cyt. C treated	7	3.2	2.1-4.0	0.7
Sodium pentothal (38 mg/kg)	Control	12	2.4	1.5-4.2	0.6
	Cyt. C treated	13	2.9	0.8-5.5	1.6
Sodium barbital (270 mg/kg)	Control	7	19	13-28	5.7
	Cyt. C treated	6	20	16-23	3.8

between cytochrome C and depressant drugs, 3 such drugs were employed: ethyl alcohol, sodium barbital, and sodium pentothal. The experimental mice were injected intraperitoneally with one ml of the dialyzed cytochrome C solution one hour prior to intraperitoneal injection of the drug. The control mice were similarly injected with an equal volume of distilled water. For each mouse the time interval following injection of the drug up to loss of the righting reflex was determined. The dosages employed are given in Table I.

Results and Comment. The cytochrome C treated mice at autopsy showed that the cytochrome C had been practically all absorbed from the peritoneal cavity. Moreover, when terminal failure of the bladder sphincter occurred during decompression, the urine was colored red. These observations indicate that the experimental mice were "saturated" with cytochrome C. It is apparent that the critical pressure observed with cytochrome C treated mice is the same as for control mice (Table I). This is true even when each mouse serves as its own control. Furthermore, cytochrome C treatment had no effect on the response of mice to alcohol or to 2 types of barbiturates (short-acting and long-acting). Practically the same length of time was required for loss of the righting reflex in both experimental and control groups with each drug (Table I).

These observations do not support the hypothesis that cytochrome C therapy can increase the efficiency of tissue oxygen utilization and are in line with all other negative findings(7,8).

Summary. Cytochrome C did not alter the critical barometric pressure below which mice could not survive and was without effect on the depressant action of ethyl alcohol and of barbiturates.

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8. Levey, S., *Science*, 1950, v111, 13.

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