Comments and Communications

Effect of Anoxia and Cytochrome C on Readily Hydrolyzable Phosphate of Rat Tissues

Proger, *et al.* (*Bull. New Engl. med. Center*, 1945, **7**, 1, 149; 1947, 9, 211; *J. biol. Chem.*, 1945, **160**, 233; *J. clin. Invest.*, 1945, **24**, 864; *Science*, 1946, **104**, 389; 1947, **106**, 367) have found that in the presence of anoxia the readily hydrolyzable phosphate (adenosine triphosphate) of tissue decreases, but the previous administration of cytochrome C prevents such a decrease. Several workers have confirmed the decrease in the presence of anoxia, but Scheinberg and Michel (*J. biol. Chem.*, 1947, **169**, 277; *Science*, 1947, **105**, 365) and, more recently, Stadie and Marsh (*J. clin. Invest.*, 1947, **26**, 899) found no significant effect of cytochrome C.

According to Proger, the low concentration of the cytochrome in tissue may be the limiting factor in the rate of oxygen utilization; hence, the administration of cytochrome should permit an increased utilization in the presence of a deficient oxygen supply. Stadie and Marsh have presented data on the basis of which they question the theoretical validity of Proger's hypothesis. Christensen and Pearson (*J. clin. Invest.*, 1947, **26**, 1046) have found no increase in oxygen uptake by heart and kidney slices equilibrated at oxygen tensions of 2.5–100% when cytochrome was added. Potter (*Science*, 1947, **106**, 342) has also criticized the hypothesis, but has offered no additional data.

The data Proger published in 1945 show a statistically significant effect of injected cytochrome C. Although Scheinberg and Michel and Stadie and Marsh could not confirm this finding, Proger has recently repeated his experiments and confirmed his earlier results.

In view of the importance of establishing whether or not cytochrome C can prevent the anoxia-induced decrease in readily hydrolyzable phosphate, it seems of importance to add to the literature the data obtained in this laboratory.

Albino rats weighing 150–230 gm, maintained on Purina Laboratory Chow, were used. They were divided by weight and sex into 11 groups of 3 each; each group was kept in a separate cage and used as the basis of one experiment. All animals used were females with the exception of two groups (6 animals).

The cytochrome C was prepared according to the method of Keilin and Hartree (*Proc. roy. Soc. Lond.*, 1937, **B122**, 298) and standardized spectroscopically and by analysis for iron. The iron content was 0.34%.

After being anesthetized by ether, two rats were injected intravenously in the tail with 0.7 ml of isotonic saline, and the third animal was injected with 0.7 ml of a solution of cytochrome C containing 8 mg/ml—a total of 5.6 mg of cytochrome. Ten minutes after injection, the cytochrome-injected rat and one saline-injected rat were put in a desiccator through which a mixture of 3% oxygen and 97% nitrogen was passed at a rate of 10 liters/min. After 8 min, these rats were decapitated and the heart and right kidney dropped into an absolute alcohol and solid carbon dioxide bath at −70°. Thirty to 50 sec were required for this operation. The other saline-injected animal (control) was left in the open air and similarly killed 18 min after injection.

The organs were then weighed and homogenized in 25 ml of 5% trichloroacetic acid in a Waring blender. The solution was filtered, and 9 ml was mixed with 1 ml of 10 *N* sulfuric acid and hydrolyzed at 100° for 7 min.

Phosphate determinations were done before and after hydrolysis by the method of Fiske and Subbarow (*J. biol. Chem.*, 1925, **66**, 375), using a Coleman Junior Spectrophotometer at 540 mμ. All solutions were adjusted to contain 0.3 *N* sulfuric acid and 4% trichloroacetic acid in the 10 ml of solution to be read.

TABLE 1

EFFECTS OF ANOXIA AND CYTOCHROME C ON READILY HYDROLYZABLE PHOSPHATE*

Experiment	Kidney			Heart		
	Control	Anoxia plus saline	Anoxia plus cytochrome C	Control	Anoxia plus saline	Anoxia plus cytochrome C
1	7.3	3.3	7.2	14.2	10.8	11.4
2	5.8	4.8	4.6	13.1	5.8	5.3
3	7.3	7.0	2.8	10.9	10.2	9.9
4	12.9	5.1	4.8	14.5	10.5	7.5
5	9.3	6.8	6.6	9.1	8.9	10.8
6	5.0	6.9	5.9	8.3	9.4	11.7
7	12.5	5.6	7.0	18.2	11.8	10.9
8	9.9	6.7	7.7	15.3	10.4	12.6
9	10.2	5.5	6.3	18.4	13.8	15.8
10	15.5	8.8	8.0	20.3	15.5	14.1
11	9.4	12.3	6.3	10.9	8.7	12.5
Mean	9.6	6.6	6.1	13.9	10.5	11.1

* Data expressed as mg of phosphate/100 gm of wet tissue.

The data are given in Table 1. No effect of cytochrome C on the readily hydrolyzable phosphate was found. Applying the *t* test of Fisher (*Statistical methods for research workers*, 9th ed., 1944) to the data, the decrease in hydrolyzable phosphate in anoxic animals was found to be highly significant ($P < 0.01$), but the difference between the cytochrome-treated and non-cytochrome-treated anoxic animals was not significant.

Conclusions: (1) Anoxia reduces the amount of readily hydrolyzable phosphate in rat kidney and heart. (2) Previous intravenous injection of cytochrome C in doses of 5.6 mg/animal has no effect in preventing this reduction.

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