

Effect of Cytochrome C Therapy on Altitude Tolerance of Normal Rats.

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Previous studies have indicated that the ability of normal rats to withstand severe anoxia can be improved by a variety of procedures. Animals rendered polycythemic by the administration of cobalt were capable of increased work performance under conditions of severe anoxia.¹ Administration of a concentrated extract of adrenal cortex in sesame oil was followed by a marked improvement in ability to withstand short exposures to ordinarily lethal altitudes.² Animals given a 2-hour conditioning exposure to pressures equivalent to 18,000 or 26,000 feet altitude daily for 7 or 30 days developed improvement in their ability to withstand exposure to ordinarily lethal altitudes.³ In these later studies, the improvement was most marked after conditioning at the higher altitude, and was accompanied by both polycythemia and adrenal gland hypertrophy. However, the improvement could not be explained solely on the basis of either polycythemia or adrenal gland hypertrophy, since it occurred following conditioning at lower altitudes, which failed to influence organ weight or morphology, and there was a lack of correlation between hematocrit increase and the survival rate of the conditioned animals. It is apparent, therefore, that the mechanisms responsible for the increased altitude tolerance found in the above-noted experiments are not entirely clear. In view of this circumstance, it has been considered advisable to appraise the effect of parenterally administered cytochrome

C upon altitude tolerance. This was undertaken because of the recently demonstrated influence of adrenalectomy on cytochrome oxidase and cytochrome C concentration⁴ and because of the beneficial influence of parenterally administered cytochrome C on patients suffering from inadequate oxygenation of their tissues.^{5,6,7}

Methods. Young, male, white rats of approximately 200 g average weight were used in these studies. The animals were maintained in wire mesh cages on a standard diet of Purina dog chow. Access to food and water was unrestricted until 5 hours prior to exposure to anoxia; after this, water only was allowed. Five mg of cytochrome C in 2 cc of isotonic saline were administered to the experimental animals in a single intraperitoneal injection 45 minutes prior to altitude exposure.* The control animals were handled in the same manner as the treated group, except that an equal volume of physiological saline was substituted for the cytochrome C solution.

The cytochrome C was isolated according to the technique of Keilin and Hartree which yields a product containing .34% Fe.⁸ The concentration of cytochrome in the injected material was determined by spectrophotometric analysis. The amount given is com-

¹ Dorrance, S. S., Thorn, G. W., Clinton, M., Jr., Edmonds, H. W., and Farber, S., *Am. J. Physiol.*, 1943, **139**, 399.

² Thorn, G. W., Clinton, M., Jr., Davis, B. M., and Lewis, R. A., *Endocrinology*, 1945, **36**, 381.

³ Thorn, G. W., Clinton, M., Jr., Farber, S., and Edmonds, H. W., *Bull. Johns Hopkins Hosp.*, 1946, **79**, 59.

⁴ Tipton, S. R., *Endocrinology*, 1944, **34**, 181.

⁵ Proger, S., *Bull. N. E. Med. Center*, 1945, **7**, 1.

⁶ Proger, S., and Dekaneas, D., *Bull. N. E. Med. Center*, 1945, **7**, 149.

⁷ Proger, S., and Dekaneas, D., *Science*, 1946, **104**, 389.

* It has been reported by Proger and Dekaneas⁷ that intravenous, intramuscular, and intraperitoneal injections of cytochrome C increase the blood and organ content of cytochrome C.

⁸ Keilin, D., and Hartree, E. F., *Biochem. J.*, 1945, **39**, 289.

parable to that used by Proger and Dekaneas in their studies.^{5,6}

The acute altitude survival test has been described in detail elsewhere.^{2,3} Exposure was carried out in a decompression chamber with sufficient air flow to prevent any perceptible increase in carbon dioxide content. The survival rates reported represent the percentage of animals alive at the conclusion of the test, since no delayed deaths were noted. All animals were exposed for one hour at the test altitude. Moreover, they were exposed simultaneously, thus eliminating any possible differences in survival rate due to variations in temperature and humidity within the chamber or such other unpredictable variables as handling and feeding.

Results and Discussion. The results of exposure with and without parenteral cytochrome C were as shown in Table I. A graphic presentation is given in the accompanying chart.

It is apparent that cytochrome C was not effective in protecting the intact rat against severe anoxia. If injected cytochrome C is capable of increasing the ability of tissues to utilize oxygen under conditions of anoxia, one might expect administration of this respiratory enzyme to increase the ability of the intact organism to withstand the anoxia of high altitude exposure. The present experiments give no indication as to why this expected action did not occur. It is quite possible that the cytochrome C, being a pro-

EFFECT OF INJECTED CYTOCHROME C
ON ALTITUDE TOLERANCE OF NORMAL RATS

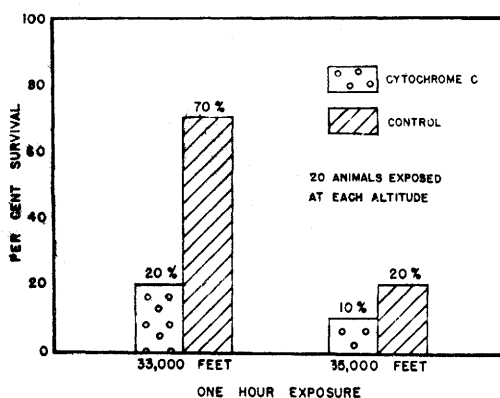


FIG. 1.

tein whose molecular weight is at least 13,000, was unable to penetrate intact cells. However, there are indications that even had this occurred, it would not have affected the oxygen consumption of the cells.⁹

Summary. This report presents data concerning the ability of rats treated with cytochrome C to withstand the anoxia of high altitude exposure. The survival rates of experimental animals given an intraperitoneal injection of 5 mg of cytochrome C in isotonic saline were compared with those of control animals treated with a similar amount of saline. Groups of control and experimental animals were exposed simultaneously for periods of one hour. Altitudes of 33,000 and 35,000 feet were employed. In both instances, the survival rate was higher in the control group. It is concluded, therefore, that the intraperitoneal injection of cytochrome C is not effective in protecting rats against the anoxia of high altitude exposure.

TABLE I.
10 animals in each experiment.

Exposure altitude	Treatment	% survival
ft		
33,000	Normal saline—2 cc	70
"	Cytochrome C—5 mg	20
35,000	Normal saline—2 cc	20
"	Cytochrome C—5 mg	10

⁹ Shack, J., *J. Nat. Cancer Inst.*, 1943, **3**, 389.