# Cobalt Polycythemia and Cytochrome C[1]

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It has been reported by Proger and Dekaneas (*4, 5*) that intravenous administration of cytochrome C into both rats and men will reduce the effect of anoxemic conditions, e.g., reverse the effect of a 10% oxygen atmosphere and change the electrocardiographic pattern to normal after myocardial anoxemia. Zelman and Gilbert (*7*) noted a case of hepatolenticular degeneration in man, presenting indications of tissue anoxemia, which responded to the administration of cytochrome C. Scheinberg and Mitchel (*6*) found that administration of cytochrome C did not prolong the life of rats in an atmosphere of 2.8 or 3.9% oxygen. This concentration of oxygen is considerably below that used by Proger and may be a limiting concentration. On the other hand, it has been reported recently by Beinert and Reissmann (*1*) that cytochrome C labeled with radioactive iron did not enter the tissue cells of rats following intravenous injection.

TABLE 1

EFFECT OF CYTOCHROME C ON COBALT-INDUCED POLYCYTHEMIA OF THE RAT

| Supplement | Number of animals | Hemoglobin—g/100 ml | | |
|---|---|---|---|---|
| | | Before supplement | Weeks after supplement | |
| | | | 1 | 2 |
| None ..... | 9 | 14.2 ± 1.0* | 14.9 ± 0.9 | 13.4 ± 0.8 |
| Cytochrome C† . | 6 | 13.5 ± 0.5 | 13.8 ± 0.9 | 13.4 ± 0.6 |
| Cobalt‡ ..... | 7 | 18.3 ± 0.9 | 18.2 ± 0.9 | 17.6 ± 0.9 |
| Cobalt‡ plus cytochrome C† | 8 | 18.8 ± 0.8 | 18.2 ± 0.9 | 18.4 ± 0.9 |

* Mean plus standard deviation.

† Animals receiving cytochrome C were given 7.5 mg per day intravenously for the test period.

‡ Cobalt was given intraperitoneally, 0.5 mg per day.

It has been suggested (*2*) that the polycythemia produced by cobalt may be a compensation to internal anoxemia produced by the inhibition of cellular respiration systems. It was considered of interest to determine whether intravenous administration of cytochrome C would counteract this effect.

Polycythemia was produced in hooded rats maintained on dog chow by the intraperitoneal injection of 0.5 mg of cobalt per day 6 days a week. When the level of hemoglobin became constant, the test animals received 7.5 mg of cytochrome C, dissolved in physiological saline, into one of the tail veins. This amount of cytochrome C is approximately one half that contained in the body of the rat, according to Drabkin and Crandall (*3*). Cobalt was given to the test animals during the entire study.

Results are presented in Table 1. The intravenous administration of cytochrome C did not appear to affect the hemoglobin concentration in either normal animals, those not receiving cobalt, or polycythemic animals. Perhaps this is to be expected in light of the work of Beinert and Reissmann in which intravenously administered cytochrome C did not appear to enter the tissue cells.

[1] The cytochrome C used in this study was generously supplied by Dr. C. E. Graham of the Wilson Laboratories, Chicago, Illinois.

### References

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# Rapid Method of Preparing Schiff's Reagent for the Feulgen Test

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The following reagents are commonly used to reduce fuchsin base to the leuco compound: sodium bisulfite, potassium metabisulfite, sodium thiosulfate, and sulfur dioxide (*1, 2*). To obtain satisfactory results with these reagents, the solutions should stand for about 24 hr. We have found that by the use of a readily obtainable reagent, sodium hydrosulfite, Schiff's reagent may be prepared in a few minutes. A summary of the procedure follows:

Prepare a 0.5% solution of fuchsin base as usual. To 100 ml of this solution add 0.5 g of active and undecomposed sodium hydrosulfite, and at the same time add 0.25 g of activated charcoal (Nuchar). Shake thoroughly in a closed flask, and filter through a coarse filter paper. The finished product is of a light amber tinge.

The tissue section to be examined is hydrolyzed in normal HCl for 4 min at 60° C, washed, and allowed to stand in the leuco-base solution for 2 hr. To prevent oxidation, the solution is preferably layered with xylol. The slides are then washed, first in a 0.25% solution of sodium hydrosulfite, next with distilled water; then they are cleared and mounted.

### References

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