tribution of nonelectrolytes across living membranes. However, it is suggested that the phenomenon might be of importance wherever a diffusible nonelectrolyte passes through a membrane with different concentrations of nondiffusible solute on the two sides as, for example, in the passage of oxygen and carbon dioxide through the lung membrane, of gases and of diffusible solids from the blood to the body tissues, and of gases and of diffusible solute through plant membranes, and in the formation of urine and other body fluids.

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The Use of Cytochrome C in Combating Tissue Anoxia[1]

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We have been interested in attacking the problem of tissue anoxia by attempting to improve the efficiency of the tissue utilization of oxygen. In this connection it is important to recall that even under conditions of anoxia there is considerable oxygen in the venous blood. Thus, under conditions of anoxia which are barely compatible with consciousness (about 10 per cent oxygen) there may still be in the venous blood returning to the heart some 4–6 vol. per cent oxygen. Under such conditions there is therefore an available supply of oxygen which needs only to be utilized. Theoretically, respiratory enzymes might favor such utilization.

Our earlier studies (*5*) were with the group of C_4 dicarboxylic acid substances in the so-called Krebs cycle, particularly succinic acid. These are probably among the less important of the respiratory catalysts, but our studies with these substances served to demonstrate that one could, by means of such substances, apparently improve the tissue utilization of oxygen in the living animal under conditions of anoxia.

Among the most important of the substances which promote tissue oxidation are the cytochromes. Of these, only cytochrome C can be readily prepared (*2*). We have used beef heart as the source of cytochrome C and have found that, by reprecipitation and passage through a Seitz filter, it is apparently nontoxic. The fact that it has been demonstrated to be not only nontoxic but stable makes it clinically utilizable. Despite the fact that cytochrome C is a protein (iron porphyrin protein), it appears to be nonantigenic.

[1] This work was done with the aid of grants from Brewer and Company, Inc., and the Charlton Fund.

Certain facts make cytochrome C potentially useful. Among these is the fact that its organ content can be significantly increased by parenteral injection (*8, 9*). Also, the organs normally contain considerably more cytochrome oxidase than can be activated by the cytochrome C present (*4, 11*). Hence, if additional cytochrome C can be supplied to the organs, additional cytochrome oxidase is present for activation for the final linkage with molecular oxygen. Also, we find that the magnitude of increase of cytochrome C which can be produced in organs by parenteral injection is such as to produce *in vitro* an increase of oxygen consumption of 50–100 per cent (*9*). The final fact of importance in this connection is, as indicated above, that there is even under conditions of anoxia considerable unused oxygen in the venous blood returning to the heart, so that if the tissue uptake of oxygen can be increased, there is an available supply of oxygen.

On the basis of these considerations it might have been anticipated that conditions associated with anoxia *in vivo* might be benefited by the injection of cytochrome C. This seems, in fact, to be the case, as indicated by the following observations.

The easily hydrolyzable phosphorus fraction, particularly adenylpyrophosphate, is thought to play an important role in the tissue transfer of energy (*3*). This compound continuously donates phosphoric acid radical to other metabolites and hence requires continuous resynthesis. The continual release of this so-called "phosphate-bond" energy is only possible through a continual supply of the energy released by the cell oxidations which are catalyzed by the cytochrome-cytochrome oxidase system and which serve to resynthesize the adenylpyrophosphates. We have demonstrated in rats that the organ content of these phosphates is markedly diminished under conditions of severe anoxia (*10*). When, however, some additional cytochrome C has been supplied to the rats beforehand, the anoxia produces little or no change in the organ content of the phosphates concerned (*10*). If such important biochemical effects of anoxia can be largely prevented by cytochrome C, it is not unlikely that other effects, in addition to those associated with phosphorylation, can also be favorably influenced.

The effects of anoxia on the living heart can be easily studied by means of the electrocardiogram. We have found that such effects on the electrocardiogram as can be produced by moderately severe anoxia (10 per cent oxygen and 90 per cent nitrogen) can be regularly prevented by the previous intravenous injection of cytochrome C (*6*). Also, those patients who experienced subjective distress with such anoxia were free from the distress under the anoxia when they had been injected previously.

These facts naturally lead to some observations on

patients with angina pectoris and coronary thrombosis. Our limited observations thus far have indicated that with the dosages employed and in short, acute experiments, cytochrome C seems to have a moderately beneficial effect in angina pectoris and no immediate effect in acute myocardial infarction (*6*). The latter may well be due to the fact that the total occlusion of the vessel makes it impossible for the cytochrome to reach the infarcted area.

Studies of certain cerebral functions have demonstrated that cytochrome C is effective in preventing some of the consequences of anoxia on the brain (*6*). The electroencephalographic changes produced by anoxia (varying from 10 to 12 per cent oxygen) can be largely prevented by cytochrome. By injection of cytochrome C the impairment of visual discrimination induced by anoxia can be entirely overcome within about 5 minutes, and such a complex cerebral function as code transliteration can be strikingly influenced, in that the slowing in time required to decode certain standard material under conditions of low oxygen tension (pressure chamber) can be quickly and entirely overcome by the cytochrome C (*6*).

Anoxia may be considered an important factor in shock, and it may even be the chief factor leading to those chemical changes which probably result in producing the state of irreversibility in hemorrhagic shock. These considerations led us to study the influence of injected cytochrome C in shock. In traumatic (tourniquet) shock in rabbits the cytochrome seemed to have no effect. In hemorrhagic shock in dogs, however, the fatal irreversible state which usually followed the experimental conditions was prevented in 9 of 14 dogs when a large dose of cytochrome C was injected a few hours after the hemorrhage (*7*). These experiments were done under the same conditions, in the same laboratory, and by the same workers who had previously demonstrated that, despite employing several theoretically desirable treatments, 107 of 108 dogs died of "irreversible" shock (*1*).

There seems to be a peculiar reciprocal relationship between the organ and blood content of cytochrome C after injection (*9*). Normally there is no cytochrome C in the circulating blood. After injection (intramuscularly, intraperitoneally, or intravenously) there is a considerable amount in the blood as well as an increase in the organ content. When anoxia is induced, the level of cytochrome C in the blood declines sharply, while in the organs it simultaneously increases. When the anoxia is discontinued, however, the organ content decreases and the blood level rises. It is as though the blood stream served as a reservoir from which the organs take up additional cytochrome C when they need it and to which the organs release the cytochrome C when it is no longer needed. We have used this phenomenon as a guide to dosage, which is made sufficiently large so that under conditions of anoxia there is still some cytochrome C in the blood. (This can be quickly and easily determined qualitatively by noting the presence in the serum of a pinkish color not unlike that of hemolyzed blood.) Under these circumstances it is assumed that there is a maximum supply in the organs where it is needed—that is, there is an "overflow." If, under the conditions of anoxia, there is no cytochrome C in the blood, one cannot be certain that the organ content of cytochrome C has been raised to an adequate level for effectiveness. Employing such a guide, we find that the effective dose may vary from 50 mg. for the purpose of preventing anoxia changes in the electrocardiogram in human beings to 350 mg. to prevent irreversibility in hemorrhagic shock in dogs.

The fate of the injected cytochrome C in the body is not clear. It is not recoverable in the urine (except following excessive doses), and it is assumed that it is broken down before excretion. If such is the case, the breakdown rate is fairly uniform. For, whereas 50 mg. injected intravenously will gradually disappear from the blood stream in 24–48 hours (man and dog), 350 mg. injected intravenously will result in gradually diminishing blood levels over a period of 5–6 days. It is conceivable, therefore, that if further studies continue to show cytochrome C to be nontoxic in unlimited doses, a single large injection may be used to serve as a supply for several days or longer.

Obviously, it is desirable to explore the possible usefulness of such a substance as cytochrome C in all conditions in which anoxia, acute or chronic, is thought to play a role. Such conditions, in which we have already made some preliminary observations, may be as diverse as Raynaud's disease, pulmonary emphysema, hemorrhagic shock, certain cerebral dysfunctions, etc. Among the more intriguing possibilities for future study are those degenerative diseases associated with arteriosclerosis in which mild chronic anoxia may be a significant factor.

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