

ENERGY ACTION OF COCARBOXYLASE IN RESPIRATORY BLOCKAGE INDUCED BY CYANIDE AND ITS THERAPEUTIC PROJECTION

—DEMONSTRATIVE INVESTIGATION—

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It has been repeatedly shown that alkaline salts of cyanhydric acid have particular affinity to combine with molecules which contain the heme group such as hemoglobin and cytochrome-oxide reductase.

The distal end of the respiratory chain is occupied by a cytochrome named A_3 , which is actually the direct donor of electrons to molecular oxygen. Therefore, the bond between the cyanide ion and the iron atom inhibits the electronic flow towards the oxygen thus blocking the activity of the aerobic respiratory cycle which is the main source of cellular energy.

When some oxidants such as sodium nitrite, sodium tiosulphate or methylene blue (all of which are capable of oxidizing the ferrous ion into ferric ion) intervene, they likewise transform hemoglobin into methemoglobin, which in turn gives rise to the formation of cyano-methemoglobin whose capacity to carry oxygen is practically inhibited. On the other hand, the cytochrome A_3 —cyanide complex, in which the cuprous ion has been transformed into a cupric ion by an oxidizing action, is not completely impeded from donating electrons to the molecular oxygen.

The opposite happens if cocarboxylase is used when respiratory blockage by cyanide occurs. It is then understood that the desired protection, evidenced by the survival of the poisoned animals, is due to the donating capacity of this coenzyme, since energy is ultimately the real goal in any type of respiration.

Based on this concept, a biological assay was carried out with cocarboxylase (more precisely named pyruvic cocarboxydehydrogenase), in order to ascertain whether the donor mechanism of pyrophosphate favored the survival of an animal which had received a lethal dose of cyanide. To this end, doves were used.

MATERIAL AND METHOD

1. Biological material.—Fifty healthy doves were used with an average weight of 250 grams.

2. Pharmacological material.—0.16% sodium cyanide solution and 4% pyruvic cocarboxydehydrogenase solution (stabilized).

3. Instruments.—Precision scale; 2.5 and 5.0 c.c. disposable syringes with needles (num. 22).

4. Method.—All doves were weighed and separated into two homogeneous groups named A and B.

Group A doves were injected in the left pectoral muscle with 3.47 mg. of sodium cyanide per kg. of body weight. Group B doves also received a dose of 3.47 mg. of sodium cyanide per kg. of body weight. However, these latter doves were injected after five seconds with 640 mg. of pyruvic cocarboxydehidrogenase per kg. of body weight in the right pectoral muscle. Researchers recorded all signs and symptoms minute by minute for each bird, including mortality rate.

RESULTS

Group A doves showed signs of poisoning in a lapse of 2 minutes, with loss of equilibrium and falling to one side; within 3 minutes tonicoclonic seizures appeared accompanied by opisthotonos in the majority of cases. Tachypnea was followed by short periods of apnea; rapid and progressive depressions led to immobility and the birds died in an average time of 4 minutes.

Group B doves also showed loss of equilibrium in 2 minutes, falling to one side. Within 3 minutes they also began to have tonicoclonic seizures accompanied by opisthotonos in the majority of cases; tachypnea was also observed, as well as short periods of apnea with progressive depressions.

Nevertheless, after 5 minutes all of them had survived and they presented a lengthening of the described picture; i.e., respiratory distress, loss of equilibrium and depression. Approximately 2 minutes later, i.e., in a 7 minutes lapse, the recovery phase began. The respiratory rate which had been severely accelerated (80-110 respirations per minute) declined slowly until it reached normalcy in the next 15-20 minutes, during which time the seizures also disappeared and equilibrium was restored. Over the next 30 minutes the doves were depressed, but alterations in breathing did not occur, and 60 minutes after the test was started they seemed totally recovered.

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DISCUSSION

Oxydized ferrous ion and cuprous ion prevent the cyanhydric compound from totally blocking the biological activity of hemoglobin and cytochrome A₃ respectively. The hemoglobin can thus continue precariously transporting oxygen, and the cytochrome can sparingly supply electrons to the molecular oxygen. At the same time the detoxification of the organism occurs through the natural mechanism of transformation of cyanhydric radical to thiocyanate which is non-toxic and is eliminated. This process explains on the one hand the possibly spontaneous recovery of animals which in spite of a lethal dose survived and resisted the most intense period of poisoning; they somehow compensated for the necessary vital energy normally taken from the aerobic respiratory mechanism whose prolonged depressions through cyanide in a lethal dose must culminate in death.

For the above reasons, we think that the action of pyruvic cocarboxydehydrogenase occurs at the level of the oxydative decarboxylation of pyruvic acid. This donates pyrophosphate as a source of energy normally provided by aerobic respiration and explains the survival of the doves poisoned with cyanide and yet protected by the coenzyme.

In a more detailed analysis the symptomatology of the doves showed unquestionably that the poisoning process was not interrupted nor altered by the presence of the coenzyme, since this picture was practically indistinguishable for both groups in the course of the first four minutes, until the recovery of animals protected by the coenzyme started, and death occurred to the other group. We must remember that the cyanide ion has a similar action to that of cocarboxylase in its ability to decarboxylate alpha-ceto acids, thus establishing a competitive role with each other.

On the other hand, it is comprehensible that the unblocking of the Pasteur effect is to no avail if the molecular oxygen which is at the end of the respiratory chain becomes non-usable for any reason whatsoever. Therefore, the only possible explanation for the function of cocarboxydehydrogenase is its ability to provide pyrophosphate and thus to furnish energy.

Regarding the positive results obtained from this experiment, we would like to stress that in our opinion we have corroborated our thesis that the coenzyme molecule used has a macroergic charge capable of

prolonging the life of an animal deprived of indispensable oxygen, the lack of which could otherwise entail death, while the animal's inherent homeostatic mechanisms detoxicate the system.

It is our thinking that the pyruvic cocarboxydehydrogenase manufactured according to Heberto Alcazar's technique, stable in solution and a potent pyrophosphate donor, is the ideal substance for this research.

SUMMARY

The results of this research proved that animals poisoned with lethal doses of cyanide can be protected through the use of pyruvic cocarboxydehydrogenase. This is due to its marked capacity as an energy donor through pyrophosphate, whose function is actually the essential goal of any respiratory mechanism.

From the above discussion it follows that the protecting mechanism obtained through the action of pyruvic cocarboxydehydrogenase can in no way be compared with the classical and well-known mechanism attained by those oxidants capable of transforming the metallic ions from their bivalent to their trivalent form in hemoglobin as well as in the cytochrome-oxide reductase.

THERAPEUTIC PROJECTION

In pathology, acute cases of hypoxia with the same consequences of respiratory blockage as that caused by cyanide can be observed: regional, such as those produced by cerebral thrombosis and myocardial infarction; and systemic such as anoxia in the newborn, and accidents by anesthesia or by asphyxia. Thus, the immediate usage of stable cocarboxylase in its active form can be considered, both as an agent of oxidative decarboxylation, and as a direct donor of pyrophosphate. This maintains the function of the biochemical cycle of Krebs while a sufficient contributor of oxygen is attained, and thus breaks the vicious circle caused by insufficient oxygen—low level of ATP—non-phosphorylated thiamine—blocking of the Pasteur effect—progressive loss of energy.

Finally, we must stress the fact that if a new contribution of oxygen of regional or systemic consequences were attained without the prior action of cocarboxylase to break the vicious circle, its effects would be to no avail whatsoever.