0.15 M acetate buffer pH 5.5 and the volume adjusted to contain 20 mg of protein per ml. Aliquots of these preparations were used for determination of enzyme activity and of protein.

The results presented in Table I show DβH activity in the serum of various human control subjects and in patients with some neurological disorders. DβH activity varies within a certain range in the serum of the control subjects. Preliminary results indicate that the DβH activity depends on the age and physical activity of the human subjects. The DβH activity in the serum of some patients with Parkinson's disease and with amyotrophic lateral sclerosis was lower as compared with the activity in the serum of the controls. However, these patients have been physically inactive for a long period of time and the low DβH activity in the serum may be due to the physical inactivity and may not necessarily be associated with the disease. Furthermore, the prolonged treatment of some patients with L-Dopa may affect the DβH activity. Studies are now in progress to determine the factors which are responsible for the low DβH activity in the serum of these patients.

The presence of DβH activity in human serum was also assayed with immunochemical techniques. The addition of purified rabbit anti-DβH antiserum[7] to human serum resulted in an inhibition of DβH activity. This antiserum exhibited a specific effect on DβH activity and had no effect on PNMT activity. DβH activity was also measured in the serum of various species. In rats, mice and monkeys the DβH activity was much lower than in humans. In C-1300 mice bearing neuroblastoma tumors the DβH activity in the serum was significantly higher than in the corresponding serum of control mice.

The results presented in Table II show the DβH activity in various tissues of rats. The adrenal gland shows the highest DβH activity, while in the heart and salivary glands the DβH activity is much lower. DβH activity was determined in various regions of the brain, but at present reproducible results were obtained only in the brain stem. It is noteworthy that DβH was also localized with immunofluorescence techniques in the cell bodies in the medulla oblongata and the pons[7,8]. Thus, the presence of DβH in the brain stem was confirmed with 2 different procedures. Studies on the effects of stress and of psychoactive drugs on DβH activity in serum and in tissues are now in progress.

Zusammenfassung. Eine isotopische Methode zur Bestimmung der DβH-Aktivität wird beschrieben. Die Methode wurde verwendet zur Bestimmung der DβH-Aktivität im menschlichen Serum und in verschiedenen Organen der Ratte. Das sympatische Nervensystem des Menschens kann mit dieser Methode weiter erforscht werden.

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The Effect of Pyruvate on Cyanide-Inhibited Respiration in Intact Ascites Tumor Cells

Cyanide is known to inhibit mitochondrial electron transport by combining with cytochrome oxidase[1]. The cyanide inhibition is considered not readily reversible. Indeed, even the previously accepted reversibility by physical methods of this inhibition[1-5] has been later questioned on the basis of results obtained by spectrophotometric techniques[6].

The combination of cyanide with different substances, such as fructose, acetaldehyde, pyruvic and oxalacetic acids, results in the formation of cyanhydrins which can mask the expected poisoning effect[7-10]. In this respect the only reports in biological systems were those relative to cyanide inhibition in long-term experiments. Acetaldehyde and pyruvic acid, employed as substrates for bacteria[8] and kidney slices[9], respectively, were added from the beginning of the experiments together with the inhibitor. In this way, because of the early formation of cyanhydrins, the inhibitory effect of cyanide was obviously not feasible.

In this communication we report how the effect of cyanide on the endogenous respiration of ascites tumor cells may be readily and almost completely removed by the further addition of pyruvate even when the binding of cytochrome oxidase to the inhibitor is already established. It has therefore been of interest to investigate under these conditions the real efficiency of the mitochondrial respiratory chain in these cells. The results obtained show that the possibility exists of blocking and releasing quickly the phosphorylating electron flow through the whole respiratory chain.

Material and methods. ELD (Ehrlich-Lettré hyperdiploid) ascites tumor cells were grown in albino Swiss mice by weekly transfer of 0.2 ml of ascites fluid. The cells were harvested 7–8 days after the inoculation, washed in an isotonic phosphate-buffered medium and resuspended in the same medium for the experiments. Rat liver mitochondria were prepared by the method of Chance and Hagihara[11]. Oxygen uptake was measured

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polarographically with a Clark oxygen electrode. Nicotinamide adenine dinucleotides were measured fluorimetrically in an Eppendorf filter fluorometer. Cytochrome kinetics were followed in a Dual wavelength/Split-beam Aminco-Chance spectrophotometer. All the experiments were performed at room temperature.

Results and discussion. Figure 1 shows the effect of pyruvate on the cyanide-inhibited endogenous respiration of ELD cells. In trace A), oxygen consumption is blocked by 0.65 m*M* KCN and addition of 6.7 m*M* pyruvate gives a recovery of 85% of the respiration in about 2 min. Subsequent addition of antimycin A inhibits oxygen uptake by about 60%. The antimycin A effect is then relieved by N,N,N′,N′-tetramethyl-*p*-phenylene diamine (TMPD). As in isolated mitochondria[13], the latter agent is able in these cells[14,15] to by-pass electrons from the reduced cytochrome *b* to cytochrome *c* in the presence of antimycin A. The final addition of sulphide almost completely inhibits oxygen consumption. The effectiveness of sulphide in blocking the mitochondrial electron flow in this system clearly demonstrates that cytochrome oxidase can still react with chemical agents, when binding to cyanide is removed by pyruvate, presumably with formation of an inactive cyanhydrin (see ref.[1]). In other words, the use of pyruvate affords a chemical method for readily reversing the inhibition of the mitochondrial terminal oxidase. Trace B) of Figure 1 shows that coupling between electron transport and energy conservation is not seriously impaired as a result of the pyruvate effect. Indeed, oligomycin, when added to ascites cells respiring under conditions of endogenous metabolism, inhibits the respiration by 70–80%[16,17], and in this case the inhibition is only slightly lower (about

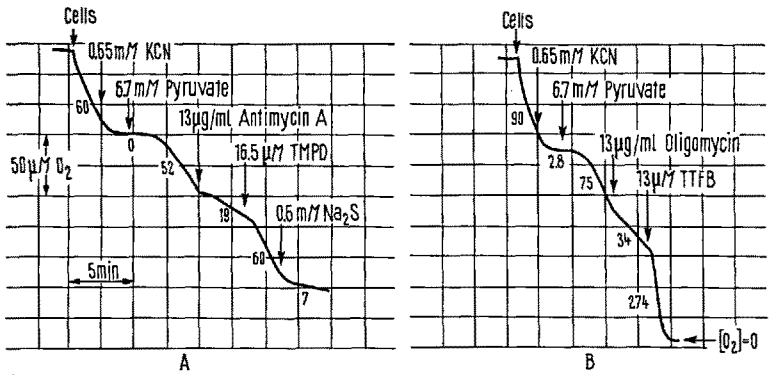

Fig. 1. Polarographic recordings of oxygen uptake in ELD ascites tumor cells showing the effect of pyruvate on the cyanide-blocked respiration. 0.4 ml of cell suspension, containing 24.5 (A) and 25.9 (B) mg dry weight cells, were added to 2.6 ml of isotonic saline medium (154 m*M* NaCl, 6.2 m*M* KCl, 11 m*M* Na-phosphate buffer, pH 7.4)[12]. The numbers along the traces indicate O_2 consumption in nmoles/min.

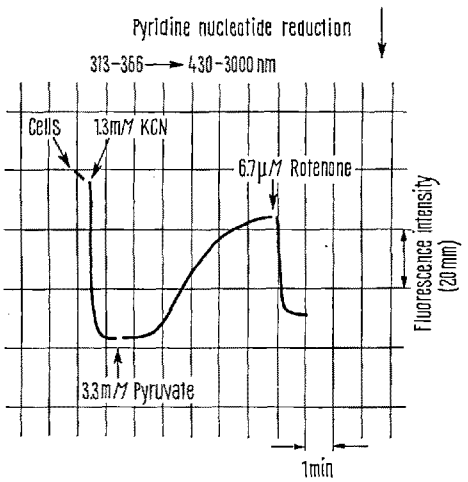

Fig. 2. Fluorimetric measurements of steady state changes of nicotinamide adenine dinucleotides in cyanide-treated ELD cells induced by pyruvate and rotenone. The excitation was at 313–366 nm (primary filter) and the emitted fluorescence was measured, after passing through another filter (secondary filter), above 430 nm. The cells were suspended in the oxygen saturated saline medium (see Figure 1) at the final concentration of 7.0 mg dry weight/ml.

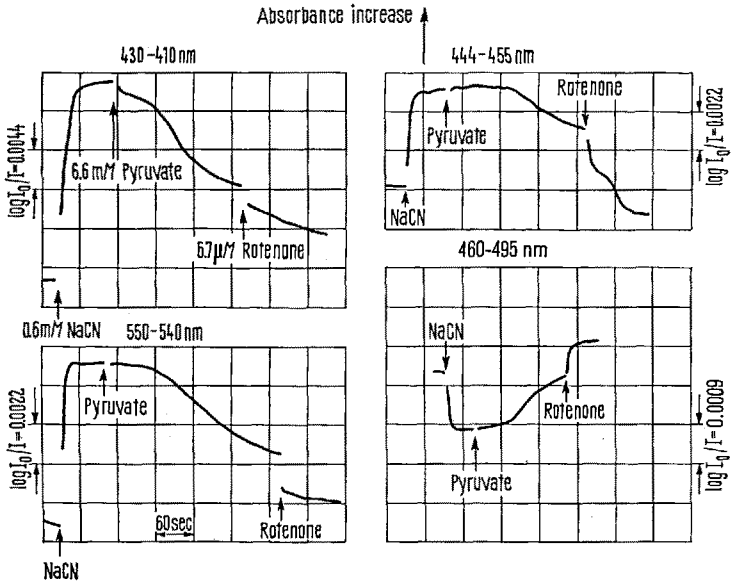

Fig. 3. The traces show the effect of pyruvate on the reduced steady-state of the mitochondrial respiratory carriers of ELD ascites cells treated with cyanide. The kinetics of cytochromes and flavoproteins were followed by the double-beam technique[20]. Cytochrome *b* and $a + a_3$ were measured in the Soret region at 430–410 and 444–455 nm respectively, whereas cytochrome *c* was measured in the α region at 550–540 nm. For flavoproteins the wavelength pair 460–495 nm was used. The cells were suspended in the oxygen saturated saline medium at the final concentration of 7.5 mg dry weight/ml.

55%). 4, 5, 6, 7-Tetrachloro-2-trifluoromethylbenzimidazole (TTFB) strongly stimulates oxygen uptake by uncoupling oxidative phosphorylation[18]. A similar effect of pyruvate on the cyanide-inhibited respiration was observed in rat liver mitochondria supplemented with succinate plus rotenone, phosphate and ADP (State 3[19]).

Figure 2 shows the reversal, induced by pyruvate addition, of the cyanide effect on the redox state of nicotinamide adenine dinucleotides in ELD cells. KCN causes reduction of the nucleotides which are mainly mitochondrial. The further addition of pyruvate induces, after a lag of about 1 min, rapid reoxidation of a large part of the nucleotides reduced in the presence of the inhibitor. Finally, the increased level of reduced steady-state which follows the addition of rotenone indicates that the mitochondrial electron flow has been restored, after pyruvate addition, from the NADH-cytochrome *b* segment of the respiratory chain.

Figure 3 gives a picture of the behaviour of all the other electron carriers (cytochromes and flavoproteins) when the cyanide block is removed by pyruvate. Once again the effect of pyruvate, in reoxidizing the respiratory chain reduced in the presence of cyanide, is very fast, starting within about 1 min from its addition. Rotenone has, in all the cases, the same effect, which is that of further reoxidizing the electron carriers.

In conclusion, the use of rapid and sensitive techniques for monitoring short-term phenomena occurring in the mitochondrial respiratory chain of intact ascites tumor cells has allowed us to find out that the use of pyruvate may represent a suitable tool for removing quickly the functional block by cyanide of cytochrome oxidase. The integrity of oxidative phosphorylation seems to be unaffected when such a removal is obtained, as indicated by the specific efficiency of the inhibitors tested. Moreover, preliminary experiments on mice indicate that the supply of pyruvate removes the cyanide effect even in vivo[21].

Riassunto. È stato osservato che l'aggiunta di piruvato a cellule ascite di Ehrlich del topo è capace di rimuovere rapidamente l'inibizione della respirazione indotta da cianuro, restaurando l'integrità della fosforilazione ossidativa.

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Correlation of β-Lipoprotein Levels and Serum Cholesterol Concentration

In the fasting state approximately 60% of the total serum cholesterol is transported by the β-lipoprotein fraction. This same transport system is used by both exogenous and endogenous cholesterol.

Plasma triglycerides are transported by 2 systems. Exogenously ingested triglycerides are removed from the intestine into the blood as chylomicra which consist mainly of unchanged triglycerides, but also as small amounts of α- and β-lipoproteins. The plasma concentration of chylomicra varies and decreases with time following ingestion of a fatty meal. Endogenous triglycerides are synthesized in the liver, either from carbohydrate or from re-esterification of free fatty acids. These endogenously synthesized triglycerides are transported largely by the pre-β-lipoprotein fraction (very low density lipoprotein) which consists mainly of β-lipoprotein (low density lipoprotein) with a small amount of α-lipoprotein (high density lipoprotein).

The amount of both chylomicra and pre-β-lipoprotein present in the serum is directly related to the diet, both fractions increasing as the fat in the meal increases. The amount remaining after the time elapsed since the meal was ingested is highly variable from individual to individual.

The chylomicra and the pre-β-lipoprotein contain both the A and B apoprotein antigens. Other fractions are determined by a single antigen. The α-lipoprotein contains only the A antigen, and the β-lipoprotein contains only the B antigen[1].

Among the methods used to determine serum β-lipoprotein level is that based on an immunologic assay. This test consists of measuring the width of immunological reaction of an antibody against the B antigen to determine the level of β-lipoprotein. It is readily apparent that this will also measure pre-β-lipoprotein because of the large amount of β-lipoprotein (determined by the B antigen) in the pre-β-lipoprotein fraction.

The immunologic method therefore measures fractions which are responsible for the transport of both cholesterol and triglycerides. The following study was carried out to determine whether or not the immunologic test could be used to predict both hypercholesterolemia and hypertriglyceridemia as Allard and Goulet[2] have asserted.

Methods. A total of 161 adult patients, 86 males and 75 females were selected randomly from consecutive hospital admissions to have triglyceride and cholesterol levels determined. Sera was obtained following a 12 h fast and stored at −20 °C before analysis.

1. *β-lipoprotein test.* β-lipoprotein levels were determined by the immunologic test called 'β-L-test' utilizing the anti-human β-L precipitin serum supplied by Hyland Laboratories, Los Angeles, California. This serum was processed specifically for precipitation of low-density lipoprotein. The test was performed as described by the