

55%). 4, 5, 6, 7-Tetrachloro-2-trifluoromethylbenzimidazole (TTFB) strongly stimulates oxygen uptake by uncoupling oxidative phosphorylation¹⁸. 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 3¹⁹).

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 con-

tains only the A antigen, and the β -lipoprotein contains only the B antigen¹.

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 GOULET² 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

manufacturer and the results were recorded as the mm value of precipitation zone in a microcapillary tube.

2. *Total cholesterol test.* The procedure for determining the total cholesterol in the blood is based upon the reaction of concentrated sulfuric acid and ferric acid in acetic acid upon steroids having the 5-ene 3-ol groupings.

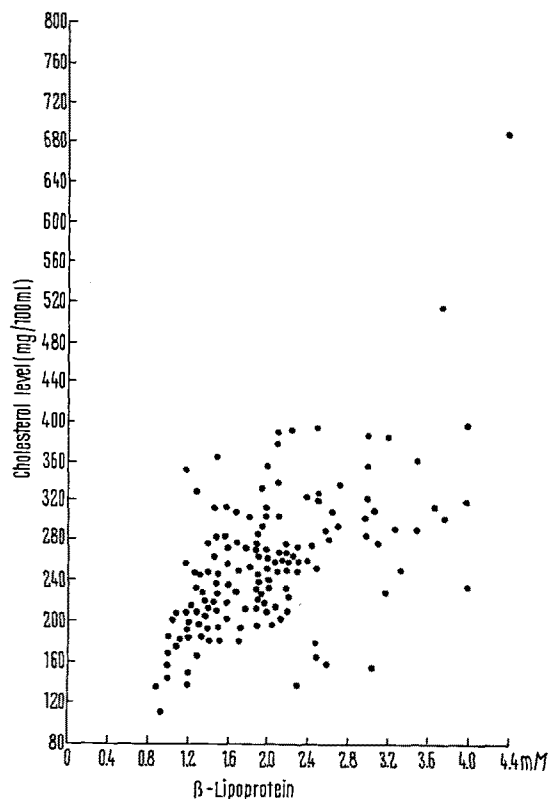


Fig. 1. Serum cholesterol plotted against serum β -lipoprotein as determined by the immunocrit method.

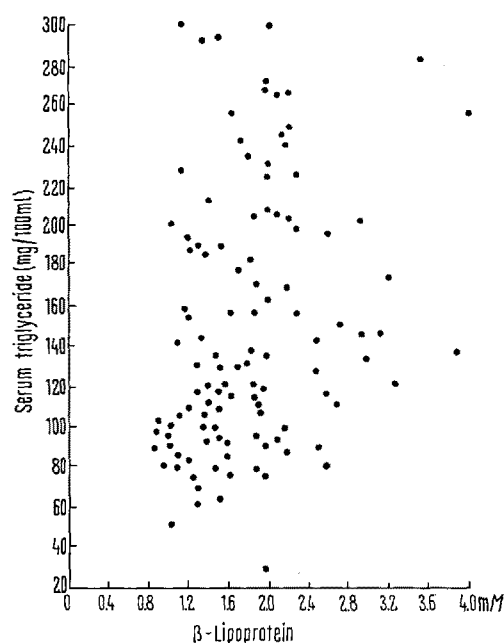


Fig. 2. Serum triglyceride plotted against serum β -lipoprotein as determined by the immunocrit method.

Cholesterol levels were measured at 550 nm using the modified 'auto analyzer' methodology as described by LEVINE and ZAK³.

3. *Total triglyceride test.* The isopropanol extract of triglyceride was saponified into glycerol which was subsequently oxidized with periodate reagent to form formaldehyde. The formaldehyde was then allowed to react with a fluorescent reagent which was quantitatively measured with the use of a fluorometer. This procedure was applied on the 'auto-analyzer' as modified from KESSLER and LEDERER⁴.

Results and discussion. Figure 1 shows the serum cholesterol plotted against serum β -lipoprotein, as determined by the immunocrit method. When this data was subjected to regression analysis, a β -lipoprotein value of 2.3 mm or greater was often associated with a hypercholesterolemia of 250 mg/100 ml ($r > 0.52$). On the other hand the scatter is variable enough that hypercholesterolemia is not always predictable.

Figure 2 depicts serum triglyceride plotted against serum β -lipoprotein. The immunocrit method did not reliably predict an elevated triglyceride level (> 150 mg/100 ml) although there was a positive correlation ($r > 0.30$).

We have shown a direct correlation between the cholesterol and β -lipoprotein level but an inability to consistently predict hypercholesterolemia or hypertriglyceridemia.

WARBURTON and NIXON⁵ have shown that there is poor predictive ability in relating elevated cholesterol to increased β -lipoprotein in patients who have recently suffered an acute myocardial infarction.

However, they imply that in other causes of hypercholesterolemia there is better predictive ability. ALLARD and GOULET² go further and state: 'the β -L-test' is a reliable tool for screening hyperlipoproteinemia (hypercholesterolemia and/or hypertriglyceridemia).

In the random group of patients studied we were unable to verify a reliable predictive ability of the β -L-test for hypercholesterolemia or hypertriglyceridemia. However, the immunocrit method may be used in conjunction with cholesterol and triglyceride level determinations to follow patients with hyperlipidemia.

Zusammenfassung. Mit Hilfe der immunologischen Methode wurden der β -Lipoproteinspiegel wie auch der Serum-, Cholesterol- und Triglyceridspiegel von 161 Erwachsenen bestimmt. Der Gehalt an Cholesterol und Triglyceriden stand in direktem Verhältnis zum β -Lipoproteinwert, wobei jedoch Unsicherheit in der Voraussage der Hypercholesterolaemie auf Grund der Hypertriglyceridaemie besteht.

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