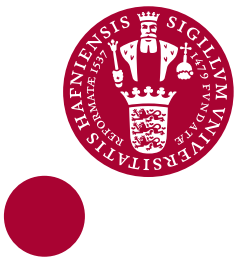

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Abstracts

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Three-Dimensional Mapping of Metabolic State of Rat Liver. Effects of High and Low Alcohol Concentrations

The metabolic state of liver and other tissues may be trapped in vivo by different ways of rapid cooling to low temperature. Furthermore, the quenching process does not appreciably affect the tissue morphology at the light microscopic level. It is therefore possible to apply a newly developed low-temperature scanning technique by which three-dimensional recording of the redox state of a frozen tissue sample may be performed[1]. The redox state is measured as the ratio between the fluorescence intensity of flavoprotein and pyridine nucleotides[2]. The resolution of the instrument is about 5×10^{-4} mm^3, corresponding to 50 - 100 hepatocytes, which provides for a detailed comparison of morphology and metabolism.

The instrument is a modification of the Johnson Foundation time-shared filter fluorometer built together with a low-temperature precision miller. The fluorescence of reduced pyridine nucleotide and oxidized flavoprotein is recorded by surface fluorometry in a series of two-dimensional scans on consecutive surfaces formed by milling of the tissue block. The optical coupling between the tissue surface and the fluorometer is obtained by a micro light guide with 7 fibres, 80 μ in diameter in a 6 + 1 concentric assembly. Fluorescence is excited via the peripheral fibres at 366 nm and 436 nm for pyridine nucleotides and flavoprotein, respectively, and sampled at 460 nm and 540 nm via the central fiber. The milling and the fluorescence recording is performed at 77 °K, which prevent heat induced changes in the redox state during cutting and improves the signal-to-noise ratio considerably due to the temperature conditioned fluorescence quantum yield increase. The instrument is computerized, PDP 11/10, which allows for a display of the data, e.g. as redox ratio images or as redox ratio frequency distribution histograms.

A tissue volume of 6 x 6 x 0.5 mm has been reconstructed from 36 000 single point measurements of the flavoprotein/pyridine nucleotide ratio in control livers as well as in alcohol perfused livers (6 and 60mM alcohol). The results may be summarized as follows: 1) There is a striking similarity between the redox ratio pattern and liver morphology, suggesting a close relation between metabolic function and location in the microcirculatory unit of the liver. 2) There is a pronounced redox heterogeneity in the control as well as in the alcohol treated livers. Changes of 120% may be observed within the area of the single liver acinus. 3) The steepness of the redox ratio transitions are enhanced by alcohol; in most locations the transition will take place within the resolution of the instrument, i.e. 100 μm. 4) The average redox ratio is about 2-fold greater in the control than in the livers receiving alcohol (0.48 and 0.23, respectively), whereas the range is almost unchanged. 5) Although high alcohol concentration (60mM) does not change the average redox ratio significantly compared with 6mM alcohol, the population of relatively oxidized cells seems to increase. 6) In the alcohol treated livers, but not in the controls, the frequency distribution histograms of the redox state measurements become bi-modal in a scanning depth of 2 - 300 μm suggesting the existence of two populations of liver cells reacting differently to alcohol. This effect is more pronounced with high alcohol concentration.

1 Quistorff, B. & Chance, B. (1977) in Oxygen and physiological function, F. F. Jöbsis addiction. Professional information library, Chicago., in press.
2 Chance, B. et al. (1975) 10th FEBS Meeting, abstract 1957, Paris.

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Proteolytic Activation of AMP Aminohydrolase in Crude Extract of Trout Gill

The AMP aminohydrolase (EC 3.5.4.6) activity present in crude extract of Trout (*Salmo gairdneri*) gill increases with time. The rate of this activation is temperature dependent. At 4 °C, the enzyme activity increases to about 150% within 10 - 12 h, and then decreases. This process is initiated by the homogenization of the tissue. The observed activation is unlikely to be due to the breakdown of an inhibitor initially present in the extract. Soybean trypsin inhibitor and mainly α_2-macroglobulin strongly inhibit the activation process. The increase of AMP aminohydrolase activity is also markedly reduced in the presence of 10^{-3}M *N*-ethylmaleimide. Diisopropylfluorophosphate is inactive. $Mg^{2\oplus}$ enhances the rate of decrease of AMP aminohydrolase activity initiated after about 10 h, and EGTA lowers, it. Both compounds are without effect on the first part of the activation process. Addition of 0.5 mg/trypsin/ml of a crude extract induces a sharp increase in the activity of the enzyme within 5 min. The action of trypsin is prevented by diisopropylfluorophosphate. Trypsin has no activity on fully activated extracts and there is a proportionality between the state of activation of AMP aminohydrolase and its sensitivity to trypsin. These results suggest the presence, in trout gill extracts, of 2 types of proteinases which have an activation and an inhibitory action, respectively, on AMP aminohydrolase.