Increase of Cholinesterase Content in Mouse Liver Homogenates after Incubation

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The rate of hydrolysis of acetylcholine by mouse liver homogenates increases by about 100% when the homogenates are left either overnight at room temperature (20°C) or two to three hours at 37°C.

Of the organs investigated (brain, kidney, plasma, erythrocytes and liver) only the liver presented a real "autoactivation". This increase in activity takes place in liver homogenates from mice of different ages and both sexes, but seems to be more progressive in the female. There seems to be a greater "activation" in liver homogenates from infantile mice than mature ones, although there are large fluctuations.

The "activation" does not disappear with dialysis and seems to be localized to the particles of the liver cell upon "high speed" centrifuging. Calcium ions have no influence, neither is the increase in activity dependent on the isotonicity of the medium.

Kinetic experiments with a selective inhibitor of "pseudo"-cholinesterase, Nu 683, suggest that one and the same enzyme, viz. a "pseudo"-cholinesterase, is responsible for the major part of the activity with acetylcholine as substrate, both prior to and after the "activation".

The increase of hydrolysis takes place with acetylcholine and butyrylcholine as substrates, while deactivation results when tributyrin and benzoylcholine are employed. With acetyl-β-methyl-choline the rate of hydrolysis is quite slow, but has a slight tendency to increase with time; too small, however, to be of any experimental significance. These experiments indicate that we are dealing with a butyrocholinesterase.

Benzoylcholine, often used as a specific substrate for "pseudo"-cholinesterase, is not hydrolysed at an increased rate after incubation. On the contrary, the hydrolysis decreases with time. Thus there might perhaps be two "pseudo"-cholinesterases in the mouse liver; one butyrocholinesterase and one "benzoylcholinesterase". As known, "benzoylcholinesterases" are occasionally found in the plasma of rabbits and in the liver of guinea pigs.


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On the Question of a Cysteinylglycinase Activity of RNA

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Binkley reported that cysteinylglycinase should be a protein-free RNA and put forward the hypothesis that RNA should interfere in protein synthesis through this activity. Because of the remarkable implications of these statements (among others, that an enzyme should be non-protein in nature), an attempt was made at purifying further the cysteinylglycinase (through chromatography on calcium phosphate, Dowex 2 and DEAE cellulose) and at studying some of its chemical properties.

A separation of cysteinylglycinase activity from RNA was achieved, and Folin-positive, orcinol-negative, P-negative, chloroform-sensitive preparations of enzyme were obtained, showing a maximum in UV absorption spectrum at about 290 nm.


A Method for the Isolation of 2-Deoxy-D-ribose from Thymidine

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It has always been assumed that the sugar component of thymidine is in analogy with the purine nucleosides 2-deoxy-D-ribose. Good evidence in support of this assumption has recently been provided 1-3, although the conclusive chemical proof was lacking. The present work describes the isolation of crystalline 2-deoxy-D-ribose from thymidine, and also provides a very convenient micro-method for the isolation of this sugar for tracer studies, which was the main purpose of this work. Thymidine is reduced by 3% NaHg in aqueous solution at room temperature. The reduction mixture was shown by paper electrophoresis to contain two acidic 2-deoxy-D-ribose components formed by breakage of the reduced pyrimidine ring under the prevailing alkaline conditions. Removal of alkali in the reduction mixture with Amberlite IR 120(H) followed by gentle acid hydrolysis with dilute sulphuric acid and passage through a column of weakly basic resin such as Amberlite IR 4B(OH) afforded crystalline 2-deoxy-D-ribose. The yield was almost 100%. For tracer work it is advisable to purify it by recrystallization or paper chromatography.


Glucose Inhibition of Respiration
in Ascites Tumor Cells

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Glucose exerts a pronounced inhibitory effect upon the respiration of ascites tumor cells, a phenomenon also called the Crabtree effect. In an attempt to approach this problem the glycolysis and the tricarboxylic acid cycle of the ascites tumor cells were studied under various conditions. Ascites cells (30-40 mg protein) incubated in phosphate buffer for 1 h at 37°C with excess glucose as substrate were found to accumulate 1-2 \( \mu \)M of a barium insoluble fructose ester, assumed to be fructose-diphosphate.

In the presence of glucose (10 \( \mu \)M/ml), the removal of inorganic phosphate (20 \( \mu \)M/ml) from the medium increased the oxygen uptake by 25-50% and decreased the glucose uptake.

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