DNA synthesis as measured by $^{32}\text{P}_i$ incorporation on the nucleotide pool of the cells. Under the conditions used there seems, however, to be an interdependence between accumulation of cordycepin diphosphate and a decrease in the ribotide pool of the cells. When these two events have proceeded to a certain degree, complete inhibition of DNA synthesis is observed. A similar decrease of the ribotide pool could be obtained with 2-deoxyglucose instead of cordycepin. It was again found that when the ribotide pool had reached the same critical level as above, complete inhibition of DNA synthesis was obtained.

The effect of cordycepin on DNA synthesis may, therefore, partially be due to a general change in the intermediary metabolism of the tumor cells through replacement of adenosine phosphate by cordycepin phosphates. The cordycepin phosphates are not able to substitute for the adenosine phosphates in metabolic reactions of importance for DNA synthesis.

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Studies on the Nature of the Prosthetic Group of Glucose Dehydrogenase of *Bacterium anitratum*

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Glucose dehydrogenase (GDH) of *B. anitratum* has in its oxidized state a broad absorption band at about $360\text{ m}\mu$, a band which in the presence of glucose is intensified and moved to $337\text{ m}\mu$ ¹. This finding, which recalls similar observations with muscle triosephosphate dehydrogenase², together with the acceptor specificity pattern found, prompted us to postulate that the prosthetic group was a tightly bound form of niacinamide. Attempts to positively identify this group with DPN or TPN failed, and other evidence suggested that the niacinamide, if indeed niacinamide was involved, was bound in a manner that in several respects was unusual. These studies have now been extended.

The highly purified enzyme has an ultraviolet spectrum that does not allow the presence of adenine in significant amounts, and from this a prosthetic group of the usual dinucleotide variety is ruled out. In order to ascertain whether we were dealing with a phosphorylated group at all, the organism was grown in the presence of ^{32}P -labelled phosphate. The amount of phosphate found in the purified enzyme was less than 2 % of that expected on the assumption that the $337\text{ m}\mu$ band represented niacinamide.

It was conceivable that GDH even in the absence of the normal pyridine nucleotide moieties adenine and phosphoric acid might still utilize the niacin nucleus. If this was the case, absorption bands in the region $300\text{--}350\text{ m}\mu$ should be observed upon addition of cyanide or sulfite to the enzyme preparation. No such absorption could be observed. These negative tests for an N-substituted niacin derivative were supported by negative microbiological tests for niacin on hydrolyzates of the enzyme.

A final experiment was then designed that was felt would conclusively establish the presence or the absence of niacin in this enzyme. A niacinless mutant of the organism was produced by ultraviolet irradiation and isolated by penicillin treatment followed by replica plating on selective media. This mutant was grown on a large scale with ^{14}C -labelled niacin. When the enzyme was isolated from this batch of cells and the radioactivity measured, less than 1 % of the counts expected on the basis of the $337\text{ m}\mu$ absorption was detected.

GDH of *B. anitratum* thus appears to fall completely outside the known groups of dehydrogenases: neither flavin, nor niacin is involved in the primary dehydrogenation, but a prosthetic group of a yet unknown nature.

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The Effect of Cordycepin 1-N-oxide on DNA and RNA Synthesis

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Cordycepin consist of adenine coupled to a branched 3-deoxyribose called cordycepose^{1,2}. It is produced by the mold *Cordyceps militaris*.

The 1-N-oxide has been prepared by the method described previously³. Cordycepin 1-N-oxide (2 μ moles per ml suspension) blocks RNA synthesis in Ehrlich ascites cells *in vitro* after 1½–2 h of incubation and DNA synthesis after about 3 h. In the cells cordycepin 1-N-oxide is slowly reduced to cordycepin which in turn is phosphorylated to the mono-, di- and triphosphates of cordycepin. This latter conversion is within experimental error quantitative. This is confirmed by the finding that no significant amount of either cordycepin or its product of deamination, hypoxanthine cordyceposide, could be detected. The adenine nucleotide pool is slowly decreasing during experimental time (7 h). No radioactivity is found in DNA or RNA when (³H) cordycepin 1-N-oxide is used. These experiments and others performed with cordycepin (Klenow⁴) indicate that cordycepidiphosphate may inhibit the nucleic acid synthesis in Ehrlich ascites cells *in vitro*.

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Distribution of Esterases in the Intracellular Fractions of Rat Liver

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In a study of the intracellular esterases of rat liver, it was found that the nuclear, mitochondrial, microsomal and the supernatant fractions took up large amounts of radioactive diisopropyl phosphorofluoridate (DF³²P) and that the labelled proteins could be separated into a number of fractions by DEAE-cellulose chromatography¹. Phosphorylserine could be isolated from each of the labelled peaks. In a series of parallel experiments without DF³²P in which esterase activity was determined using *p*-nitrophenyl acetate as the substrate, a close relationship between the relative positions of the radioactive and esteratic peaks was observed. There was no fraction with a marked

cholinesterase activity as eserine produced only a slight and uniform inhibition throughout.

To correlate the various radioactive peaks with the esteratic peaks and to detect any esterases not taking up the label (A-esterases)², experiments have been performed in which the determinations of the two activities were carried out on the same fractions by the following procedure:

The four cellular components, from four rat livers at a time, were isolated by the differential centrifugation technique³ and the particulate fractions were solubilised with sodium desoxycholate. After removing the latter on Sephadex G-25 (Pharmacia, Sweden) the proteins were fractionated on DEAE-cellulose by the gradient elution technique using 0.01 M Tris-acetic acid buffer, pH 7.4, containing sodium acetate of increasing concentration from 0 to 1 M. About 200 fractions of 10 ml each were collected in all. Aliquots from each fraction were treated with DF³²P (Radiochemical Centre, Amersham) and the labelled protein was separated from the excess DF³²P on a column of Sephadex G-50 (Medium) under standardised conditions. Aliquots were also analysed for esterase activity using *p*-nitrophenyl acetate, acetylcholine chloride, butyrylcholine iodide and acetyl- β -methylcholine iodide as substrates. Colorimetric methods were used for these estimations^{4,5}.

It was found that fractions showing intense DF³²P binding always coincided with those having maximal esterase activity on *p*-nitrophenyl acetate. Though there was proportionality between the two activities within each enzyme peak, the amount of DF³²P taken up by different peaks was different, some regions of low esterase activity on *p*-nitrophenyl acetate being found with comparatively large amounts of the label and *vice versa*. Thus a separation of esterases seems to have been achieved on the basis of their specificities. There were four large reproducible enzyme peaks in each of the three particulate fractions, the microsomal fraction having the highest activity. There were no esterase peaks which did not take up DF³²P and the activity was completely inhibited by this reagent at a concentration of 10⁻⁶ M. The supernatant fraction had the lowest esterase activity distributed over more than 6 peaks, some of which were possibly derived from the particulate fractions in the course of their isolation. This fraction also contained a small amount of DFP-insensitive enzyme admixed with those taking up the label.

In none of the peaks in the particulate or the supernatant fractions could any cholinesterase