New Pyrimidines Derived from Thymidine and 5-Fluorouracil and Some of their Biochemical Properties

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During continued studies of reduction products of pyrimidines and pyrimidine nucleosides [1], some new compounds with characteristic spectral properties have been detected. This report deals with products formed when thymidine and 5-fluorouracil, respectively, is treated with 3 % NaHg in 0.2 M aqueous acetic acid.

Our investigations strongly indicate that these compounds are 5-methyl-2-pyrimidone-2′-deoxyriboside and 5-fluoro-2-pyrimidone [2].

The 5-methyl-2-pyrimidone-2′-deoxyriboside was purified by chromatography on a Dowex 1 x 4 column in the borate form[3], followed by paper chromatography in the butanol:ethanol:ammonia(conc.):water (4:1:2:1) system. The substance was crystallized from absolute ethanol as white platelets. Its absorption maxima in 0.1 N NaOH, distilled water, and 0.1 N HCl are, respectively, 321, 314, and 325 mμ. The corresponding values for ε_{max} are 7.1×10^3, 6.1×10^3, and 8.2×10^3. Using the spectrophotometric method of Fox and Shugar [4], the p*K*a values were determined to be 2.2−2.4 and >12. The latter is probably due to dissociation in the sugar moiety. Contrary to thymidine, the glycoside bond is very labile under acid condition.

5-Fluoro-2-pyrimidone was purified by chromatography on a Dowex 1 x 8 column followed by paperchromatography in the butanol:ethanol:ammonia(conc.):water (4:1:2:1) system. The compound was recrystallized from ethyl acetate and obtained as white needles. The 5-fluoro-2-pyrimidone exhibits a shift in absorption maxima. In 0.1 N NaOH, distilled water and 0.1 N HCl the absorption maxima are respectively 311, 316, and 319 mμ. The spectrophotometrically determined dissociation constants are 1−2 and 7.2−7.3. The molecular extinction coefficient is 4.4×10^3 in 0.1 N HCl.

5-Methyl-2-pyrimidone-2′-deoxyriboside has been tested as a growth factor for the organism *Lactobacillus acidophilus* R 26 Orla Jensen, which requires a deoxyriboside for growth. By using the method described by Hoff-Jörgensen [5], the 5-methyl-2-pyrimidone-2′-deoxyriboside was determined to have the same activity as thymidine in promoting growth in this organism. The substance has no activity as substrate for the thymidine phosphorylase, isolated from horse liver [6].

In agreement with the finding that 5-methyl-2-pyrimidone-2′-deoxyriboside served as a growth factor for *Lactobacillus acidophilus*, the 5-fluoro-2-pyrimidone could be converted to the corresponding deoxyribose-compound by a transglycosidase prepared from the same organism according to MacNutt [7].

In contrast to 5-fluorouracil which inhibits the growth of *E. coli* K-12 strain at very low concentrations, 5-fluoro-2-pyrimidone has no effect on the growth even at relatively high concentrations.

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Effect of Cordycepin on DNA Synthesis and the Ribotide Pool of Ehrlich Ascites Tumor Cells *in vitro*

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The adenine nucleoside cordycepin [1] has been found not only to inhibit incorporation of $^{32}P_i$ into DNA of Ehrlich ascites tumor cells *in vitro* but also to give rise to accumulation of phosphorylated derivatives in the cells [2].

Investigation of the effect of increasing concentrations of cordycepin has revealed that the presence of cordycepin triphosphate (ACTP) in the cells has no great effect on either

DNA synthesis as measured by $^{32}P_i$ incorporation or 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 mμ, a band which in the presence of glucose is intensified and moved to 337 mμ [1]. This finding, which recalls similar observations with muscle triosephosphate dehydrogenase [2], 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 adenin 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 mμ 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 300–350 mμ 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 mμ 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*.