The increase in the excretion of N'-methylnicotinamide by methylthiouracil-treated rats, which is even more pronounced than the decrease observed in the case of the hyperthyroid animals, is, therefore, to be expected since it represents an effect opposite to that produced in hyperthyroidism. However, on the basis of the incomplete present day knowledge of the mechanism of action of the thyroid hormone in the normal living system, no precise explanation of this pronounced enhancement of methylation in hypothyroidism can be given. It may, however, be mentioned that a similar influence of thyroid imbalance on the acetylation of sulphanilamide has been observed by FRAENKEL-CONRAT AND GREENBERG[17] and on the synthesis of acetylcholine by GUZMAN[18].

The decreased methylation of nicotinamide in hyperthyroidism, observed in the present study, does not, therefore, favour the postulate of CALVO *et al.*[10] that the thyroid hormone stimulates transmethylation.

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Occurrence of free nucleotides in *Penicillium chrysogenum*

In the course of studies on the metabolism of *Penicillium chrysogenum* it was thought of some importance to investigate the nature of the nucleotides occurring in the mycelium of this organism. Only adenosine-5'-monophosphate and adenosine-5'-triphosphate have hitherto been identified in *P. chrysogenum*[1].

The mycelium used in this study (strain Wis 49–133) was grown in a stirred fermenter in the synthetic medium of JARVIS AND JOHNSON[2], containing 22.5 g lactose, 7.5 g glucose, 3 g ammonium acetate and 5 g ammonium lactate, per liter. The mycelium was removed from the fermentation after 50 hours when the reducing sugar content was approximately 0.5%, filtered by suction, washed with water and kept at −20° C. The nucleotides were extracted with 50% ethanol and precipitated with mercuric acetate, as described by CAPUTTO, LELOIR, CARDINI AND PALADINI[3]. The supernatant obtained after decomposition of the mercury salts, with hydrogen sulphide was fractionated by chromatography on Dowex-1 formate with the formic acid system of HURLBERT, SCHMITZ, BRUMM AND POTTER[4]. Eleven peaks were detected by measuring the optical density of the fractions at 260 mμ. The fractions comprising each peak were pooled and lyophilized, either directly or after adsorption and elution on charcoal[5], and then further fractionated by large-scale paper chromatography on Whatman No. 1 or 3 MM using ethanol-ammonium acetate (pH 3.8) as solvent[6]. Each ultraviolet-absorbing band was cut out, washed with ethanol, and then eluted with water at 5° C. The solutions thus obtained were used to identify the compounds on the basis of the following criteria: (a) type of U.V. spectrum in acid, alkaline and neutral solution, (b) mobility on paper with four different solvents in parallel with corresponding authentic nucleotides, (c) type of U.V. spectrum and mobility on paper (using two different solvents) of the base obtained after hydrolysis with perchloric acid, (d) ratio of base to organic phosphate to ribose (for most of the purine-nucleotides), (e) hydrolysis with 5'-nucleotidase from bull seminal plasma (for monophosphates)[7], (f) type of sugar released on mild acid hydrolysis

(for guanosine-diphosphate-mannose, uridine-diphosphate-glucose, uridine-diphosphate-galactose and uridine-diphosphate-N-acetyl-glucosamine), (g) U.V. spectrum shift in cyanide[8] and ability to act as coenzyme for alcohol dehydrogenase or for glucose-6-phosphate dehydrogenase (for diphosphopyridine-nucleotide and triphosphopyridine-nucleotide).

Thus the following sixteen compounds, listed in the order they emerge from the column, have been fully identified: diphosphopyridine-nucleotide (DPN), cytidine-5'-monophosphate (CMP), adenosine-5'-monophosphate (AMP), triphosphopyridine-nucleotide (TPN), guanosine-5'-monophosphate (GMP), inosine-5'-monophosphate (IMP), uridine-5'-monophosphate (UMP), adenosine-5'-diphosphate (ADP), guanosine-5'-diphosphate-mannose (GDPM), uridine-5'-diphosphate-N-acetylglucosamine (UDPAG), uridine-5'-diphosphate-glucose (UDPG) and uridine-5'-diphosphate-galactose (UDPGa), uridine-5'-diphosphate (UDP), adenosine-5'-triphosphate (ATP), guanosine-5'-triphosphate (GTP) and uridine-5'-triphosphate (UTP).

Several unidentified compounds have also been detected by the above procedure. Two are eluted from the column in the first peak, just before DPN and CMP; both show a typical uridine spectrum and give UMP after mild acid hydrolysis. A compound contained in the peak which immediately follows AMP shows a pyridine-nucleotide spectrum, characterised by the appearance of a maximum at 315 mμ on addition of potassium cyanide; in contrast to DPN and TPN this compound is unable to act as coenzyme either for alcohol dehydrogenase or for glucose-6-phosphate dehydrogenase. Two adenosine phosphates emerge with IMP and a third with ATP; none corresponds to AMP, ADP or ATP. Finally two other unknown compounds show a spectrum with a maximum at 267 mμ in acid solution, shifted to 270 mμ in neutral and alkaline solution; one of them appears together with the above mentioned pyridine-nucleotide-like substance and is phosphorus-free, the other is contained in the ADP fractions, gives a positive reaction for phosphorus and is stable to mild acid hydrolysis. These unknown compounds will be the subject of further studies.

The isolation of cytidine, uridine, adenosine and guanosine phosphates from *P. chrysogenum* represents the first direct evidence for the simultaneous occurrence of all these substances in a mould. It shows yet again how wide is the distribution of these cellular constituents in nature, and indicates the possibility that some of them, apart from their role as coenzymes and phosphorylating agents, may take part in other metabolic reactions of general importance. For instance, as several authors have assumed, they may act as precursors in the biosynthesis of the nucleic acids. Furthermore the presence of UDPAG, UDPG, UDPGa and GDPM in a mould which contains chitin[9,10] and polysaccharides composed of glucose, galactose and mannose[11,12], suggests that these nucleotides may represent the activated forms of glycosyl residues involved in the biosynthesis of polysaccharides[13,14,15].

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