These results indicate that a complex between the uridyl group and the enzyme is formed, but a more obvious proof of the reaction would be to apply a C^{14}-labelled uridine compound to the same experiment with subsequent isolation of a C^{14}-labelled uridyl enzyme compound. For this purpose C^{14}-labelled uridine di- and triphosphate can be prepared from C^{14}-labelled uridylic acid by a new enzyme, found in extracts from *Saccharomyces fragilis* (grown on galactose). This enzyme brings about a phosphorylation of UMP to UDP and UTP in the presence of ATP. The separation of the uridine compounds from the adenine compounds is accomplished by paper ionophoresis in citrate buffer, pH 4.2. The uridine polyphosphate compound formed gives positive reaction in the hexokinase test for triphosphates [1].

This phosphorylation process is most likely a phosphorylation of UMP to UDP (with subsequent action of nucleoside diphosphokinase to form UTP from UDP) since equimolar amounts of UMP and ATP give UDP exclusively, whereas excess of ATP yields both UDP and UTP. Experiments with ATP, labelled in the terminal phosphate group, agree with this explanation.

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Metabolism of Uridinediphosphoglucose (UDPGlucose) in *Hemophilus Influenza* B.

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Hemophilus influenza (obtained through the courtesy of Drs. Alexander and Zamenhof, College of Physicians & Surgeons, New York) was grown on bloodagar and harvested (our thanks are due to Dr. M. Pittmann, Department of Pathology, National Institutes of Health, and Dr. N. Sloane, Lederle Laboratories, New York). The cells were ruptured in the sonic vibrator and lyophilized. Enzyme fractions could be isolated which brought about a rapid splitting of UDPglucose from the cell extracts. The split products were mainly 5-uridylate, uridine and α-glucose-1-phosphate. Unlike the usual nucleotide pyrophosphatases pyridine nucleotides did not undergo any splitting in the presence of the *Hemophilus* enzyme. UDP-N-acetyl glucosamine was not attacked to any detectable degree. Also, unlike correspondent preparations from yeast or *E. coli* (extract from sonic lysates) the *Hemophilus* enzyme operates independent of addition of inorganic pyrophosphate or 1-esters (galactose-1-phosphate, ribose-1-phosphate). Moreover, addition of pyrophosphatase does not interfere in any way with the enzymic splitting of UDPglucose. The formation of glucose-1-phosphate is preceded by an induction period which has attracted our special attention. The enzyme reaction may be either a simple specific hydrolysis or a combined hydrolysis and transfer reaction, or finally a two step reaction. The two latter possibilities are under investigation in an attempt to find a possible relation between the peculiar UDPglucose metabolism in this organism and the presence of polyglycophosphates in the capsules [1]. Dr. E. Maxwell and the author are investigating the presence of the UDPglucose splitting enzyme in various strains and mutants of the *Hemophilus* group with special reference to growth requirements and capsule formation.

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On the Interaction between Glucose-1,6-diphosphate and Ribose-1-phosphate

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Previous experiments[1] have shown that glucose-1,6-diphosphate (GDP) can serve as a coenzyme for the conversion of ribose-1-phosphate (R-1-P) to ribose-5-phosphate (R-5-P), and that this reaction can be catalysed by phosphoglucomutase from muscle. From incubation mixtures of GDP, R-1-P and phosphoglucomutase a ribose-1,5-diphosphate has been isolated. This led to the formulation of the reaction as follows: GDP + R-1-P $\rightleftharpoons$ RDP + G-6-P. This reaction has now been studied in details by following the formation of G-6-P spectrophotometrically in the presence of TPN and excess of Zwischenferment. The K_m value of R-1-P was found to be about 2×10^{-3} *M*. The optimum concentration of GDP was about 4×10^{-5} *M*, and higher concentrations inhibited the reaction in a competitive way. The K_m of GDP as inhibitor was about 2×10^{-3} *M*. The equilibrium of the

reaction between R-1-P and GDP has been determined by following the formation of G-6-P. The optimum conditions for the preparation of RDP have been worked out. With the same enzyme system evidence has also been obtained for a reaction between GDP and deoxyribose-1-phosphate or galactose-1-phosphate. Preliminary experiments with pigeon liver acetone powder confirm the indications obtained by Saffran and Scarano [2] for RDP to be an intermediary in 5-adenylic acid formation from ^{14}C-adenine.

1. Klenow, H. *Arch. Biochem. Biophys.* **46** (1953) 186.
2. Saffran, M. and Scarano, E. *Nature* **172** (1953) 1949.

Studies on the Inhibition of D-Amino Acid Oxidase

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Studies on D-amino acid oxidase from pig kidney purified according to Negelein and Brömel [1] have been made. The flavineadenine dinucleotide (FAD) used was a pure sample obtained from baker's yeast by inophoresis on paper as the final step in the purification. The coupling between the apoenzyme and FAD caused no shift in the absorption band and no quenching of the fluorescense. Thus, D-amino acid oxidase is fluorescent and is similar to Straub's diaphorase in this respect.

Inhibition of enzyme activity by different anions and by partial structural analogs of the FAD molecule has been demonstrated. Anions were inhibitory in the following order:

$$J^- > Br^- > NO_3^- > Cl^- > PO_4^{--} > SO_4^{--} > CH_3COO^- \gg F^-$$

Thus, the anions of strong acids were most effective as inhibitors. At a concentration of 1.4×10^{-7} *M* FAD 50% inhibition was exerted by 8.5×10^{-2} *M* NaCl. The inhibition was reversible in the presence of high FAD concentration, suggesting competition of the inhibitor with the prosthetic group.

In similar experiments strong inhibition by adenylic acid, ATP and FMN was observed. 50 % inhibition was given by concentration of approximately 5×10^{-4} *M*. The inhibition by DPN was somewhat weaker, while adenosine, adenine and hypoxanthine exerted inhibition of the same order of magnitude at concentrations of 6×10^{-3} *M*. Cytosin and uracil were slightly inhibitory while riboflavin was without effect.

The experiments indicate that the phosphoric ester groups and the adenine part of the FAD molecule are of importance in the coupling to the apoenzyme. A possible physiological significance by some of these inhibitors in regulation of enzyme activity is suggested.

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Distribution in Rabbit Livers of Intravenously Injected Iron

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Rabbits weighing from 1.5 to 1.7 kg were injected with various amounts of a high molecular weight iron-carbohydrate complex (Astra) and sacrifized after different time intervals. The livers were homogenized and fractionated according to Hogeboom, Schneider and Pallade [1] and the iron determined in the different fractions.

The results show a considerable increase in ferritin in all the fractions. In the nuclei and the mitochondria there was also a large increase of a water-insoluble hydrolyzeable fraction (hemosiderin). Notable also was a very marked increase of bound hemin (cytochrome b) in the microsome fraction.

1. Hogeboom, G. H., Schneider, W. C. and Pallade, G. E. *J. Biol. Chem.* **172** (1948) 619.

The Combination of Flavin Mononucleotide and Riboflavin with the Protein of "The Old Yellow Enzyme"

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In a forthcoming paper *(Acta Chem. Scand.)* we have presented some kinetic data on the reversible dissociation of the old yellow enzyme (OYE) into FMN and apoenzyme obtained by the aid of fluorescence measurements. In water, the dissociation constant *K* of this