Ergebnisse, die Vermutung zu, dass sich die Larven sei es zur Bahnung ihres Weges oder sei es zur Nahrungsaufnahme, dieses enzymatischen Hilfsmittels bedienen. Die Resultate lassen auch daran denken, dass die Wanderlarven nach pharmakotherapeutischer Hemmung des Kollagen-Kollagenasesystems, eine Möglichkeit zur Unterbrechung des Entwicklungskreises der Dasselfliege bieten.

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Enzymatic Synthesis of Ureidosuccinic Acid

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Ureidosuccinic acid (USA) has been shown to be a normal intermediate in the biogenesis of orotic acid from aspartic acid and CO_2 in rat liver slices [1]. After preparation of cell fractions by differential centrifugation of homogenized rat or rabbit liver in 0.25 *M* sucrose-versene the enzymatic synthesis of USA could now be localized to the mitochondrial fraction. Aspartic acid, bicarbonate, ammonia, $Mg^{\cdot\cdot}$, ATP and glutamate were required for USA synthesis. Glutamate served the double function of regenerating ATP and being the source of a catalyst for USA synthesis. When small amounts of carbamyl glutamate or acetylglutamate were present during the reaction, glutamate could be substituted by succinate. Optimal synthesis was obtained only in the presence of the substituted glutamates. The requirements for optimal USA synthesis are strongly reminiscent of the enzymatic mechanism leading to citrulline synthesis [2].

USA synthesis could also be obtained in solution after freezing and thawing of the mitochondria followed by high speed centrifugation. These extracts showed optimal USA synthesis from aspartate, ammonia and bicarbonate in the presence of acetyl glutamate, $Mg^{\cdot\cdot}$, ATP, phosphoglyceric acid and a muscle enzyme fraction [3] (for regeneration of ATP). When ornithine was substituted for aspartic acid, citrulline synthesis could readily be demonstrated in the extract. However, extracts of acetone powder of rat liver prepared according to Cohen and Hayano [4] had almost completely lost the ability to synthesize USA, while showing slightly better capacity to synthesize citrulline than the mitochondria extracts. It is concluded that at least not all the enzymatic steps are the same in USA and citrulline synthesis.

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Metabolism of Uridine Triphosphate in Yeast

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Experiments have been performed to investigate the mode of action of the uridyl transferase found in yeast, which catalyzes the reaction:

Uridinediphosphoglucose + pyrophosphate ⇌ uridinetriphosphate + α-glucose-1-phosphate

In accordance with the concept of the enzyme as acceptor of anhydride groups, the reaction could be formulated as follows:

UPPG + enzyme ⇌ enzyme-UP + G-1-P
Enzyme-UP + P—P ⇌ enzyme + UPPP

Attempts to bring P^{32}-labelled G-1-P or P—P into equilibrium with UPPG or UPPP respectively failed, when a crude preparation of the uridine transferase was used, but the possibility exists that other reactions may take place here and disturb the equilibrium. If a highly purified enzyme preparation is applied, however, a significant incorporation of the radioactive reactant into the nucleotide can be demonstrated.

The main feature in the purification was a fractionation with ethanol (active fraction obtained between 24—28 % ethanol) succeeded by an ammonium sulfate fractionation (active fraction precipitated at 54—60 % saturation. Activity: 1 μg of protein converted 0.008 μmole UPPG per minute). By incubating this enzyme solution with UPPP and P^{32}-labelled PP followed by deproteinization and chromatography of the reaction mixture, a significant amount of the labelled pyrophosphate could be detected in the UPPP, showing that the following reaction has taken place:

$$UPPP + \overset{*}{P}\overset{*}{P} \rightleftharpoons UP\overset{*}{P}\overset{*}{P} + PP$$

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