It appears that a G6P-dehydrogenase is present in all species, except in the larval stage of *Echinococcus*. This enzyme is TPN-linked. In the few cases where a weak reaction with DPN^+ was obtained, this must probably be attributed to the fact that G6P is also decomposed by way of the Embden-Meyerhof glycolytic pathway. GA6P-dehydrogenase is also present in all species, except in *Echinococcus*-larvae. It is specifically TPN-linked and always less active than G6P-dehydrogenase. The amount of both enzymes is relatively low in planarians and trematodes and very variable among cestodes. *Annelida* and especially parasitic nematodes have a higher content of both dehydrogenases. The enzyme content in the latter group is comparable to that of the bacterium *Aerobacter cloacae*, which is known to have an active system of the hexose-monophosphate oxidative route[6]: these bacteria contain about 1.2 units soluble G6P-dehydrogenase and about 0.3 units of soluble GA6P-dehydrogenase per gram living material[7].

In a system containing 0.05 *M* Tris buffer pH 7.46, 35 μmole sodium gluconate-6-phosphate, 0.8 μmole TPN^+, 44 μmole pyruvate, 40 units rabbit-muscle lactic dehydrogenase and 0.2 units GA6P-dehydrogenase from the female genital tract of *Ascaris lumbricoides*, sedoheptulose was formed, and detected by paper chromatography.

These results show that a number of worms, free living and parasitic, contain the enzyme system for the hexose-monophosphate oxidative route, or at least a closely related one.

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Enzymic synthesis of guanosine and cytidine triphosphates: A note on the nucleotide specificity of the pyruvate phosphokinase reaction

The recent isolation of the phosphorylated derivatives of uridine, cytidine and guanosine raises the question of the exact nucleotide specificity of a number of enzymes which have been studied employing usually adenosine, and sometimes inosine, nucleotides. Pyruvate phosphokinase, the enzyme catalyzing the transfer of phosphate from phosphopyruvate to a nucleoside diphosphate, has already been shown to be active with ADP*, IDP and UDP[1-3]. It has now been found that phosphorylation of GDP and CDP is also catalyzed by this enzyme.

In this study pyruvate phosphokinase was prepared from rabbit muscle as described by KORNBERG AND PRICER[4]. The material obtained by this preparation approximated in purity the material reported by them. Activity was measured by coupling the reaction to lactic dehydrogenase[4]. The suitability of a nucleotide as an acceptor in this reaction was, therefore, observed as the oxidation of reduced diphosphopyridine nucleotide (DPNH) measured at 340 mμ, in the coupled reaction. Phosphopyruvic acid (barium salt) was the gift of Mr. W. E. PRICER, Jr., and DPNH was prepared chemically with sodium hydrosulfite[5].

It is believed that each of the nucleotides employed was free of other nucleotides, except where indicated. Where possible, several independent samples of each nucleotide were employed. The sources of the nucleotides were as follows:

ADP, obtained from Sigma Chemical Co., St. Louis;

IDP, (1) from Sigma Chemical Co., and (2) kindly supplied by Drs. K. KURAHASHI AND M. UTTER[6];

UDP, (1) prepared from UTP[7] (Pabst Laboratories, Milwaukee, Wisconsin) by incubating with a crude yeast hexokinase preparation and isolating the products by anion exchange chromatography**, and (2) prepared by hydrolysis of UDP N-acetylamino sugar compounds from *Staphylococcus aureus*[8];

GDP, (1) by hydrolysis of guanosine diphosphate mannose from hen's oviduct[9], and (2) from Sigma Chemical Co***;

* AMP, ADP, ATP, IMP, IDP, ITP, UMP, UDP, UTP, CMP, CDP, CTP, GMP, GDP, GTP are the 5'-mono-, di- and triphosphates of adenosine, inosine, uridine, cytidine and guanosine respectively.
** This preparation of UDP contained about 5% UMP.
*** Both preparations of GDP contained 5–10% GMP.

CDP, prepared by transphosphorylation between ATP and CMP*[10] and isolating the products by anion exchange chromatography.

Each of the nucleoside diphosphates employed served as a phosphate acceptor in the spectrophotometric test (Fig. 1). The relative rates of reaction with ADP, GDP, IDP, UDP and CDP (each at a concentration of about $4 \cdot 10^{-4}$ *M* and with pyruvate phosphokinase limiting) were 100, 19, 12, 3 and 2 respectively.

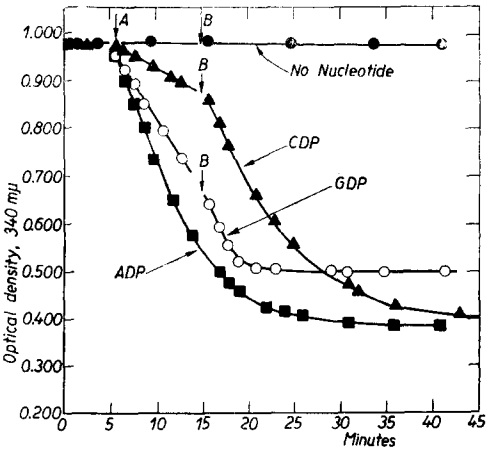

Fig. 1. Reaction of phosphopyruvate with ADP, GDP and CDP catalyzed by pyruvate phosphokinase. Each cuvette contained the nucleoside diphosphate indicated, 0.2 μM phosphopyruvate, 0.075 μM DPNH, and lactic dehydrogenase in 0.5 ml. 0.1 *M* potassium phosphate, 0.004 *M* $MgCl_2$, pH 7.0. At A, pyruvate phosphokinase was added in the relative amounts of 1 (ADP), 2.5 (GDP), 12.5 (CDP) and 12.5 (no nucleotide). At B, an excess of pyruvate phosphokinase was added to the latter three. The amounts of nucleoside diphosphate added and DPNH oxidized were as follows: 0.052 μM ADP, 0.049 μM DPNH oxidized; 0.043 μM GDP, 0.039 μM DPNH oxidized; 0.053 μM CDP, 0.049 μM DPNH oxidized.

In order to demonstrate that the corresponding triphosphates were formed in the reaction, incubation mixtures were electrophoresed in 0.02 *M* citrate buffer, pH 5.1[11]. In each case a nucleotide was formed which had a greater mobility than the nucleoside diphosphate (relative mobilities about 1.1:1). The compounds formed with ADP, IDP, UDP and CDP had the same electrophoretic mobilities as the corresponding triphosphates*. No sample of GTP was available for comparison.

In order to isolate GTP, a larger scale incubation was carried out with 48 μM GDP (Sigma), 85 μM phosphopyruvate and enzyme in 30 ml 0.004 *M* potassium phosphate, 0.003 *M* $MgCl_2$, pH 6.9. The course of the reaction was followed by removing aliquots and assaying for pyruvate with DPNH and lactic dehydrogenase. At the completion of the reaction (30 minutes) the incubation mixture was run onto a column of Dowex-1 Cl (2% cross-linked), and the products eluted by gradient elution employing mixtures of NaCl in 0.01 *N* HCl.

Two ultraviolet absorbing peaks were observed. The first, eluted at about 0.05 *M* NaCl in 0.01 *N* HCl, contained 3 μM of nucleotide. This compound had the same chromatographic and electrophoretic behavior as GMP, and was also found as an impurity in the starting material. A second peak, eluted at about 0.25 *M* NaCl in 0.01 *N* HCl, contained 39 μM nucleotide. The compound recovered from this peak by charcoal absorption and elution had an R_F in ethanol-ammonium acetate-versene[12] smaller than GDP, and a mobility in 0.02 *M* citrate, pH 5.1, greater than GDP. It contained 2.8 moles of total phosphorus per mole of guanosine, 1.9 moles of which were labile in 1 *N* HCl in 15 minutes. Guanosine was calculated from the 257 mμ absorption using 12,400 as the molar extinction coefficient. Ultraviolet absorption spectra between 220 and 310 mμ in 0.01 *N* HCl and 0.1 *N* NaOH corresponded to the acid and alkaline spectra of guanosine. By this evidence the compound has been characterized as guanosine triphosphate**.

Nucleoside diphosphokinase[11,14,15] was absent from the enzyme preparations employed. It is, therefore, believed that the several phosphorylations catalyzed by pyruvate phosphokinase are direct reactions, and do not involve the intermediacy of catalytic amounts of adenosine nucleotides. Recently several other reactions have been found for phosphorylation of UDP, CDP, IDP, and GDP[6,10,11,13–19].

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** The isolated GTP has also been found to react with AMP in the presence of an enzyme purified from calf liver with formation of two nucleoside diphosphates[13].

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Increased liver glucose-6-phosphatase activity after cortisone administration*

The behavior of liver glucose-6-phosphatase under physiological and pathological conditions has been under investigation in this laboratory. Studies of the glucose-6-phosphatase reaction in normal fasting animals[1], and in precancerous, neoplastic[2], regenerating, foetal and new born liver[3] have focussed our attention on the regulation of this enzymic activity in tissues.

It has been known that high doses of cortisone resulted in permanent diabetes in rat[4], characterized by hyperglycemia and glycosuria. Insulin resistant "cortisone diabetes" showing very high blood sugar levels and glycosuria was also described in human[5]. The increase in the quantity of carbohydrate after cortisone administration has been attributed to increased gluco-neogenesis and there is also evidence that some phases of the carbohydrate utilization are inhibited[6]. Recently however, it was demonstrated that cortisone administration caused a striking increase in the rate of glucose production[7]. Since glucose production from the liver is related to the rate of hydrolysis of the glucose-6-phosphate ester, it seemed of interest to study the glucose-6-phosphatase reaction in the liver of animals treated with high doses of cortisone. The preliminary results of this investigation are reported here.

Young male Wistar rats of 100 grams of weight were injected with 1 ml (25 mg) of cortisone acetate ("Cortone", Merck**) intramuscularly, daily for five days and were killed by decapitation on the sixth day. Control animals received injection of the vehicle**, in which the cortisone is dissolved. Animals were maintained on Purina Fox Chow and water *ad libitum* until sacrificed. Livers were pooled and the homogenates were prepared for enzymic studies as described in a previous communication[2]. The glucose-6-phosphatase activity was measured by the method of CORI AND CORI[8] using a 15 minutes incubation time. Liver glycogen was determined by the method of GOOD, KRAMER AND SOMOGYI[9] employing the NELSON'S adaptation of the SOMOGYI method for glucose[10]. The enzymic activities are expressed per wet weight, nitrogen, per average cell basis and also per liver weight/body weight ratio. Total nitrogen was determined by the micro-Kjeldahl procedure. Nuclear counts were done by the method of PRICE AND LAIRD[10] as modified by ALLARD *et al.*[11]. Blood glucose was determined by the method of FOLIN-WU.

The preliminary results are shown in Figs. 1 and 2. The results are presented as percentage changes from the control values which are taken arbitrarily as 100%.

Fig. 1 shows that cortisone injection increased the blood glucose level by 78%. The well known glycogenic effect of cortisone on the liver is also illustrated in Fig. 1. When liver glycogen is expressed on per cell basis the increase in glycogen content after cortisone administration is higher than when data are expressed on the conventional gram per cent wet weight basis.

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