fraction and incorporated into the DNA fraction, while in embryos treated with cycloheximide the uptake of [^{3}H]thymidine remained unchanged but thymidine incorporation into the DNA fraction was inhibited (table 1). In embryos cultured in the absence of cycloheximide (control), chromatographic analysis showed that the acid-soluble fraction consisted mainly of [^{3}H]thymidine ([^{3}H]TdR) and the amounts of its phosphorylated derivatives ([^{3}H]thymidine tri-, di-, and monophosphate, abbreviated as [^{3}H]dTTP, [^{3}H]dTDP and [^{3}H]dTMP, respectively) were relatively low, whereas in embryos treated with cycloheximide, the acid-soluble fraction consisted mainly of [^{3}H]dTTP and [^{3}H]TdR, the former accounting for almost all the radioactivities in phosphorylated derivatives and the latter decreasing to nearly half of that of the control (table 1). From the above and previous results[13], it is suggested that blastulae synthesize DNA very actively, and thus [^{3}H]dTTP is incorporated into DNA as soon as formed so that it does not accumulate in the acid-soluble fraction in the control. However, in embryos treated with cycloheximide, the step of polymerization to form DNA is partially inhibited, and the requirement of dTTP for polymerization decreases so that [^{3}H]dTTP accumulates in the acid-soluble fraction. The results are consistent with those described previously[13]. The in vitro activities of the enzymes involved in DNA synthesis were compared in the presence and absence of cycloheximide. Table 2 indicates that only DNA polymerase activity is inhibited by cycloheximide. Purified DNA polymerase from sea urchin nuclei was not inhibited at all by cycloheximide at 4 mM. These results, therefore, suggest that only the step of polymerization of deoxyribonucleoside triphosphate to form DNA requires concomitant protein synthesis, and the other phosphorylating enzymes do not.

Table 2. Effect of cycloheximide on the in vitro activities of DNA polymerase, thymidine kinase and thymidylate kinase in blastulae

Enzyme	Control (cpm/μg protein)	%	10 mM cycloheximide (cpm/μg protein)	%
DNA polymerase	25.4	100	15.5	61
Thymidine kinase	4.79	100	4.83	101
Thymidylate kinate	8.99	100	8.64	96

Insoluble zinc-precipitated phosphomonoesterase from rat kidney

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Summary. The insoluble form of phosphomonoesterase precipitated by means of Zn^{2+}-ions was prepared from the partially purified extract of rat kidney. The freeze-dried preparation of the precipitated enzyme is highly active and is stable on heating at 100 °C.

Recently the insoluble form of uridine kinase of a high stability and enzyme activity was prepared by the precipitation of the enzyme by divalent metal ions[1]. Uridine kinase and other enzymes taking part in the course of metabolic conversions of pyrimidine analogues used as cytostatics, are of primary importance in determining the biological activity of these compounds. However, tumour cells resistant to these drugs often lack the enzymes necessary for their conversion[2]. The aim of our study is to substitute the missing enzymes and to enhance the deleted pathway in mutant cell-lines.

In this communication, evidence is presented that zinc-precipitated protein fraction from rat kidney displays the activity of non-specific phosphomonoesterase. Since heterogeneous alkaline phosphatase is extremely active in the kidney[3] and zinc-containing phosphatases were isolated from various sources (e.g.[4]), the technique described can be used for the simple enzyme preparation in the insoluble form.

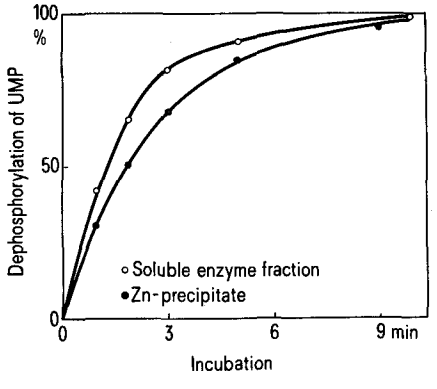

Fig. 1. Time course of UMP dephosphorylation using soluble and Zn-precipitated forms of phosphomonoesterase from rat kidney. 0.5 m*M* Uridine-2-^{14}C 5′-monophosphate was incubated at 37 °C in 0.3 ml of 66 m*M* *tris*-HCl buffer (pH 7.4) with 1.06 mg proteins in the soluble enzyme fraction or 1 mg of Zn-precipitated and lyophilized enzyme preparation.

Material and methods. The preparation of an enzyme extract from rat kidney homogenate in 25 m*M* *tris*-HCl (pH 7.4) containing 25 m*M* KCl and 5 m*M* $MgCl_2$ was the same as described earlier[1]. The partially purified enzyme fraction was precipitated under cooling with Zn^{2+}-ions added to 5 m*M* concentration, the precipitate centrifuged and freeze-dried[1]. The activity of phosphomonoesterase was assayed at 37 °C, using uridine-2-^{14}C 5′-monophosphate (spec. radioactivity 44 mCi/mmol) as the substrate. Separation of incubation mixture (66 m*M* *tris*-HCl, pH 7.4, total volume 0.3 ml) was carried out chromatographically on Whatman paper No. 1 in a solvent system composed of isobutyric acid-ammonium hydroxide-water

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(44:1:22). The radioactive zones were cut out and their radioactivity was measured in a Packard liquid-scintillation spectrometer.

Results and discussion. Zinc-precipitated and lyophilized protein fraction, partially purified from rat kidney cytosol by 20–35% ammonium sulfate saturation[5], retains its activity to dephosphorylate UMP as the soluble enzyme. The insoluble fraction of precipitated proteins is able to phosphorylate in the presence of ATP uridine and pyrimidine nucleoside analogues to corresponding 5′-nucleotides (active uridine kinase[1]) but not to transform uridine to uracil or to catalyze its synthesis from uracil and ribose-1-phosphate. The fraction of precipitated proteins displays the activity of nonspecific phosphomonoesterase and catalyzes also the splitting of uridine 2′(3′)-phosphate and 2,4-dinitrophenyl phosphate.

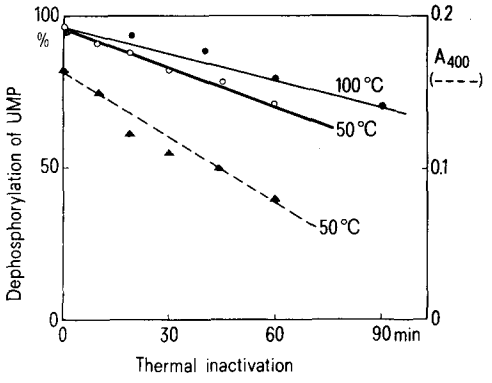

Fig. 2. Thermal stability of zinc-precipitated phosphomonoesterase in solution at 50 °C and in dry state at 100 °C. 0.25 m*M* UMP-2-^{14}C was incubated 5 min at 37 °C in 0.3 ml of 66 m*M* *tris*-HCl buffer (pH 7.4) containing Zn-precipitated enzyme (1 mg) heated for different period of time at 100 °C (●—●) or preincubated in the above buffer at 50 °C (○—○). The degradation of 12 m*M* 2,4-dinitrophenyl phosphate by the enzyme preincubated at 50 °C was measured (A_{400}) at pH 7.4 after 10 min incubation at 37 °C and dilution with 0.5 *M* NaOH (▲—▲).

The time course of UMP dephosphorylation using soluble and zinc-precipitated fractions of the kidney enzyme (Figure 1) indicates that both enzyme preparations split UMP to the same degree. Also kinetic constants of UMP dephosphorylation by the two forms of the enzyme are similar. Since in our system the inhibition by excess of the substrate occurred and a complex character of UMP degradation by the present enzymes was observed, the dephosphorylation was characterized only by the substrate concentration (2.7 m*M*) at which the rate of enzyme reaction was equal to one-half of maximal velocity of the substrate disappearance.

In contrast to the soluble from of phosphomonoesterase, zinc-precipitated enzyme is highly resistant to thermal inactivation. While the soluble enzyme fraction is completely inactivated by a 5 min incubation at 100 °C, the activity of zinc-precipitated and lyophilized proteins after their heating at 100 °C (in unsealed ampules using a sterilizing oven) is almost unchanged (Figure 2). Also the activity of metal complexed enzyme preincubated at 50 °C in a buffered medium is 30–40% higher than that of the soluble one. For comparison, the decomposition of 2,4-dinitrophenyl phosphate by precipitated and freeze-dried enzyme fraction preincubated at 50 °C is presented (Figure 2).

The partially purified extract of rat kidney used for the precipitation contains a number of proteins obviously participating in the final form of the precipitate. Although it is difficult to speculate about the form of active enzymes in coagulated precipitates, the precipitation by zinc ions offers a simple method to prepare a stable protein fraction possessing phosphomonoesterase activity.

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The action of D-penicillamine on cytochrome oxidase in vivo and in vitro

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Summary. The activity of cytochrome oxidase greatly decreases in the organs of rats treated with D-penicillamine for 20 days (30 mg/100 g/day). Although the drug does not affect cytochrome oxidase in vitro, it readily reduces oxidized cyt. c.

D-penicillamine is the well-known chelating agent used in the treatment of Wilson's disease[1] and of several heavy metal intoxications[2,3]. In a previous paper[4] we have reported that D-penicillamine decreases the content of iron, zinc and copper of most organs and tissues of the rat, and that this reduction significantly affects the concentration of blood ceruloplasmin and of the superoxide-dismutase activity of some tissues. With the aim of finding out whether D-penicillamine acts also upon other metalloproteins, we have studied the activity of cytochrome oxidase in rats treated with the drug. It has been reported[5] that cytochrome oxidase is very sensitive to copper deficiency and that the evaluation of its activity is a satisfactory indicator of the clinical copper status in mammals.

The marked effect observed in vivo suggested to us to extend the study to liver mitochondria preparations to investigate whether D-penicillamine affects cytochrome oxidase also in vitro.

Materials and methods. 12 male Wistar rats of about 250 g, fed with pellets of standard composition, were injected parenterally with D-penicillamine (15 mg/100 g body weight every 12 h) for 20 days. 10 rats were used as controls. The organs of each animal were homogenized with 9 vol. of 0.25 M sucrose in 0.1 M phosphate buffer pH 7; the suspended particles were solubilized by adding Tween 80, then centrifuged at 15,000 × g and the clear supernatant was employed for the determination of the

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