column. Elution was then performed with 0.01 *M*, pH 4.6 citrate buffer, 1 *M* in NaCl. Enzymatic activity was assayed with the 4-methyl-umbelliferone substrate as previously described[5].

Double diffusion analyses showed the precipitin line with crude extracts of human liver but not with rabbit (the source of the antibody), sheep, calf, mouse or rat liver. Nonetheless, we suspected that a cross-reaction with some of these species might be present but that the avidity of the antibody would be insufficient for a precipitation reaction (cross-reaction with a single hapten per enzyme molecule seemed less likely). Such low avidity antibodies may allow elution from immunoadsorbent columns at milder conditions. As seen in the figure, rat and mouse liver *N*-acetyl-$\beta$-hexosaminidase were retained by the immunoadsorbent column and readily eluted in 1.0 *M* NaCl. However, calf, sheep (not shown), and rabbit (as expected) liver *N*-acetyl-$\beta$-glucosaminidase were not retained by the column. Despite large variations in the amount of enzyme applied to the columns, similar amounts were retained by the immunoadsorbent column suggesting that each of the antigenic preparations saturated the available antibody-combining sites about equally well. As seen in the table, the immunoadsorbent column capacity was roughly similar for human, mouse and rat liver *N*-acetyl-$\beta$-glucosaminidase. The 79-(mouse) and 44-(rat)fold purifications achieved with the heterologous antigens were actually higher than those achieved with the homologous antigen but this is explained because the human liver starting material was partially purified.

Immunoadsorption purification of enzyme from other species extends the usefulness of an antibody prepared to a purified enzyme of one species. Similar capacities and purification factors are achieved and elution may occur more readily[6].

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## Reaction step requiring protein synthesis in DNA synthesis in sea urchin embryos

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*Summary.* In the acid-soluble fraction extracted from embryos treated with cycloheximide, thymidine triphosphate was largely accumulated and DNA polymerase activity decreased, while both activities of thymidine kinase and thymidylate kinase remained unchanged.

It has been demonstrated that the initiation of in vivo DNA synthesis is dependent on protein synthesis[2–12]. The present study was conducted to investigate which step in the reaction sequence of DNA synthesis from deoxyribonucleosides requires protein synthesis.

*Materials and methods.* Embryos of the sea urchin, Hemicentrotus pulcherrimus, were cultured to the blastula stage at a population density of $3.5 \times 10^4$ per ml in artificial sea water containing 300 units of penicillin and 50 $\mu$g of streptomycin per ml for 20 h at 20 °C. Then the suspension was divided into 2 parts. One part was allowed to develop as before (control) and the other was incubated with 10 mM cycloheximide. 60 minutes after adding cycloheximide, samples (10 ml, $3.5 \times 10^5$ embryos) of each suspension were exposed to 25 $\mu$Ci, 2.38 nmoles of [$^3$H]-thymidine for 10 min at 20 °C. After reaction, radioactivities in the medium, the acid-soluble and DNA fractions were measured, and then the acid-soluble fraction was chromatographed and the fractions separated were analyzed as described previously[13]. The activities of DNA polymerase, thymidine kinase and thymidylate kinase were assayed as described previously[13].

*Results and discussion.* In normal embryos (control), much added [$^3$H]thymidine was taken up into the acid-soluble

Table 1. Effect of cycloheximide on the uptake and incorporation of [$^3$H]thymidine in blastulae and relative proportions of [$^3$H]thymidine and its phosphorylated derivatives in the acid-soluble fraction

| | Control (cpm $\times 10^{-5}$) | % | 10 mM cycloheximide (cpm $\times 10^{-5}$) | % |
|---|---|---|---|---|
| Medium after incubation | 45.06 | 23.4 | 67.82 | 35.6 |
| Acid-soluble fraction | 96.25 | 50.0 | 98.25 | 51.5 |
| dTTP | 8.76 | (9.11) | 45.85 | (46.60) |
| dTDP | 2.32 | (2.41) | 3.55 | (3.61) |
| dTMP | 2.52 | (2.62) | 4.38 | (3.45) |
| TdR | 82.50 | (85.80) | 44.92 | (45.65) |
| DNA fraction | 51.02 | 26.6 | 24.39 | 12.9 |
| Total | 192.34 | | 190.46 | |

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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.

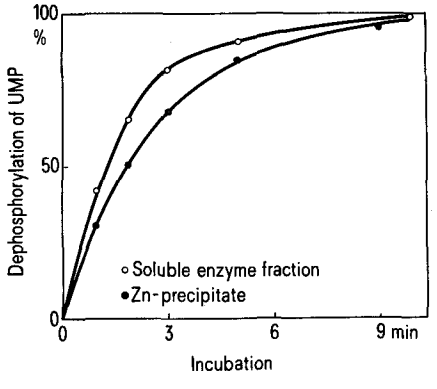


Fig. 1. Time course of UMP dephosphorylation using soluble and Zn-precipitated forms of phosphomonoesterase from rat kidney. 0.5 m*M* Uridine-2-$^{14}$C 5′-monophosphate was incubated at 37 °C in 0.3 ml of 66 m*M* *tris*-HCl buffer (pH 7.4) with 1.06 mg proteins in the soluble enzyme fraction or 1 mg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