The Effect of Isoproterenol upon the Activity and Intracellular Distribution of Pyrimidine Nucleoside Kinases in the Mouse Parotid Gland*

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SUMMARY

The pyrimidine deoxyribonucleoside kinase activities were very low in the normal mouse parotid gland, while uridine kinase activity was high. Isoproterenol administration increased uridine, deoxycytidine, and thymidine kinase activities 2-, 10-, and 30-fold, the enzymes reaching maximum activity at 12 to 14, 27, and 30 hours after isoproterenol injection, respectively. DNA synthesis was maximal at 27 hours.

In control glands 25% of the deoxycytidine and thymidine kinase activities were present in a nuclear fraction and 40% of the uridine kinase activity in the microsomal fraction. Most of the remainder of their activities was present in the high speed supernatant fraction. After isoproterenol injection the activities of these enzymes in the nuclear, mitochondrial, and microsomal fractions changed only slightly, while 97% of the increased activities was present in the cytosol fraction.

Sedimentation of crude homogenates on sucrose gradients showed that the nuclear and cytoplasmic forms of the deoxyribonucleoside kinases had the same molecular weights, being 65,000 for deoxycytidine kinase and 70,000 for thymidine kinase. Isoproterenol produced no change in the sedimentation characteristics of deoxycytidine kinase, but three new thymidine kinase species with molecular weights of 55,000, 95,000, and 130,000 were present under varying conditions.

A single intraperitoneal injection of isoproterenol produces, after a lag period of about 20 hours, a marked increase in DNA synthesis in the salivary glands of both rats (1) and mice (2). This organ is the only one in rodents with the exception of the kidney (3) to respond to isoproterenol with cellular proliferation.

Changes in protein synthesis (2, 4, 5), RNA synthesis (6), glycolipid synthesis (7), glycoprotein concentration (8), cyclic adenosine 3' : 5'-monophosphate levels (9), and the activity of several enzymes (2, 9, 10) have been described in mouse salivary gland cells in the prereplicative period between the administration of isoproterenol and the onset of DNA synthesis.

The activities of thymidine kinase (EC 2.7.1.21) (11) and deoxycytidine kinase (12) are extremely low in resting tissues, but their activity increases markedly in tissues which are stimulated to proliferate. Uridine kinase (EC 2.7.1.48) activity is also higher in proliferating tissues (13). It has been suggested that changes in either the activity (14) or the intracellular distribution (15) of the pyrimidine deoxyribonucleoside kinases might be important mechanisms for the control of DNA synthesis. Thymidine kinase has been shown to increase in activity in the salivary gland after isoproterenol injection (1, 11), but its distribution within the acinar cells of this gland is not known.

In this study, the subcellular distribution of the pyrimidine nucleoside kinase activities in the nonstimulated mouse parotid gland and the effect of isoproterenol on both the activity and distribution of these enzymes are described.

EXPERIMENTAL PROCEDURE

Methods

Tissue Fractionation by Differential Centrifugation—Fels A or Porton female mice were used when between 4 and 5 months old and weighing 24.5 to 28.5 g. The animals were kept on a 12-hour light and dark schedule and fed ad libitum. Isoproterenol-HCl (4.5 mg per animal) was dissolved in water and administered by intraperitoneal injection in a volume of 0.2 ml. The animals were killed by cervical dislocation and the parotid glands removed and chilled in ice-cold Medium A (0.32 M sucrose in 0.05 M Tris-HCl, pH 7.4 at 20°C, 0.025 M KCl, 0.003 M MgCl₂, and 0.002 M CaCl₂). Calcium was added to the isolation medium as it has been shown to aid in the retention of soluble enzymes in the nucleus during
tissue fractionation (16). The pooled glands from 6 to 12 animals were blotted dry, mixed with a razor blade, and homogenized with twelve strokes at 1000 rpm in 8 volumes (w/v) of Medium A by means of a glass Potter-Elvehjem homogenizer fitted with a Teflon pestle (clearance 0.028 cm). The homogenate was passed through two layers of cheesecloth and an aliquot retained for the assay of the total homogenate. The remainder of the filtrate was then subjected to tissue fractionation as summarized in Fig. 1. Only the supernatants from the first washings of the nuclear sediment and the mitochondrial fraction were combined with the supernatant from the previous centrifugation.

Pure nuclei were prepared from the crude nuclear pellet by means of the method of Blobel and Potter (17) and in nonaqueous media with the use of the procedure previously described (18), except that the maximum cyclohexane-carbon tetrachloride density used was 1.315. The yield of nuclei was determined as previously described (18).

All particulate fractions were finally resuspended in Medium A. It has previously been shown that it is necessary to break open the subcellular organelles for the maximum activity of the pyrimidine nucleoside kinase to be expressed (18, 19). Therefore, before assaying for these enzymes the subcellular fractions were routinely subjected to three cycles of freezing and thawing.

**Sucrose Density Gradient Centrifugation**—Enzyme preparations (2 to 6 mg of protein in 1 ml) were layered onto 38 ml of a 5 to 20% linear sucrose gradient containing 50 mM Tris-HCl, pH 7.9, at 4°C and 5 mM β-mercaptoethanol and centrifuged at 26,500 rpm in an SW 27.1 Spinco rotor for 48 hours at 4°C. Fractions (1 ml) were collected and aliquots assayed for enzyme activity. Supernatant enzyme fractions contained sufficient hemoglobin for it to act as an internal marker, and hemoglobin was added to nuclear preparations. The distribution of hemoglobin in the gradient after centrifugation was determined by measuring the optical density at 430 nm, and separate gradients were aligned by matching the hemoglobin peaks. Aldolase was also added to some gradients as a molecular weight marker.

**Measurement of Protein Purity**—Purity of the individual subcellular fractions was evaluated both by electron microscopic study and the use of the following enzymes as specific markers. NAD-pyrophosphorylase for nuclei, β-hydroxybutyrate dehydrogenase for mitochondria, glucose 6-phosphatase for microsomes, (Na+,K+)-ATPase for the plasma membrane, and glucose 6-phosphate dehydrogenase for the cytosol (20).

**Enzyme Assays**—Nucleoside kinase activities were determined by means of the assay procedure of Ives et al. (21). The optimal conditions for the assay of thymidine and deoxyadenosine kinase with the use of this assay have been determined previously (21, 22). The final concentrations of reagents common to both reaction mixtures, in a total volume of 80 μl, were ATP, 10 mM; β-mercaptoethanol, 10 mM; MgCl2, 10 mM; NaF, 12.5 mM; phosphoenolpyruvic acid, 15 mM; Tris-HCl, pH 8.0 at 37°C, 100 mM; pyruvate kinase, 2 μg; myokinase, 1 μg; and [5-3H]deoxythymidine or [5-3H]deoxyadenosine, 0.02 μM, or [5-3H]uridine, 0.2 μM (0.5 μCi per assay).

Deoxyadenosine deaminase activity was determined as described previously (12).

**Materials**

[^-3H]Deoxyadenosine (10 Ci per mmole), [5-3H]deoxythymidine (26.7 Ci per mmole), [5-3H]UMP (6.63 Ci per mmole), [5-3H]uridine (28.4 Ci per mmole), [5-3H]deoxyguanosine (8.8 Ci per mmole), and [5-3H]thymidine (33 Ci per mmole) were purchased from Amersham-Searle. Isoproterenol was kindly provided by Sterling-Winthrop. Pyruvate kinase (330 e.u. per mg of protein), myokinase (1070 e.u. per mg of protein), and all other reagents for the enzyme assays were products of Sigma Chemical Co. All chemicals were of reagent grade.

**RESULTS AND DISCUSSION**

**Parameters of Nucleoside Assay System**

Initial studies were conducted with a crude homogenate of the parotid gland to confirm that the assay conditions previously shown to be suitable for the determination of the pyrimidine nucleoside kinases from other tissues (18, 21, 22) were appropriate for this system. The concentrations of ATP and MgCl2 and the pH employed were all found to be at or near optimal. An ATP-regenerating system was essential to obtain a linear reaction course over any extended period.

The rate of formation of nucleotides from deoxyadenosine and deoxythymidine by deoxyadenosine and thymidine kinases was linear with respect to time up to at least 30 min and from uridine by uridine kinase for 15 min (Fig. 2A). Assays were carried out over a time period of 30 min from the former two kinases and 15 min for the latter kinase. Linearity of reaction with changing protein concentration was observed up to 300 μg protein per assay (Fig. 2B).

Since a number of mouse tissues contain high levels of deoxycytidine deaminase (12), the possibility that apparent deoxycytidine phosphorylation might result from the deamination of deoxycytidine to deoxyuridine and the phosphorylation of this latter compound by thymidine kinase was investigated. Two lines of evidence suggest that this pathway was not operative to any significant extent. Firstly, although deoxycytidine deaminase activity was detectable in homogenates of the parotid gland, its activity was very low (<10 pmoles of deoxycytidine deaminated per 30 min per mg of protein) and increased only slightly 27 hours after isoproterenol administration. Secondly, the addition of 1 mM deoxyuridine to the assay system for deoxycytidine kinase did not reduce the measured degree of phosphorylation of deoxyadenosine.

**Effect of Isoproterenol on Pyrimidine Nucleoside Kinase Activities**

The time course of the effect of a single injection of isoproterenol upon the pyrimidine nucleoside kinase activities is shown in Fig. 3. In the control parotid gland the activities of thymidine and deoxycytidine kinases were extremely low. Upon stimulation of the gland by isoproterenol there was a lag phase followed by large increases in the specific activity of these kinases. Deoxycytidine kinase activity began to increase at about 12 hours, reached a peak at 27 hours, and then fairly rapidly declined. Thymidine kinase activity showed a longer lag period, only starting to rise at 20
FIG. 2 (left). Parameters of the assay system for nucleoside kinases. A, time course of nucleoside phosphorylation. B, variation in the rate of reaction with enzyme concentration. The combined total homogenates from glands 27 and 30 hours after isoproterenol stimulation were used as the enzyme source. A, assay mixtures, total volume 400 μl, were set up containing components as described under "Methods." Enzyme (1.6 mg of protein) was added at zero time and the reaction mixtures incubated at 37°. At the times indicated 50-μl aliquots were removed, the reaction terminated, and the formation of nucleotide determined as described under "Methods." B, varying amounts of homogenate were added to reaction mixtures set up as detailed.

hours, and reaching a peak at 30 hours, which was again followed by a rapid decline. DNA synthesis began to increase at 20 hours and was maximal at 27 hours. Thus the peaks of DNA synthesis and deoxycytidine kinase activity coincide, but maximal thymidine kinase activity is only reached after the rate of DNA synthesis has begun to decline. A similar phenomenon has been observed in synchronized L cells (25) and HeLa cells (26), and in phytohemagglutinin-stimulated human lymphocytes (27). In contrast, deoxyadenosine and deoxyguanosine kinase activities were high in the normal parotid gland and were not altered 27 hours after isoproterenol administration (Table I). Therefore, the increase in deoxycytidine and thymidine kinases are not part of a completely general mechanism whereby all the enzymes of the DNA precursor salvage pathways are elevated in activity.

Uridine kinase activity was not increased at 27 hours after isoproterenol injection, but there was a small, but significant, rise in activity at 12 and 16 hours (Fig. 3). This increase corresponds to the period of rapid ribosomal RNA synthesis in the parotid and the time of the increase in the specific activity of total RNA when [3H]uridine is used as the label (6).

Composition of Subcellular Fractions

The distribution of a variety of marker enzymes among the subcellular fractions of the parotid gland is seen in Table II. The distribution of glucose 6-phosphate dehydrogenase indicates that the nuclear fraction was contaminated with no more than 3% whole cells. Observation of the nuclear fraction by both light and electron microscopy revealed an even smaller contamination with whole parotid cells. The preponderance of NAD-pyrophosphorylase in this fraction indicates that few of the nuclei were disrupted upon homogenization.

The mitochondrial fraction contained only a partial enrichment of mitochondria, the rest of this fraction consisting of rough endoplasmic reticulum as judged by electron microscopy. The activity of mitochondrial enzymes was therefore low, but β-hydroxybutyrate dehydrogenase activity was only measurable in this fraction. The mitochondria in the parotid gland are concentrated mainly in the striated duct epithelium, with the acinar cells which form the bulk of the tissue containing only scattered mitochondria (28). The striated duct epithelium is situated in close conjunction to connective tissue, and during the mild homogenization conditions employed these cells are likely to remain unbroken and be retained preferentially on the cheesecloth, along with the connective tissue. That this occurred is supported by the observations that the specific activity of DNA is higher in filtered homogenate than in the whole gland after [3H]deoxythymidine labeling following isoproterenol stimulation1 and that the stimulation of DNA synthesis by

1 R. Baserga, personal communication.
isoproterenol occurs primarily in the acinar cells (2). Thus the procedure used would indeed appear to select for the acinar cells.

The microsomal fraction appeared to contain a strong enrichment of plasma membrane fragments, as indicated by the concentration of (Na+, K+)-ATPase in this fraction. This observation has been made the starting point of a method for the purification of plasma membranes.

Both the activity and distribution of the marker enzymes were identical in control and isoproterenol-stimulated parotid glands.

### Comparison of Nuclear Preparations

Although thymidine and deoxyuridine kinases are often thought of as soluble enzymes which will readily enter and leave nuclei, recent evidence shows that nuclei isolated in aqueous sucrose media contain the same level of thymidine kinase activity as those isolated by nonaqueous techniques (18, 19). Table III shows that this is also the case with the mouse parotid nuclei prepared in cyclohexane-carbon tetrachloride having a thymidine kinase activity very similar to that present in the crude nuclear fraction prepared in sucrose media. However, if the aqueous nuclear fraction was purified by means of the technique of Blobel and Potter (17) the nuclei then contained less than 10% of the thymidine kinase activity present in the original fraction. Similar losses of activity on preparing purified nuclei in concentrated sucrose solutions have been observed with rat liver (29). Electron microscopy showed that the purified nuclei had lost a large proportion of their contents. Loss of material from the nuclei was also indicated by the recovery of only 9% of the cellular protein in the Blobel and Potter nuclei. This is considerably less than the 13 to 15% which is commonly reported in the literature for nuclei purified in hypertonic sucrose and the 17% observed in nuclei purified in nonaqueous media. It was decided, therefore, to use the crude nuclear fraction in studies on the subcellular localization of the pyrimidine kinases.

### Intracellular Distribution of Nucleoside Kinases

The intracellular localization of the pyrimidine nucleoside kinases has been investigated in both control glands and at the times of the stimulation of enzyme activity by isoproterenol. Any change in the distribution of the pyrimidine nucleoside kinases that is related to the increases in DNA and RNA synthesis produced by isoproterenol would be expected to be manifest at these times.

#### Uridine Kinase—The distribution of uridine kinase activity among the subfractions of mouse parotid gland homogenates

Given in Table IV. Activity in the nuclear fraction was within the bounds of whole cell contamination. This contrasts with both rat thymus and liver in which an appreciable proportion of the total cell activity was found in purified nuclear fractions (18, 30). The mitochondrial fraction contained no measurable activity, but there was a considerable increase in the specific activity of uridine kinase in the microsomal fraction. This fraction contained 40% of the total homogenate activity, while the remaining activity was located in the supernatant. Although the microsomal fraction appears to contain plasma membrane fragments as judged by the presence of (Na+, K+)-ATPase activity, the uridine kinase is not located on this structure as a membrane-bound enzyme. Complete sedimentation from the homogenate of a molecule of this size will occur under our conditions of centrifugation so that the distribution is not an artifact of the subfractionation procedure. The reason for this extremely large size is not clear, but it would seem probable that it results from the association of the enzyme with other molecules, perhaps in a ribonucleoprotein complex.

Isoproterenol stimulation had a marked effect upon the distribution of uridine kinase. At 14 hours after isoproterenol administration there was a 2-fold increase in uridine kinase activity in the whole homogenate. However, activity in the microsomal fraction fell to 60% of the control value, while there was a 4-fold increase in the specific activity of the supernatant

### Table II

**Distribution of marker enzymes among subcellular fractions of mouse parotid glands.**

Conditions were as described under "Experimental Procedure." The figures in parentheses are the percentage of total recovered activity present in each fraction.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>NAD pyrophosphorylase</th>
<th>β-Hydroxybutyrate dehydrogenase</th>
<th>(Na+, K+)-ATPase</th>
<th>Glucose 6-phosphatase</th>
<th>Glucose 6-phosphate dehydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total homogenate</td>
<td>1</td>
<td>&lt;0.04</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Nuclei</td>
<td>4.1 (95)</td>
<td>0.05 (4)</td>
<td>0.7 (14)</td>
<td>2.4 (58)</td>
<td>0.11 (2.6)</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>&lt;0.04</td>
<td>1 (96)</td>
<td>6.4 (19)</td>
<td>4.7 (16)</td>
<td>0.08 (0.3)</td>
</tr>
<tr>
<td>Microsomes</td>
<td>&lt;0.04</td>
<td>&lt;0.04</td>
<td>14.5 (67)</td>
<td>2.8 (14)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Supernatant</td>
<td>0.00 (5)</td>
<td>&lt;0.04</td>
<td>&lt;0.2</td>
<td>0.2 (12)</td>
<td>1.38 (97.1)</td>
</tr>
</tbody>
</table>

### Table III

**Activity of thymidine kinase in crude and pure nuclei preparations**

Sixteen mice were used for each method of preparation of purified nuclei. Other conditions were as described under "Experimental Procedure."

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein</th>
<th>Specific activity</th>
<th>Total activitya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude nuclei</td>
<td>22.6</td>
<td>19.3</td>
<td>436</td>
</tr>
<tr>
<td>Blobel and Potter purified nuclei</td>
<td>3.0</td>
<td>4.3</td>
<td>40b</td>
</tr>
<tr>
<td>Nonaqueous media purified nuclei</td>
<td>1.4</td>
<td>27.0</td>
<td>454c</td>
</tr>
</tbody>
</table>

* a Per 100 mg of total homogenate protein as starting material.
* b Corrected for 32% recovery of nuclei.
* c Corrected for 8.2% recovery of nuclei.
fraction. By 27 hours after isoproterenol injection the situation had almost returned to that of the control, but there were still slight indications of increased activity in the homogenate and supernatant and decreased activity in the microsomal fraction. The interrelationships of the particulate and soluble forms of this enzyme are at present under study.

Deoxycytidine and Thymidine Kinases The distributions of thymidine and deoxycytidine kinases after fractionation of mouse parotid gland are shown in Table V. In control animals 23% of the total cellular activity of both enzymes was found in the crude nuclear fraction. This is similar to the distribution in pure nuclei from rat liver (19) and thymus (18), but quite different from the almost complete concentration of thymidine kinase in the nuclear fraction of mouse fibroblasts (31). The mitochondrial fraction contained only a small amount of these kinase activities, but this probably represents true mitochondrial activity as it is too high to be accounted for by cytosolic contamination (as measured by glucose 6-phosphate dehydrogenase distribution). A small amount of thymidine kinase activity has also been found in mitochondria from rat tissues (18). The deoxycytidine kinase activity in this fraction was considerably greater than the total activity measurable in the initial homogenate. Assays for phosphatase action with the use of [4C]ATP and [3H]dCMP revealed no significant breakdown of either substrate or product. Recombination of particulate fractions with the supernatant gave the activity to be expected from the addition of the activities in the separate fractions. Thus the increase in activity in the supernatant fraction does not appear to be due either to the removal during the fractionation of some inhibitor of deoxycytidine kinase activity of the sort that have been described for uridine kinase (39) and thymidine kinase (40), or to phosphatases breaking down dCMP or ATP. Rather, it seems that some physical change to the enzyme is occurring during the fractionation which results in an increased activity.

Twenty-seven hours after stimulation with isoproterenol the deoxycytidine and thymidine kinase activities in the total homogenate had risen 10- and 33-fold, respectively. However, the nuclear activity did not rise at all for deoxycytidine kinase, and thymidine kinase showed only a doubling in both specific and total activities. The percentage of total cellular activity of these enzymes present in the nuclear fraction fell to 3 and 2%, respectively. In the microsomal fraction the activity of both enzymes rose 6-fold, but the percentage of the total cellular activity in this fraction also fell. The supernatant fraction showed the same the cell and is still found in cells deficient in the cytoplasmic enzyme (36-38).

The greater proportion of the kinase activities was present in the supernatant fraction. The activity of deoxycytidine kinase in this fraction was considerably greater than the total activity measurable in the initial homogenate. Assays for phosphatase action with the use of [4C]ATP and [3H]dCMP revealed no significant breakdown of either substrate or product. Recombination of particulate fractions with the supernatant gave the activity to be expected from the addition of the activities in the separate fractions. Thus the increase in activity in the supernatant fraction does not appear to be due either to the removal during the fractionation of some inhibitor of deoxycytidine kinase activity of the sort that have been described for uridine kinase (39) and thymidine kinase (40), or to phosphatases breaking down dCMP or ATP. Rather, it seems that some physical change to the enzyme is occurring during the fractionation which results in an increased activity.

Each value is the average of four experiments. Numbers in parentheses are the percentage of total activity in each fraction. The distribution of protein among the various fractions is detailed in Table IV. Other conditions were as described under "Experimental Procedure."
activation as observed in control animals. Thus the increase in activity of this enzyme after isoproterenol administration is not the result of the same kind of molecular alteration of activity that appears to occur upon fractionation of the parotid gland.

Sucrose Density Gradient Centrifugation

The sedimentation behavior on sucrose density gradients of deoxycytidine and thymidine kinases from the cytosol and nuclei of control animals and the cytosol of isoproterenol-stimulated animals are shown in Figs. 4 to 6. Deoxycytidine kinase activity from all three preparations exhibited the same sedimentation profile, consisting of a single, almost symmetrical peak moving very slightly faster than hemoglobin, indicating a molecular weight of approximately 65,000. This is slightly larger than the deoxycytidine kinase of calf thymus, which has a molecular weight of 56,000 (22). Recovery of activity from the gradient was almost complete. This suggests that the nuclear and cytosolic activities of the control gland represent compartmentalized forms of the same enzyme and that the elevated deoxycytidine kinase activity induced by isoproterenol stimulation may represent an increase in the normal parotid gland enzyme.

The control gland nuclear and cytosolic thymidine kinase activities also sedimented as identical single peaks moving slightly faster than hemoglobin, indicating a molecular weight of approximately 70,000, which is very similar to the size of the enzyme from calf thymus (41) and regenerating rat liver (42) (Fig. 5). In contrast, the isoproterenol-stimulated gland cytosol enzyme activity exhibited a quite markedly different sedimentation profile (Fig. 6). Initially, a wide variation in the profile was observed between different preparations. The reason for this appears to be that although the enzyme activity is stable to freezing and thawing, this process alters the molecular form of the enzyme. Thymidine kinase which has been frozen has a major peak of activity associated with a protein of molecular weight of about 55,000 (Fig. 6A, Peak I), with aldolase and

Fig. 4. Sucrose density gradient centrifugation of deoxycytidine kinase from mouse parotid gland. Aliquots of the nuclear (4.7 mg of protein) and supernatant (5 mg of protein) fractions from control animals and the supernatant (3.1 mg of protein) fraction from isoproterenol-stimulated animals were placed onto sucrose gradients and centrifuged as described under “Methods.” The nucleoside concentration in the assays for kinase activity was 0.002 mM, and 60 μl aliquots were used for assay; other conditions were as described under “Experimental Procedure.” O—O, control cytoplasmic (k = 3); •—•, control nuclear (k = 3); □—□, isoproterenol-stimulated cytoplasmic (k = 4).

Fig. 5. Sucrose density gradient centrifugation of thymidine kinase from mouse parotid gland. Aliquots of the supernatant (9 mg of protein) and supernatant (9.9 mg of protein) fractions of control animals and the supernatant (9.9 mg of protein) fraction from isoproterenol-stimulated animals, which were given actinomycin D (1 μg per g of body weight) 4 hours after isoproterenol, were placed onto sucrose gradients and centrifuged as described under “Methods.” Other conditions were as described in Fig. 4 and under “Experimental Procedure,” except that 1 μCi of [3H]deoxythymidine was used in each assay. O—O, control cytoplasmic; •—•, control nuclear; □—□, isoproterenol-stimulated cytoplasmic + actinomycin D.

Fig. 6. Sucrose density gradient centrifugation of thymidine kinase from isoproterenol-stimulated mouse parotid gland. A, the supernatant fractions were quick frozen in Dry Ice and acetone and stored at −80° for 1 week prior to centrifugation. Aliquots of the control (9.1 mg of protein) and isoproterenol-stimulated (5.6 mg of protein) supernatant fractions were used. B, the supernatant fraction was centrifuged immediately upon preparation. Aliquots of the control (12.5 mg of protein) and isoproterenol-stimulated (8.9 mg of protein) supernatant fractions were used. Aliquots of 60 and 20 μl were used for the assay of the control and isoproterenol-stimulated gradient fractions, respectively. Other conditions were as described under “Methods,” except that 1 μCi of [3H]deoxythymidine was used per assay. •—•, control; O—O, isoproterenol-stimulated.
hemoglobin as the standards and applying the method of Martin and Ames (43) for the determination of molecular weights. There is also a minor peak with a molecular weight of about 95,000 (Fig. 6A, Peak II). Thymidine kinase which is applied straight to the gradient, on the other hand, shows activity associated solely with molecules of high molecular weight (Fig. 6B). Peak II now becomes a major peak, and there is a new peak (Peak III) of even higher molecular weight (mol wt approximately 130,000). There is also evidence of higher aggregational states as thymidine kinase activity does not fall off rapidly toward the bottom of the gradient, but rather it tails off slowly with activity present almost to the bottom of the gradient. Actinomycin D, administered at any time up to 10 hours after isoproterenol, completely prevented the appearance of these new molecular forms of thymidine kinase (Fig. 5).

Hashimoto et al. (44) have found that thymidine kinase in Yoshida sarcoma cells exhibits two peaks of activity with molecular weights of 70,000 and 120,000, and the enzyme induced in the adrenocorticotrophic hormone-stimulated adrenal gland (34) sediments faster in glycerol gradients than the thymidine kinase from the unstimulated gland. Thus it seems to be a quite general phenomenon that the thymidine kinase induced in conditions of stimulated proliferation is of higher molecular weight than the enzyme which is present in differentiated tissues.

The role of deoxycytidine and thymidine kinases in cells is still not clear. Presumably in most normal tissues they play a part in the salvage pathways (45). Their level of activity in a tissue might, therefore, be expected to reflect the extent of turnover and breakdown of DNA and thus the amount of substrate available to the salvage pathways in that tissue, as well as perhaps reflecting the rate of new DNA synthesis in the tissue. However, there is no apparent quantitative relationship between the levels of activity of these enzymes and the rate of DNA synthesis in normal (12) and neoplastic tissues (46), rather, in lymphoid origin (12). Nonetheless, both enzyme activities are almost always elevated when a tissue or cell culture is stimulated to a higher rate of DNA synthesis (11, 12). Again, these increases may reflect the fact that in such systems as virus-infected cell cultures (47), the isoproterenol-stimulated salivary gland (48), regenerating liver (49), and neoplasms (50) there is an increased rate of DNA breakdown as well as an increased rate of DNA synthesis.

The fact that the increases in the pyrimidine deoxyribonucleoside kinase activities in the isoproterenol-stimulated salivary gland (Table IV), the adrenocorticotrophic hormone-stimulated adrenal gland (34), and in regenerating liver (19) are predominantly in the cytoplasmic fractions also raises serious questions about the in vivo relevance of these changes with regard to DNA synthesis. Adams (31) has suggested that cytoplasmic thymidine kinase may not even be an active enzyme in vivo but a storehouse of metabolically inactive enzyme. Moreover, it would seem clear that the final end product of these enzymes’ action, that is the deoxyribonucleoside triphosphates, are going to be utilized primarily in the nucleus.

However, it is probable that a major source of deoxythymidine and deoxycytidine for reutilization comes from DNA breakdown in cells that are not making new DNA and are presumably dying. Thymidine and deoxycytidine (or their nucleotides) will be released from these cells to be reutilized by other cells as the nucleosides. The phosphorylation of these nucleosides in the cytoplasm of cells taking them up might thus provide a trapping mechanism (51), facilitate their transport through the cytoplasm, or simply be the most efficient way of converting nucleosides to nucleotides. Indeed, although nucleoside kinases may not be involved in the actual physical process of transport across the cell membrane (51, 52), there is evidence that the actual amount of deoxymethionine transported is in some way affected by thymidine kinase levels (53, 54). The nuclear activity of these enzymes would serve to scavenge any nucleoside resulting from phosphatase action on de novo synthesized nucleotides or nucleosides resulting from DNA breakdown or repair within the cell. Thus the nuclear to cytoplasmic ratio of these enzymes’ activities might be expected to vary widely depending upon whether nucleoside for the salvage pathway is being supplied endogenously or exogenously.

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