planted by the same carrier in normal lymphocytes (11). Table 2 shows that exposure of corticoid-sensitive P1798 lymphocytes to cortisol resulted in marked inhibition of ^{3}H-uridine uptake (15% at 2 hr; 30% at 3 hr) with no significant decrease in 2-deoxyglucose transport. Inhibition of uridine incorporation into tumor RNA paralleled the reduction of nucleoside uptake and was of similar magnitude (not shown). The specificity of this decrease is demonstrated by the absence of inhibitory effects when the inactive isomer, epicortisol, is used instead of cortisol. Furthermore, cortisol did not inhibit uridine uptake in cell suspensions prepared from corticoid-resistant P1798, suggesting that inhibition of uridine transport plays an important role in glucocorticoid-induced lymphocytolysis.

Discussion

Our results clearly demonstrate that glucocorticoid-induced inhibition of uridine transport in corticoid-sensitive P1798 lymphosarcoma does not depend on prior reduction of glucose uptake. Gabourel and Aronow reported that mouse lymphoma cells cultured in the presence of cortisol show a decreased content of protein and DNA with no concomitant reduction in glucose utilization or lactate production (2). Stevens *et al.* (12) demonstrated that inhibition of *in vivo* thymidine incorporation into mouse lymph node DNA and of leucine incorporation into protein occurred prior to cortisol effects on glucose uptake. Drews and Wagner (13) in a comparative study on the effects of glucose deprivation and prednisolone treatment on rat thymocytes conclu-

The TrT activity is routinely measured by polymerization of rATP into acid-insoluble form in the presence of $rA(pA)_5$ as initiator. rATP polymerization is carried out by incubating the enzyme at 35° in 0.2 M Tris Cl (pH 8.3), 4 mM 2-mercaptoethanol, 10 μM $rA(pA)_5$, 100 μg per ml of bovine serum albumin, 0.5 mM $MnCl_2$ and 0.5 mM [^{14}C]-rATP. The amount of product formed is measured as acid-insoluble radioactivity by the filter paper disk technique (13). One unit of enzyme activity is defined as one nmole of [^{14}C]-rATP polymerized per hour. Sucrose density gradient centrifugation of various fractions from our TrT preparation was carried out as previously described (12).

RESULTS

Purification. A crude soluble extract (Fraction 1) and phosphocellulose concentrate (Fraction 2) are prepared from calf thymus gland as previously described for the preparation of DNA polymerase (14) and terminal deoxynucleotidyl transferase (12). Fraction 2 contains most of TrT recoverable from fraction 1, as well terminal transferase and high molecular weight DNA polymerase. Residual nucleic acid is removed from Fraction 2 by passing through DEAE-cellulose equilibrated with 0.2 M KPi, pH 7.2, and then the protein is concentrated by adsorption and elution from phosphocellulose (cf 14). The phosphocellulose concentrated material, still containing TrT, terminal transferase and DNA polymerase, is dialyzed against 50 mM Tris-Cl, pH 8.0, containing 4 mM 2-mercaptoethanol. When the dialyzed material is passed through DEAE-cellulose (Whatmann DE-11) equilibrated with dialysis buffer the TrT is not adsorbed to this column whereas terminal transferase and DNA polymerase are. The flow through from the DE-11 column is concentrated by precipitation at 70% $(NH_4)_2SO_4$ saturation and dissolved in 0.1 M KPi, pH 7.5 (Fraction 3). Fraction 3 is then filtered through a 2.54 x 90 cm column of Sephadex G-100 run in 0.1 M KPi, pH 7.5. The active TrT fractions, appearing in the retarded volume of the column, are pooled (Fraction 4) and adsorbed directly onto a 1 x 4 cm column of hydroxylapatite (Biorad HTP) equilibrated with 0.1 M KPi, pH 7.5. A linear gradient to 0.3 M applied to the

TABLE I

Purification of Terminal Riboadenylate Transferase from Calf Thymus Gland*

Fraction	Protein mg	Activity Units $X10^{-6}$	Specific Activity Units per mg
1 Crude, Soluble	76797	10.21	133
2 Phosphocellulose	3722	4.76	1,279
3 $(NH_4)_2SO_4$	64.9	0.95	14,607
4 G-100	12.2	0.61	50,000
5 Hydroxylapatite	0.8	0.34	425,000

*All values referred to 2 Kg of thymus gland

hydroxylapatite column produces homogeneous TrT (Fraction 5), eluting at about 0.2 M KPi in the gradient. As seen in Table 1, our best preparation has a specific activity of 400,000 nmoles per hour per mg. It stores well at -20° in 50% glycerol but slowly loses activity. The final product runs as a single band of protein and activity on acrylamide disc electrophoresis at pH 8.9, and on electrophoresis in SDS shows a single band with a molecular weight of about 60,000. Optimum activity is seen at around pH 8.3.

The Mg^{++}-dependent activity (1) was isolated with bound polynucleotide. We therefore examined the sedimentation rate of the Mn^{++}-TrT on 5-20% sucrose gradients (0.1 M in KPi, pH 7.5) through the course of purification. A single peak of activity sedimenting at about 3.5 S was observed in crude soluble fraction (Fraction 1), the concentrated $(NH_4)_2SO_4$ fraction (Fraction 3), and the homogeneous hydroxylapatite fraction (Fraction 5). From this result, and the extremely low rate of self initiation (see Table II, below) it appears that the activity is not associated with any large fragments of nucleic acid.

Properties. We have used oligoribonucleotide initiators during purification

TABLE II

Oligonucleotide Acceptors

Oligomer	Polymerization Rate (nmoles/hr/μg)
--	12
rA(pA)	43
$rA(pA)_2$	220
$rA(pA)_3$	229
$rA(pA)_4$	224
$rA(pA)_5$	221
$r(Ap)_2$	<1
$r(Ap)_3$	5
$r(Ap)_4$	<1
$r(Ap)_5$	<1
$r(Ap)_6$	<1

Each reaction mixture (.25 ml) contained 0.5 mM [^{3}H]-rATP (6794 cpm per nmole), 0.5 mM $MnCl_2$, 200 mM Tris-Cl pH 8.25, 4 mM mercaptoethanol, TrT and 10 μM oligomer as indicated.

and Table II shows that a trinucleoside diphosphate with a free 3'-OH is sufficient for initiation to occur. If a 3'-PO_4 is present on the oligonucleotide it is inactive. The enzyme also seems to self-initiate slowly, but we have not analyzed the product for 5'-triphosphate ends, nor have we attempted purification of ATP to remove any endogenous acceptors that may be present. ADP is not polymerized, ruling out polynucleotide phosphorylase activity.

Only rATP is polymerized (Table III) and Mn^{++} is about 5 times as effective as Mg^{++} for activation. Direct tests with radioactive GTP, CTP, and UTP show less than 1% of ATP polymerization rates. Deoxy ATP is not polymerized nor are oligodeoxynucleotides utilized as initiators to any appreciable extent, ruling out terminal deoxyribonucleotidyl transferase activity. Other experiments (not presented) show that ribonu-

TABLE III

Specificity of Nucleotide Polymerization

Nucleotide	Metal	Polymerization Rate (nmoles/hr/μg
rATP	$MnCl_2$	160
rATP	$MgCl_2$	27.5
rGTP	$MnCl_2$ or $MgCl_2$	<1
rCTP	$MnCl_2$ or $MgCl_2$	<1
rUTP	$MnCl_2$ or $MgCl_2$	<1
rADP	$MnCl_2$ or $MgCl_2$	<1
dATP	$MnCl_2$ or $MgCl_2$	<1
dATP + $dA(pA)_4$	$MnCl_2$ or $MgCl_2$	<1

Each reaction mixture (0.25 ml) contained 0.5 mM nucleotide: either [^{14}C]-rGTP (1674 cpm per nmole); [^{14}C]-rCTP (1493 cpm per nmole); [^{3}H]-rUTP (12670 cpm per nmole); [^{14}C]-rADP (2566 cpm per nmole); or [^{3}H]-dATP (6411 cpm per nmole); 0.5 mM $MnCl_2$ or 4.0 $MgCl_2$ as indicated, 200 mM Tris-Cl pH 8.25, 4 mM mercaptoethanol, TrT, and 10 μM $rA(pA)_4$. Reaction mixtures were incubated at 35° and 50 μl aliquots were taken for rate determination.

cleotides are not copolymerized to any appreciable degree and that RNA polymerase type activity is absent. The addition of other ribonucleotides does product some inhibition of rATP polymerization (Table IV), indicating some competition, but they do not polymerize or copolymerize.

Initiators. Our homogeneous TrT does not contain any detectable contamination or endogenous activities of either RNase or RNase H. For this reason we feel that we can use this enzyme to make an assessment of the initiator function of any RNA molecule. At low concentrations of an initiator such as $rA(pA)_5$ the rate of rATP polymerization is directly proportional to $rA(pA)_5$ concentration at a fixed TrT concentration. We have also tested poly A, poly U, tRNA (adenylated and deadenylated, from yeast and *E. coli*), 16 S and 23 S ribosomal RNA, and Qβ and MS-2 viral RNA as initiators. On the basis of 3'-OH

TABLE IV

Inhibition of ATP Polymerization by Other Nucleotides

Nucleotide Added	Polymerization Rate (nmoles/hr/μg)	Inhibition %
None	147	0
rGTP	102	31
rCTP	128	13
rUTP	130	12
dATP	91	38
3rXTP's	120	18

Each reaction mixture (0.25 ml) contained 0.25 mM [^{14}C]-rATP (3446 cpm per nmole); 10 μM $rA(pA)_4$, 0.5 mM $MnCl_2$, 200 mM Tris-Cl pH 8.25, 4 mM mercaptoethanol, and TrT. Where indicated, 0.25 mM nucleotide or the mixture of rGTP, rCTP, and rUTP at 0.25 mM total nucleotide was added.

concentration the rate obtained with poly A is nearly equal to $rA(pA)_5$, poly U is very poor and the rest somewhat less effective. That is, of a variety of stable RNA species all will initiate, however, none have especially good initiator properties. It will now be of interest to search for "super initiators' in other classes of cellular RNA.

DISCUSSION

The availability of a homogeneous, RNase-free preparation of TrT allows an unambiguous demonstration of the biochemical properties of this interesting and ubiquitous enzyme. The Mn^{++}-dependent TrT polymerizes rATP only but is democratic in its use of RNA species. If an RNA species with special acceptance properties could be found then the biological consequences of this enzyme activity might become of major importance.

In the absence of special biological properties we simply note that the enzyme has capabilities for modification of RNA molecules that might be useful in other synthetic and analytical studies.

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