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NUCLEOSIDE TRIPHOSPHATE PHOSPHOTRANSFERASE

A NEW ENZYME ACTIVITY OF ONCOGENIC AND NON-ONCOGENIC "BUDDING" VIRUSES

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SUMMARY

A new enzyme activity of viruses which have cell membrane components is described. The activity is a phosphotransferase activity exchanging the γ-phosphates of ribonucleoside triphosphates (preferring purines). It is stimulated by the nonionic detergent Triton N101. The enzyme activity was found in vesicular stomatitis virus, avian myeloblastosis virus, influenza and Rauscher leukemia virus but not in polio virus. A comparison with the NTPase activity of these viruses was made.

INTRODUCTION

Host cell enzymes have been demonstrated to be present in the viral envelopes of many viruses, *e.g.* avian myeloblastosis virus[1,2], herpes virus[3], influenza viruses[4]. Recently viral enzymes concerned with replicating at least part of the viral genome have been demonstrated in all oncogenic viruses examined[5–7] and also vesicular stomatitis virus[8].

It was the purpose of the authors to look at the product synthesized by vesicular stomatitis virus (VSV) in terms of its size and function. One of the first approaches used was to employ γ-^{32}P-labelled triphosphates to label the 5′ terminus of the product RNA. We found that not only was the majority of the label incorporated into a phospholipid, but also that [γ-^{32}P]UTP, [γ-^{32}P]GTP and [γ-^{32}P]ATP all supported the [^{32}P]phosphate incorporation. We then found that in a reaction mixture containing all four triphosphates (including [γ-^{32}P]ATP), after 60 min of incubation in the presence of the nonionic detergent Triton W101 (needed to demonstrate the replication enzyme[8]), the ^{32}P label was randomized between three triphosphates GTP, ATP and, to a lesser extent, UTP. The enzyme responsible for the randomization—which we call NTP phosphotransferase—has been characterized

The present paper describes the NTP phosphotransferase activity of VSV, and

Abbreviation: VSV, vesicular stomatitis virus.

compares its activity with the NTPase activity of VSV as well as avian myeloblastosis, influenza, Rauscher leukemia and polio viruses.

MATERIALS AND METHODS

Chemicals

[β,γ-$^{32}P_2$]ATP, [^{32}P]phosphate and [^{32}P]pyrophosphate were obtained from I.C.N., Irvine, Calif. [^{3}H]ATP was purchased from Schwartz Bioresearch Inc., Orangeburg, N.Y. [α-^{32}P]UTP was synthesized by the procedure described by HARUNA *et al.*[9].

Viruses and cells

Influenza virus was a gift from Dr. R. Simpson, Rutgers University. Rauscher leukemia and avian myeloblastosis virus were gifts from Dr. S. Spiegelman. Baby hampster kidney cells (BHK-21) were initially obtained from Dr. D. Dubin, Rutgers University, and vesicular stomatitis virus (VSV), Indiana serotype, was initially obtained from Drs. R. Simpson and J. Obejeski of Rutgers University. VSV was grown in BHK-21 adapted for suspension culture. Cells were maintained at a concentration of between $2 \cdot 10^5$ and $10 \cdot 10^5$ per ml of Eagles' minimal essential medium, with added 1% glutamine and 10% calf serum, in which medium they doubled every 15 h. For a viral infection the cells were centrifuged at 1000 rev./min for 15 min in an MSE mistral 6L centrifuge, and washed by suspension in Eagles' minimal essential medium and recentrifuged. The cells were then suspended in Eagles' minimal essential medium–glutamine at a concentration of $5 \cdot 10^7$ per ml and infected with a virus stock containing $2 \cdot 10^9$ p.f.u./ml at a multiplicity of infection of 0.5–1.0. After stirring for 30 min at 37° the cells were diluted with Eagles' minimal essential medium–glutamine to a concentration of $1 \cdot 10^6$ per ml and fetal calf serum added to give final concentration of 5%. The cells were grown for 24 h at 37° before harvesting. When [^{3}H]-uridine was used to label the cells and virus, it was added 3 h after the initiation of infection at a concentration of 5 μC per ml of culture.

Purification of virus

The virus was purified from the culture supernatant by ammonium sulphate precipitation and subsequent sucrose gradient centrifugation. The cells were harvested by centrifugation at 1000 × *g*. $(NH_4)_2SO_4$ (300 g/l of supernatant) was added slowly (with stirring) at 4° and the pH kept at 7–8 by adding 1 M Tris–HCl buffer (pH 8). The precipitate was collected by centrifugation at 1500 × *g* for 30 min in an MSE mistral 6L, dissolved in 0.4 M $(NH_4)_2SO_4$–0.1 M Tris–0.01 M EDTA (pH 7.4) and centrifuged for 70 min at 82 000 × *g* over a 1-ml pad of 100% glycerol in a Spinco SW27 rotor. The supernatant above the band of virus was carefully removed and the virus on the pad collected by adding $(NH_4)_2SO_4$–Tris–EDTA buffer and swirling as minimally as possible to suspend the particles. The suspension was adjusted to a density of less than 1.05 g/ml, then layered over a 30–15% linear gradient of sucrose in 0.1 M NaCl–0.01 M Tris–0.002 M EDTA (pH 7.4) and centrifuged for 50 min at 82 000 × *g*. Usually three bands were clearly visible—the lower infectious virus band (VSV-I) and two defective particle bands (VSV-II and VSV-III). The presence or amount of each defective band was in correspondence with the initial multiplicity

of infection. With low multiplicities of infection (0.5 or less) little or no defective particles were evident. Each band was collected by pipet, diluted with 0.1 M NaCl–0.01 M Tris–HCl buffer, and concentrated by centrifugation at 82 000 × *g* for 90 min over a 100% glycerol pad. The three viral preparations were centrifuged a second time through sucrose gradients to obtain better particle purity. Finally they were centrifuged over a glycerol pad and suspended in 0.1 M NaCl–0.01 M Tris–HCl buffer (pH 7.4). This suspension, which also contained some glycerol from the pad, was frozen in 0.2-ml aliquots at −70°. Some viral preparations were only centrifuged through a gradient once (see text).

Synthesis of ribonucleoside [γ-^{32}P]triphosphates

The synthesis of ribonucleoside [γ-^{32}P]triphosphates (NTP) was based on that described by GLYNN AND CHAPPELL[10]. The reaction mixture (10 ml) contained 2 ml 1 M Tris–HCl (pH 7.4); 0.03 ml 1 M $MgCl_2$; 0.03 ml 1 M mercaptoethanol; 0.03 ml 0.005 M NAD; 1.00 ml 0.1 M sodium 3-phosphoglycerate; 0.06 ml 0.01 M phosphoric acid; 0.12 ml 0.05 M nucleoside triphosphate; 5.00 ml $H_3{}^{32}PO_4$, carrier-free, 50 mC (neutralized to pH 7 with NaOH); 0.06 ml 3-phosphoglycerate kinase (Sigma, 10 mg/ml); 0.10 ml glyceraldehyde phosphodehydrogenase (Sigma, 10 mg/ml); water to 10 ml. For the synthesis of [γ-^{32}P]ATP and [γ-^{32}P]CTP the enzymes were dialysed for 4 h against 0.4 M NH_4Cl–0.01 M Tris–HCl buffer (pH 7) to remove $(NH_4)_2SO_4$, prior to use. Otherwise the $(NH_4)_2SO_4$ eluted with the CTP and ATP from the Dowex-1 (Cl^-) column and precipitated at the $BaCl_2$ stage. The mixture was incubated at 37° for 20 min, diluted to 20 ml with water and loaded on a 10 ml column of Dowex-1-X8 (Cl^-) (200–400 mesh). After washing with 20 ml of water the ribonucleoside triphosphates were separated from [^{32}P]phosphate by a salt gradient. A 400-ml linear gradient of 0.05–0.28 M LiCl in 0.01 M HCl was used for all triphosphates. Elution was monitored by the $A_{260\ nm}$ and the fractions containing triphosphate pooled, neutralized with NaOH and evaporated to about 5 ml. After addition of 0.10 ml of saturated $BaCl_2$ and 5 vol. of methanol, the insoluble barium salt of the triphosphate was collected by centrifugation, suspended in 3 ml of water and the barium exchanged for hydrogen using Dowex 50 (H^+) beads (Dowex 50W-X8, 20–50 mesh). The soluble triphosphoric acid was finally neutralized with NaOH, lyophilized and dissolved in water. Usually the specific activity, depending on the batch of carrier-free $H_3{}^{32}PO_4$, was between 5 and 10 mC/μmole and the yield around 3–4 μmoles.

Assay of ATP, GTP, UTP phosphotransferases

The transfer of the γ-^{32}P-labelled phosphate from ATP to GTP and UTP, or GTP to ATP and UTP, or UTP to GTP and ATP was monitored by paper electrophoresis, or Dowex-1 (Cl^-) column chromatography. Reaction mixtures contained the following: 0.01 μmole of the [γ-^{32}P]triphosphate; 0.1 μmole of the other three triphosphates; 1 μmole $MgCl_2$; 8 μmoles Tris–HCl buffer (pH 7.5); 13 μmoles NaCl; 0.5 μmoles mercaptoethanol; 20 μg Triton N101; 20 μg virus, in a total volume of 125 μl. Aliquots of 1 μl were removed at intervals for paper electrophoresis at pH 3.5 in 0.05 M sodium citrate. Electrophoresis was for 40 min at 40 mA and 6000 V. The resolved ATP, GTP, free phosphate, UTP and free pyrophosphate were identified by autoradiography. CTP, although present in the reaction mixture, was usually not labelled by the phosphotransferase reaction (*vide infra*); during the electro-

phoresis it migrated with the ATP. After autoradiography the ^{32}P content of each spot was determined by counting in a scintillation counter.

Alternatively the reaction mixture was mixed with 0.10 ml of 10% sodium dodecyl sulfate, then 0.12 ml of each ribonucleoside triphosphate added and the mixture loaded and resolved by a salt gradient on Dowex 1 (Cl^-). Under these chromatography conditions the GTP and UTP eluted very close together. However, as GTP has a negligible 5% absorption at $A_{290\ nm}$ in comparison to its $A_{260\ nm}$, its position could be determined easily since UTP has a 45% absorption at $A_{290\ nm}$ in comparison to its $A_{260\ nm}$. Fractions (4 ml) eluted from the gradient were therefore monitored for radioactivity as well as $A_{290\ nm}$ and $A_{290\ nm}$.

Assay of the ATPase, CTPase, GTPase and UTPase activities

The release of free [^{32}P]phosphate from each γ-^{32}P-labelled ribonucleoside triphosphate was measured by incubating the virus and each [γ-^{32}P]triphosphate, in the absence of the other three triphosphates, under the same conditions as described for the phosphotransferase assay. The amounts of triphosphate and free phosphate were determined after paper electrophoresis.

RESULTS

NTPase activity of VSV

The ATPase, CTPase, GTPase and UTPase (NTPase) activity of VSV-I particles in the presence of the nonionic detergent Triton N101 was measured by monitoring the release of γ-^{32}P from the triphosphate using paper electrophoresis. Under these conditions the four activities were essentially equivalent as seen in Fig. 1. In the absence of detergent the four activities were much greater (Fig. 1). Evidently the detergent suppresses the NTPase activity for each of the ribonucleoside triphosphates.

In order to determine if both the β and γ phosphates were removed during the incubation in the presence of triton, [α-^{32}P]UTP was incubated alone with the virus particles and the release of unlabelled phosphate from the β and γ positions monitored by looking for the appearance of [α-^{32}P]UDP, [^{32}P]UMP or [^{32}P]phosphate. The results, shown in Table I, indicate that UTP is degraded to UDP but not further. Hence the UTPase activity operates by removing the γ-phosphate and not the β- or α-phosphates. This UTPase activity is similar to the ATPase activity of avian myeloblastosis virus which also removes only the γ-phosphate[11].

The phosphotransferase activity of VSV

When one γ-^{32}P-labelled triphosphate was incubated with triton-treated VSV particles in the presence of ten times the amount of the other three unlabelled triphosphates, it was found that a transfer of the γ-phosphate occurred except in the case of CTP (Figs. 2, 3). No transfer of the [γ-^{32}P]CTP label to the other three triphosphates was detected by 60 min of incubation. Nor did the other three triphosphates transfer their γ-phosphates with CTP to any significant extent. UTP transferred its γ-phosphate to a limited extent with ATP and GTP. Both purines exchanged γ-phosphates readily, and also with UTP—although to a lesser extent.

The kinetics of γ-phosphate labelling of GTP, UTP and free phosphate by

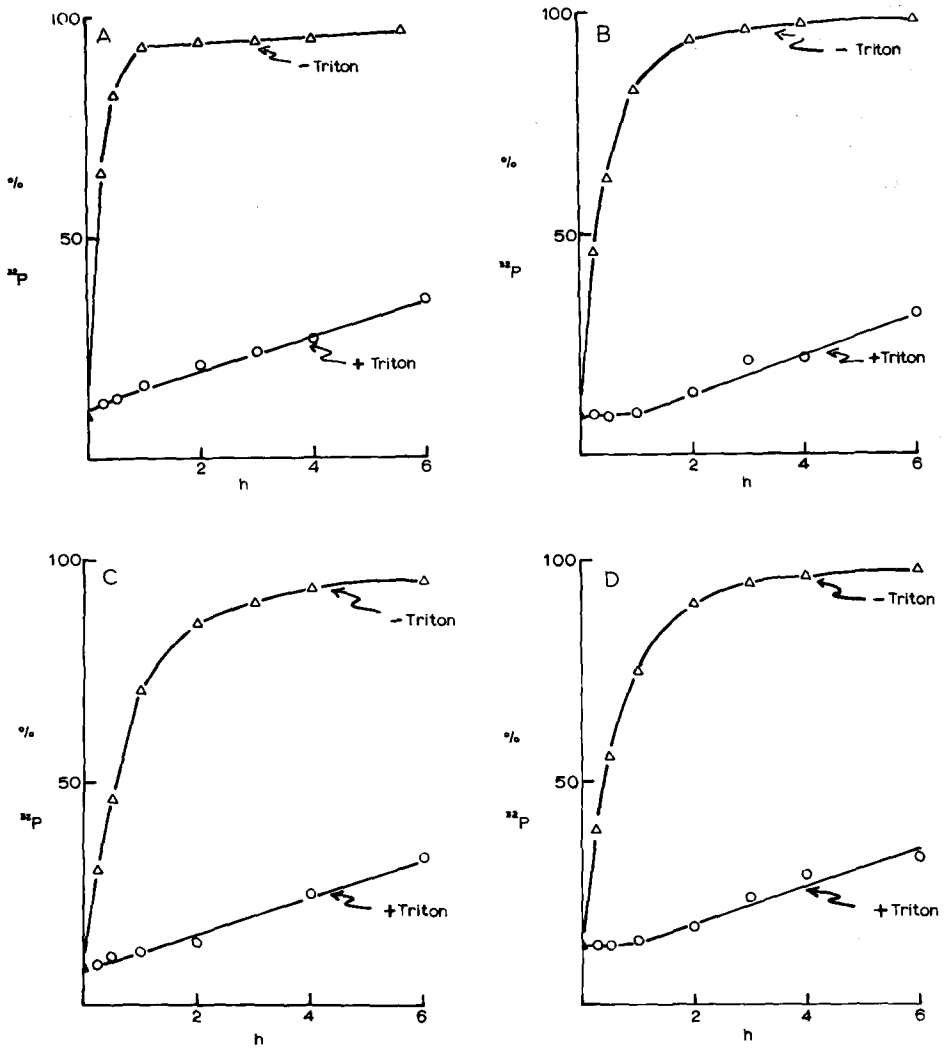

Fig. 1. The NTPase activity of VSV particles. (A) A reaction mixture containing 0.01 μmole [γ-^{32}P]ATP (5 mC/μmole)—but no other triphosphate—was incubated in the presence or absence of Triton N101 with VSV-I as described in MATERIALS AND METHODS. Aliquots (1 μl) were removed as indicated, subjected to paper electrophoresis and autoradiography to determine the distribution of label. The percentage of [^{32}P]phosphate recovered is recorded. Alternatively, reaction mixtures containing (B) [γ-^{32}P]GTP (5 mC/μmole), or (C) [γ-^{32}P]UTP (4 mC/μmole), or (D) [γ-^{32}P]CTP (4 mC/μmole) were similarly incubated with VSV to determine the GTPase, UTPase or CTPase activity, respectively.

[γ-^{32}P]ATP is shown in Fig. 4A. Although the GTP that became labelled showed a precursor–product relationship with the label incorporated into UTP, in a similar experiment in which GTP was left out, the UTP was readily labelled (Fig. 4B). GTP is therefore not an obligatory intermediate in the labelling of UTP. Comparison of the kinetics of UTP and GTP labelling from [γ-^{32}P]ATP indicated that GTP was the preferred recipient (Figs. 4A and 4B).

TABLE I

MODE OF ACTION OF VSV UTPASE

A reaction containing 0.01 μmole [α-^{32}P]UTP (1 mC/μmole) was incubated with VSV-I, —no Triton—and no other triphosphate and aliquots (1 μl) removed at the indicated time intervals and subjected to paper electrophoresis to resolve the UMP, UDP, UTP and phosphate. The percentages of label in each spot are recorded.

Substrate	*Time (min)*	*% ^{32}P*			
		UMP	*UDP*	*Phosphate*	*UTP*
[α-^{32}P]UTP	0	0	0	0	100
	10	6.6	19.9	1.5	72.0
	20	4.1	31.2	1.7	63.0
	30	7.3	45.3	2.9	44.5
	40	4.0	54.8	3.4	37.8
	50	3.6	60.7	3.3	32.4
	60	4.0	65.7	3.8	26.5

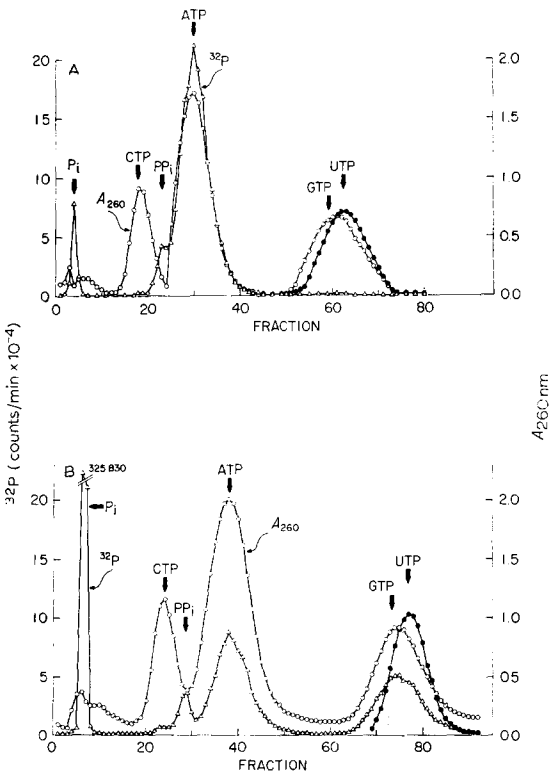

Fig. 2. The phosphotransferase activity of VSV. Donor: [γ-^{32}P]ATP. A reaction mixture containing 0.01 μmole [γ-^{32}P]ATP (5 mC/μmole) was incubated for 60 min at 37° in the presence of Triton with VSV-I and three unlabelled triphosphates (0.1 μmole each) (see MATERIALS AND METHODS for details). After addition of sodium dodecyl sulfate and 6 μmoles of all four triphosphates the mixture was resolved on Dowex 1 (Cl^-) and eluted with a salt gradient. Fractions were monitored for radioactivity and the $A_{260\ nm}$ and $A_{290\ nm}$ (see MATERIALS AND METHODS for details). The positions and amounts of UTP and GTP were determined from the $A_{290\ nm}$ readings (see text). UTP is given as the $A_{260\ nm}$ (●—●). (A) Unincubated control, [γ-^{32}P]ATP alone. (B) [γ-^{32}P] ATP reaction.

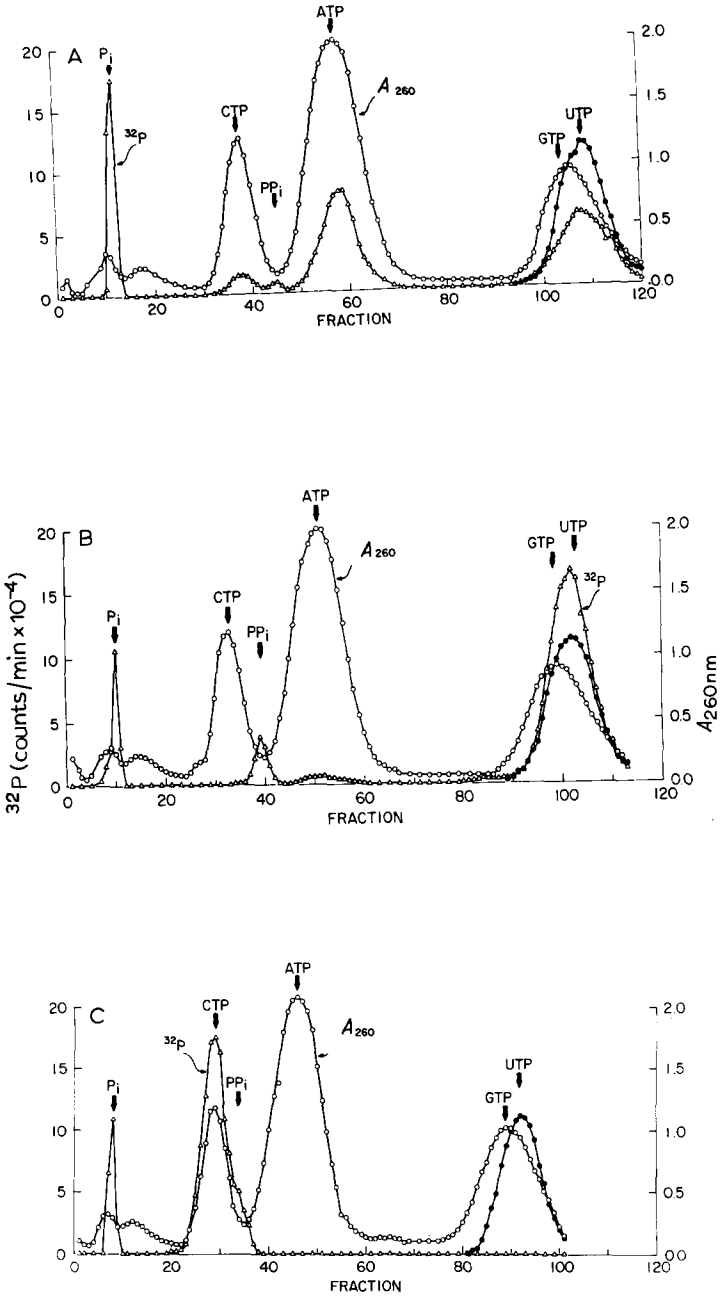

Fig. 3. The phosphotransferase activity of VSV. (A) Donor: [γ-^{32}P]GTP. A reaction mixture containing 0.01 μmole [γ-^{32}P]GTP (5 mC/μmole) was processed as described in Fig. 2. Alternatively, the donor was (B) [γ-^{32}P]UTP ,or, (C) [γ-^{32}P]CTP.

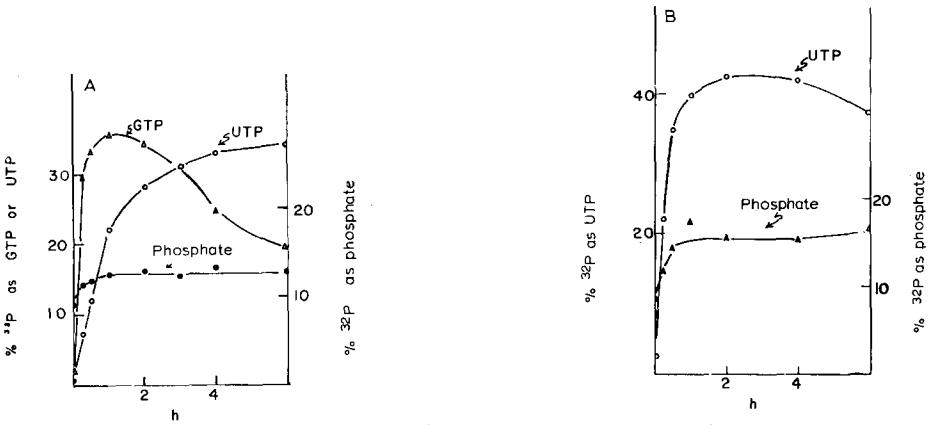

Fig. 4. The kinetics of VSV phosphotransferase. Donor: [γ-^{32}P]ATP. Reaction mixtures containing 0.01 μmole [γ-^{32}P]ATP (5 mC/μmole), UTP and CTP (0.1 μmole) and either no GTP (B), or with GTP (0.1 μmole) (A) and the other ingredients—including Triton— as described in MATERIALS AND METHODS, were incubated with VSV-1. Aliquots (1 μl) were removed at intervals and analysed by paper electrophoresis. The percentages of label in UTP, GTP and phosphate are given.

Similarly the kinetics of γ-phosphate transfer between [γ-^{32}P]GTP and ATP, UTP and free phosphate, which is shown in Fig. 5, indicate that ATP is the preferred recipient of the γ-phosphate. It should be noted that at 2 h of incubation, 60% of

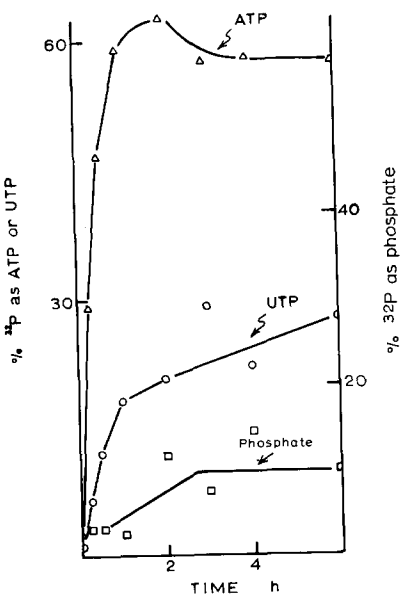

Fig. 5. The kinetics of VSV phosphotransferase. Donor: [γ-^{32}P]GTP. A reaction mixture containing 0.01 μmole [γ-^{32}P]GTP (5 mC/μmole) was incubated with the other three unlabelled triphosphates, Triton and VSV-I particles. Aliquots (1 μl) were resolved by paper electrophoresis (see MATERIALS AND METHODS for details). The percentages of label in UTP, ATP and phosphate are given.

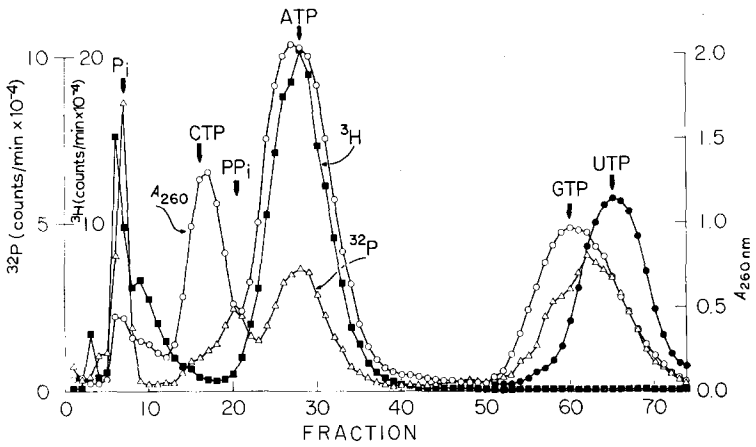

Fig. 6. The phosphotransferase of VSV. Donors: [^{3}H]ATP, [γ^{32}-P]ATP. A reaction mixture containing 0.005 μmole [^{3}H]ATP (20.7 mC/μmole), 0.005 mole [γ-^{32}P]ATP (5 mC/μmole) was processed as described in Fig. 2.

the GTP [^{32}P]phosphate was incorporated into the ATP, when in the reverse experiment (Fig. 4A), 35% of the ATP [^{32}P]phosphate was incorporated into GTP. In both experiments the amounts incorporated into UTP were approximately similar.

In an experiment initially set up to determine if the β-, γ-phosphates are both

TABLE II

COMPARISON OF VSV PHOSPHOTRANSFERASE ACTIVITY USING [γ^{32}P]ATP OR [β,γ-$^{32}P_2$]ATP AS SUBSTRATES

Standard reaction mixtures were set up with 0.01 μmole of [β,γ-$^{32}P_2$]ATP (1 mC/μmole) or [γ-^{32}P]-ATP (3 mC/μmole) and the other components (including Triton) as described in MATERIALS AND METHODS. Aliquots (1 μl) were removed at the indicated intervals and resolved by paper electrophoresis. The percentages of label in ADP, ATP, GTP, UTP, phosphate or pyrophosphate are given.

Substrate	*Time (min)*	*% ^{32}P*					
		ADP	*ATP*	*GTP*	*UTP*	*Phosphate*	*Pyrophosphate*
[β,γ-$^{32}P_2$]ATP	0	1.7	96.8	0.7	0	0.8	0
	15	5.6	87.6	4.0	0	2.8	0
	30	6.7	82.6	6.4	0.3	4.0	0
	60	7.3	74.2	12.9	2.3	3.3	0
	120	6.6	64.2	19.0	5.5	4.7	0
	180	6.5	59.7	20.6	7.9	5.6	0
	240	6.1	62.8	17.1	8.5	5.5	0
	360	6.2	60.3	15.0	12.1	6.4	0
[γ-^{32}P]ATP	0	0	85.4	1.1	0	9.7	3.8
	15	0	76.0	7.7	0.5	12.5	3.3
	30	0	65.6	15.4	1.2	13.7	4.1
	60	0	53.1	24.1	4.3	14.7	3.8
	120	0	36.2	37.2	9.0	14.6	3.0
	180	0	29.2	37.9	15.4	13.5	4.0
	240	0	24.7	39.0	13.7	18.1	4.5
	360	0	23.5	31.8	26.5	15.0	3.2

transferred or if only the γ is moved, [α-^{32}P]GTP was incubated with the other three triphosphates and the reaction monitored for the appearance of labelled GMP and GDP. Very little loss of GTP to mono- and diphosphate was observed. This suggested therefore that the transfer of γ-[^{32}P]phosphate was not accompanied by breakdown of the donor triphosphate. If this was true, then the phosphotransferase activity is an exchange activity—either exchanging phosphates between triphosphates, or removing and transferring a γ-phosphate and replacing it with a free phosphate. In order to determine by another method if the exchange was accompanied by triphosphate breakdown, the following experiment was set up. [γ-^{32}P]ATP and [^{3}H]-ATP in equal amounts (0.005 μmole each per reaction) were incubated with the other triphosphates under standard reaction conditions and the fate of both labels examined by Dowex-1 (Cl^-) chromatography after 1 h of incubation. As seen from Fig. 6, the majority (75%) of the ^{3}H label remained in the ATP region, whereas only 28% of the ^{32}P label was left with ATP. The rest of the ^{32}P label was associated with GTP, UTP, free phosphate and pyrophosphate. This experiment confirms therefore that the triphosphate is kept although its original γ-phosphate is transferred to other triphosphates.

In an experiment in which [^{32}P]phosphate (carrier-free) or $Na_4{}^{32}P_2O_7$ (6 mC/μmole) were added to a reaction mixture containing unlabelled triphosphates, no label was incorporated into any triphosphates. Although a negative experiment does not prove that free phosphate and pyrophosphate are not intermediates in the exchange, it does suggest that the exchange is a process mediated directly between triphosphates by an enzyme.

In order to examine if the β- as well as the γ-phosphate is exchanged between triphosphates, a reaction containing [β,γ-^{32}P$_2$]ATP was incubated with the other three unlabelled triphosphates and the exchange of label compared to that in which [γ-^{32}P]ATP was the donor. The comparison of these two experiments is shown in Table II. With [β,γ-^{32}P$_2$]ATP as the triphosphate donor, about 30% of the ^{32}P was incorporated into GTP and UTP, when with [γ-^{32}P]ATP as donor, about 60% of

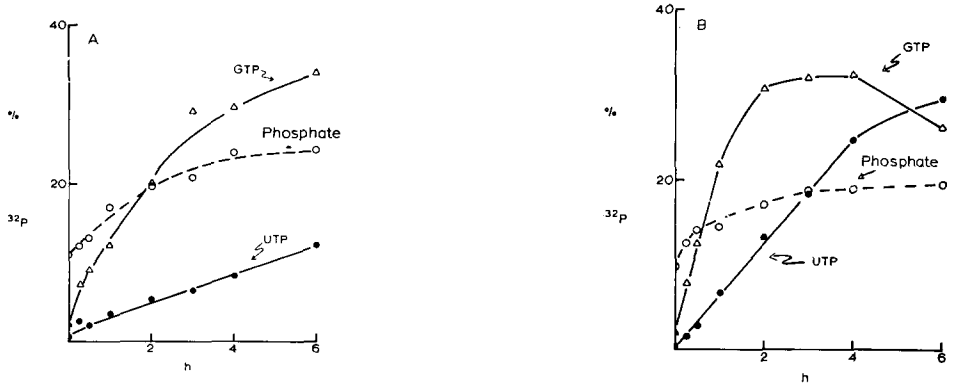

Fig. 7. The phosphotransferase of VSV. Effect of Triton. Reaction mixtures containing 0.01 μmole [γ-^{32}P]ATP (5 mC/μmole), and the three other unlabelled triphosphates were incubated with VSV particles (crude virus preparation) in the absence (A) or presence (B) of Triton and aliquots (1 μ) resolved by paper electrophoresis (see MATERIALS AND METHODS for details). The percentages of label incorporated into GTP, UTP and phosphate are given.

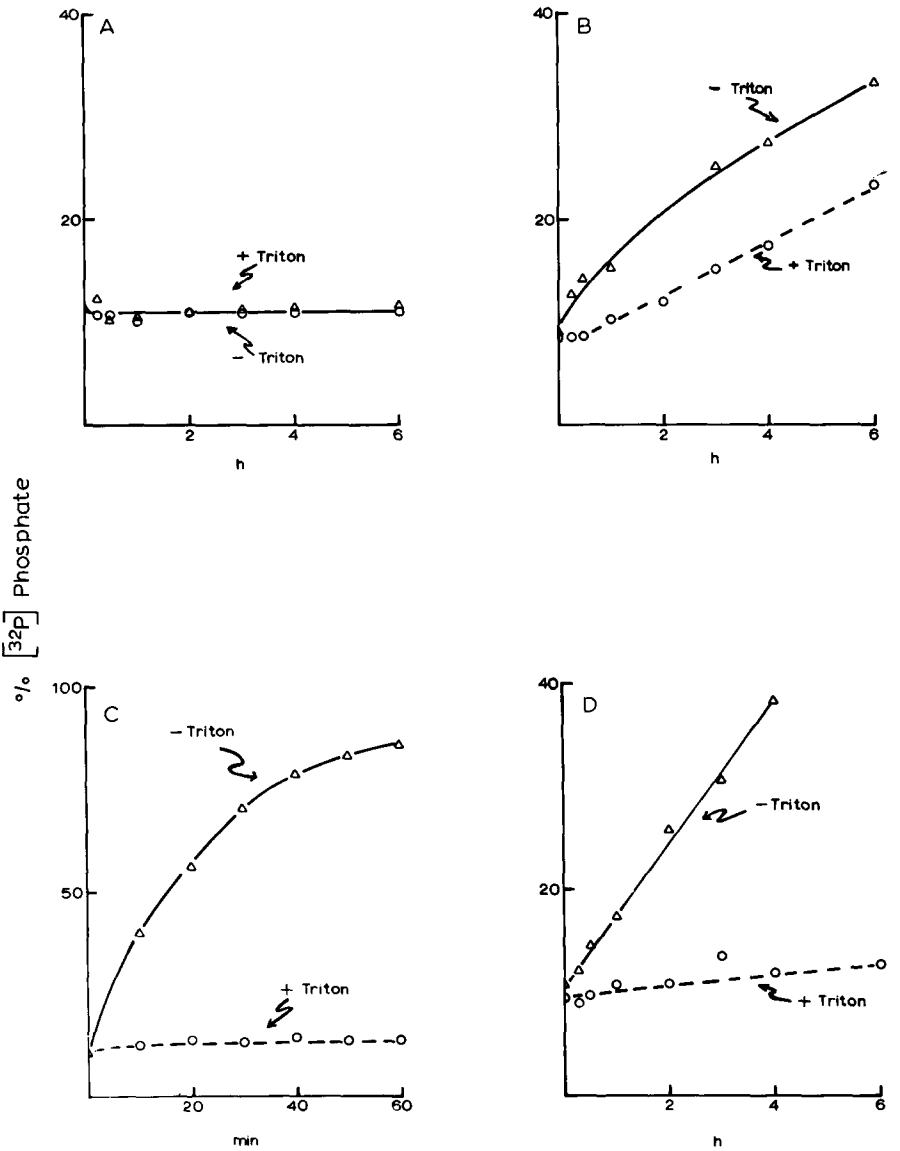

Fig. 8. The ATPase activity of polio, influenza, avian myeloblastosis and Rauscher leukemia viruses. Reaction mixtures containing 0.01 μmole [γ-^{32}P]ATP (5 mC/μmole)—no other triphosphate—with or without triton and either polio virus (10 μg, A) or influenza virus (5 μg, B) or avian myeloblastosis virus (5 μg, C) or Rauscher leukemia virus (45 μg, D), were incubated as indicated and aliquots resolved by paper electrophoresis to determine the percentages free phosphate.

the ^{32}P ended up in GTP and UTP. The amount of [^{32}P]ADP which was recovered from the [β,γ-$^{32}P_2$]ATP experiment was about equivalent to the increase in free phosphate but not equivalent to the label incorporated into GTP and UTP; therefore the ADP labelling is probably the result of residual ATPase activity. These results

indicate therefore that the γ-phosphate alone is involved in the exchange. They also confirm that the donor triphosphate is conserved during the exchange process.

So far, in the experiments described above, the NTP phosphotransferase activity was examined in Triton-treated particles. The exchange of [γ-^{32}P]ATP to other triphosphates in the presence and absence of Triton is shown in Figs. 7A and 7B. Evidently the exchange process in the absence of Triton is slower than with Triton. It should be noted when comparing the ATPase activity in this experiment and that recorded in Fig. 1, that there is 31 times the amount of total triphosphate here, although the ATP levels are the same for the two experiments. Therefore, the ATPase activity is not a distinct activity from the CTPase, GTPase and UTPase activities because the presence of the other three unlabelled triphosphates substantially reduces the ATPase activity.

The ATPase activity of polio, influenza, avian myeloblastosis and Rauscher leukemia viruses

The ATPase activity of four other viruses in the presence and absence of Triton is shown in Fig. 8. Other than polio, which did not have an ATPase activity, three viruses showed an ATPase activity which was reduced in the presence of Triton N101.

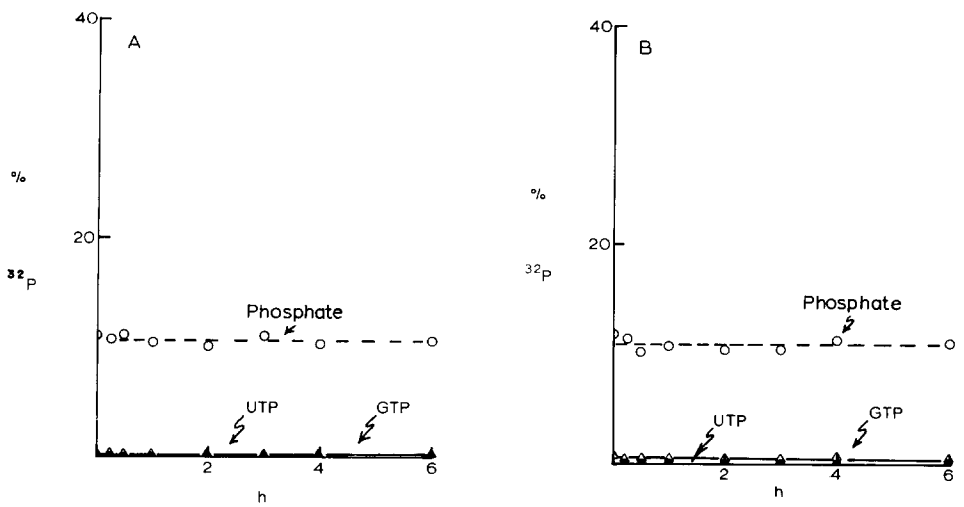

Fig. 9. The NTP phosphotransferase activity of polio virus. Reaction mixtures containing [γ-^{32}P]-ATP (5 mC/μmole), three unlabelled triphosphates, polio virus (10 μg), without (A) or with Triton (B) were processed as described in Fig. 7. The percentages of label in GTP, UTP and phosphate are given.

The NTP phosphotransferase activity of polio, influenza, avian myeloblastosis and Rauscher leukemia viruses

The NTP phosphotransferase activity was found in three viruses but not in polio virus (Figs. 9–12). The activity was stimulated for each virus by the presence of Triton N101. Evidently, like the NTPase activity, the transferase activity is associated with viruses which bud from the cell membranes.

DISCUSSION

The NTPase activity of viruses which bud out from the host cell surface and have host antigens, has been documented and studied over many years[1,2,11–17]. The effects of detergents on this activity for avian myeloblastosis virus has been studied by KORB AND RIMAN[12]. The anionic detergent deoxycholate reduces by 95% the ATPase activity whilst the nonionic detergents digitonin and Tween 80 reduce it 30–60%.

From the experiments described here it can be seen that the nonionic detergent Triton N101 substantially reduces the ATPase activity of avian myeloblastosis virus, influenza virus, Rauscher leukemia virus and vesicular stomatitis virus. Polio virus

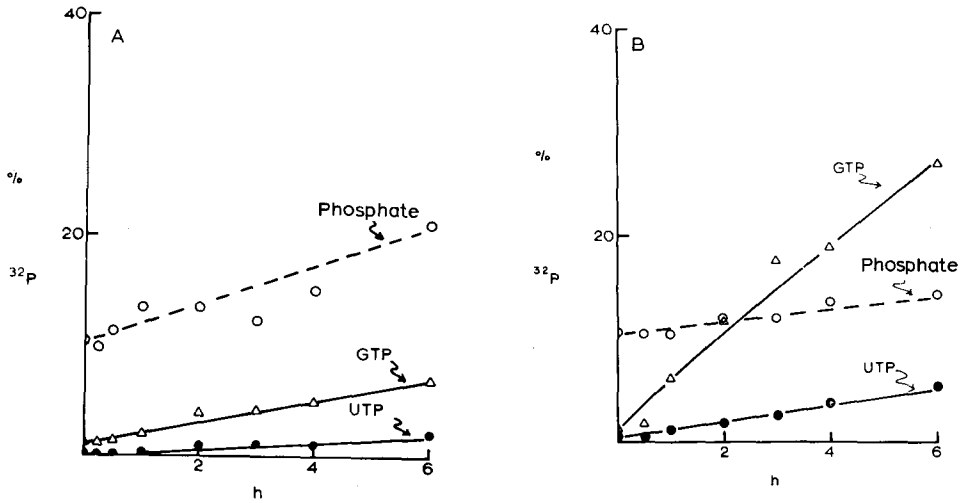

Fig. 10. The NTP phosphotransferase activity of influenza virus. Reaction mixtures containing [γ-^{32}P]ATP (5 mC/μmole), three unlabelled triphosphates, influenza virus (5 μg) without (A) or with Triton (B) were processed as described in Fig. 7. The percentages of label in GTP, UTP and phosphate are given.

does not have an ATPase activity or NTP phosphotransferase activity. The NTPase activity of VSV, which acts by removing the γ-phosphate but leaving the β- and α-phosphates, is probably mediated by one enzyme for all four triphosphates since the presence of unlabelled CTP, GTP and UTP substantially reduces the ATPase activity of VSV. Evidently, though, the enzyme has different affinities for the four triphosphates (Fig. 1).

The NTP phosphotransferase activity has been found in influenza, avian myeloblastosis, Rauscher leukemia and vesicular stomatitis virus, but not in polio virus. The enzyme is stimulated by the presence of Triton and acts by transferring the γ- but not the β- or α-phosphates between triphosphates. Apparently in the transfer there is an exchange of phosphates since the donor triphosphate is not converted to a mono or diphosphate.

The result of using mono or diphosphates as acceptor ribonucleosides in the

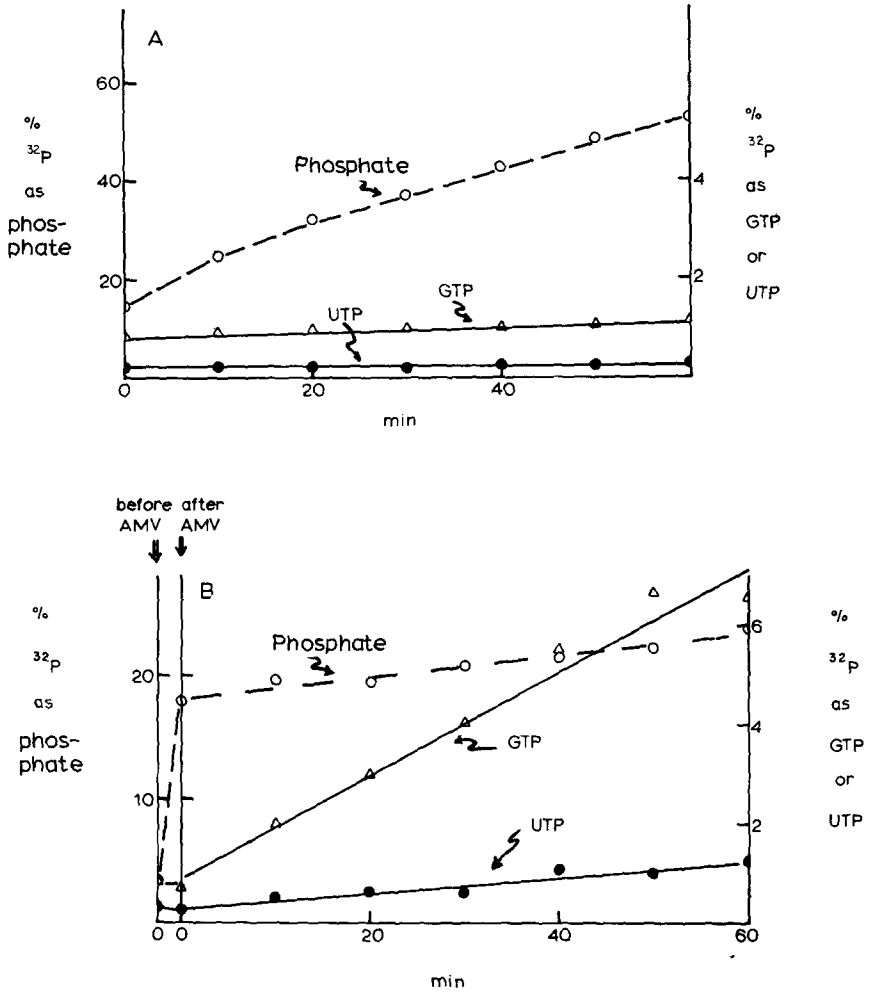

Fig. 11. The NTP phosphotransferase activity of avian myeloblastosis virus. Reaction mixtures containing [γ-^{32}P]ATP (5 mC/μmole), three unlabelled triphosphates, avian myeloblastosis virus (AMV, 5 μg) without (A) or with Triton (B) were processed as described in Fig. 7. Note that zero time aliquots were taken before and after avian myeloblastosis virus addition. The 14% increase in free phosphate which was found after avian myeloblastosis virus addition reflects the ATPase activity of avian myeloblastosis virus before inhibition by Triton. Evidently it would be preferable to mix avian myeloblastosis virus with Triton before addition to the reaction. However, in the experiments described here the virus was always added last.

phosphotransferase reaction will be described in a later communication. The separation of both the NTPase and NTP phosphotransferase activities from the nuclear cores and replication enzyme of VSV will also be described in another communication.

It will be of interest to know if the NTPase and phosphotransferase activities are mediated by the same enzyme. There are striking differences between the two activities. The NTPase activity is depressed by Triton when the phosphotransferase is stimulated. Moreover, the phosphotransferase prefers purines although there are

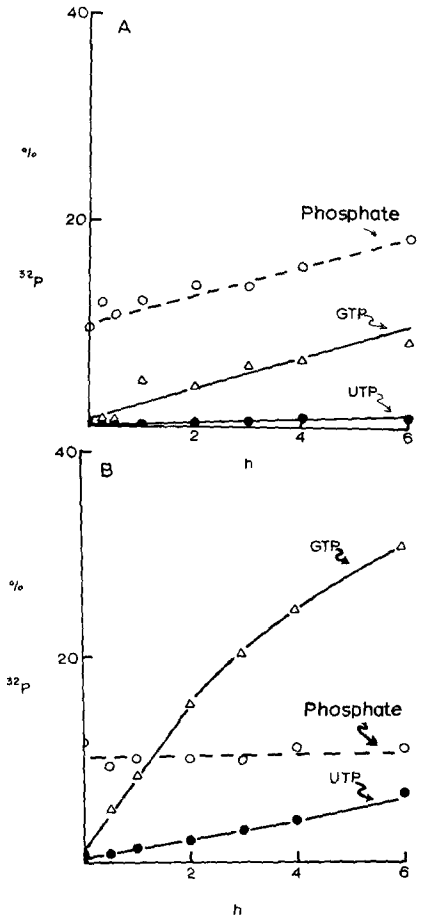

Fig. 12. The NTP phosphotransferase activity of Rauscher leukemia virus. Reaction mixtures containing [γ-^{32}P]ATP (5 mC/μmole), three unlabelled triphosphates, Rauscher leukemia virus (45 μg, purity unknown) without (A) or with Triton (B) were processed as described in Fig. 7. The percentages of label in GTP, UTP and phosphate are given.

pronounced CTPase and UTPase activities of VSV particles. Both activities only use the γ-phosphate of the triphosphate. To determine if these two activities are mediated by one enzyme will require further purification of the virus proteins.

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