Title

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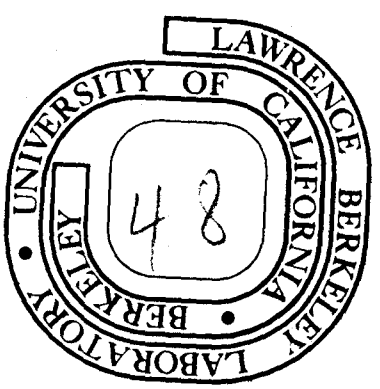

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DISCLAIMER

EFFECT OF GTP AND OTHER NUCLEOSIDE TRIPHOSPHATES ON GMP INCORPORATION BY ISOLATED CORTICAL NUCLEI

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Running Title: Substrate Concentration and RNA Synthesis.

ABSTRACT

The concentration of GTP was found to be critically important in determining the characteristics of incorporation of GMP by DNA-dependent RNA polymerase from rat brain nuclei. The linearity of the incorporation rate was related to the log of the GTP concentration. Three hundred μM GTP in the presence of the other nucleoside triphosphates (1 mM) was near to the optimal conditions in terms of maximum incorporation and linearity. The concentration of ammonium sulfate was an important factor in determining the optimum GTP and UMP concentration. The U/G ratio was less than one at low concentrations of substrate and increased with increasing substrate or ammonium sulfate concentration. α-Amanitin strongly inhibited the reaction, indicating that RNA polymerase II is the effective enzyme.

The influence of the concentration of nucleoside triphosphate on RNA synthesis in nuclei has not been studied sufficiently. Novello and Stirpe (1969) have studied briefly the effect of varying the concentration of all nucleoside triphosphates on the DNA-dependent RNA polymerase (EC 2.7.7.6) in rat liver nuclei but we are unaware of any such study in brain. The concentrations of nucleoside triphosphates used by different authors have extended over a wide range. This is particularly true in the case of the labeled nucleoside triphosphate for which the concentration has extended over nearly four orders of magnitude, from 0.35 μM (Banks & Johnson, 1973) to 1 mM (Austoker et al., 1972). Apparently the concentrations have been chosen arbitrarily and comparison of the various studies is difficult. In the present study, the effect of the concentration of GTP on GMP incorporation was studied and found to be critically important in determining the rate, duration, base ratio of incorporation, and inhibition by actinomycin D.

MATERIALS AND METHODS

Nuclear preparation. Sprague-Dawley male rats from Horton Laboratories, Ca. Oakland, (200-350 g) were killed by cervical dislocation and decapitated. The cerebral cortices were removed, rinsed in saline, and the nuclei were prepared essentially following the method of Løvtrup-Rein and McEwen (1966). All operations were performed at ice temperature. The cortices were homogenized by hand immediately after sacrifice in a loose Teflon-glass homogenizer in 10 volumes of 0.32 M sucrose. (All sucrose solutions contained 1 mM $MgCl_2$ and 1 mM potassium phosphate buffer, pH 6.5.)

The homogenate was filtered through several layers of cheesecloth and centrifuged for 10 min at 1000 g, the crude nuclear pellet was washed twice with 0.32 M sucrose, drained and suspended in 2 M sucrose. The suspension was layered over a gradient of 10 ml of 2 M sucrose and 5 ml of 2.2 M sucrose. The gradients were prepared 24 h before the experiment and kept at 0°C until used. To assure that all the types of nuclei are present in the final preparation, the centrifugation was extended to a period of 60 min (Løvtrup-Rein and McEwen, 1966) at 26,500 rpm in a SW-27 rotor in a Spinco L2-65B centrifuge at 4°C. The pellet of purified nuclei was suspended in 0.32 M sucrose and washed once to eliminate the concentrated sucrose. Finally the pellet was resuspended in 0.32 M sucrose and used within 2 h. Microscopic examination showed little cytoplasmic contamination and the presence of all types of nuclei as characterized by shape, size, and number of nucleoli (Austoker et al., 1972). The low degree of cytoplasmic contamination was confirmed by the very low RNA/DNA ratio, 0.166 $\pm$5% determined by the method of Morimoto et al. (1974). This value can be compared to the ratio 0.46 reported by Løvtrup-Rein and McEwen (1966). Other values which have been reported for purified nuclei from brain range from 0.60 to 0.32 (Austoker et al., 1972).

Determination of RNA synthesis

The method of McEwen et al. (1972) was used with some modifications described below. The nuclei suspended in 100 μl of 0.32 M sucrose were added to 400 μl of concentrated medium at 37°C. The final concentration was 2 mM $MnCl_2$, 330 mM $(NH_4)_2SO_4$, 100 mM Tris-HCl, pH 8.9, 70 mM KCl, 40 mM β-mercaptoethanol, 1 mM each ATP, CTP and UTP, final pH 8.2. This medium is called standard medium; to it the appropriate amount of 8-^{3}H-GTP

and unlabeled GTP was added. The final volume was 500 µl. In the preincubation experiments the concentrations of some components were higher during the preincubation but were diluted to the standard concentration before the incubation. When the preincubations were done in 0.32 sucrose the solution also contained 1 mM $MgCl_2$ and 1 mM potassium phosphate, pH 6.5. The incubations and preincubations were done at 37°C with mild shaking. The reaction was terminated by the addition of one volume (0.5 ml) of 8% w/v $Na_2P_2O_7$, frozen in dry ice and kept overnight. The samples were allowed to thaw while shaking in the presence of 250 µl of ice cold 5% CCl_3COOH, then 6 ml more of 5% CCl_3COOH were added. This procedure gave a dispersed precipitate which later could be efficiently washed on a glass fiber fiber filter (GF/C Whatman). The samples were kept at ice temperature for more than 15 min prior to filtering. Subsequently, the samples were washed 4 times with 5 ml of cold 5% CCl_3COOH, and finally twice with cold absolute ethanol. The samples on the filters were placed in scintillation vials and digested with 500 µl of Solvene-100 (Packard Instruments Co.) in order to avoid self-absorption as observed by us in preliminary experiments and also reported by Silverman and Mirsky (1973).

DNA determination

The quantity of nuclei was estimated by DNA determination using the method of Morimoto et al. (1974). In most cases the incorporation was expressed as picomoles incorporated per 100 µg of nuclear DNA.

Standard deviation

The standard deviation was expressed as percent of the value of the respective average.

Materials

Unlabeled ribonucleoside triphosphates were purchased from Calbiochem (San Diego, California); ATP and CTP were disodium salts and GTP and UTP were trilithium salts. The radioactive 8-^{3}H-GTP and the 5-6-^{3}H-UTP had a specific activity of 5.6 Ci/mmole and 22 Ci/mmole respectively and were purchased from New England Nuclear (Boston, Mass.). The bovine serum albumin was from Metrix (Chicago, Ill.). α-Amanitan was a gift from Prof. T. Wieland.

RESULTS

In preliminary experiments it was found that the incorporation of GMP was proportional to the amount of nuclear DNA added beyond the range used in these experiments. No incorporation was found when the nuclei were preincubated with DNAase. More than 80% of the counts were solubilized by pancreatic RNAase. At least 70% of the incorporation was dependent on the presence of all four nucleoside triphosphates.

Effect of GTP concentration on GMP incorporation

In Fig. 1 is shown the influence of GTP concentration on the GMP incorporation at 2 and 10 min. The number of picomoles incorporated between 0.35 μM and 0.71 μM ^{3}H-GTP was proportional to the concentration of GTP and then increased more slowly with further increases in concentration. The percent of GMP actually incorporated in 10 min was 0.57% for 0.35 μM and 0.14% for 300 μM GTP when the nuclear DNA concentration was approximately 200 μg/ml. Similar results were obtained in other experiments using six GTP concentrations below 300 μM (results not shown). Therefore, the dependence of incorporation on the concentration of GTP was not due to depletion of substrate.

The linearity of the incorporation with time was dependent on the concentration of GTP; this is shown in Fig. 1B, where we have calculated the "percent deviation from linearity". The "percent deviation from linearity" is the percent difference between the hypothetical incorporation for a reaction in which the incorporation at 10 min is 5 times that at 2 min and the actual incorporation at 10 min. When this "percent deviation from linearity" is plotted against log of the GTP concentration, a straight line is obtained (Fig. 1B). The dependence of linearity on the GTP concentration was also observed in several preliminary experiments not shown here.

Investigation of the basis for non-linearity

The next series of experiments were designed to test alternative explanations for the non-linearity with time observed for the incorporation of GTP. Two principal reasons for the non-linearity were considered. The first (a) was that changes in composition of the incubation medium due to destruction or other losses of substrates, changes in pH, or accumulation of inhibitors caused a decrease in incorporation rate. The other possible reason (b) was that the nuclear template or enzymes were changed during the reaction.

(a) Experiment to test alteration of medium. To test the first explanation, aliquots of nuclei were incubated in the medium with 0.71 μM ^{3}H-GTP. After 10 min, additional nuclei were added to some of the tubes. The results are shown in Fig. 2. As expected, the usual non-linear reaction was observed. However, the addition of new nuclei resulted in a brief period of rapid incorporation of GMP followed by a reduction in rate. This brief period of rapid incorporation clearly showed that the decrease in rate is not due to alteration of the medium.

(b) Enzyme or template inactivation by the incubation medium. The experiments done to study the effect of preincubations in non-complete media on the later incorporation of GMP by nuclei are summarized in Tables 1 and 2. It was found that only a moderate loss of activity (9 to 18%) took place when nuclei were preincubated in 0.32 M sucrose containing $MgCl_2$ and potassium phosphate buffer, pH 6.5. A similar loss of activity took place if one or more of the nucleoside triphosphates (1 mM) were also present during this preincubation. However, 50% of the activity was lost when preincubation for 10 min was in the standard medium without nucleoside triphosphates. This inactivation of the incorporation was increased to 61% when ATP was present and to about 75% when ATP, CTP, and UTP were present and only GTP was omitted, and was 59% in the presence of all four substrates. The addition of either glycerol or bovine serum albumin had a moderate inhibitory activity on the incorporation but did not protect the activity from the effect of preincubation (Table 2).

Effect of different substrate concentrations on GMP incorporation

A wide range of substrate concentrations was investigated (Table 3). The concentrations of ATP, CTP, and UTP were kept equal but varied from 0 to 1 mM, while the concentration of ^{3}H-GTP was varied from 0.24 µM to 300 µM.

The results showed that 300 µM GTP in the presence of 1 mM of the other triphosphonucleosides was near to the optimal conditions in terms of maximum incorporation and linearity. A further increase of the concentration of the three nucleoside triphosphates to 2 mM resulted in a decrease (20%) of the incorporation (Table 4). But this inactivation by substrates was compensated for by a further increase in the concentration of GTP. An

additional increase of the concentration of ATP, CTP, and UTP resulted again in inhibition of about 12%.

Probably there is a complicated relationship between concentration of the substrates and the rate and linearity of the incorporation. This needs to be studied more extensively.

Effect of ionic strength on GMP incorporation at high and low GTP concentration

1) In Table 5 the effect of ammonium sulfate concentration on GMP incorporation is shown. At low GTP concentration the maximum stimulation was at 50 mM and a further increase in ionic strength caused a decreased incorporation. This pattern was nearly the same at both 2 and 10 min. The linearity of the incorporation was not clearly affected by the ionic strength.

At high GTP concentration the effect of ammonium sulfate was different; the maximum stimulation was observed at 330 mM. Also the linearity of the incorporation was clearly improved by the high ammonium sulfate.

2) The effect of ammonium sulfate was studied also on the incorporation of UMP from UTP at low and high concentration (Table 6). The results with UMP incorporation were similar to the ones obtained with GMP. That is, at low concentration of UTP the maximum incorporation was at 50 mM ammonium sulfate and at high UTP concentration the maximum was at 330 mM.

The U/G incorporation ratio, obtained in the conditions described in Table 6, was markedly dependent on the substrate concentration, suggesting that actually the type of RNA synthesized is dependent on the substrate concentration. At low concentration of the labeled substrate (either UTP or GTP) the U/G incorporation ratio was smaller than one and was increased by

raising the ammonium sulfate concentration. However, the effect of substrate concentration seems to outweigh the effect of ionic strength on the U/G incorporation ratio.

Inhibition by α-amanitin and actinomycin-D at low and high GTP concentration

The effect of α-amanitin and actinomycin-D on the incorporation of GMP is shown in Table 7. At both concentrations of GTP, α-amanitin is equally effective, indicating that RNA polymerase II is responsible for the incorporation (Kedinger et al., 1970). On the other hand, the actinomycin-D shows different inhibition at the two GTP concentrations.

DISCUSSION

As was noted in the Introduction, the concentration of nucleoside triphosphates used by different authors for studying RNA synthesis in vitro by brain nuclei has extended over a wide range; this is particularly true for the labeled nucleoside triphosphate (Slagel & Akers, 1972; Banks & Johnson, 1973; Austoker et al., 1972). The effect of this wide range of substrate concentration has not been previously reported. From a practical point of view, the use of very low concentrations of the labeled substrate is very convenient because it is possible to use higher final specific activities so that the total radioactivity incorporated is considerably higher.

The GMP incorporated increases with the concentration of GTP, especially at low GTP concentrations, but the saturation is not reached at 300 μM. The saturation with GTP depends on the concentration of the other three substrates as well.

It is important to note that the incorporation at low GTP concentrations is less linear with time than at higher GTP concentrations (Fig. 1B). This different linearity could not be explained in terms of exhaustion of substrates.

Another characteristic of the RNA polymerase in nuclei that was investigated was its inactivation when preincubated in the absence of selected components of the medium. The preincubation experiments indicated that the polymerase activity is moderately labile when the nuclei are preincubated in sucrose solution; we found a smaller inactivation (10-20%) than had previously been reported by Thompson (1973). Ammonium sulfate was found to cause a rapid loss of activity, up to 50% with 10 min preincubation. The addition of ATP, CTP, and UTP to this high ionic strength preincubation medium resulted in a further inactivation to approximately 70%. Partial and similar results were reported by Novello and Stirpe (1969).

Sarkar and Paulus (1972) reported an inactivation by ATP of a purified bacterial RNA polymerase. However, there are several differences between their report and our results. In their system the ATP is more inhibitory than any other of the nucleoside triphosphates, while we found that ATP did not contribute more to inactivation than the other nucleoside triphosphates. Also glycerol did not provide protection from inactivation, contrary to what they reported. So it seems that the two phenomena are not closely related, but comparisons are difficult because of differences between the procedures used.

The inactivation by ammonium sulfate can be explained by the reports of Chambon et al. (1965,1967) that the RNA polymerase can be associated

with DNA with or without RNA. When the enzyme is associated with DNA only, it is less stable than when it is also associated with RNA. Once the enzyme is dissociated from the DNA it cannot reassociate in the presence of ammonium sulfate. However, the DNA enzyme complex is stabilized when exposed to all four substrates before addition of ammonium sulfate because it transforms into the DNA-enzyme-RNA complex. We believe that the stability increases when the enzyme is engaged in RNA synthesis because then the concentration of the ternary complex would be higher than in the absence of RNA synthesis. In the presence of extremely low GTP concentrations, the conditions are very similar to the absence of RNA synthesis, and probably many complexes of enzyme-template break into free enzyme and template. As this process is irreversible in the presence of ammonium sulfate, the decreased concentration of active enzyme complexes results in loss of enzyme activity and therefore a less linear reaction than at higher GTP concentration. In that case, the results shown in Fig. 1B reflect the relative stability of the enzyme template complex at different GTP concentrations.

There are some other differences between the reaction at low and high GTP concentrations, for example, the stimulation by ammonium sulfate. Usually the optimal concentration of ammonium sulfate has been reported to be between 0.3 M and 0.4 M both in liver (Pogo et al., 1967; Windell & Tata, 1966) and brain (Kato & Kurokawa, 1970, and Thompson, 1973). All these authors used relatively high concentrations of the labeled nucleoside triphosphate. Slagel and Akers (1972) and Banks and Johnson (1973), using a low concentration of GTP, reported a lower optimal ammonium sulfate concentration. We found at low GTP concentration in the standard

medium that the stimulation by ammonium sulfate was maximal at 50 mM. In contrast, at high GTP concentration the stimulation was maximal at 300 mM. This may explain the apparent contradiction in the previous reports. The concentration of the remaining three substrates seems also to influence the optimal ammonium sulfate concentration.

Kato and Kurokawa (1970) reported that the single nucleoside triphosphate reaction is maximal at 30 mM ammonium sulfate. They also reported that actinomycin D stimulated rather than inhibited the single nucleotide reaction as shown by the incorporation of ATP.

Thus the reduced inhibition by actinomycin D of GTP incorporation that we observed at low GTP concentration (Table 7) suggests that this GMP incorporation is partially by the single nucleotide reaction. We also showed that in the absence of ATP, CTP, and UTP substantial incorporation of GTP occurred, which provided more direct evidence of single nucleoside triphosphate incorporation (Table 3).

In this context, it is probably important to mention the reports of Johnson et al. (1969,1971) that 40 mM ammonium sulfate under certain conditions stimulates the synthesis of ribosomal RNA in rat liver nuclei. Despite the fact that in our report, at low GTP, the GMP incorporation is stimulated by low ammonium sulfate concentration and the U/G incorporation ratio is low, the failure of actinomycin D to inhibit is an indication that it is not ribosomal RNA synthesis. The only other reported inhibition by actinomycin at low GTP concentration and high ionic strength was in the presence of 400 µg/ml of the inhibitor (Slagel and Akers, 1972), so the results are not comparable. The extent of inhibition by actinomycin at high GTP is in agreement with other reports (Thompson, 1973; Gomez et al., 1971).

From these experiments, it is evident that the substrate concentrations are of crucial importance. The changes of the characteristics of the incorporation at the different substrate concentrations could indicate some form of regulation of RNA synthesis *in vivo*. It is known that the concentrations of nucleotides change with different metabolic states of the brain (Piccolo *et al.*, 1969) and so does the RNA synthesis (Glassman, 1969). While it would be difficult to maintain that the conditions of incubation used *in vitro* could possibly reflect the physiological conditions of the "*in vivo*" nucleus, nevertheless in many occasions the results of *in vitro* incorporation reflected the "*in vivo*" physiological state of the animal (Salaman *et al.*, 1972; Pogo *et al.*, 1966; Franze-Fernandez and Pogo, 1971; Haywood *et al.*, 1970; Windell and Tata, 1968).

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TABLE 1.--EFFECT OF PREINCUBATION ON THE INCORPORATION OF GMP BY NUCLEI

Components of the standard medium omitted during pre-incubation	GTP concentration during incorporation: 0.71 µM, Time of incubation: 10 min — GMP incorp. pmoles/100 µg DNA	% Inactivation	GTP concentration during incorporation: 300 µM, Time of incubation: 20 min — GMP incorp. pmoles/100 µg DNA	% Inactivation
Non-preincubated control*	3.3 ± 12%		321 ± 7%	
All components omitted, preincubated in 0.32 M sucrose	2.7 ± 4%	18	291 ± 5%	9
$(NH_4)_2SO_4$, GTP			225 ± 13%	30
ATP, CTP, UTP, GTP	1.5 ± 11%	55	156 ± 8%	51
CTP, UTP, GTP			126 ± 13%	61
8-^{3}H-GTP	0.67 ± 19%	80		
GTP			131 ± 9%	59

*Preincubations were at 37°C for 10 min. The preincubation volume was 480 µl in tubes with final 0.71 µM GTP. However, the preincubation volume was 100 µl for tubes of both GTP concentration when preincubated in 0.32 M sucrose, 1 mM $MgCl_2$, 1 mM potassium phosphate, pH 6.5. For the tubes with a final concentration of 300 µM GTP, the preincubation volume was different in every case, as follows: In medium without $(NH_4)_2SO_4$ and GTP, 410 µl; in medium without all nucleotide triphosphates, 400 µl;

TABLE 1 (Cont.)

without CTP, UTP, and GTP, 420 µl; and in the medium without 8-^{3}H-GTP, 480 µl. With the exception of preincubation in sucrose, the pH was 8.2 during preincubation and incubation. At 0.71 µM GTP the incorporations at both 2 and 10 min were done, but as the percent inactivation was similar at 10 and 2 min, the latter are not shown. At 0.71 µM GTP and at 300 µM GTP, 2 and 4 µCi of 8-^{3}H-GTP were present respectively. The volume during the incorporation was 500 µl. All determination were done in quadruplicate.

TABLE 2.--EFFECT OF PREINCUBATION IN MEDIUM WITH OR WITHOUT NUCLEOTIDES ON THE INCORPORATION OF GMP AND THE EFFECT OF ALBUMIN AND GLYCEROL ON THE ACTIVITY.

Components of the medium omitted	Media during incorporation					
	Standard medium		Medium with 100 µg/tube albumin		Medium with glycerol, 10% v/v	
	GMP incorp. pmoles/100 µg DNA	% Inactivation	GMP incorp. pmoles/100 µg DNA	% Inactivation	GMP incorp. pmoles/100 µg DNA	% inactivation
Non-preincubated controls	341.6±11%		300.0±12%		247.5±7%	
ATP, CTP, UTP, GTP	178.0±27%	48	169.5±8.5%	43	117.6±18%	52
GTP	91.0±11.4%	73	99.5±23%	67	57.4±43%	77

Nuclei were either incubated in the standard medium for 20 min (see METHODS) or were preincubated 10 min at 37°C in the medium without nucleoside triphosphates or with the medium containing 1.2 mM of ATP, CTP, and UTP. The preincubation volume was 440 µl; after 10 min, 80 µl of the remaining components necessary to complete the medium were added and the incorporation of GMP was allowed for 20 min. The volume during the incorporation was 520 µl, the concentration of GTP (4 µCi) was 300 µM, and the concentration of DNA was 146 µg/ml. The pH was 8.2. The determinations were done in quadruplicate, in the presence of 4 µCi of 8-^{3}H-GTP and 77.6 µg of DNA.

TABLE 3.--EFFECT OF CONCENTRATION OF NUCLEOSIDE TRIPHOSPHATES ON THE INCORPORATION OF GMP

GTP concentration μM	Time of incubation min	Concentration of ATP, CTP, UTP, μM each: 0	0.35	10	300	1000
		picomoles incorporated per 100 μg of DNA				
0.24	2		.21	.49	.38	
	10		.61	1.36	.98	
	Deviation from linearity		41%	45%	48%	
10	2	.45			9.6	7.7
	10	1.06			28.6	23.5
	Deviation from linearity	53%			41%	39%
300	2	11.0	5.70	26.3	66.4	56.2
	10	11.6	5.70	24.8	157.6	214.5
	Deviation from linearity	79%	80%	81%	52%	23%

The nuclei were incubated in the standard medium but with different nucleotide concentrations, as shown in the table. The values are picomoles incorporated per 100 μg DNA at 2 min and 10 min. The deviation from linearity is given as percent and is defined in the legend to Fig. 1B. The determinations were done in duplicate and 4 μCi of ^{3}H-GTP were added to 10 μM and 300 μM GTP containing tubes.

TABLE 4.--INHIBITORY EFFECT OF HIGH CONCENTRATION OF ATP, CTP, AND UTP ON THE GMP INCORPORATION

GTP, mM	picomoles incorporated per 100 µg DNA in 10 min ATP, CTP, and UTP concentration			
	1 mM	1.5 mM	2 mM	3 mM
0.3	186.1 ± 13.8	185.3 ± 8.4	149.9 ± 6.6	
1.0			228.9 ± 12.5	164.2 ± 10.1

The determinations were done in quadruplicate; 4 µCi of ^{3}H-GTP were added to each tube. Unless otherwise stated, the conditions were as in Table 3.

TABLE 5.--EFFECT OF DIFFERENT AMMONIUM SULFATE CONCENTRATIONS ON THE INCORPORATION OF GMP FROM EITHER 0.35 μM ^{3}H-GTP OR FROM 300 μM

$(NH_4)_2SO_4$ conc. (mM)	picomoles of GMP incorp. per 100 μg DNA in 2 min	picomoles of GMP incorp. per 100 μg DNA in 10 min	Deviation from linearity (%)
	0.35 μM GTP		
0	0.18 ± 4.6%	0.41 ± 25%	59
50	0.40 ± 6.3%	0.67 ± 8.4%	67
150	0.33 ± 2.5%	0.57 ± 10.2%	65
330	0.24 ± 2.5%	0.54 ± 9.2%	56
	300 μM GTP		
0	17.4 ± 7.1%	25.8 ± 14%	70
50	21.0 ± 12%	28.8 ± 7.1%	72
150	33.8 ± 5.2%	53.8 ± 8.3%	68
330	39.1 ± 8%	125.9 ± 4.1%	35

Nuclei from rat cerebral cortex were added in 100 μl of 0.32 M sucrose, 1 mM $MgCl_2$, 1 mM KH_2PO_4, pH 6.5, to 400 μl of solution, so that the final concentration expressed as mM was Tris 100, KCl 70, β-mercaptoethanol 40, $MnCl_2$ 2, ATP, CTP, and UTP each 1, and GTP either 0.35 μM or 300 μM. When the GTP concentration was 0.35 μM, each tube contained 1 μCi of 8-^{3}H-GTP; when the concentration was 300 μM, each tube contained 4 μCi per tube. The pH during the incubation was 8.2. The incubation was done at 37°C. The results are expressed as pmoles incorporated per 100 μg of nuclear DNA. The actual amount of DNA added to each tube was 102 μg. All determinations were done in quadruplicate.

TABLE 6.--EFFECT OF DIFFERENT AMMONIUM SULFATE CONCENTRATIONS AND LABELED NUCLEOSIDE TRIPHOSPHATE CONCENTRATIONS (EITHER UTP OR GTP) ON THE RATIO OF LABELED UMP AND GMP INCORPORATED BY RAT CORTICAL NUCLEI

$(NH_4)_2SO_4$ conc. (mM)	pmoles of GMP incorp. during 10 min/100 µg DNA	pmoles of UMP incorp. during 10 min/100 µg DNA	U/G
	Concentration of labeled nucleoside triphosphate, 0.4 µM		
0	0.345 ± 5%	0.131 ± 3.4%	0.38
50	0.679 ± 4.8%	0.366 ± 5.5%	0.54
330	0.535 ± 1%	0.339 ± 2.4%	0.63
	Concentration of labeled nucleoside triphosphate, 300 µM		
0	28.5 ± 4.4%	35.3 ± 15%	1.24
50	38.3 ± 17%	54.1 ± 11%	1.41
330	150.0 ± 2%	224.2 ± 4.5%	1.51

The conditions were the same as in Table 5 unless otherwise stated. The actual amount of DNA per tube was 68 µg. At 0.4 µM of the labeled nucleoside triphosphate 1.14 µCi were present, and at 300 µM, 4 µCi were present.

TABLE 7.--INHIBITION OF GMP INCORPORATION BY α-AMANITIN AND ACTINOMYCIN D AT LOW AND HIGH GTP CONCENTRATION

	GTP concentration			
	0.35 μM		300 μM	
	picomoles incorp.	Inhibition %	picomoles incorp.	Inhibition %
Control	2.27 ± 11%		331 ± 14%	
α-Amanitin	0.16 ± 18%	93	18.9 ± 30%	94
Actinomycin D	1.89 ± 8%	16	45.3 ± 37%	86

The incubation was done in the standard medium; 1 μCi of 8-^{3}H-GTP was present in tubes with 0.35 μM GTP and 4 μCi in 300 μM. The incubation time was 10 min; to appropriate tubes 1.28 μg of α-amanitin or 1.5 μg of actinomycin were added. The final volume was 500 μl with 47 μg of nuclear DNA per tube.

FIGURE LEGENDS

Fig. 1.--(A) Effect of GTP concentration on GMP incorporation at 2 and 10 min. Nuclei (corresponding to 108 µg of DNA) were incubated for 2 min (-●-●-) or 10 min (-o-o-) in the standard medium with different concentrations of GTP as indicated. The 0.35 µM GTP had 1 µCi 8-^{3}H-GTP per tube; the 0.71 µM GTP had 2 µCi. In both cases labeled GTP was the only GTP added. At 10, 100, and 300 µM GTP, 4 µCi of ^{3}H-GTP were added with an appropriate amount of non-labeled GTP. The incorporations are expressed as picomoles incorporated per 100 µg of DNA. The vertical bars are the standard deviations. The incorporations at 0.35 µM and 0.71 µM GTP are shown in an expanded scale in the inset.

(B) Effect of GTP concentration on the linearity of GMP incorporation. Percent deviation of linearity, between 2 and 10 min, is the percent difference between the hypothetical incorporation at 10 min and the actual incorporation at 10 min. The hypothetical incorporation at 10 min is calculated assuming that the incorporation is linear between 2 and 10 min; that is, at 10 min the incorporation is 5 times the incorporation at 2 min.

Fig. 2.--Nuclei containing 28 µg of DNA were incubated for different times up to 20 min; to some of the tubes additional nuclei were added (15 µg of DNA in 25 µl of 0.32 M sucrose 1 mM), and the incubation was continued for 2, 5, or 10 more minutes. The standard medium was used. The concentration of GTP was 0.71 µM and 2 µCi were present in each tube. The values shown are means of duplicates.

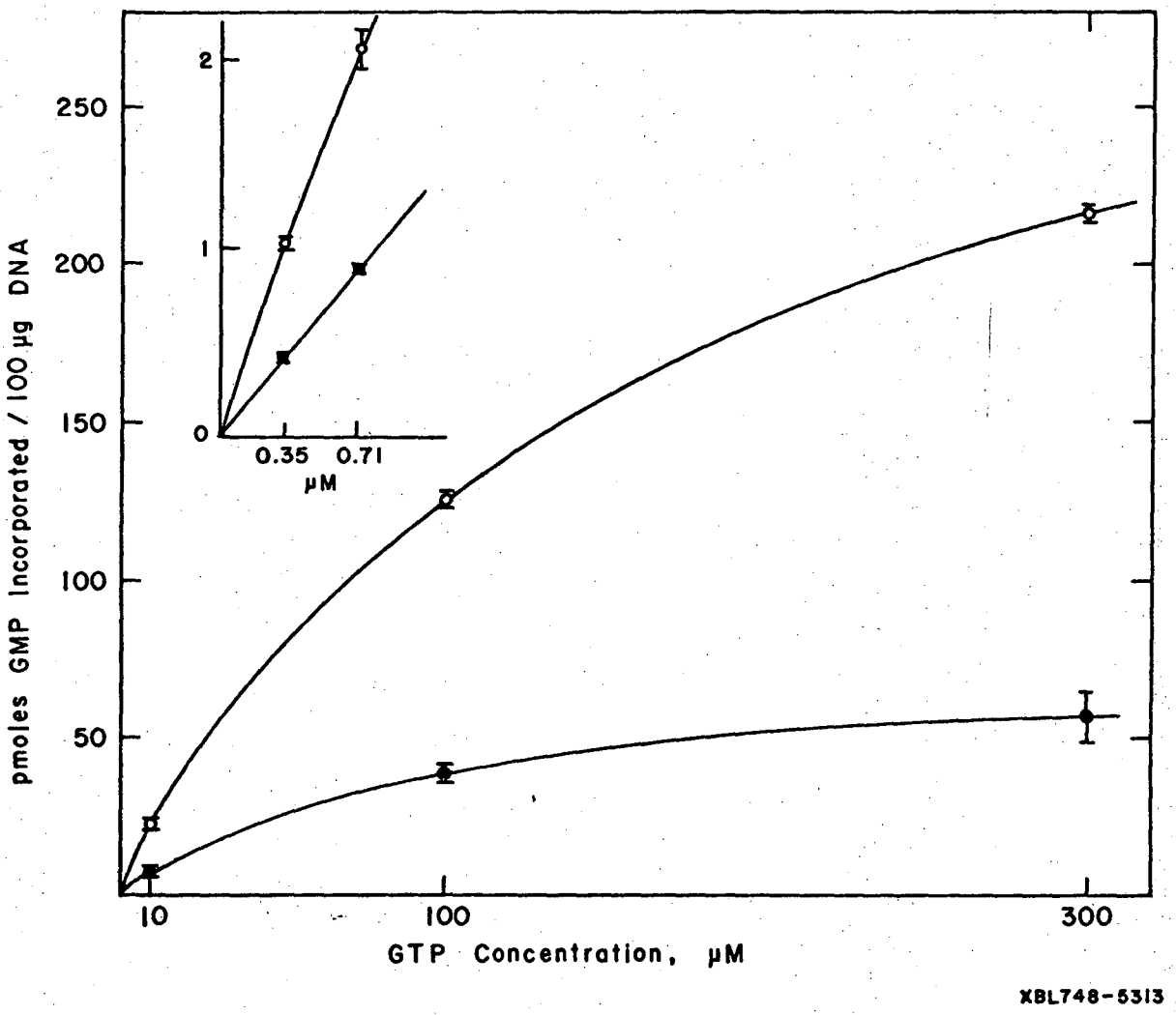
pmoles GMP Incorporated / 100 µg DNA
250
200
150
100
50
10
100
300
GTP Concentration, µM
2
1
0
0.35
0.71
µM
XBL748-5313

Fig. 1a.

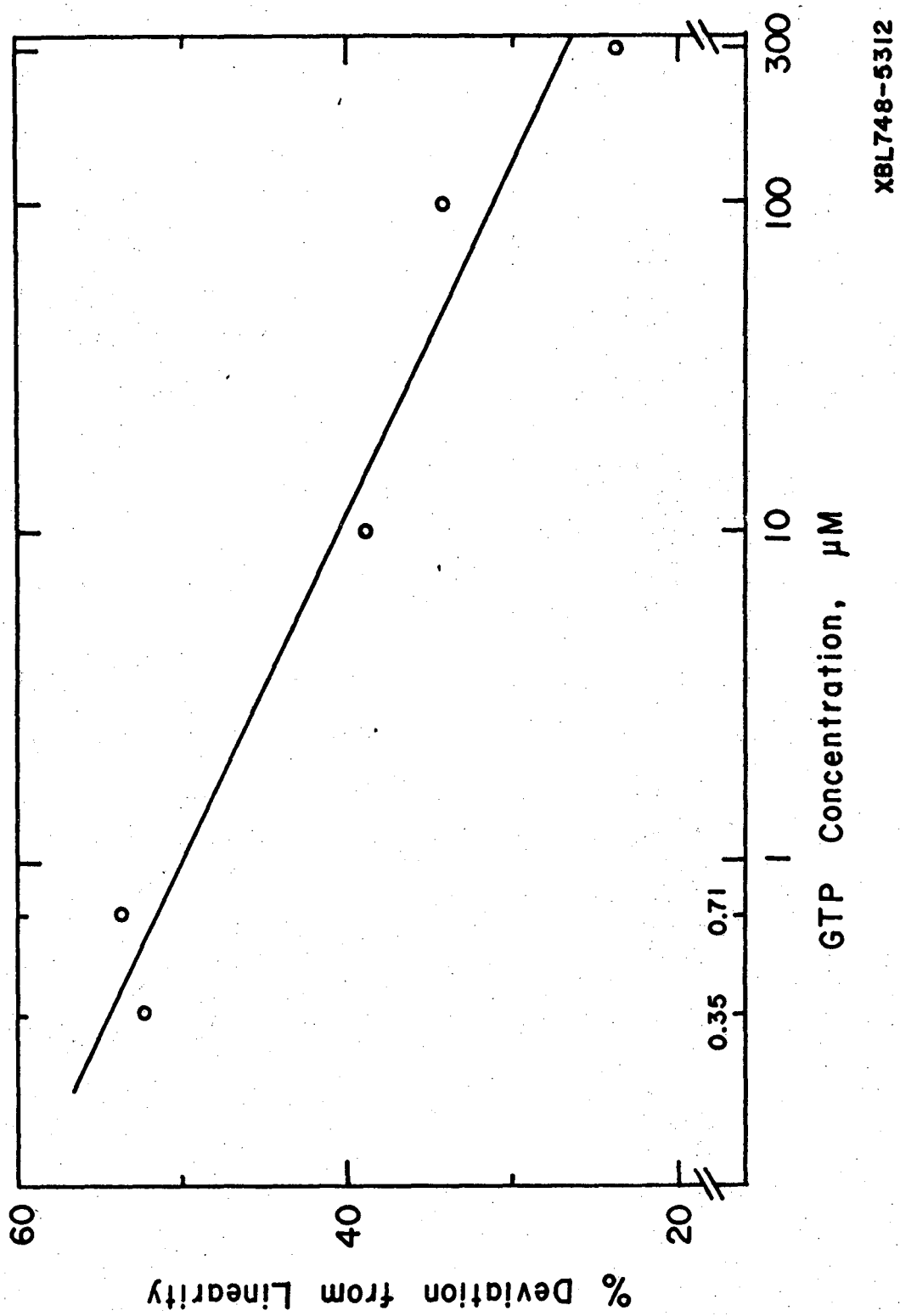
% Deviation from Linearity
60
40
20
GTP Concentration, μM
0.35
0.71
1
10
100
300
XBL748-5312

Fig. 1b.

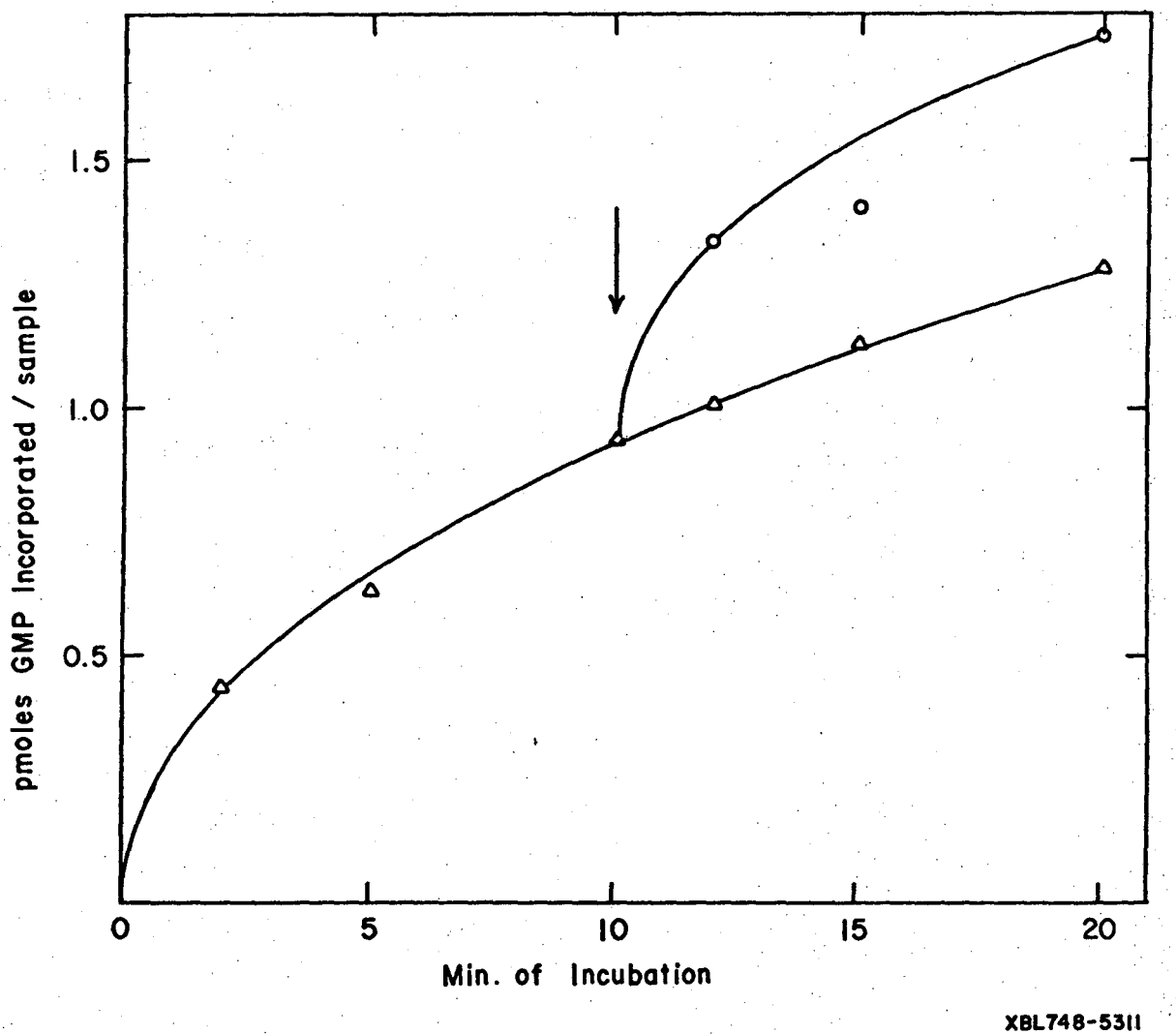
pmoles GMP Incorporated / sample
1.5
1.0
0.5
0
5
10
15
20
Min. of Incubation
XBL748-5311

Fig. 2.

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