# Effects of $N^2, O^{2'}$-dibutyril cyclic GMP on the nucleoside phosphotransferase activity of the retina of the chick embryos

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*Summary.* In the retina of the chick embryo, 2 different forms of nucleoside phosphotransferase take part in the phosphorylation of thymidine. One is an unstable form with higher molecular weight. The other with lower m. wt is a stable form. This paper shows that $N^2, O^{2'}$-dibutyril cyclic GMP causes a marked decrement of the activity of the unstable nucleoside phosphotransferase.

The phosphorylation of thymidine is catalyzed in mammalian tissues and E. coli by thimidine kinase. In plants and in certain microorganisms[1-5], this reaction depends upon another activity, a nucleoside phosphotransferase which catalyzes the reversible transfer of ester phosphate from nucleoside monophosphate or diphosphate to nucleoside.

Previously[6] we found in the retina of the chick embryos 2 different forms of nucleoside phosphotransferase which are able to phosphorylate thymidine. Both these forms have a non-specific activity and they prefer the nucleosides monophosphate as phosphate donors. The form with a higher m.wt is very unstable to dilution, storage and gel filtration, and it is unable to utilize the adenine nucleotides as phosphate donors. UTP seems to protect this form by the inactivation. The other form with lower m.wt is stable and utilizes also the adenine nucleotides as phosphate donors. AMP seems to be the substrate more active. In this paper, we examine the effects of $N^2, O^{2'}$-dibutyril GMP (DBcGMP) on the nucleoside phosphotransferase activity of the chick embryo retina.

Effects of $N^2$, $O^{2'}$-dibutyril cyclic GMP (DBcGMP) on the thymidine phosphorylating activity of the chick embryo retina

| Substrate | Thymidine phosphorylated nmoles/mg of protein | | |
|---|---|---|---|
| | | DBcGMP | |
| | | 1 mM | 2 mM |
| No addition | 0.18 ± 0.02 | 0.15 ± 0.02 | 0.10 ± 0.02 |
| ADP | 1.18 ± 0.12 | 1.41 ± 0.14 | 1.65 ± 0.18 |
| AMP | 1.65 ± 0.18 | 2.24 ± 0.25 | 3.08 ± 0.30 |
| UDP | 2.85 ± 0.30 | 1.99 ± 0.22 | 1.56 ± 0.17 |
| UMP | 3.60 ± 0.26 | 1.90 ± 0.18 | 1.58 ± 0.19 |
| GMP | 3.30 ± 0.24 | 2.21 ± 0.20 | 2.14 ± 0.18 |
| CMP | 3.10 ± 0.28 | 1.86 ± 0.20 | 1.52 ± 0.17 |
| TMP | 3.75 ± 0.32 | 2.25 ± 0.24 | 1.76 ± 0.20 |

Data are the mean ± SE of 6 separate experiments.

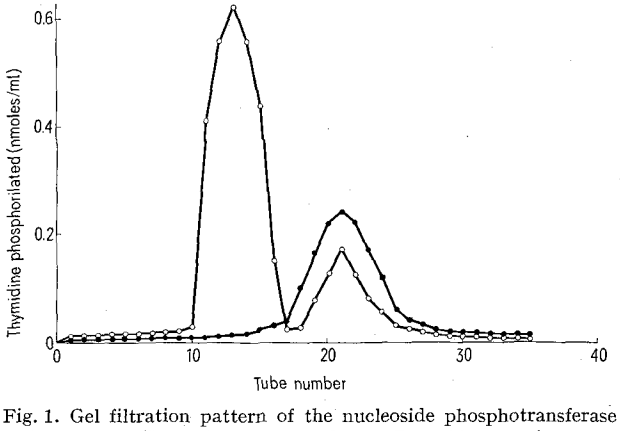


Fig. 1. Gel filtration pattern of the nucleoside phosphotransferase activity on a Sephadex G-200 column. For this experiment, a homogenate was obtained from 40 retinas and the resulting 105,000 g supernatant was applied to a column (32 × 2 cm) of Sephadex G-200. The column was equilibrated and eluted with 5 mM tris-HCl buffer (pH 8.0) containing 0.2 mM UTP and 2 mM $MgCl_2$. Fractions of 5 ml were collected. 200 µl of each fraction were utilized for the measurement of the nucleoside phosphotransferase activity by using as substrate UMP (○—○) or AMP (●—●).

The retinas were rapidly removed from 12-day-old chick embryos, washed gently in cold 0.1 M tris-HCl buffer (pH 8.0) and homogenized in Potter-Elvehjem in the same medium (0.25 ml/retina). The homogenate was centrifuged at 105,000 × g for 30 min and the supernatant was utilized for the incubation samples. The standard reaction mixture contained, in a final volume of 500 µl, 40 mM tris-HCl (pH 8), 5 mM $MgCl_2$, 10 mM substrate, 20 µM (0.5 µCi) (Me-$^3$H) thymidine and 200 µl of the enzyme extract. After incubation at 37 °C for 1 h, the reaction was stopped by boiling the samples for 3 min. For the evaluation of the thymidine nucleotides formed, we have employed columns (3 × 1 cm) of Dowex-1 formate, prepared according to Hurlbert et al.[7]. At first thymidine was eluted from the column by 38 ml of 2 N formic acid. Successively the thymidine nucleotides were eluted by 10 ml of 1 N ammonium formate-4 N formic acid. This last fraction was evaporated 'in vacuo' at 10 °C and the residue was dissolved in 0.5 ml of water. Aliquots were evaluated for radioactivity in a Nuclear-Chicago scintillation counter. Corrections for quenching were made by using external standardization. As determined by chromatographic procedure, thymidilic acid accounted for at least 95% of the phosphorylated products.

Data reported in the table show the effects of DBcGMP on the thymidine phosphorylating activity when the reaction was measured in the 105,000 g supernatant. DBcGMP causes an increment of this activity when ADP and particularly AMP are employed as phosphate donors, while it has an inhibitive effect using the other substrates reported in the table. This inhibition is greater when UMP was employed. The elution pattern of nucleoside phosphotransferase on Sephadex G-200 column is

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reported in figure 1. For this experiment, the Sephadex was swelled and eluted with a solution of 5 mM tris-HCl buffer (pH 8.0) containing 0.2 mM UTP and 2 mM $MgCl_2$, because $Mg^{++}$ and particularly UTP stabilize the unstable form of nucleoside phosphotransferase[6]. The figure shows 2 peaks of activity: the first corresponds, as previously demonstrated[6], to the unstable nucleoside phosphotransferase and it prefers UMP as phosphate donor, while the second is represented by a stable form which employes preferentially AMP as substrate.

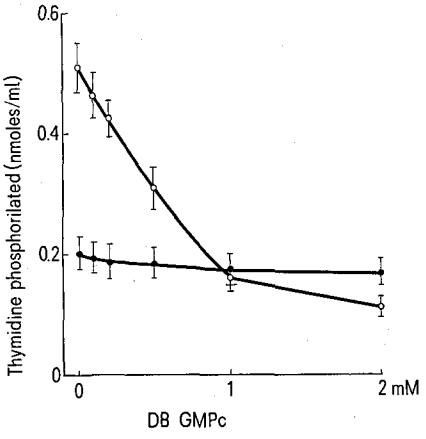


Fig. 2. Effects of DBcGMP on the 2 forms of nucleoside phosphotransferase of chick embryo retina. Peak I (tube numbers 11–15) and peak II (tube numbers 19–23) were collected separately and 200 µl of each peak were utilized for the incubation sample. The activity was measured by using as phosphate donor UMP (○—○) for peak I and AMP (●—●) for peak II. Data are the means ± SE of 6 separate experiments.

As shown in figure 2, DBcGMP markedly inhibits the nucleoside phosphotransferase of peak I, while any significative effect was not observed for the activity of peak II. Previously[6] we have hypothesized that the nucleoside phosphotransferase is present in the chick embryo retina at least in 2 different forms, which could be an expression of the same enzyme at different aggregation states.

It is possible that DBcGMP facilitates the conversion of the form with higher m.wt into a disaggregated state. This state could be represented by the stable nucleoside phosphotransferase, an enzymatic activity which is able to utilize as phosphate donors also the adenine nucleotides. These considerations could explain why the DBcGMP causes an increment of the thymidine phosphorylating rate when the reaction is measured, by using AMP as phosphate donor, in the 105,000 g supernatant. Furthermore, because it seems that the nucleoside phosphotransferase takes part in the control of the endogenous pools of nucleosides and nucleotides, the effects of DBcGMP on this activity could indicate the participation of this compound in the regulation of nucleotide metabolism.

## Absorption and biotransformation of L(+)-tartaric acid in rats

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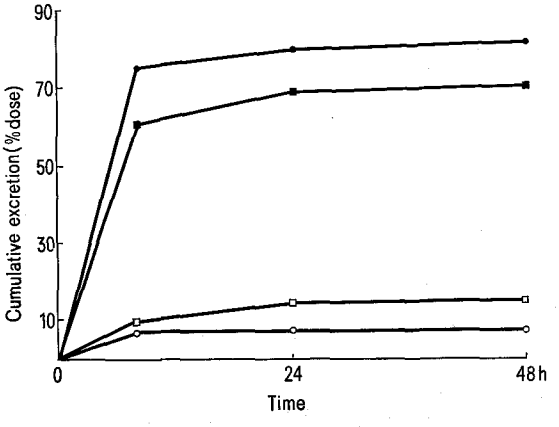
*Summary.* Oral or parenteral doses of monosodium $^{14}$C-L(+)-tartrate (400 mg/kg) are rapidly excreted by rats and a proportion completely metabolized to $CO_2$. The oral dose was well-absorbed.

Tartaric acid and its salts are used in medicine and in the food industry. In humans, the acid is thought to be poorly absorbed[1] and when given orally, to be metabolized by the gut flora[2,3], since it is readily metabolized by microorganisms such as Pseudomonas putida[4] and Penicillium charlesii[5], which convert it to glycerate and $CO_2$. Studies in dogs and rabbits have shown that oral doses of tartaric acid were excreted in the urine as unchanged compound, the proportion of which decreased with increasing doses[6]. Much of the tartaric acid used is obtained as a byproduct of wine manufacture and is therefore the naturally-occurring L(+) form[7]. Thus the absorption and biotransformation of tartaric acid has been evaluated using the $^{14}$C-L(+) form.




Cumulative excretion of radioactivity in the urine (■, ●) and expired air (□, ○) of rats dosed orally or i.v. respectively with monosodium $^{14}$C-L(+)-tartrate (400 mg/kg).

*Materials.* (1,4-$^{14}$C)-DL-Tartaric acid of specific activity 2–10 mCi/mmoles was obtained from The Radiochemical Centre, Amersham, England, and was resolved into the L(+)-isomer[8]. The resulting monosodium $^{14}$C-L(+)-

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