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# ENZYMATIC SYNTHESIS OF RIBONUCLEOSIDE-5'-PHOSPHATES FROM SOME $N^6$-SUBSTITUTED ADENOSINES

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**Key Word Index**—*Acer pseudoplatanus; Schizosaccharomyces pombe;* Saccharomycetaceae; cytokinins nucleotides biosynthesis; adenosine kinase.

**Abstract**—Adenosine kinase catalyses ribonucleoside-5'-monophosphate synthesis from various $N^6$-substituted analogues of adenosine. The nature of the $N^6$-substituent sharply influences the rate of the synthesis with enzymes from different plant systems. Attempts to synthesize nucleoside triphosphates from several purified $N^6$-substituted adenosine-5'-monophosphates using a number of different enzyme systems, were not successful. The significance of the results to our understanding of the *in vivo* metabolism of cytokinin is discussed.

## INTRODUCTION

$N^6$-SUBSTITUTED analogues of adenosine are of interest because of their cytokinin activity. When a cytokinin is supplied to a plant organ, it is at least partially converted into several metabolites, among which the cytokinin ribosides are often observed. McCalla *et al.*[1] and Fox *et al.*[2] have shown that 6-benzylaminopurine (BAP) is transformed into its related nucleoside by senescing leaves of *Xanthium pensylvanicum* and by soybean tissue. Guern obtained the same result with *Cicer arietinum* epicotyls, using either BAP[3,4] or kinetin.[5] Sondheimer and Tzou[6] have shown that zeatin supplied to bean hypocotyls is partially transformed into zeatin and dihydrozeatin ribosides. Some of these authors[1,2,6] also obtained the related nucleoside-5'-phosphates as metabolites. Formation of the corresponding nucleotides from PAP (6-propylaminopurine), BAP, FAP (6-furfurylaminopurine) was also described by Doree *et al.*[7,8] in *Acer pseudoplatanus* cell suspension.

It is possible to perform the *in vitro* enzymatic synthesis of cytokinin nucleosides[9] by using an *E. coli* nucleoside phosphorylase (E.C. 2.4.2.1) to catalyse the reaction but similar synthesis of cytokinin nucleotides has not yet been obtained. We investigated the possibility

[1] MCCALLA, D. R., MOORE, D. J. and OSBORNE, D. (1962) *Biochim. Biophys. Acta* **55**, 522.
[2] FOX, J. E., DYSON, W. D., SOOD, C. and MCCHESNEY, J. (1972) in *Plant Growth Substances* 1970, *Proc. 7th Intern. Conf. Plant Growth Substances, Canberra, Australia* (CARR, D. J., ed.), p. 449, Springer, Berlin.
[3] GUERN, J. (1966) *Compt. Rend.* **262**, 2340.
[4] GUERN, J., DOREE, M. and SADORGE, P. (1968) in *Biochemistry and Physiology of Plant Growth Substances, Proc. 6th Intern. Conf. Plant Growth Substances, Ottawa, Canada,* 1967 (WIGHTMAN, F. and SETTERFIELD, G., eds.), p. 1155, Runge Press, Ottawa.
[5] GUERN, J. personnal communication.
[6] SONDHEIMER, E. and TZOU, D. S. (1971) *Plant Physiol.* **47**, 516.
[7] DOREE, M., TERRINE, C. and TRAPY, F. (1972) *Compt. Rend.* **275**, 1131.
[8] DOREE, M., TERRINE, C. and GUERN, J. (1973) in *Modern Aspects of Hormonal Regulation of Plant Growth and Development* (KALDEWEY, O. K., ed.), Springer, Berlin.
[9] SIVADJIAN, A., SADORGE, P., GAWER, M., TERRINE, C. and GUERN, J. (1969) *Physiol. Veg.* **7**, 31.

that adenosine kinase (E.C. 2.7.1.20) might be able to catalyse the synthesis of the corresponding nucleotides from several $N^6$-substituted adenosines (MAPR, 6-methyladenosine; PAPR, 6-propyladenosine; FAPR, 6-furfuryladenosine; BAPR, 6-benzyladenosine), using ATP as the phosphoryl donor. The results presented here show that this synthesis is actually possible.

Since the synthesis of nucleoside triphosphates of $N^6$-substituted adenosines would be a prerequisite for further incorporation in polynucleotidic structures, we tried to perform this synthesis from the related nucleoside-5′-monophosphates but failed.

## RESULTS

### *Evidence of the Enzymatic Synthesis of the Nucleoside-5′-monophosphates from the Corresponding* N$^6$*-substituted Adenosines*

The ability of *Schizosaccharomyces pombe* extracts to catalyse the phosphorylation of APR (adenosine), MAPR, PAPR, FAPR and BAPR in the presence of ATP as phosphoryl donor, has been investigated (see Experimental). APR is fully transformed into products well identified by their electrophoretic properties as nucleotides. ATP is the main product (more than 95%). When $N^6$-substituted adenosines were used as substrates, a rapid and complete transformation into a single product, well defined by its electrophoretic mobility and chromatographic behaviour, was observed in each case (Table 1). The reaction products were assumed to be the related nucleotides of the $N^6$-substituted adenosines used as substrates. Their sensitivity to *E. coli* alkaline phosphatase was tested (see Experimental); the $N^6$-substituted nucleoside starting material was recovered in each case. The same result was obtained by using *Crotalus adamantus* 5′-nucleotidase; therefore the products synthesized from $N^6$-substituted adenosines are indeed $N^6$-substituted adenosine-5′-phosphates. The electrophoretic mobilities of these nucleotides are closer to the mobility of AMP (2·6 cm/hr) than to those of ADP (5·8 cm/hr) or ATP (7·0 cm/hr). The products from $N^6$-substituted adenosines are therefore the corresponding nucleoside-5′-monophosphates. The enzyme responsible for this synthesis is really an adenosine kinase. Arima *et al.*[10] have shown that in sprouting potatoes and *Tetrahymena pyriformis*, the thymidine phosphorylation reaction in the presence of ATP is as follows: ATP is first converted to AMP by an ATP hydrolysing enzyme, then thymidine is phosphorylated by nucleoside phosphotransferase with AMP as phosphate donor. $N^6$-substituted nucleotide synthesis may be different, since AMP cannot replace ATP as phosphoryl donor (see Table 2). On the other hand one can discard a more sophisticated hypothesis involving the transformation of the nucleoside into the base, followed by a phosphoribosyl group transfer from phosphoribosylpyrophosphate (PRPP) to the base catalysed by a purine phosphoribosyltransferase (E.C. 2.4.2.7). In fact, it is not possible to synthesize the $N^6$-substituted nucleotides under the same experimental conditions when the corresponding bases are used as substrates instead of nucleosides, as the addition of PRPP is required for this synthesis.[11]

We have also demonstrated that adenosine kinase activity is present in the axillary buds of *Cicer arietinum* and in cells of *Acer pseudoplatanus* grown in liquid medium.

### *Influence of the* N$^6$*-substituent Group upon the Rate of Nucleotide Synthesis Catalysed by* S. pombe *and* A. pseudoplatanus *Adenosine Kinase*

With the same concentration ($4 \times 10^{-6}$ M) of different $N^6$-substituted adenosines, we

[10] ARIMA, T., MASAKA, M., SHIOSAKA, T., OKUDA, H. and FUJII, S. (1971) *Biochim. Biophys. Acta* **246,** 184.
[11] PETHE-SADORGE, P., SIGNOR, Y. and GUERN, J. (1972) *Compt. Rend.* **275,** 2493.

TABLE 1. MOBILITIES OF ADENOSINE, $N^6$-SUBSTITUTED ADENOSINES AND RELATED NUCLEOSIDE-5′-MONOPHOSPHATES

| Compound | Separation procedure: *n*-BuOH–HOAc–$H_2O$ (17:3:5) Whatman 3 paper $R_f$s | Separation procedure: Tris-citric acid pH 3,5 Whatman 3 MM electrophoresis migration rates (cm/hr)* |
|---|---|---|
| Adenosine | | |
| nucleoside | 0·43 | −2·48 |
| nucleotide | 0·07 | +2·64 |
| 6-Methyladenosine | | |
| nucleoside | 0·65 | −2·48 |
| nucleotide | 0·14 | +2·64 |
| 6-Propyladenosine | | |
| nucleoside | 0·83 | −2·45 |
| nucleotide | 0·27 | +2·72 |
| 6-Furfuryladenosine | | |
| nucleoside | 0·85 | −1·32 |
| nucleotide | 0·25 | +3·14 |
| 6-Benzyladenosine | | |
| nucleoside | 0·90 | −1·64 |
| nucleotide | 0·29 | +2·92 |

* 70 V/cm electrophoresis at 0°. Negative values mean migration to the cathode direction, positive values migration to the anode direction.

measured the initial rate of nucleotide synthesis catalysed either by the *S. pombe* enzyme or by that of *A. pseudoplatanus*. These rates depend upon the nature of the $N^6$-substitution,

TABLE 2. REQUIREMENT OF ATP FOR ADENOSINE AND 6-FURFURYLADENOSINE PHOSPHORYLATION BY *A. pseudo platanus* AND *S. pombe* ENZYMES

| Substrate | Phosphoryl group donor | $^{14}$C nucleotides obtained as % of the amount synthesized with ATP as phosphoryl group donor: *A. pseudoplatanus* enzyme | *S. pombe* enzyme |
|---|---|---|---|
| 8-$^{14}$C-Adenosine | ATP | 100 | 100 |
| | AMP | 12 | 0 |
| | No added | 10* | 0 |
| 8-$^{14}$C-Furfuryladenosine | ATP | 100 | 100 |
| | AMP | 3·9 | 0 |
| | No added | 3 | 0 |

* The very low yield of $^{14}$C nucleotides obtained without phosphoryl group donor added is likely due to the presence of a small amount of ATP in the crude extracts used.

and are the same for the two enzyme systems (Table 3). MAPR, which has the closest structure to that of APR, is a better substrate than FAPR, PAPR and BAPR; FAPR is more easily transformed than PAPR and BAPR. Nevertheless, no systematic study has

been made of the influence of the riboside concentration upon the phosphorylation rate, and it could be possible that the differences in rates depend upon the concentration of the substrates.

TABLE 3. SPECIFIC ACTIVITIES* OF *S. pombe* OR *A. pseudoplatanus* ADENOSINE KINASE TOWARD APR AND SEVERAL $N^6$-SUBSTITUTED APR

| Substrate | *S. pombe* enzyme I† | *S. pombe* enzyme II | *A. pseudoplatanus* enzyme I | *A. pseudoplatanus* enzyme II |
|---|---|---|---|---|
| APR | 0·63 | 0·57 | 0·19 | 0·21 |
| MAPR | 0·33 | 0·25 | 0·14 | 0·19 |
| PAPR | 0·029 | 0·018 | 0·033 | 0·048 |
| FAPR | 0·055 | 0·073 | 0·041 | 0·086 |
| BAPR | 0·027 | 0·015 | 0·024 | 0·020 |

* Specific activities are expressed as $10^{-4}$ μmol of nucleotide formed per min per μg of protein.
† Two different extracts were independently prepared and used for specific activities determination.

*Attempts to Synthesize the Nucleoside-triphosphates of* $N^6$*-substituted Adenosines*

We have shown that the enzymatic system for ATP synthesis from AMP is present in *S. pombe* extracts as well as in extracts of *A. pseudoplatanus*. When 8-$^{14}$C-APR and $^{12}$C-ATP are added to these extracts, the labelled substrate is converted mainly into 8-$^{14}$C-ATP. This is probably due to the presence in the extracts of an adenylate kinase, catalysing ADP formation from AMP and ATP, associated with another enzyme responsible for the ATP formation from ADP. Such a system is not able to synthesize the nucleoside triphosphates from all the corresponding nucleoside monophosphates; for example, it does not synthesize GTP from GMP. Nevertheless the enzyme responsible for this synthesis is actually present in our extracts; 8-$^{14}$C-GTP is formed when 8-$^{14}$C-GMP and $^{12}$C-ATP are added to both extracts. Still the only nucleotides we obtained from $N^6$-substituted adenosines were the nucleoside monophosphates.

The enzymes for nucleoside triphosphate synthesis from common nucleoside monophosphate may not be able to accept the nucleoside monophosphates of $N^6$-substituted adenines as substrate. Alternatively, the nucleoside triphosphates are synthesized but are then immediately converted back to the monophosphate by hydrolytic enzymes. In order to decide between these two possibilities, we performed the synthesis and purification of nucleoside-5′-monophosphates of several $N^6$-substituted adenines (see Experimental) and then used them as substrates for the purified or semi-purified enzyme systems commonly used for the phosphorylation of nucleoside-5′-monophosphates.

The first, purified system to be used was the adenylate kinase–pyruvate kinase system (E.C. 2.7.4.13 and E.C. 2.7.1.40 respectively). The specificity of this system for the *common* nucleoside-monophosphates is well known: only AMP can be further phosphorylated to the triphosphate level.[12] The second, semipurified, system we used was an *E. coli* extract prepared according to Canellakis,[13] together with creatine kinase (E.C. 2.7.3.2), ATP and

[12] BOYER, P. D. (1962) in *The Enzymes* (BOYER, P. D., LARDY, H. and MYRBÄCK, K., eds.), Vol. 6, p. 95, Academic Press, New York.
[13] CANELLAKIS, E. S., GOTTESMAN, M. E. and KAMMEN, H. O. (1962) in *Biochemical Preparations* (COON, M. J., ed.), Vol. 9, p. 120, Wiley, New York.

creatine phosphate; the *E. coli* extract readily synthesizes nucleoside-triphosphates from many purine or pyrimidine nucleoside-monophosphates, such as AMP, dAMP, GMP, dGMP, IMP, UMP, CMP, dCMP and TMP. Creatine kinase catalyses the phosphate group transfer from creatine phosphate to ADP, accounting in this way for the ATP renewal in the incubation medium. Neither system catalysed the further phosphorylation of nucleoside-5′-monophosphates of $N^6$-substituted adenines, irrespective of the nature of the $N^6$-substitution. (Table 4). Our experimental conditions were precise enough to measure meaningfully a phosphorylation rate of these substrates 21000 times less than that of AMP for the adenylate kinase–pyruvate kinase system, and 10000 times less than that of AMP, 1320 times less than that of GMP, 148 times less than that of IMP for the second enzyme system.

TABLE 4. ATTEMPTS TO SYNTHESIZE NUCLEOSIDE TRIPHOSPHATES AND DIPHOSPHATES FROM VARIOUS $N^6$-SUBSTITUTED AMP USING EITHER THE ADENYLATE KINASE-PYRUVATE KINASE SYSTEM OR THE *E. coli* EXTRACT-CREATINE KINASE SYSTEM

| Enzyme system | Substrate | Distribution of the radioactivity (cp 1 hr) between the products of the reaction: Monophosphate | Diphosphate + triphosphate ($n_t$) | $n_t$/Standard deviation† | Sp. act. of the 5′-monophosphate phosphorylating system* |
|---|---|---|---|---|---|
| Adenylate kinase | 8-$^{14}$C-MAMP | 4895 | 22 | 0·80 | 0 |
| + | 8-$^{14}$C-PAMP | 1080 | 06 | 0·10 | 0 |
| pyruvate kinase | 8-$^{14}$C-FAMP | 1470 | 16 | 0·40 | 0 |
| | 8-$^{14}$C-BAMP | 1572 | 10 | 0·25 | 0 |
| *E. coli* extract | 8-$^{14}$C-MAMP | 2400 | 30 | 0·75 | 0 |
| + | 8-$^{14}$C-PAMP | 866 | 10 | 0·25 | 0 |
| creatine kinase | 8-$^{14}$C-FAMP | 864 | 18 | 0·45 | 0 |
| | 8-$^{14}$C-BAMP | 1520 | 36 | 0·55 | 0 |

* Expressed as arbitrary units proportional to the amount of substrate further phosphorylated per min and $\mu$l of enzyme solution, with our standard conditions of incubation. The value 1 would correspond to the further phosphorylation of 5% of the substrate in these conditions. The sp. act. of the adenylate kinase–pyruvate kinase system for AMP is 21000. The sp. act. of the second system for AMP, GMP and IMP are respectively 10060, 1320 and 148.

† The probabilities of an error exceeding the $n_t$ value for the following values of $n_t$/standard deviation: 0·67–1–1·64–1·96, are respectively: 0·5–0·32–0·1 and 0·05.[14]

## DISCUSSION

The present results show that it is possible to synthesize enzymically the nucleoside-5′-monophosphates from several $N^6$-substituted adenosines. Furthermore the enzyme activity responsible for this synthesis seems to be ubiquitous, since we obtained evidence of its presence in yeasts, plant cells suspension and buds of higher plants. Nevertheless, one can only speculate whether this enzyme is actually involved in the *in vivo* synthesis of cytokinin nucleotides. At least in the case of *A. pseudoplatanus* cells, the well documented *in vivo* biosynthesis of cytokinin nucleotides from the corresponding bases,[7] cannot take place via the formation of the nucleoside from the base, followed by the formation of the nucleotide

[14] WANG, C. H. and WILLIS, D. L. (1965) in *Radiotracer Methodology in Biological Sciences* (MCELROY, W. D. and SWANSON, C. P., eds.), p. 186, Printice Hall, New Jersey.

from the nucleoside. According to such a pathway, the first step would involve the transfer of ribose from ribose-1-phosphate to the $N^6$-substituted adenine, catalysed by a nucleoside phosphorylase (E.C. 2.4.2.1), and the second one, the transfer of a phosphoryl group from ATP to the nucleoside, catalysed by adenosine kinase. In fact we failed in all our attempts to synthesize the $N^6$-substituted adenosine-5′-phosphates from the corresponding bases, with ribose-1-phosphate and ATP, using extracts of *A. pseudoplatanus* cells. On the other hand it is possible to perform this synthesis from the base, using PRPP as phosphoribosyl group donor.[11] This reaction is catalysed by a purine phosphoribosyltransferase.

It is well known that several *t*RNAs species contain $N^6$-substituted adenosines which exhibit a cytokinin activity. An hypothesis often accepted to account for the biological activity of theses substances is to consider them as *t*RNA precursors. If cytokinins really were *t*RNA precursors, it should be possible to synthesize their nucleoside-5′-triphosphates since such synthesis would be a prerequisite for purine and pyrimidine incorporation into polynucleotidic structures. Shigeura *et al.*[15] failed in their attempts to phosphorylate further *in vitro* the MAPR-5′-monophosphate using the rabbit adenylate kinase as enzyme. Our results support the view that not only in the case of MAPR-5′-monophosphate, but also in those of PAPR, FAPR and BAPR, this enzymatic system cannot actually perform such a phosphorylation. We also failed with other enzyme systems, such as the various *E. coli* monophosphokinases. On the other hand, we have shown that plant cells extracts (*A. pseudoplatanus*; *S. pombe*) do not seem to be able to perform the phosphorylation of the $N^6$-substituted adenosines beyond the monophosphate level, although they successfully catalyse ATP and GTP synthesis from the corresponding nucleoside-monophosphates.*

The failure to demonstrate the synthesis of $N^6$-substituted nucleoside triphosphates could be due to their lability, and not to the failure of the enzyme systems. We consider this as unlikely, since we used mild fixation procedures. A second hypothesis would be that the enzyme system responsible for the $N^6$-substituted adenosine-triphosphates synthesis is really present in the cells, but cannot be extracted, or else is destroyed during extraction, (in fact, control experiments using whole homogenates of *Acer* cells gave the same results), or that it requires other experimental conditions than those used. If this is the case, then this phosphorylation system would be different from those which catalyse the further phosphorylation of the common purine nucleoside-monophosphates. The simplest explanation is that the enzymatic synthesis is absent from plant cells.

## EXPERIMENTAL

*Radioactive substrates.* 8-$^{14}$C-$\beta$-adenosine (sp. act. 20 mCi/mM) was purchased from the CEA, France 8-$^{14}$C-6-Chloropurine (sp. act. 11·2 mCi/mM) and 8-$^{14}$C-6-furfurylaminopurine (sp. act. 16·5 mCi/mM) were obtained from the Radiochemical Center, Amersham, England. The other labelled $N^6$-substituted adenines have been synthesized by Dr. J. Guern from 8-$^{14}$C-6-chloropurine by condensation with the corresponding amines.[17] $N^6$-substituted adenosines were synthesized from the corresponding bases, using a purified nucleoside phosphorylase from *E. coli* and ribose-1-phosphate,[9] then isolated and purified by PC by descent (Whatman 3, with *iso*-PrOH–$H_2O$, 5:1).

* Just before this paper was submitted for publication, it has come to our knowledge that Elliott *et al.*[16] also failed in their attempts to perform *in vitro* the further phosphorylation of the BAP-nucleoside-5′-monophosphate.

[15] SHIGEURA, H. T., SAMPSON, S. D. and MELONI, M. L. (1966) *Arch. Biochem. Biophys.* **115,** 462.
[16] ELLIOTT, D. C., MURRAY, A. W., SACCONE, G. T. and ATKINSON, M. R. (1972) in *Plant Growth Substances* 1970, *Proc. 7th Intern. Conf. Plant Growth Substances, Canberra, Australia* (CARR, D. J., ed.), p. 459, Springer, Berlin.
[17] FILIPPI, J. and GUERN, J. (1965) *Bull. Soc. Chim. Fr.* 2617.

*Enzymes.* S. pombe *extracts.* The cells were grown overnight in liquid medium (yeast extract 5 g, glucose 20 g, $KH_2PO_4$ 4·25 g, $K_2HPO_4$ 5·5 g for 1 l. of medium), harvested by centrifugation, washed in a small vol. of phosphate buffer 0·2 M pH = 6·8. The cells were ground with glass beads in a Virtis homogenizor. After centrifugation (70000 *g* for 15 min), the supernatant was collected and used as 'enzymic extract'. A. pseudoplatanus *extracts.* The cells were grown as described before[18,19] and the crude enzymic extract was prepared in the same way as for *S. pombe. Semi-purified* E. coli *extracts prepared according to* Canellakis[13] were gifts from Dr. Lecoq, CEA France and Dr. Tempe, CNRA France, whom we thank. *Commercial enzymes.* Pyruvate kinase (10 mg proteins/ml), adenylate kinase (5 mg/ml), *E. coli* alkaline phosphatase (10 mg/ml) and *Crotalus adamantus* 5′-nucleotidase were purified enzymes obtained from Sigma, U.S.A. Pure creatine kinase was purchased from Boehringer, Germany.

*Enzymatic synthesis of nucleosides-5′-phosphates.* Reaction mixtures containing 50 $\mu$l of 8-$^{14}$C labelled nucleoside $10^{-5}$ M, 20 $\mu$l of ATP $2 \times 10^{-3}$ M, 5 $\mu$l of $MgCl_2$ 0·1 M and 50 $\mu$l of enzyme extract (eventually diluted with phosphate buffer 0·2 M pH = 6·8) were incubated at 37°. From time to time samples were collected and injected into an equal vol. of 100% EtOH, in order to stop the reaction.

*Separation of the reaction products.* The samples were applied onto very small slips of paper, which were then placed on a Whatman 3 MM paper sheet. Electrophoresis was then carried out at 0°, for 1 hr at 70 V/cm (current intensity 50 mA) using a Tris-citric acid buffer (0·05 M pH 3·5). Electrophoregrams were cut into segments of 1·0 cm width, whose radioactivity was counted (proportional detectors RA 12 and RA 15 Intertechnique).

*Identification of the* $N^6$*-substituted adenine-nucleotides.* Nucleotides obtained from $N^6$-substituted nucleosides were eluted with dist. $H_2O$, concentrated to dryness under low pressure, then solubilized into 100 $\mu$l Tris-HCl buffer 0·1 M pH 8·6, and 5 $\mu$l *E. coli* alkaline phosphatase was added. After 3 hr of incubation at 37° the nucleotides were fully hydrolysed into nucleosides, as shown by PC in *n*-BuOH–$NH_4OH$–$H_2O$ (86:5:14), which well separates the various $N^6$-substituted adenosines (BAPR: $R_f$ 0·88; FAPR: $R_f$ 0·89; PAPR: $R_f$ 0·89; MAPR: $R_f$ 0·45) from adenosine ($R_f$ 0·30), inosine ($R_f$ 0·09) and guanosine ($R_f$ 0·05). These chromatogramms were compared with those obtained prior to phosphatase treatment. Reaction mixture used for nucleotide hydrolysis with *Crotalus adamantus* enzyme was: 20 $\mu$l nucleotide $10^{-5}$ M, 200 $\mu$l glycine–NaOH buffer 0·5 M pH 8·6, 200 $\mu$l $MgCl_2$ 0·05 M and 0·1 mg enzyme. Incubation was made at 37° for 2 hr.

*Attempts to perform the synthesis of* $N^6$*-substituted adenosine-triphosphates.* The reaction mixture used in the case of the adenylate kinase–pyruvate kinase system consisted of 50 $\mu$l 8-$^{14}$C-nucleoside-5′-monophosphate $10^{-5}$ M, 10 $\mu$l ATP $10^{-2}$ M, 50 $\mu$l of phosphoenolpyruvate $10^{-3}$ M (prepared in the following solution: 40 mM Tris, 4 mM $MgSO_4$, 32 mM $K_2SO_4$, 6 mM EDTA for 1 l.), 5 $\mu$l pyruvate kinase, 5 $\mu$l adenylate kinase (not diluted in the case of $N^6$-substituted substrates, adequately diluted in the case of AMP, in order to get less than 10% of the initial amount of 8-$^{14}$C-AMP converted to 8-$^{14}$C-ATP within the first 5 min of incubation at 37°. Control experiments were designed in the case of $N^6$-substituted substrates, using also diluted enzyme, and no change was observed in the results. In the case of the Canellakis *E. coli* extract—creatine kinase system, the reaction mixture was as follows: 50 $\mu$l nucleoside-5′-monophosphate $10^{-5}$ M, 20 $\mu$l $MgCl_2$ 0·1 M, 20 $\mu$l Tris-HCl buffer 0·1 M pH 7·8, 20 $\mu$l ATP $10^{-2}$ M, 20 $\mu$l creatine phosphate 4·4 mg/ml, 20 $\mu$l of a 0·66 mg/ml creatine kinase solution, 50 $\mu$l *E. coli* extract (not diluted for the $N^6$-substituted adenosine-monophosphates, adequately diluted in the case of AMP, IMP and GMP (controls with diluted enzyme preparation gave the same results in the case of the $N^6$-substituted substrates). The reaction mixture was incubated at 37° for 5 min. To stop the reactions, 2 procedures were used; (a) adding 1 vol. of 100% EtOH to 1 vol. of reaction mixture: the heavy precipitate was discarded by centrifugation and the clear supernatant analysed; and (b) adding 1 vol. of perchloric acid 0·1 N to 1 vol. of reaction mixture: the pH drops to below 2 and the reaction is stopped, apparently without protein precipitation. When a $N^6$-substituted adenine nucleotide was used as substrate, 8 separate 20 $\mu$l aliquote fractions were collected after stopping the reaction, and independently analysed (only 2 aliquot fractions for the other substrates). These samples, applied onto small paper slips were carefully dried without heating. Products were then separated using the electrophoretic procedure described above; electrophoregrams were dried and cut into 1·0 cm with segments, which were counted for 1 hr, using a low background proportional detector. The countings above the background obtained for segments located nearer to the anode than the nucleoside monophosphate used as substrate accumulated, and the average background was substracted, giving $n_t$ (see Table 4). The ratio of $n_t$ to the standard deviation was calculated, in order to check its statistical signification.

[18] Doree, M., Leguay, J. J., Terrine, C., Sadorge, P., Trapy, F. and Guern, J. (1970) in *Les Cultures de Tissus de Plantes*, p. 345, C.N.R.S., Paris.

[19] Doree, M., Leguay, J. J. and Terrine, C. (1972) *Physiol. Veg.* **10**, 115.