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METABOLISM OF EXOGENOUS ADENINE BY *ACER PSEUDOPLATANUS* CELLS

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Key Word Index—*Acer pseudoplatanus*; Aceraceae; adenine metabolism.

Abstract—The cells of *Acer pseudoplatanus* convert exogenous adenine to various metabolites. The balance between synthesis and degradation of adenine nucleotides has been studied for different adenine concentrations and different periods of incubation. The enzymic pathway mediating the synthesis of adenylic nucleotides from exogenous adenine, and those accounting for the degradation of adenine are discussed, and the deamination of AMP as a possible regulatory mechanism governing the size of the pool of adenylic nucleotides is considered.

INTRODUCTION

IT HAS been shown by various authors that the addition of adenine to the culture medium of various plant tissues or plant organs results in some cases in a stimulation of cell division or bud neoformation.[1-6] Therefore to progress in the understanding of these biological effects, a study of the metabolic utilization of exogenous adenine by plant tissues was undertaken. In the work presented here, we investigated the utilization, and the possible regulatory mechanisms linked to the utilization, of adenine by *Acer pseudoplatanus* cells cultivated as cell suspensions.

RESULTS AND DISCUSSION

Analysis of the Metabolites Produced from Exogenous Adenine

The radioactivity distribution between the various metabolites 10 min after adenine-[8-^{14}C], at the initial concentration 0·2 μM, was added to the growth medium of *A. pseudoplatanus* cells is shown on Table 1. At this time 80% of the radioactivity had been absorbed from the medium, and could be completely extracted from the cells by 0·5 N perchloric acid at 0°. Only a very small part of the radioactivity remained as unchanged adenine. The major labelled adenine derivatives were adenylic nucleotides, mainly ATP. There was no radioactivity in compounds resulting from degradation of the purine ring (allantoic acid,

[1] SKOOG, F. and TSUI, C. (1948) *Am. J. Botany* **35**, 782.
[2] PLUMMER, T. H. and LEOPOLD, A. C. (1957) *Proc. Am. Soc. Hort. Sci.* **70**, 442.
[3] PAULET, P. and NITSCH, J. P. (1959) *Bull. Soc. Bot. Fr.* **106**, 425.
[4] NITSCH, J. P., NITSCH, C., ROSSINI, L. M. E. and BUI DANG, H. A. (1967) *Phytomorphology* **17**, 446.
[5] EARLE, E. D. and TORREY, J. G. (1965) *Plant Physiol.* **40**, 520.
[6] MILLER, C. O. (1968) in *Biochemistry and Physiology of Plant Growth Substances*, Proc of the 6th. Int. Conf. on Plant Growth Substances, Ottawa (1967) (WIGHTMAN, F. and SETTERFIELD, G., eds.), p. 33, Runge Press, Ottawa.

allantoin, urea). Analyses were also carried out a30 sec after denine, at the initial concentration of 0·01 μM, was added to the medium: in this case the only labelled compounds present in the cells were adenine and adenyllic nucleotides. The main compound, ATP, accounted for 80% of the intracellular radioactivity.

TABLE 1. PARTITION OF THE RADIOACTIVITY* BETWEEN THE VARIOUS INTRACELLULAR COMPOUND PRODUCED FROM EXOGENOUS ADENINE-[8-^{14}C] FOR DIFFERENT INITIAL CONCENTRATIONS AND DIFFERENT PERIODS OF INCUBATION

Compound	Adenine concentrations and periods of incubation				
	0·2 μM		0·5 μM		100 μM
	10 min	5 min	30 min	3 hr	4 hr
Adenine	<1	7	4	<0·5	9
Adenylic nucleotides	92	82	82	76	59
Guanylic, Inosinic } nucleotides	<2	7	4	6	3
Xanthine + hypoxanthine (bases and ribosides)	5	2	6	5	14
Allantoic acid	<0·5	<1	2	10	10
Allantoin + urea	<0·5	<1	2	2	5

* Expressed as per cent of the total radioactivity of the cold-acid soluble fraction. The total counts supplied in the growth medium at zero time were 24 000 cpm/ml for the 0·2 μM assay (80% of the radioactivity was absorbed by the cells after 10 min of incubation), 120 000 cpm/ml for the 0·5 μM assay (30, 98 and 100% of the radioactivity were respectively absorbed by the cells after 5 min, 30 min and 3 hr of incubation) and 240 000 cpm/ml for the 10^{-4} M assay (40% of the radioactivity was absorbed after 4 hr).

Figure 1 shows the time course of adenine uptake by *Acer* cells when its initial concentration in the medium was 0·1 mM, the kinetics of radioactivity partition between the cold-acid soluble fraction and the nucleic fraction, as well as the increase of the pool of adenylic nucleotides resulting from adenine uptake. The main part of the radioactivity absorbed by the cells remained in the cold-acid soluble fraction, at least for short-time absorptions. Table 1 shows how the radioactivity was distributed between the various metabolites of this fraction 4 hr after the beginning of the adenine absorption (experimental conditions of Fig. 1). Most of the radioactivity (59%) is in the nucleotidic fraction, mainly as adenylic nucleotides. Xanthine and hypoxanthine (or their ribosides) stand for an important part of the total intracellular radioactivity (14%), as well as allantoic acid, allantoin and urea (15%), which originate from the breakdown of the purine ring. The nucleic acid fraction is probably labelled without lag after adenine is added to the medium, but the rate of its incorporation reaches a constant and maximal value only 1 hr after starting the incubation. This constant rate of radioactivity incorporation implies probably that the specific radioactivity of nucleic acids precursors and the rate of nucleic acid synthesis remain constant during this period. The pool of adenylic nucleotides reaches its maximal size 1 hr after the beginning of adenine uptake. It is well known that the *de novo* synthesis of purine compounds is depressed when cells are supplied with exogenous adenine.[7,8] Henderson[8] has shown that supplying Ehrlich ascites tumor cells with 10^{-5} M adenine

[7] KOCH, A. L., PUTNAM, F. W. and EVANS, E. A. (1952) *J. Biol. Chem.* **197**, 105.
[8] HENDERSON, J. F. (1962) *J. Biol. Chem.* **237**, 2631.

results in a 40% decrease of the *de novo* synthesis of purine compounds: a 10^{-4} M concentration of adenine entirely suppresses this synthesis. If this is the case for *A. pseudoplatanus* cells, the specific radioactivity of ATP reaches its constant value when all the ATP molecules are produced from exogenous ^{14}C-adenine. This seems to be the case as the size of the intracellular pool of adenylic nucleotides (measured by an enzymic procedure) 4 hr after supplying the cells with 10^{-4} M adenine-[8-^{14}C] is quite similar to the amount of labelled adenylic nucleotides within the cells (enzymic procedure: 15 pmol/10^6 cells; radioactivity counting: 16·5 pmol/10^6 cells).

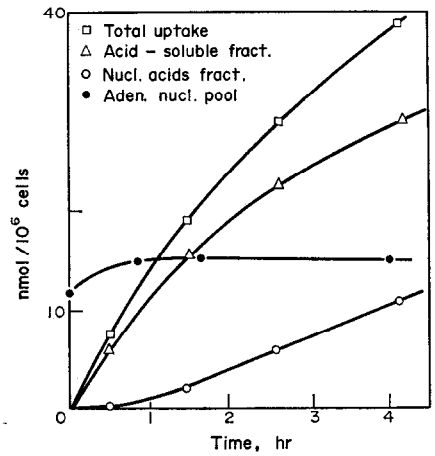

FIG. 1. KINETIC STUDY OF ADENINE-[8-^{14}C] UPTAKE AND INCORPORATION INTO NUCLEIC ACIDS. CORRELATIVE EXPANSION OF THE POOL OF ADENYLIC NUCLEOTIDES.

The initial concentration of adenine in the medium was 10^{-4} M. The radioactivity absorbed and incorporated either in the cold-acid soluble fraction or in the nucleic acid fraction is expressed by the corresponding amount of adenine molecules (nanomoles for 10^6 cells). The size of the pool of adenylic nucleotides (*labelled or not*) has been measured by an enzymic procedure and is also expressed as nmol/10^6 cells.

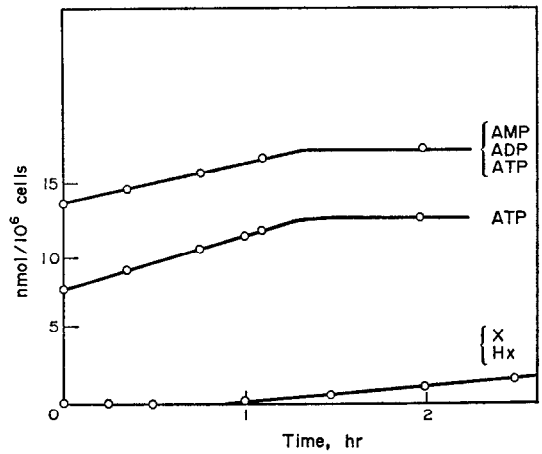

FIG. 2. TIME COURSE OF THE EXPANSION OF THE INTRACELLULAR POOL OF ADENYLIC NUCLEOTIDES AND OF THE ACCUMULATION OF XANTHINE OXIDASE SUBSTRATES IN THE GROWTH MEDIUM BROUGHT ABOUT BY THE ADDITION OF 10^{-4} M ADENINE IN THE MEDIUM.

The total amounts of xanthine (X) and hypoxanthine (Hx) in the medium are expressed as nmol/thine (Hx) in the medium are expressed as nmol/ml of medium. There were about 10^6 cells/ml cell suspension. Control experiments showed that there was no variation of the size of the intracellular pool of adenylic nucleotides, and no detectable accumulation of xanthine or hypoxanthine in the medium, when the cells were incubated under the same experimental conditions in a medium not supplemented with adenine.

The results of a pulse experiment are also shown in Table 1. The radioactivity distribution between the compounds of the cold-acid soluble fraction was analysed 5 min, 30 min and 3 hr after injecting adenine at the initial concentration 0·5 μM into a cell suspension. The amounts of adenine remaining in the medium are respectively 70, 2 and 0% of the amount supplied at zero time. The radioactivity incorporated in the adenylic nucleotides remains in these compounds even when the exogenous adenine has been fully exhausted. Nevertheless the amount of compounds resulting from the purine ring breakdown becomes significant for a 3 hr incubation (12% of the total radioactivity). This result suggests there may be a rapid turn over of the adenylic nucleotides in *A. pseudoplatanus* cells.

What is the Metabolic Pathway Mediating the in vivo *Synthesis of Adenylic Nucleotides from Exogenous Adenine?*

As the major metabolites synthesized from exogenous adenine are adenylic nucleotides, we have been interested in elucidating the pathway of their synthesis. The main possible ways are the following:

pathway I: adenine → hypoxanthine → IMP → AMP →
pathway II: adenine → adenosine → AMP →
pathway III: adenine → AMP →

Pathway I involves first the formation of hypoxanthine from adenine, catalyzed by adenase (E.C. 3.5.4.2), then the synthesis of IMP from hypoxanthine, catalyzed by phosphoribosyltransferase (E.C. 2.4.2.8.). Hypoxanthine-[8-^{14}C] absorption by *A. pseudoplatanus* leads to a reduced synthesis of adenylic nucleotides compared to the guanylic nucleotides synthesis.[9] Furthermore, important amounts of compounds resulting from the purine ring breakdown are produced in this case, even for short-time incubations. As these results sharply contrast with those obtained for adenine uptake, it seems likely that pathway I cannot account for the *in vivo* adenylic nucleotides formation from exogenous adenine by *A. pseudoplatanus* cells. However it is still possible that exogenously supplied hypoxanthine may not be metabolized in the same way as endogenous hypoxanthine that could be eventually produced from adenine.

Pathway II involves the transient formation of adenosine, resulting from the transfer of a ribose group from ribose-1-phosphate on adenine, catalyzed by nucleoside phosphorylase (E.C. 2.4.2.1), then adenosine kinase (E.C. 2.7.1.20) catalyzed synthesis of AMP from adenine, using ATP as phosphoryl group donor. Although the amount of labelled adenosine formed when the cells absorb adenine-[8-^{14}C] is rather small, this pathway would not seem unlikely. We have shown that the cells of *A. pseudoplatanus* exhibit a high adenosine kinase activity, which could possibly account for the non-accumulation of adenosine.[10] Nevertheless no nucleoside phosphorylase can be demonstrated in those cells.[11] We checked that our failure to perform the *in vivo* synthesis of adenosine from adenine cannot be accounted for by a phosphatasic hydrolysis of ribose-1-phosphate.[11] However, it might be due to the continuous conversion of adenosine to adenine, catalyzed by nucleoside hydrolase (E.C. 3.2.2.1). This enzyme is actually present in the cell extracts. As a coupling between nucleoside phosphorylase (so far as this enzyme is really present in *Acer* cells) and adenosine kinase would possibly prevent, at least partially, conversion of adenosine back to adenine, we incubated a dialysed homogenate of *A. pseudoplatanus* cells with a mixture of adenine-[8-^{14}C], ribose-1-phosphate and ATP.

Table 2 shows that it is possible, under such experimental conditions, to perform the synthesis of labelled adenine nucleotides (mainly ATP-[8-^{14}C]). If no ATP is added to the reaction mixture, no labelled nucleotides are formed. If the first step of the reaction is really mediated by nucleoside phosphorylase, ribose-1-phosphate has to be present in the reaction mixture. In fact, it is possible to synthesize adenylic nucleotides from adenine and ATP without adding ribose-1-phosphate. Klenow has demonstrated in skeletal muscles the enzymic formation of ribose-1-phosphate from ribose-5-phosphate;[12] on the other

[9] DOREE, M. unpublished results.
[10] DOREE, M. and TERRINE, C. (1973) *Phytochemistry* **12**, 1017.
[11] SADORGE, P., DOREE, M., TERRINE, C. and GUERN, J. (1970) *Physiol. Vég.* **8**, 499.
[12] KLENOW, E. (1963) in *Methods in Enzymology* (COLOWICK, S. P. and KAPLAN, N. O., eds.), Vol. I, p. 361, Academic Press, New York.

hand, ribose-5-phosphate synthesis from ATP has been obtained using enzyme extracts from various origins.[13,14] Nevertheless, even if the cells extracts of *A. pseudoplatanus* were actually able to perform ribose-1-phosphate synthesis from ATP, it is likely that they would not allow nucleoside phosphorylase to remain saturated with respect to ribose-1-phosphate during the time-course of the incubation. According to pathway II, adenosine formation from adenine is necessarily rate-limiting in adenylic nucleotides synthesis, as there is no accumulation of adenosine. Consequently ribose-1-phosphate addition to the reaction mixture would be expected to increase the rate of AMP synthesis. Table 2 shows that this is not the case: AMP synthesis from adenine does not depend upon ribose-1-phosphate addition.

TABLE 2. ATP-DEPENDENT SYNTHESIS OF ADENYLIC NUCLEOTIDES FROM ADENINE CATALYZED BY *Acer* CELLS HOMOGENATES

Reaction mixture		Amount of adenylic nucleotides formed (pmol/min/μg protein)
(M) 50 μl adenine	2×10^{-5} M	
50 μl R-1P*	2×10^{-3} M	
50 μl ATP	2×10^{-3} M	2×10^{-2}
20 μl $MgCl_2$	10^{-1} M	
50 μl dialysed homogenate (110 μg protein)		
(M) without R-1P		2×10^{-2}
(M) without R-1P and ATP		$< 10^{-4}$
(M) without R-1P and ATP		$< 10^{-4}$
(M) with 50 μl AMP 2×10^{-3} M		

* Ribose-1-phosphate.

Another kind of evidence argues for the absence of nucleoside phosphorylase activity in *Acer* cells. We have shown elsewhere[10] that their adenosine kinase catalyses the kinetic nucleotide synthesis from the corresponding nucleoside. On the other hand Sivadjian *et al.*[15] have demonstrated that substituting a furfuryl group at the N6-position of adenine does not modify the rate of nucleoside synthesis from the base catalyzed by the nucleoside phosphorylase of *E. coli*. If a nucleoside phosphorylase (with a specificity like to that of the *E. coli* enzyme) functions in *Acer* cells homogenates, ATP-dependent synthesis of kinetin nucleotide from kinetin should be possible. However, this synthesis cannot be performed. This result is not due to the continuous hydrolysis of the transient kinetin nucleoside to kinetin, as the *Acer* cells homogenates catalyse the ATP-dependent conversion of kinetin nucleoside to kinetin nucleotide without formation of kinetin.[10] The preceding evidences makes it unlikely that nucleoside phosphorylase activity is involved in AMP formation from exogenous adenine in *A. pseudoplatanus* cells.

Pathway III involves the direct transfer of a phosphoribosyl group from PRPP (5-phospho-αD ribosylpyrophosphate) to adenine, catalyzed by adenine phosphoribosyltransferase (E.C. 2.4.2.7). As the extracts of *A. pseudoplatanus* exhibit a very high adenine

[13] HEPPEL, L. A. (1963) in *Methods in Enzymology* (COLOWICK, S. P. and KAPLAN, N. O., eds.), p. 117, Academic Press, New York.
[14] DUCET, G. and SEMERE, A. (1962) *Bull. Soc. Fr. Physiol. Vég.* **8**, 61.
[15] SAVADJIAN, A., SADORGE, P., GAWER, M., TERRINE, C. and GUERN, J. (1969) *Physiol. Vég.* 31.

phosphoribosyltransferase activity, this pathway seems to be the most likely for the immediate formation and accumulation of adenylic nucleotides from exogenous adenine. As PRPP cannot be replaced by ATP for this reaction, adenylic nucleotides synthesis from adenine and ATP shown in Table 2 would be due to the previous PRPP synthesis from ATP and ribose-5-phosphate catalyzed by PRPP-synthetase (E.C. 2.7.6.1). To check whether the adenine phosphoribosyltransferase activity of *A. pseudoplatanus* is high enough to account for the intensive adenylic nucleotides synthesis recorded when the cells are incubated in a medium supplied with adenine concentrations as high as 10^{-4} M, 2 identical cell suspensions were used, either for *in vivo* or *in vitro* investigations. For *in vivo* experiments, the growth medium was made 75 μM with respect to adenine at zero time, and aliquots were harvested at 0·5, 4·5 and 9 min. in order to measure adenine incorporation into adenylic nucleotides. The rate of *in vivo* adenylic nucleotides synthesis from adenine was about 40 pmol/min/mg of cells (dry wt). For *in vitro* experiments, the adenine phosphoribosyltransferase activity of the other cell suspension was extracted and measured at the same temperature with the same adenine concentration (75 μM) and 20 μM PRPP. The rate of *in vitro* adenylic nucleotides synthesis from adenine was about 77 pmol/min/mg of cells (dry wt). Thus the adenine phosphoribosyltransferase activity of the cells is high enough to account for the *in vivo* synthesis of adenylic nucleotides from exogenous adenine.

As the *in vivo* synthesis might be possibly limited by PRPP availability, further investigations were carried out to estimate the PRPP level of the cells. An aliquot of a cell suspension supplied with 10^{-4} M adenine was compared with another supplied with distilled water. After 1·5 hr, the PRPP contents of the cells were measured: they were 63 pmol/10^6 cells and 61 pmol/10^6 cells, respectively. As adenine uptake brought about an adenylic nucleotides synthesis as high as 12 nmol/10^6 cells during the incubation period, it is clear that the intensive PRPP consumption related to nucleotide synthesis does not cause any decrease of the PRPP content of the cells. This indicates that PRPP turnover must be very rapid in those cells.

What is the Pathway of Adenine Degradation?

The above results show that various compounds other than adenylic nucleotides are formed in significant amounts when adenine at high concentration is supplied in the growth medium. It may be assumed that xanthine and hypoxanthine are precursors of allantoin, allantoic acid and urea.[17] We have checked that the latter compounds are the main metabolites formed when *Acer* cells absorb hypoxanthine.[9] When the growth medium is supplied with adenine at high concentration, the cells accumulate relatively high amounts of xanthine and hypoxanthine compared to the products formed from the breakdown of the purine ring. This result indicates that the rate of xanthine and hypoxanthine formation is higher than the rate of their further degradation. We have shown that under the same experimental circumstances there is an important loss of xanthine and hypoxanthine from the cells. Figure 2 shows that this loss begins only when the pool of adenylic nucleotides reaches its maximal value: this suggests that a new biochemical type of adenine utilization leading to the formation and to the release of hypoxanthine and xanthine from the cells arises when the pool of adenylic nucleotides reaches this critical size. It might be assumed that xanthine and hypoxanthine are produced directly from exogenous adenine; an alternative hypothesis is that adenine conversion to adenylic nucleotides occurs prior to xanthine

[16] DOREE, M. (1973) *Physiol. Vég.* in press.
[17] TERROINE, E. F. (1960) in *Le Métabolisme Nucléique* (C.N.R.S., ed.), C.N.R.S., Paris.

and hypoxanthine formation. We believe that the former hypothesis cannot account for the evidence presented above. For example, the incorporation of adenylic nucleotides with a constant specific radioactivity into nucleic acids does not cause any decrease of the size of the pool of adenylic nucleotides: this implies that xanthine and hypoxanthine synthesis from exogenous adenine does not suppress the pool renewal from exogenous adenine. According to the second hypothesis, the extracellular hypoxanthine originates from IMP, which is formed within the cells by deamination of AMP, whereas xanthine originates from the excess of XMP (itself produced from IMP) which fails to be converted to GMP. Such a pathway of xanthine and hypoxanthine formation from exogenous adenine has been depicted by Hershko *et al.*[18] in rabbit erythrocytes.

Two different interpretations where AMP deaminase (E.C. 3.5.4.6) fulfils a central role may be proposed to account for the noteworthy coincidence between the end of the expansion of the pool of adenylic nucleotides and the beginning of xanthine and hypoxanthine release from the cells (so far as this release is actually correlated with the increase of their rate of synthesis). According to the first, the increase of the ATP content of the cells will induce a decrease of the rate of its own synthesis from AMP, and the resulting increase of AMP availability would be correlated with an increase of the net AMP deaminase activity, from which an over-production of IMP would originate. Alternatively, AMP deaminase may be activated by ATP (specially efficient when the ATP content of the cells reaches its maximal value). Setlow and Lowenstein have shown that the AMP deaminase activity of highly purified enzymic extracts is regulated by the ATP concentration in the reaction mixture.[19] Razin and Mager also recorded such a AMP deaminase stimulation by ATP, and an inhibition by phosphate ions.[20] According to these authors, AMP deaminase would have a regulatory role by preventing too large an expansion of the size of the adenylic nucleotides pool in all circumstances where an enhanced production of these nucleotides increases the ATP:P_i ratio. It is difficult to choose between these two hypotheses, since the effective presence of AMP deaminase in *A. pseudoplatanus* cells remains to be demonstrated.

EXPERIMENTAL

Cell suspension culture. Acer pseudoplatanus cells were grown in a synthetic medium, according to the procedure already reported.[21,22] Every week the cells were transferred to a new medium by adding 80 ml of the preceding culture to 200 ml of a freshly prepared medium. The exponential growth of the cells was not interrupted (generation time, *ca.* 72 hr).

Uptake experiments. 7 days after the last renewal of the medium the cells were used for the uptake experiments (cell population density: about 850 000 cells/ml). The cells were aseptically equilibrated for 20 hr on a reciprocal shaker.[22] At zero time, adenine-[8-^{14}C] (50 mCi/mM) was added to the cell suspension. From time to time 1 ml aliquot fractions were removed and injected into 5 ml of cold H_2O. Then the mixture was filtered through a Millipore membrane, and the radioactivity absorbed by the cells was measured by liquid scintillation.

Biochemical analysis. The collected cells were dropped into $HClO_4$ 0·5 N at 0°, to achieve a rapid fixation and an efficient extraction of the cold-acid soluble fraction. After centrifugation and neutralization, the metabolites were separated by paper electrophoresis (Whatman 3MM, 70 V/cm, 50 mA, 1 hr, 5°) using a Tris–citric acid buffer (0·05 M pH 3·5). Purine bases, their nucleosides, allantoïne and urea migrate towards the cathode, whereas nucleotides, uric acid and allantoic acid migrate towards the anode. The cationic and the

[18] Hershko, A., Razin, A., Shosmani, T. and Mager, J. (1967) *Biochim. Biophys. Acta* **149,** 59.
[19] Setlow, B. and Lowenstein, J. M. (1967) *J. Biol. Chem.* **242,** 607.
[20] Razin, A. and Mager, J. (1966) *Israel J. Med. Sci.* **2,** 614.
[21] Doree, M., Leguay, J. J., Terrine, C., Sadorge, P., Trapy, F. and Guern, J. (1970) in *Les Cultures de Tissus de Plantes* (C.N.R.S., ed.), p. 345, C.N.R.S., Paris.
[22] Doree, M., Leguay, J. J. and Terrine, C. (1972) *Physiol. Vég.* **10,** 115.

anionic fraction were then separately eluted and analyzed. The metabolites of the cationic fraction were separated by Whatman III paper chromatography using a dilute NH_4OH solution (pH 11) as solvent system. To estimate the urea and allantoin content of this fraction, the radioactivity located at the front of the chromatogram, where these compounds migrate, was measured. The remaining part of the chromatogram was eluted, the eluate concentrated to dryness under low pressure, solubilized again with 1 N $HClO_4$ and boiled for 1 hr. After neutralization, the resulting bases were identified either by PC, using two different solvent systems (solvent *a*: NH_4OH solution pH 11; solvent *b*: BuOH–H_2O–NH_4OH, 86:14:5) or electrophoresis (as described above). The metabolites of the anionic fraction were treated with alkaline phosphatase (SIGMA), then the fraction was chromatographed with solvent a. Allantoic acid migrates to the front and is readily separated from the other compounds. The latter was eluted, concentrated and identified using paper chromatography with solvents *a* and *b* or electrophoresis. After performing the total extraction of the cold acid soluble fraction (3× with $HClO_4$ 0·5 M at 0° during 10 min), the pellet was treated 3× again with $HClO_4$ 0·5 M at 70° during 30 min; after centrifugation the supernatants were combined and their total radioactivity (radioactivity of the nucleic acid fraction) was counted. The amount of adenylic nucleotides (labelled or not) in the cells has been measured by using the luciferine–luciferase method, according to Pradet.[25] To measure the PRPP content of the cells, 2 ml aliquot fractions were removed from the cell suspension and filtered through a Millipore membrane. The cells were immediately dropped (together with the Millipore membrane) into a boiling solution of Tris-$MgCl_2$ (Tris 0·01 M; $MgCl_2$ 0·02 M) for 1 min, then immediately chilled in ice. The Millipore membrane was removed and the cell suspension sonicated for 3 min (Sonatron 80 kilocycles/sec) and centrifuged. The PRPP content of the supernatant was measured by the amount of AMP-[8-^{14}C] formed when the sample is added together with adenine phosphoribosyltransferase to adenine-[8-^{14}C]. The enzyme solution used is an extract of *Acer* cells (3 mg protein/ml); the procedure used to prepare the extracts and the conditions of assay have been described previously.[11] The xanthine and hypoxanthine content of the growth medium has been measured using xanthine oxidase: the corresponding procedure has been described elsewhere.[21] Each experiment shown in this paper was performed *at least* in duplicate with similar results.

[23] DOREE, M., LEGUAY, J. J. and TERRINE, C. (1970) *Compt. Rend.* **271,** 1876.
[24] DOREE, M. and GUERN, J. (1973) *Biochim. Biophys. Acta* in press.
[25] PRADET, A. (1967) *Physiol. Vég.* **5,** 209.