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STUDY OF ADENOSINE 5'-MONO-, DI- AND TRIPHOSPHATES IN PLANT TISSUES

IV. REGULATION OF THE LEVEL OF NUCLEOTIDES, *IN VIVO*, BY ADENYLATE KINASE: THEORETICAL AND EXPERIMENTAL STUDY

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SUMMARY

The purpose of this work is to demonstrate that adenylate kinase (EC 2.7.4.3) controls, *in vivo*, the equilibrium of adenine nucleotides in tissues of lettuce seeds and wheat leaves.

1. The theory of equilibria governed by this enzyme has been extensively studied. Two values were defined: $\Sigma = [\text{ATP}] + [\text{ADP}] + [\text{AMP}]$ and $\varepsilon =$ the sum of $\sim P$ of ATP and ADP ($= 2[\text{ATP}] + [\text{ADP}]$). The ratio ε/Σ has a value between 0 and 2 and indicates the energy charge of the adenylate system. If the amounts of the nucleotides are expressed in % of Σ, then their variations can be represented as functions of ε/Σ. A group of curves, one for each nucleotide, is obtained for a given value of the equilibrium constant of adenylate kinase.

2. Adenylate kinase was detected in the experimental material. The equilibrium constant of this enzyme, extracted from lettuce, was measured.

3. The levels of adenine nucleotides were studied in tissues where the energy charge was stable and in tissues where rapid changes of the energy charge were induced. In every case the results were consistent with an equilibrium maintained by adenylate kinase, although the apparent equilibrium constant varied from one experiment to another.

INTRODUCTION

Very little is known about the amounts in plant tissues of adenine nucleotides, though these compounds have to play a very important role in the energy metabolism of the cell. Our previous work on this problem has led to the hypothesis that adenylate kinase (EC 2.7.4.3, previously known as myokinase) regulates the equilibrium of adenine nucleotides in certain plant tissues[1,2]. This enzyme, widely distributed in living tissues, is known to catalyze the reaction AMP + ATP → 2ADP, which is the principal way leading to the formation of the β bond of ADP. It is well known, also, that this reaction is reversible, but very little attention has been given to the consequences of this fact. Meanwhile, ATKINSON AND WALTON[3] have begun work on this problem with a study of enzymatic regulation *in vitro*. The theoretical study of this question will be pursued here; it will be shown that different phenomena are observed *in vivo*, which can be predicted by the application of this theory.

In order to prove the hypothesis that adenylate kinase regulates the equilibria of adenine nucleotides in living tissues two requirements must be satisfied: (a) that the tissues studied contain adenylate kinase; (b) that the respective levels of the nucleotides, *in vivo*, be in accordance with the hypothesis of adenylate kinase intervention.

These two points will be examined successively.

MATERIAL AND METHODS

The conditions of the culture and of the preparation of the experimental material are the same as those which have already been described. Lettuce seeds (*Lactuca sativa*, var. Reine de Mai), after 8 h of imbibition[2], and Wheat leaves (*Triticum sativum*, var. Moisson) were used[1].

Two different methods were employed for determination of nucleotides. The first[4] carries the risk of slight interference by non-adenine nucleotides. For this reason the method of COLE AND ROSS[5] was used latterly. In this case, the phosphorylated compounds were labelled by feeding the excised leaves through their cut ends with $^{32}P_i$ in presence of 10^{-4} M KH_2PO_4 for 4 h. After that time, the leaves were kept 2 more hours with their cut ends in water before the beginning of the experiment, in order to allow the homogenization of the labelling to take place. After separation by two-dimensional chromatography the radioactivity of the different compounds was measured. Having ascertained that the specific activity of phosphorus was the same in α, β and γ, the result of the counting was brought back into a molecular base system by dividing the values obtained for ADP by 2 and those for ATP by 3.

Conditions of fixation

The object of these experiments being to discover if adenylate kinase equilibria are found *in vivo*, it is necessary that the activity of this enzyme be totally eliminated at the instant of fixation. Different techniques of fixation were used. It is certain in all cases that adenylate kinase activity stops as soon as the material is disintegrated: in effect, when important quantities of ATP are added at the moment of fixation of certain samples, all of the added ATP is found again in the extracts and it does not induce any variation in the level of the other nucleotides. It is, then, only during the disintegration of the material that some modification could eventually arise.

Lettuce seeds

After the stabilization of lettuce seeds by liquid N_2 in stainless steel pots, trichloroacetic acid (5 %) was rapidly poured into these pots which were simultaneously placed in a bath at —15°. The material was homogenized with an Ultra-Turrax homogenizer for four periods of 15 sec each.

With wheat leaves 2 techniques were used

(a) When the experimental procedure included the subsequent separation of the nucleotides by chromatography, the leaves, after stabilization by liquid N_2, were placed in a glass mortar which had been pre-cooled to —20° and contained 3 ml of formic acid (80 %), and were immediately ground while still frozen.

This technique, better adapted to work on wheat leaves which are not disintegrated by the Ultra-Turrax, seems to be as advantageous as the preceding one.

(b) When enzymatic determination of the nucleotides was planned, a quicker and very different technique was used, giving similar results. Water at 100° was introduced into the cell where the leaves were incubating; the cell was maintained at 100° for 2 min. The contents of the cell were then transferred to a Potter–Elvehjem homogenizer, rapidly cooled to 0° and the leaves homogenized. This simple, rapid heating to 100° is suffcient to inactivate the adenylate kinase of wheat, indicating that this enzyme is not very thermostable, unlike certain animal adenylate kinases.

Whatever precautions are taken for fixation, the possibility cannot be ruled out that adenylate kinase functions during the disintegration. Nevertheless, this eventuality does not seem very probable.

EVIDENCE OF THE EXISTENCE OF ADENYLATE KINASE IN THE TISSUES STUDIED

The presence of adenylate kinase has been noted in many higher plants by diverse authors[6–11]. The present study is concerned with wheat leaves and lettuce seeds.

A detailed report on this question will be the subject of a separate publication. The principal results are summarized for the present demonstration.

The adenylate kinase of lettuce seeds was isolated and purified 100 times. No phosphatase activity could be detected in the extract. The activity of the enzyme extracted from these tissues was shown to be largely sufficient to explain the rapid readjustment of the nucleotides *in vivo*. The equilibrium constant of the reaction $2\mathrm{ADP} \rightleftharpoons \mathrm{ATP} + \mathrm{AMP}$ was measured. Values varying between 0.5 and 1 were found, according to the concentration in Mg^{2+}. The enzyme was also detected in wheat leaves, and partially purified.

STUDIES OF THE EQUILIBRIA OF ADENINE NUCLEOTIDES

Theoretical study

In a system where the equilibrium between the adenine nucleotides is controlled by adenylate kinase according to the equation

$$2\,\mathrm{ADP} \rightleftharpoons \mathrm{AMP} + \mathrm{ATP}$$

the level of each nucleotide is determined if one knows the values of three parameters: ε, the sum of the energy-rich bonds of adenylate system; Σ, the sum of adenosine 5′-mono-, di- and triphosphates; K, the equilibrium constant of adenylate kinase. From these definitions one can form the three equations of Table I. This set of equations is easily resolved, yielding the results of Table II.

The formulae are, unfortunately, difficult to manipulate. It can be seen immediately that ε can vary between 0 and $2\,\Sigma$ according to the energy state of the system. It seems easiest to represent this state by ε/Σ, a concise index which could always be used to designate the energy charge of the adenylate system. The value of

TABLE I

INITIAL EQUATIONS RESULTING FROM DEFINITIONS AND ALLOWING THE CALCULATION OF THE FORMULAE OF TABLE II

$$\Sigma = [\text{ATP}] + [\text{ADP}] + [\text{AMP}]$$

$$\varepsilon = 2[\text{ATP}] + [\text{ADP}]$$

$$K = \frac{[\text{ATP}] \times [\text{AMP}]}{[\text{ADP}]^2}$$

TABLE II

GENERAL FORMULAE ALLOWING THE CALCULATION OF THE AMOUNTS OF DIFFERENT ADENINE NUCLEOTIDES

Σ, the sum of adenosine 5′-mono, di- and triphosphates; ε, the sum of the energy-rich bonds of the adenylate system; K = constant of equilibrium of adenylate kinase.

K	*ATP*	*ADP*	*AMP*
$\neq 0.25$	$\frac{\varepsilon}{2} + \frac{\Sigma - \sqrt{\Sigma^2 + (4K - 1)\varepsilon(2\Sigma - \varepsilon)}}{2(4K - 1)}$	$\frac{-\Sigma + \sqrt{\Sigma^2 + (4K - 1)\varepsilon(2\Sigma - \varepsilon)}}{4K - 1}$	$\frac{2\Sigma - \varepsilon}{2} + \frac{\Sigma - \sqrt{\Sigma^2 + (4K - 1)\varepsilon(2\Sigma - \varepsilon)}}{2(4K - 1)}$
$= 0.25$	$\frac{\varepsilon^2}{4\Sigma}$	$\frac{\varepsilon(2\Sigma - \varepsilon)}{2\Sigma}$	$\frac{(2\Sigma - \varepsilon)^2}{4\Sigma}$

this ratio will vary from 0 to 2. It represents the average number of energy-rich bonds per molecule of adenine nucleotide.

The equations of Table II have been used to construct the curves drawn in Fig. 1. The symmetry of these curves is notable: the ADP curve reaches a maximum, in terms of ε/Σ, at 1, and two points on the curve, equidistant from this maximum, will yield the same percentage. The ATP and AMP curves are symmetrical in relation to each other. This relationship is interesting as it permits us, in examining the experimental results, to represent the values obtained for these 2 compounds with one curve, by changing the ordinate ε/Σ to $2-\varepsilon/\Sigma$ for one of the two substances. As previously indicated[1], it is now clear that within wide limits of energy charge (0.5–1.5) the concentration of ADP varies only slightly. On the other hand, ADP is dramatically affected by variations of equilibrium constant. This constant can vary widely under the influence of such factors as pH and Mg^{2+} (Table III).

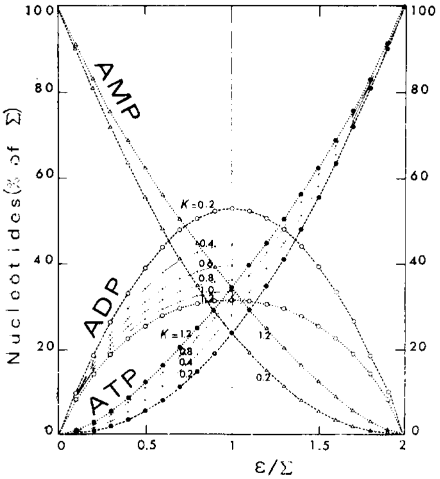


Fig. 1. The adenine nucleotide levels as a function of the ratio ε/Σ. Theoretical curves. ε, sum of energy-rich bonds of adenylate system (*cf.* Table I); Σ, sum of adenosine-5′-mono-, di- and triphosphates; K, equilibrium constant of adenylate kinase.

The important role that adenylate kinase might have in energy metabolism can now be seen. It could render the totality of the adenylic ~ P available to the energy-requiring processes, even if these processes are only capable of using the γ

TABLE III

VALUES OF THE EQUILIBRIUM CONSTANT OF ADENYLATE KINASE FOUND IN LITERATURE

Material	$K = [ATP] \times [AMP]/[ADP]^2$	*Authors*
Rabbit muscle	0.37–1	KALCKAR[12]
Rabbit muscle	0.333–1.09	BOWEN AND KERWIN[13]
Rabbit muscle	2.1–3.6	ATKINSON, BURTON AND MORTON[14]
Pigeon muscle	0.444	EGGLESTON AND HEMS[15]
Mitochondria of beef liver	0.76–0.88	MARKLAND AND WADKINS[16]
Lettuce seeds	0.5–1	PRADET AND BOMSEL

bonds of ATP. As soon as ADP is formed, it is transformed into ATP and AMP. In the Zone $0.5 < \varepsilon/\Sigma < 1.5$ (Fig. 1), where the ADP level is stable, everything seems to happen as if ATP were directly transformed into AMP. It becomes evident from this that, in a system utilizing or accumulating energy in the form of adenylic $\sim P$ in the presence of adenylate kinase, the phosphorus in β and γ have practically the same mobility. Observing this, ATKINSON AND WALTON[3] have compared the adenylate kinase system to an accumulator.

Some features of this theoretical study have already been observed *in vivo*[1,2], namely the relative stability of the ADP level. It will be seen further on that, in the tissues studied, the variations in the level of adenine nucleotides are produced according to the curves of Fig. 1.

Comparison of the equilibrium of the nucleotides in vivo with the theoretical equilibrium controlled by adenylate kinase

The data were analyzed in three ways: (1) The apparent equilibrium constant from a series of samples was calculated from the formula $k = [\text{ATP}]\,[\text{AMP}]/[\text{ADP}]^2$ and the variances compared with that expected from experimental error. (2) The values measured for the three nucleotides were compared by the pairing method[17] with values calculated using formulae from Tables I and II. (3) In order to make a graphical comparison the values observed for the nucleotides were plotted as percentages of Σ against ε/Σ, and compared with the theoretical curves of Fig. 1.

Experiments in which the nucleotide levels are approximately constant

Four sets of samples were kept under different combinations of constant conditions of lighting and oxygenation. The nucleotide levels were determined after different times of incubation. For a given combination of external conditions the levels of the different nucleotides are almost constant (Table IV), indicating that

TABLE IV

LEVELS OF ADENINE NUCLEOTIDES (nmoles/g FRESH WEIGHT) DETERMINED IN WHEAT LEAVES KEPT IN DIFFERENT CONSTANT CONDITIONS

Experimental conditions	*Nucleotide*	*Time of incubation (h)*			
		0.75	*1.5*	*2.25*	*3*
Light, N_2	AMP	42	43	69	55
	ADP	49	62	55	53
	ATP	35	27	16	12
Light, air	AMP	31	33	23	30
	ADP	53	56	57	58
	ATP	58	63	71	76
Darkness, air	AMP	26	12	18	1
	ADP	63	43	43	34
	ATP	60	57	62	82
Darkness, N_2	AMP	87	85	91	97
	ADP	37	28	22	24
	ATP	9	4.7	4.5	4.2

TABLE V

APPARENT EQUILIBRIUM CONSTANTS (k = [ATP] × [AMP]/[ADP]^2) MEASURED FROM WHEAT LEAVES KEPT IN DIFFERENT STABLE CONDITIONS

$\bar{k}_c$, average of the column; $\bar{k}_l$, average of the line; $\bar{k}_g$, general average. Analysis of variance in relation to time of incubation: one finds $s^2/\bar{k}_c = 0.0056$; $s^2_{error} = 0.0493$. Analysis of variance in relation to conditions of medium: one finds $s^2/\bar{k}_l = 0.0247$; $s^2_{error} = 0.0446$.

Conditions of mexium	*Time of equilibration (h)*				$\bar{k}_l$
	0.75	*1.5*	*2.25*	*3*	
Light, N_2	0.61	0.30	0.36	0.23	0.38
Light, air	0.64	0.66	0.50	0.67	0.62
Darkness, air	0.39	0.36	0.60	0.07	0.36
Darkness, N_2	0.56	0.51	0.84	0.71	0.66
$\bar{k}_c$	0.55	0.46	0.58	0.42	$\bar{k}_g = 0.50$

adenylate kinase equilibrium, if it exists, is attained. These results were compared with theory in the three ways listed above. The variance in k with different conditions (light, oxygen, or time of fixation) was found to be less than experimental error (Table V), and thus the values of k were taken to represent a homogeneous population. The mean value for k was 0.50 ± 0.10 with a 95 % confidence interval.

Taking the value of 0.50 for K, the values for ATP, ADP and AMP were calculated (Table VI). Measured and calculated values were statistically compared using the pairing method[17]. The fluctuations of the differences, D, between measured and calculated values were studied in relation to the theoretical average, 0. The

TABLE VI

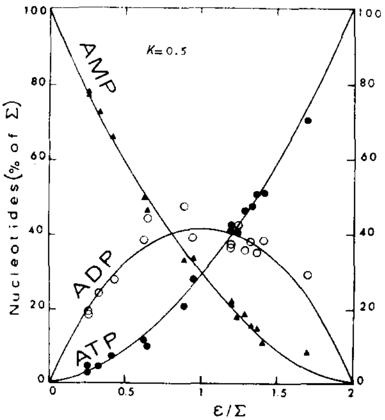
LEVELS OF ADENINE NUCLEOTIDES (nmoles/g FRESH WEIGHT) CALCULATED FOR THE EXPERIMENTS IN WHICH THE WHEAT LEAVES ARE KEPT IN DIFFERENT CONSTANT CONDITIONS

The calculation is made with the aid of formulae of Table II, on the basis of the following hypothesis: (1) for each sample, ε and Σ have the same value as that which can be calculated from the data of Table IV; (2) the equilibrium between adenine nucleotides regulated by adenylate kinase, the value of apparent equilibrium constant being 0.50.

Experimental conditions	*Nucleotide*	*Time of incubation (h)*			
		0.75	*1.5*	*2.25*	*3*
Light, N_2	AMP	41	45	70	59
	ADP	49	62	55	53
	ATP	34	31	18	16
Light, air	AMP	29	31	23	26
	ADP	57	61	57	65
	ATP	56	60	71	72
Darkness, air	AMP	27	13	17	4.5
	ADP	48	39	46	27
	ATP	62	60	59	85
Darkness, N_2	AMP	86	85	90	97
	ADP	38	28	24	25
	ATP	8.5	4.6	3.4	3.4

parameter t was calculated for the three populations of the 16 differences, D, obtained for each compound studied, and was found to be equal, for ATP 0.57, for ADP 0.54 and for AMP 0.61. These values are much less than the values 2.131 of $t_{0.05}$ for 15 degrees of freedom given by the table of Student-Fisher. Since no significant differences were found between calculated and measured values, the hypothesis of adenylate kinase regulation is not contradicted. The graphical comparison (Fig. 2) shows close correspondence of the experimental points to the theoretical curves for $K = 0.50$. The advantage with graphical comparison is not having to predetermine a value for the equilibrium constant. The method is rapid, as well, and the results revealing. Its use is particularly interesting when working with a sample series having very different values for ε/Σ, so as to place points all along the length of the curves. Working in this way only a relatively small amount of experimental data is required.

While the experiment just described supported the theory of adenylate kinase regulation of adenine nucleotide equilibrium under conditions of stable energy charge, it should now be asked whether this is also true when the energy charge of the adenylate system undergoes rapid changes.



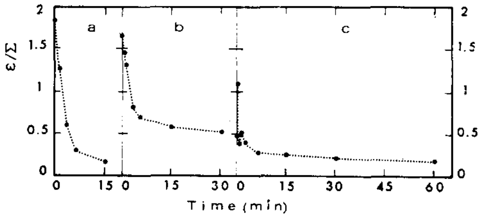


Fig. 2. Levels of adenine nucleotides. Graphic comparison of experimental values with theoretical curves. ●—●, ATP; ▲—▲, ADP; ○—○, AMP. Experiments on wheat leaves kept in different stable conditions. In darkness and with N_2: ε/Σ between 0.25 and 0.41; in light and with N_2: ε/Σ between 0.62 and 0.94; in light and with air: ε/Σ between 1.19 and 1.32; in darkness and with air: ε/Σ between 1.23 and 1.69.

Fig. 3. Evolution of energy level after suppression of phosphorylation possibilities. Expt. a: Lettuce seeds placed in N_2 at time 0; Expt. b: Wheat leaves in darkness; at time 0 they are placed in N_2; Expt. c: Wheat leaves in light and with N_2; at time 0 the light is turned off. In this experiment the leaves are infiltrated with 0.2 M glucose.

Experiments with energy-level variations

The energy level was made to rise or fall sharply by suppressing or re-establishing phosphorylation under two sets of conditions: (1) air and darkness, which allow only oxidative phosphorylation; (2) nitrogen and light, which allow only cyclic photophosphorylation. The contribution from glycolysis was neglected because of the low energy level which is reached after the exclusion of other possible phosphorylations (*cf.* Fig. 3). Three typical experiments are reported:

(a) Variations induced by the suppression of oxidative phosphorylation in lettuce seeds. The seeds, after soaking 8 h in water, were transferred from air to nitrogen. In this material a change from a very high energy level in air to a very low one in nitrogen was observed (Fig. 3). The experimental values compare favourably with the theoretical curves for an equilibrium constant, $K = 1.2$ (Fig. 4).

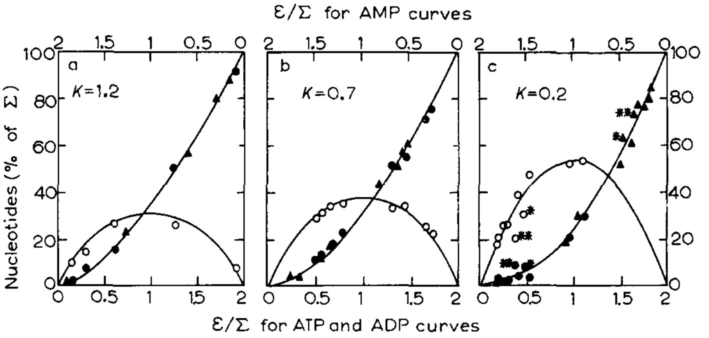


Fig. 4. Levels of adenine nucleotides. Graphic comparison of experimental values with theoretical curves. ●—●, ATP; ▲—▲, ADP; ○—○, AMP. For experimental conditions *cf.* Fig. 3. *, sample fixed after 10 sec in darkness; **, sample fixed after 35 sec in darkness.

(b) Variations induced by the suppression of oxidative phosphorylation in wheat leaves. The material, kept in darkness, was transferred from air to nitrogen. The energy level fell sharply as illustrated by Fig. 3 and AMP effectively replaced ATP, while ADP remained almost stable (as illustrated by the comparison between the autoradiographs of Fig. 5). The experimental data were seen to agree with the theoretical curves for the adenylate kinase equilibrium for a value of the constant, $K = 0.7$ (Fig. 4).

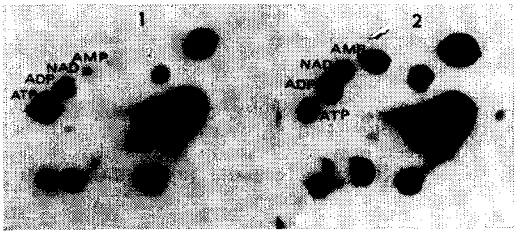


Fig. 5. Chromatographic autoradiograms of wheat leaves of Expt. b. Leaves incubated with $^{32}P_i$. 1, sample kept in air and darkness; 2, sample fixed after 30 min in N_2. The labelling being homogeneous, the activity of a molecule of ATP is 3 times greater than that of a molecule of AMP.

(c) Variations induced by the suppression of cyclic photophosphorylation in wheat leaves. In order to accentuate the fall of $\sim P$, the leaves were infiltrated with 0.2 M glucose which increases the utilization of ATP for sucrose synthesis. They were kept in a current of N_2 with light intensity of 5000 Lux. Suppression of phosphorylation was obtained by transfer of the leaves to darkness. The variations in the energy level are shown in Fig. 3, and the comparison of experimental data and theoretical curves in Fig. 4. It is seen, in this case, that there is an agreement for the theoretical curves corresponding to an equilibrium constant, $K = 0.2$. For samples fixed after

10 and 35 sec of darkness, the experimental points are rather distant from the curves. It is probable that, during such rapid variations, the activity of adenylate kinase is not sufficient to re-establish the disturbed equilibrium instantaneously. It is interesting to note that in the experiments in which oxidative phosphorylation is suppressed, this phenomenon is not observed, probably because the oxygen dissolved in the tissue buffers the transition to anaerobiosis. However, as the suppression of light in this case results in the immediate end of photophosphorylation, there is no buffering effect.

DISCUSSION

These experiments demonstrate both the presence of a very active adenylate kinase in the tissues studied, and that equilibria between the nucleotides similar to those observed *in vitro* by adenylate kinase, also occur *in vivo*. These two facts lead to the conclusion that this enzyme is effcient in regulating the level of the adenine nucleotides in the entire cell, even when the energy level varies rapidly.

An adenylate kinase equilibrium existing throughout the entire cell implies that the different cellular pools of adenine nucleotides are at the same energy level. Only under these conditions can the total of several equilibria produce a general equilibrium. It equally follows that exchanges of nucleotides between different pools must be extremely rapid. What is known about permeability of different cellular particles to adenine nucleotides makes these exchanges perfectly possible. For example, the work of SANTARIUS *et al.*[18] shows that there are very rapid exchanges of ATP and ADP between chloroplasts and the rest of the cell. JESCHKE[19] has shown, with very short time experiments, that ATP produced in chloroplasts by cyclic photophosphorylation could be used in the cytoplasm for the absorption of Cl^-. It has also been shown recently[20–22] that there is a rapid current of ADP entering the mitochondria while a current of ATP flows in the reverse direction. We have calculated the turnover of $\sim P$ in our materials[2] and have found it to be about 1 min, again implying a great mobility of $\sim P$ in the cells.

It is of the greatest importance for the economy of the cell that the β and γ bonds of ATP should have a comparable mobility.

In the course of our work with wheat leaves, we have observed, from one experiment to another, different values of the apparent equilibrium constant of the nucleotides. Though we cannot yet explain this fact, it is known that the equilibrium constant of adenylate kinase *in vitro* can vary within limits comparable to those observed and underlined above (Table III) in function of pH or concentration of Mg^{2+}. Similarly, YOSHIDA *et al.*[23] have shown that, in the leucocytes of children, the apparent equilibrium constant $[ATP] \times [AMP]/[ADP]^2$ had a value of 0.58 ± 0.15 for well-nourished children, and 0.21 ± 0.04 for those who were under-nourished.

For leaves placed in the same conditions of oxygenation or illumination, different values for the relation ε/Σ are observed from one experiment to another. This relation seems to be of a higher value in young leaves than in adult ones. The value of the ratio ε/Σ for a tissue placed in conditions not limited in regard to oxygen or light, could be a characteristic of its physiological state. It is possible to double the respiration rate and the phosphorylations of the wheat leaves by infiltrating them with glucose (unpublished data). Thus, the turnover in $\sim P$ bonds is doubled without

TABLE VII

VALUES OF THE APPARENT EQUILIBRIUM CONSTANT OF ADENYLATE KINASE AND OF THE RATIO ε/Σ FOR DIFFERENT TISSUES, CALCULATED FROM DATA OF VARIOUS PUBLICATIONS

Tissues	*Species*	*k*	ε/Σ	*Authors*
Cerebral	Guinea pig	2.5	1.86	Edel and Rein-Lovtrup[24]
	Rabbit	3.4	1.88	Edel and Rein-Lovtrup[24]
	Rat	2.9	1.92	Edel and Rein-Lovtrup[24]
	Mouse	*	1.95	Edel and Rein-Lovtrup[24]
	Mouse	0.6	1.61	Lowry *et al.*[25]
Erythrocyte	Man	1.23	1.77	Jorgensen[26]
	Man	1.30	1.74	Overgaard-Hansen, Jorgensen and Pretorius[27]
	Man	1.32	1.77	Jones and Robinson[28]
	Man	0.44	1.75	Bartlett *et al.*[29]
	Man	2	1.72	Cartier and Chedru[30]
Ehrlich ascites tumor cells	Mouse	1.58	1.75	Letnansky[31]
	Mouse	1.1–3.7	1.67–1.7	Overgaard-Hansen[32]
Liver	Rat	1.01	1.51	Wintzerith[33]
	Guinea pig	4.45	0.88	Dianzani Mor[34]
Uterus	Rat	0.50–2.96	1.23–1.43	Martray and Gautheron[35]
	Impubescent rat	0.56	1.38	Volfin, Clauser and Eboue[36]
	Adult rat	1.08	1.15	Volfin, Clauser and Eboue[36]
Striated muscle	Guinea pig	1.08	1.63	Dianzani Mor[34]
Leucocytes	Children	0.58	0.96	Yoshida *et al.*[23]
	Under-nourished children	0.21	0.84	Yoshida *et al.*[23]

* As AMP was reported as equal to 0, it was impossible to calculate *k*. We think the highly elevated energy level of the tissue implies a very low AMP level, one that would be very difficult to estimate.

changing ε/Σ. Further evidence indicating the remarkable constancy of energy charge throughout many tissues can be drawn from the literature. Values of the ratio ε/Σ and of the apparent equilibrium constant of adenylate kinase have been calculated from the data of various authors (Table VII). As far as these data reflect the state of nucleotides in living tissues, which essentially depends on the conditions of fixation, it is evident that, for a given tissue, the value of ε/Σ is more stable than that of the apparent equilibrium constant of adenylate kinase.

In plant physiology, different theories have been conceived to interpret the ratio ATP/ADP observed *in vivo*, all based on the hypothesis of the single transformation $\mathrm{ATP} \underset{\text{synthesis}}{\overset{\text{utilization}}{\rightleftharpoons}} \mathrm{ADP}$. The active intervention of adenylate kinase necessitates a re-examination of these theories.

Atkinson's theory[3,37], based on experiments *in vitro* concerning the regulation of different enzymes and the ratio ATP/ADP in relation to equilibria controlled by adenylate kinase, seems to be closer to reality. The demonstration in the present work, that this enzyme controls, *in vivo* the equilibria of certain tissues, substantiates this theory in regard to different plant tissues. It is likely that the phenomena described will be noticed again elsewhere; yet it is known that adenylate kinase activity is weak in certain plant tissues like Coleus[11].

The function of the enzymes inhibited by ADP is not efficient unless the ratio ATP/ADP is high[3], which implies very elevated values of ε/Σ as well. For a given value of the equilibrium constant of adenylate kinase, the value of ATP/ADP is a function of ε/Σ, and for a given value of ε/Σ, the value of ATP/ADP rises as that of K rises. Fig. 6 represents the variations of ATP/ADP as a function of the different values of the constant. One notices that, when ATP/ADP = 1, the values of ε/Σ fall from 1.26 to 0.94 as those of K rise from 0.4 to 1.2. It is possible that the variations of the apparent equilibrium constant, as noted above, play a part in the regulation of nucleotides.

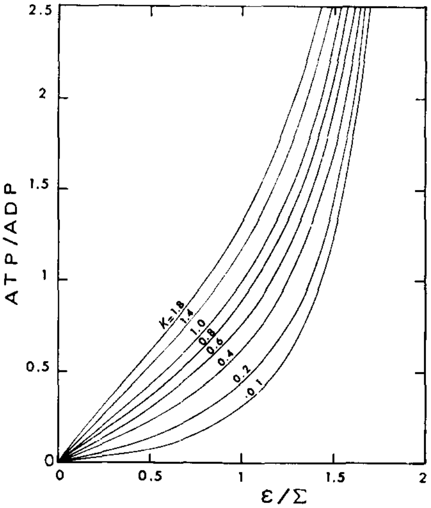


Fig. 6. Relation between the ratios ATP/ADP and ε/Σ for different values of the equilibrium constant of adenylate kinase (theoretical curves).

It is equally possible that the very wide variations of ε/Σ that we obtained in previous work[1], play a similar role. Given the data for K and ε/Σ, the speeds of the reaction will increase as Σ increases.

As ATKINSON *et al.*[3,37] have underlined, it is evident that adenylate kinase also intervenes in the control of all enzymes regulated by the different adenine nucleotides.

This study was limited to adenine nucleotides. Further experimental data to be published subsequently, indicate that, when ε/Σ undergoes profound change, energy-rich bonds of guanylic, uridylic and cytidylic pools present parallel variations. It is clear that exchanges of $\sim P$ take place between these different pools, and this could partially explain the variations observed in the apparent equilibrium constant of adenine nucleotides.

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