Biochemical Pharmacology, Vol. 38, No. 23, pp. 4261–4266, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

GENERATION OF EXTRACELLULAR ATP IN BLOOD AND ITS MEDIATED INHIBITION OF HOST WEIGHT LOSS IN TUMOR-BEARING MICE

ELIEZER RAPAPORT* and JAMES FONTAINE
Department of Microbiology, Boston University School of Medicine, Boston, MA 02118, U.S.A.

(*Received* 26 *January* 1989; *accepted* 15 *May* 1989)

Abstract—Intraperitoneal injections of adenosine 5′-monophosphate (AMP) or adenosine 5′-triphosphate (ATP), but not of adenosine, inorganic phosphate or pyrophosphate, were shown to inhibit tumor growth and host weight loss in tumor-bearing murine hosts. The inhibition of tumor growth and host weight loss did not exhibit a cause–effect relationship, though both were mediated through expansion of red blood cell (RBC) ATP pools which were promoted by administered adenine nucleotides. We then demonstrated that expansion of RBC ATP pools was preceded by expansion of liver ATP pools and that the adenosine precursor for this type of enhanced RBC ATP synthesis originated in the turnover of expanded liver ATP pools. Although adenosine, which is the primary catabolic product of ATP in the peritoneal cavity or the systemic circulation, was sufficient to yield an expansion of mouse liver ATP pools *in vivo*, external phosphate was required for the subsequent expansion of RBC ATP pools, which in turn produced elevated extracelluar (blood plasma) ATP levels. The anticancer activities which correlate with the elevated blood plasma ATP concentrations are proposed to be the result of direct action of extracellular ATP on the tumor and host tissues.

During recent years, the wide ranging physiological effects of extracellular ATP are being recognized [1, 2]. Extracellular ATP was demonstrated to have major roles in the regulation of vascular tone [1, 3], muscle contraction [4], cardiovascular functions [5], neurotransmission [6] and platelet aggregation [7]. Three sources of intravascular, extracellular plasma compartment levels of ATP have been elucidated. These are: ATP released from endothelial cells during hypoxia [1, 2], ATP released from the dense granules of blood platelets during platelet aggregation [7], and ATP released from red blood cells under conditions of hemodynamic stress [8]. The activation of cellular functions by extracellular ATP is mediated by its interaction with P_2-purinoceptors [1, 3] and intracellular biochemical reactions resulting from this interaction are being identified (for example, see Refs 9–12).

Beneficial effects of systemically administered ATP, especially for cardiovascular functions, have been reported since the 1950s (for example, see Refs 13 and 14). In addition, extracellular ATP was shown to possess growth inhibitory properties against a variety of transformed cells in several *in vitro* systems and biochemical mechanisms that mediate these activities were identified [15–19]. The effects of micromolar concentrations of ATP *in vitro* are specific towards transformed cells [15–19]. Intraperitoneal injections of adenine nucleotides into tumor-bearing mice result in the inhibition of tumor growth in the murine models [20]. These compounds were shown to yield expansions of RBC ATP pools followed by the slow release of micromolar levels of ATP into the extracellular, blood plasma compartment [21]. The elevated concentrations of extracellular ATP were suggested to account for the tumor-growth-inhibitory activities by mechanisms which were established previously during extensive *in vitro* studies [15–19]. It is important to note that the inhibition of tumor growth could also be mediated by non-immunological host functions which are affected by elevated extracellular ATP in a host through the interaction of ATP with cellular purinergic receptors [1–4, 7, 8]. In addition, the administration of adenine nucleotides into tumor-bearing mice leads to a marked inhibition of host weight loss [21]. These activities were shown to be neither the cause nor the effect of the inhibition of tumor growth. Although the rate of weight loss is related to the size of the tumors, the component of the inhibition of weight loss due to smaller size tumors in treated animals could be separated from the direct effects of adenine nucleotides on host functions, which presumably account for the other component of the inhibition of weight loss in tumor-bearing hosts [21]. Weight loss is a frequent but poorly understood aspect of cancer [22, 23]. The prognostic effect of weight loss on the response rate to chemotherapy in patients with a variety of tumor types has been established [24]. Chemotherapy response rates are lower in patients with weight loss, and reversal of poor nutritional state has been shown to affect the outcome in cases where the anticancer therapy was inherently effective [24]. An important consequence of weight loss is the slowing down of the growth rate of tumors. Thus, the slowing of tumor growth rates, which leads to a diversion of tumor cells from a proliferative to a nonproliferative state [25], results in a decreased sensitivity towards many chemotherapeutic agents which are in use today.

* Correspondence: Dr Eliezer Rapaport, Department of Microbiology, Boston University School of Medicine, 80 East Concord St., Boston, MA 02118.

As an initial step in establishing the biological effects of adenine nucleotides on host functions we have now identified the mechanism of expansion of RBC ATP pools. Since extracellular ATP has been shown to stimulate thymocyte proliferation [26–28] and to increase interleukin-2 production by a subset of T lymphocytes [27], we have also demonstrated that both the inhibition of tumor growth and host weight loss are not likely to be the result of activation of certain immunological functions in the tumor-bearing hosts.

MATERIALS AND METHODS

Experimental protocols for studies of tumor-growth inhibition and inhibition of weight loss in tumor-bearing mice by adenine nucleotides followed published procedures [20, 21]. Adenine nucleotide solutions were adjusted to pH 6.0–6.2 before injections. $CB6F_1$ mice were obtained from Jackson Laboratories, Bar Harbor, ME, and outbred athymic NCr-*nu/nu* mice were obtained from Mr. Robert Sedlacek, Laboratory for Radiation Biology, Massachusetts General Hospital, Boston, MA. CT26 tumors were inoculated (1×10^6 cells in 50 μl of phosphate-buffered saline) into the footpad of athymic *nu/nu* mice as described [20, 21], the mice were randomized, and treatment was started on day 6 when 100% of the mice carried palpable tumors.

Blood (0.25 ml) was collected from the inferior vena cava into 1-ml syringes containing 0.05 ml of citrate-dextrose as described previously [21]. Mice were anesthetized with ether during the procedure and, immediately after the removal of the blood by one person, another person excised a small portion of the liver (250–400 mg) utilizing *in situ* freeze-clamping with aluminum plates precooled in liquid nitrogen [29]. The frozen tissue was pulverized in a mortar at solid carbon dioxide temperature and extracted in 10 ml of ice-cold 7% trichloroacetic acid. Analyses of total liver, RBC and blood plasma ATP pools were performed by bioluminometry as described [21]. Conversions of ATP levels to molar concentrations are based on weight of the frozen liver portion or total volume of blood in the case of RBCs. The specific radioactivities of liver and RBC [^{3}H, ^{32}P]ATP pools and blood plasma [^{3}H]ATP levels were determined by correlation of total radioactivity with total pool size. Total radioactivities of the ATP pools were determined by thin-layer chromatography, and total pool sizes were determined by bioluminometry. Determination of total radioactivity in blood plasma [^{3}H]ATP required two-dimensional thin-layer chromatography [21].

RESULTS

Daily i.p. injections of 2 ml of 25 mM AMP or 2 ml of 25 mM ATP for 10 consecutive days, starting after the footpad CT26 tumors in athymic *nu/nu* mice became palpable, yielded significant inhibition of tumor growth and host weight loss (Fig. 1). Adenosine was ineffective in inhibiting tumor growth but inhibited weight loss in tumor-bearing animals to some extent when compared with saline-treated or untreated groups. The inhibition of weight loss in

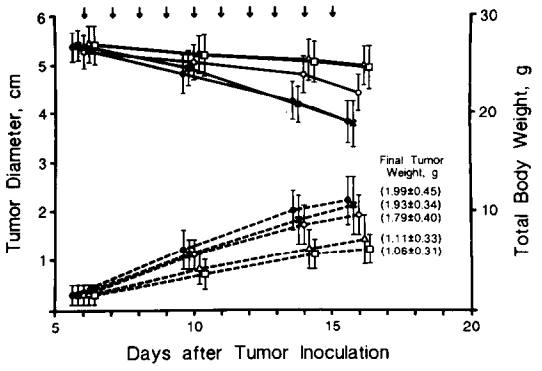

Fig. 1. Inhibition of tumor growth (– – –) and host weight loss (——) in athymic *nu/nu* mice (males, 7 weeks old) bearing footpad CT26 tumors. Treatment with saline (●), adenosine (○), AMP (△) or ATP (□) was initiated 6 days after tumor inoculation when all the animals carried palpable tumors. An untreated group was also included (×). Each group included ten mice, and data points are expressed as mean ± SD. Arrows indicate the days of injection. All other experimental details are described in the text. No deaths occurred before day 16 after tumor inoculation, the point at which the animals were killed and the tumors excised and weighed. Tumor weights in grams are shown in parentheses. The statistical significance of the inhibition of tumor growth (final tumor weight) by AMP and ATP (difference from saline-treated group by Student's *t*-test) was $P < 0.001$ for both AMP- and ATP-treated groups. No statistically significant inhibition was obtained in the adenosine-treated group. The statistical significance of the inhibition of host weight loss on day 16 after tumor inoculation (differences from saline-treated group by Student's *t*-test) was $P < 0.001$ for the ATP-treated group, $P < 0.001$ for the AMP-treated group, and $P < 0.01$ for the adenosine-treated group.

tumor-bearing hosts cannot be attributed to fluid retention in the ATP- or AMP-treated mice. Identical treatment schedules of non-tumor-bearing animals did not produce any weight gains in the ATP- or AMP-treated mice (data not shown). Furthermore, treatment schedules that were initiated 1 day after tumor inoculation yielded inhibition of weight loss that could be correlated only with the smaller size tumors in the treated mice [21]. Treatment schedules that were initiated when tumors were palpable or later, and which were thus administered when the tumors became progressively larger, resulted in a more pronounced inhibition of weight loss when compared to untreated or saline-treated animals [21].

Utilizing radioactively labeled precursors, we were able to follow the phosphorylated adenosine derivatives after single i.p. injections of 2 ml of 35 mM adenosine, AMP or ATP. The results reported in Table 1 demonstrate that adenosine, AMP or ATP expanded the total liver ATP pools by 2- to 3-fold as compared to saline treatment. However, only AMP and ATP yielded expansions of RBC ATP pools and increases in blood plasma ATP levels. Furthermore, the specific radioactivities of liver [^{3}H]ATP pools were similar to the specific radioactivities of RBC [^{3}H]ATP pools after AMP or ATP injections, suggesting that the turnover of expanded liver [^{3}H]ATP pools provides the [^{3}H]adenosine precursor which is needed for the expanded synthesis of RBC ATP pools. Mature RBCs cannot synthesize

Table 1. Mouse ($CB6F_1$) liver and RBC [^{3}H]ATP pools and specific radioactivities after i.p. injections of [^{3}H]ATP in saline, adenosine, AMP or ATP*

Compound administered	Liver		RBCs		Blood plasma	
	ATP† (mM)	ATP‡ (cpm/nmol)	ATP† (mM)	ATP‡ (cpm/nmol)	ATP† (μM)	ATP‡ (cpm/nmol)
Saline	2.78	1543	0.69	2644	0.71	3955
Adenosine	6.86	3693	0.67	2154	0.93	2637
AMP	7.63	2864	1.17	2725	1.53	3293
ATP	8.29	2196	1.52	2340	1.78	2819

* Mice ($CB6F_1$ males, 9 weeks old) were injected i.p. with 2 ml of 500 μCi of [^{3}H]ATP (30 Ci/mmol specific radioactivity) in saline, 35 mM adenosine, 35 mM AMP or 35 mM ATP, and 2 to 2.5 hr later the animals were anesthetized and analyzed as described in the text. Data are the average of two experiments.

† Total ATP pools were determined by bioluminometry.

‡ Specific radioactivities were determined by the correlation of cpm in [^{3}H]ATP pools which were determined by one- or two-dimensional thin-layer chromatography with the actual size of the [^{3}H]ATP pool determined by bioluminometry.

Table 2. Mouse ($CB6F_1$) liver and RBC [^{3}H]ATP pools and specific radioactivities after i.p. injections of 2 ml of 35 mM [^{3}H]ATP*

Time after injection (min)	Liver		RBCs	
	ATP† (mM)	ATP‡ (cpm/nmol)	ATP† (mM)	ATP‡ (cpm/nmol)
No injection	3.16	—	0.60	—
15	4.24	1743	0.93	1612
30	4.05	2408	0.99	2424
60	7.09	2766	1.31	2671
120	6.13	2815	1.73	3078
240	8.25	2513	2.39	3564

* Mice ($CB6F_1$ males, 8 weeks old) were utilized. All the experimental procedures are outlined in the text and in the footnotes to Table 1. Data are the average of two experiments.

† Total pool size.

‡ Specific radioactivity.

ATP *de novo* and require salvage precursors for ATP synthesis [30]. Injections of adenosine resulted in expansions of total liver ATP pools without any effects on RBC ATP pools or plasma ATP levels. The specific radioactivity of RBC [^{3}H]ATP pools was vastly different from the specific radioactivity of liver [^{3}H]ATP pools after injections of adenosine (Table 1). It is important to note that all the precursors, namely adenosine, AMP, ATP or high specific radioactivity [^{3}H]ATP, were incorporated into liver ATP pools as adenosine or [^{3}H]adenosine respectively (data not shown). Ectoenzymatic catabolic activities present in the peritoneal cavity and in the vascular bed, as well as enzymatic activities present in blood plasma, actively catalyze the degradation of ATP to adenosine [2, 21].

The incorporation of a radioactive precursor into liver and RBC [^{3}H]ATP pools after i.p. injections of 2 ml of 35 mM ATP was followed as a function of time (Table 2). The similarity between the specific radioactivities of liver and RBC [^{3}H]ATP pools was maintained throughout the expansion of the total ATP pools in both the liver (3 to 7 mM) and RBCs (0.6 to 1.7 mM) during the first 2 hr after injections (Table 2). Only at later times (3–4 hr after injection) did the specific radioactivity of liver [^{3}H]ATP decline at the expense of the increases in the specific radioactivity and size of the RBC [^{3}H]ATP pools (Table 2).

Further studies of the metabolic fate of i.p. injected adenine nucleotides utilizing [^{3}H, α-^{32}P]ATP as the radioactive precursor show the following. Both the phosphate and adenosine moieties of AMP or ATP were incorporated into liver ATP pools and, since the phosphate groups of AMP or ATP successfully diluted the ^{32}P radioactive label, the resulting liver [^{3}H, ^{32}P]ATP pools possessed progressively lower ^{32}P/^{3}H ratios in proceeding from adenosine to AMP to ATP as i.p. precursors (Table 3). The phosphate groups of AMP or ATP were not necessary for the expansion of total liver ATP pools. Inorganic phosphate, however, was required for the expansion of RBC ATP pools since 2 ml of 35 mM adenosine along with 105 mM inorganic phosphate produced results similar those achieved with 2 ml of 35 mM ATP (Table 3).

DISCUSSION

Recent reports suggest that extracellular ATP can act as a positive effector of thymocyte proliferation [26–28]. We now demonstrate that the anticancer

Table 3. Mouse (athymic *nu/nu*) liver and RBC [^{3}H, ^{32}P]ATP pools and specific radioactivities after i.p. injections of [^{3}H, α-^{32}P]ATP in saline, adenosine, AMP or ATP*

Compound administered	Liver			RBCs		
	ATP† (mM)	ATP‡ (^{3}H-cpm/nmol)	ATP (^{32}P/^{3}H)	ATP† (mM)	ATP‡ (^{3}H-cpm/nmol)	ATP (^{32}P/^{3}H)
Saline	2.93	1961	2.5	0.73	2874	2.5
Adenosine	6.42	2513	2.1	0.65	1063	0.4
AMP	9.10	1743	1.4	1.33	1555	0.4
ATP	9.46	2376	0.6	1.64	2240	0.5
Adenosine + P_i	7.71	1764	0.8	1.61	1899	0.6

* Mice (athymic *nu/nu* females, 9 weeks old) were injected i.p. with 2 ml of 500 μCi of [^{3}H]ATP (30 Ci/mmol) and 250 μCi [α^{32}-P]ATP (36 Ci/mmol) in saline, 35 mM adenosine, 35 mM AMP, 35 mM ATP or 35 mM adenosine along with 105 mM inorganic phosphate. Animals were analyzed 1.5 to 2 hr after injections as described in the text. The original [^{3}H, α-^{32}P]ATP solutions had a ^{32}P/^{3}H ratio of 1.27. Data are the average of two experiments.

† Total pool size.

‡ Specific ratioactivity.

activities of extracellular (blood plasma) ATP in mice are not likely to originate in the enhancement of host immunological functions. Inhibition of tumor growth and host weight loss in tumor-bearing mice after i.p. administration of adenine nucleotides was shown in athymic *nu/nu* mice carrying relatively large CT26 tumors. Athymic *nu/nu* mice are immunodeficient and have been reported to lack the capacity for interleukin-2 synthesis [31]. The CT26 tumor was shown to be poorly immunogenic, failing to either induce protection against subsequent tumor challenge or lead to a cytotoxic T-lymphocyte response in syngeneic animals [32]. It is therefore suggested that the mechanism(s) responsible for inhibition of tumor growth and host weight loss in tumor-bearing hosts by adenine nucleotides is biochemical rather than immunological in nature. The inhibition of tumor growth is presumed to be mediated by the elevated plasma ATP levels, since it is expressed in animals only when RBC ATP pools and plasma ATP levels, which originate in these RBCs, are increased markedly [20, 21]. Extensive studies from a variety of laboratories demonstrating the growth inhibitory activities of extracellular ATP with specificity toward transformed cells in *in vitro* systems [15–19] lend credence to such a mechanism. A non-immunological mediation of host response by elevated blood plasma ATP levels [1–7] cannot, however, be ruled out as accounting for the inhibition of tumor growth in mice by adenine nucleotides.

Studies reported here indicate that the expansion of RBC ATP pools after i.p. admininstration of AMP or ATP is preceded by expansion of total liver ATP pools. The expansion of liver ATP pools was promoted by adenosine which was shown previously to yield 3-fold increases in total ATP pools in isolated hepatocytes [33] or in isolated perfused rat livers [34]. Adenosine-promoted increases in hepatocyte ATP pools were reported to severely inhibit certain energy-requiring processes in these cells [33], an outcome which may be beneficial to a host under the metabolic strain of a tumor [35]. The turnover of the expanded liver ATP pools yields an increased supply of salvage precursors for the expansion of RBC ATP pools. The identical specific radioactivities of the expanded liver and RBC [^{3}H]ATP pools suggest that the adenosine precursor for the RBC ATP synthesis is taken up by the circulating RBCs in the hepatic sinusoids [30]. Adenosine, however, without the presence of inorganic phosphate, which in turn is a product of the catabolism of AMP or ATP, promoted expansion of total liver ATP pools without the subsequent expansion of RBC ATP pools. Intraperitoneal administration of ADP under the same conditions led to expansions of RBC ATP pools and elevation of blood plasma ATP levels which were, in magnitude, in between the increases achieved by the introduction of AMP and ATP respectively [20].

The role of extracellular adenosine in expanding total liver ATP pools *in vitro* [33, 34], the turnover of liver ATP pools supplying the salvage precursor for RBC ATP synthesis in rabbit liver perfused *in situ* [30], and the function of inorganic phosphate in promoting phosphorylation of adenosine to adenine nucleotides in isolated washed human erythrocytes [36] were all described in these *in vitro* systems. We have now demonstrated that a successive combination of all three steps can occur *in vivo* after i.p. administration of adenine nucleotides. The result was shown to be an expansion of RBC ATP pools and increases in the steady-state levels of blood plasma ATP. The role of elevated inorganic phosphate in promoting the expansion of RBC ATP pools after the administration of adenosine is not understood completely. We have shown previously that the incorporation of adenosine into mouse liver adenine nucleotide pools *in vivo* results in the formation of a functionally compartmentalized pool of ATP which does not mix in a diffusion-controlled process with the metabolically active hepatic adenine nucleotide pools [29]. Elevated inorganic phosphate could conceivably disrupt this form of compartmentalization. In addition, the uptake of adenosine by RBCs at the level of phosphorylation has been shown to be directly related to RBC intracellular phosphate concentration [36].

The efficacy of the anticancer activities of adenine nucleotides in humans is underestimated by the murine studies. The reason for this is that human blood and tissues possess significantly lower levels of soluble and ectoenzymatic activities that catalyze the degradation of extracellular ATP than do murine

blood and tissues [20, 21, 37 and references cited therein]. Intravenous infusions of relatively low levels of ATP into humans were shown recently to produce rapid elevations of total (cellular) blood ATP levels [38]. Thus, elevation of human RBC ATP pools and blood plasma ATP levels may be achieved with manageable systemic or toxic side effects. Increases in RBC ATP pools after intravenous administration of adenine nucleotides could be mediated by the direct uptake of adenine nucleotides by RBCs. It has been demonstrated that ADP or ATP levels of 10–100 μM could semi-permeabilize RBCs, resulting in influx and mostly efflux of adenine nucleotides [37]. The intraperitoneal mode of administration of adenine nucleotides favors the involvement of the liver because of the entry of these agents into the portal circulation. The possibility exists, however, that the expansion of RBC ATP pools could proceed by the direct incorporation of adenine nucleotides into RBCs in the systemic circulation, as well as by the uptake of liver ATP turnover products by RBCs in the hepatic sinusoids.

Clinical trials utilizing ATP following hepatic failure and in the management of various adverse circulatory conditions are currently underway (for reviews see Refs 39 and 40). The role of expanded liver and RBC ATP pools in elevating steady-state blood plasma ATP levels provides the likely basis for these treatments.

REFERENCES

1. Burnstock G and Kennedy C, A dual function for adenosine 5′-triphosphate in the regulation of vascular tone. *Circ Res* **58**: 319–330, 1986.
2. Gordon JL, Extracellular ATP: effects, sources and fate. *Biochem J* **223**: 309–319, 1986.
3. Kennedy C and Burnstock G, Evidence of two types of P_2-purinoceptor in longitudinal muscle of the rabbit portal vein. *Eur J Pharmacol* **111**: 49–56, 1985.
4. Burnstock G, Purinergic nerves. *Pharmacol Rev* **24**: 509–581, 1972.
5. Berne RM, The role of adenosine in the regulation of coronary blood flow. *Circ Res* **47**: 807–813, 1980.
6. Sneddon P and Burnstock G, ATP as a co-transmitter in rat tail artery. *Eur J Pharmacol* **106**: 149–152, 1984.
7. Holmsen H, Platelet metabolism and activation. *Semin Hematol* **22**: 219–240, 1985.
8. Born GVR, Bergquist D and Arfors KE, Evidence for inhibition of platelet activation in blood by a drug effect on erythrocytes. *Nature* **259**: 233–235, 1976.
9. Tsuda T, Kawahara Y, Fukumoto Y, Yakai Y and Fukuzaki H, Stimulation of phospholipase C-mediated hydrolysis of phosphoinositides by adenosine 5′-triphosphate via P_2-purinoceptors in cultured rat aortic vascular smooth muscles cells. *Jpn Circ J* **52**: 570–579, 1988.
10. McMillian MK, Soltoff SP, Lechleiter JD, Cantley LC and Talamo BR, Extracellular ATP increases free cytosolic calcium in rat parotid acinar cells. *Biochem J* **255**: 291–300, 1988.
11. Buisman HP, Steinberg HT, Fischbarg J, Silverstein SC, Vogelzang SA, Ince C, Ypey DL and Leight PC, Extracellular ATP induces a large nonselective conductance in macrophage plasma membrane. *Proc Natl Acad Sci USA* **85**: 7988–7992, 1988.
12. Dubyak GR, Cowen DS and Meuller LM, Activation of inositol phospholipid breakdown in HL60 cells by P_2-purinergic receptors for extracellular ATP. *J Biol Chem* **263**: 18108–18117, 1988.
13. Emmelin N and Feldberg W, Systemic effects of adenosine triphosphate. *Br J Pharmacol* **3**: 273–284, 1948.
14. Saito D, Ueeda M, Abe Y, Tani H, Nakatsu T, Yoshida H, Haraoka S and Nagashima H, Treatment of paroxysmal supraventricular tachycardia with intravenous injection of adenosine triphosphate. *Br Heart J* **55**: 291–294, 1986.
15. Rapaport E, Treatment of human tumor cells with ADP or ATP yields arrest of growth in the S phase of the cell cycle. *J Cell Physiol* **114**: 279–283, 1983.
16. Rapaport E, Fishman RF and Gercel C, Growth inhibition of human tumor cells in soft-agar cultures by treatment with low levels of adenosine 5′-triphosphate. *Cancer Res* **43**: 4402–4405, 1983.
17. Chahwala SB and Cantley LC, Extracellular ATP induces ion fluxes and inhibits growth of Friend erythroleukemia cells. *J Biol Chem* **259**: 13717–13722, 1984.
18. Weisman GA, Lustig KD, Lane E, Huang N, Belzer I and Friedberg I, Growth inhibition of transformed mouse fibroblasts by adenine nucleotides occurs via generation of extracellular adenosine. *J Biol Chem* **263**: 12367–12372, 1988.
19. Kitagawa T, Amano F and Akamatsu Y, External ATP-induced passive permeability change and cell lysis of cultured transformed cells: action in serum-containing growth media. *Biochim Biophys Acta* **941**: 257–263, 1988.
20. Rapaport E, Experimental cancer therapy in mice by adenine nucleotides. *Eur J Cancer Clin Oncol* **24**: 1491–1497, 1988.
21. Rapaport E and Fontaine J, Anticancer activities of adenine nucleotides in mice are mediated through expansion of red blood cell ATP pools. *Proc Natl Acad Sci USA* **86**: 1662–1666, 1989.
22. Dewys WD and Eastern Cooperative Oncology Group, Prognostic effect of weight loss prior to chemotherapy in cancer patients. *Am J Med* **69**: 491–497, 1980.
23. Socher SH, Martinez D, Craig JB, Kuhn JG and Oliff A, Tumor necrosis factor not detectable in patients with clinical cancer cachexia. *J Natl Cancer Inst* **80**: 595–598, 1988.
24. Van Eys J, Effect of nutritional status on response to therapy. *Cancer Res* **42**(Suppl.): 747S–753S, 1982.
25. Bruce WR and Meeker BE, Comparison of the sensitivity of hematopoetic colony-forming cells in different proliferative states to 5-fluorouracil. *J Natl Cancer Inst* **38**: 401–405, 1967.
26. Ikehera S, Pahwa RN, Lunzer DG, Good RA and Modak MJ, Adenosine 5′-triphosphate-(ATP) mediated stimulation and suppression of DNA synthesis in lymphoid cells. *J Immunol* **127**: 1834–1838, 1981.
27. El-Moatassim C, Dornand J and Mani J-C, Extracellular ATP increases cytosolic free calcium in thymocytes and initiates the blastogenesis of the phorbol 12-myristate 13-acetate-treated medullary population. *Biochim Biophys Acta* **927**: 437–444, 1987.
28. Lin J, Krishnaraj R and Kemp RG, Exogeneous ATP enhances calcium influx in intact thymocytes. *J Immunol* **135**: 3403–3410, 1985.
29. Rapaport E and Zamecnik PC, Incorporation of adenosine into ATP: Formation of compartmentalized ATP. *Proc Natl Acad Sci USA* **73**: 3122–3125, 1976.
30. Lerner MH and Lowy BA, The formation of adenosine in rabbit liver and its possible role as a direct precursor of erythrocyte adenine nucleotides. *J Biol Chem* **249**: 959–966, 1974.
31. Gillis S, Union NA and Baker PE, The *in vitro* generation and sustained culture of nude mouse cytolytic T-lymphocytes. *J Exp Med* **149**: 1460–1476, 1979.
32. Fearon ER, Itaya T, Hunt B, Vogelstein B and Frost P, Induction in a murine tumor of immunogenic tumor

variants by transfection with a foreign gene. *Cancer Res* **48**: 2975–2980, 1988.
33. Lund P, Cornell NW and Krebs HA, Effect of adenosine on the adenine nucleotide content and metabolism of hepatocytes. *Biochem J* **152**: 593–599, 1975.
34. Wilkening J, Nowack J and Decker K, The dependence of glucose formation from lactate on the adenosine triphosphate content in the isolated perfused rat liver *Biochim Biophys Acta* **392**: 299–309, 1975.
35. Young VR, Energy metabolism and requirements in the cancer patient. *Cancer Res* **37**: 2336–2347, 1977.
36. Hawkins CF, Kyd JM and Bagnara AS, Adenosine metabolism in human erythrocytes: A study of some factors which affect the metabolic fate of adenosine in intact red cells *in vitro*. *Arch Biochem Biophys* **202**: 380–387, 1980.
37. Trams EG, Kaufmann H and Burnstock G, A proposal for the role of ecto-enzymes and adenylates in traumatic shock. *J Theor Biol* **87**: 609–621, 1980.
38. Gaba S, Didier C, Cohendy R and Préfaut C, Effets vasculaires pulmonáires et systémiques de l'ATP chez l'homme. *C R Soc Biol (Paris)* **180**: 568–573, 1986.
39. Chaudry IH, Stephan RN, Dean RE, Clemens MG and Baue AE, Use of magnesium-ATP following liver ischemia. *Magnesium* **7**: 68–77, 1988.
40. Flacke WE, Adenosine and adenosine triphosphate for acute blood pressure control. *Semin Anesth* **7**: 216–225, 1988.