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REGULATION OF CYCLIC AMP LEVEL AND SYNTHESIS OF DNA, RNA AND PROTEIN BY QUERCETIN IN EHRLICH ASCITES TUMOR CELLS

Y. GRAZIANI and R. CHAYOTH

Department of Biology, Ben Gurion University of the Negev, Beer Sheva, Israel

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Abstract—Quercetin (3,3′,4′,5,7-pentahydroxy flavone) at the concentration of 10^{-4} M as well as 10^{-2} M theophylline and 10^{-3} M dibutyryl cyclic AMP caused at least 85 per cent inhibition of [^{3}H]thymidine incorporation in Ehrlich ascites tumor cells. At the same concentrations, these drugs decreased [^{3}H]uridine and [^{3}H]-L-leucine incorporation by 50–60 per cent and 35–45 per cent respectively. Ouabain (10^{-3} M), the specific inhibitor of Na^+-K^+ pump system, did not alter the incorporation of [^{3}H]thymidine and [^{3}H]uridine, but decreased the incorporation of [^{3}H]-L-leucine in these cells. Treatment of Ehrlich ascites tumor cells with the polyanion dextran sulfate did not change the inhibitory effect of quercetin, theophylline and dibutyrlyl cyclic AMP on [^{3}H]thymidine incorporation. On the other hand, this polyanion decreased the inhibitory effect of these drugs on incorporation of [^{3}H]uridine and abolished completely their effect on incorporation of [^{3}H]-L-leucine.

The role of cyclic nucleotides in the regulation of cell proliferation has been extensively investigated. It was reported that intracellular cyclic AMP levels in slowly growing non-dividing cells are significantly higher than in dividing cells [1–3]. In certain malignant cells and virus-transformed cells it was shown that the concentration of cyclic AMP is lower than in untransformed cells [2, 4]. The decreased concentrations of cyclic AMP in malignant cells may be due to reduced activity of adenylate cyclase [5], the enzyme responsible for the formation of cyclic AMP, or an increased activity of phosphodiesterase—the enzyme which catalyzes its degradation [6–8].

Recently it was shown that quercetin a potent inhibitor of cell growth *in vitro* [9], elevates cyclic AMP level in Ehrlich ascites tumor cells (EAT cells) [10], probably due to its inhibitory effect on phosphodiestrase activity [11]. On the other hand, it was suggested that in these cells bioflavonoids inhibit aerobic glycolysis by reduction of their excessive Na^+-K^+ATPase activity [12]. Evidence for this proposition was achieved by exposure of EAT cells to the polyanion dextrane sulfate which alters the permeability of the plasma membrane [13] and eliminates the inhibitory effect of quercetin on both lactic acid production and [^{14}C]valine incorporation [14].

On the basis of the above observations, the aim of the present work was to investigate whether the incorporation of [^{3}H]thymidine, [^{3}H]uridine and [^{3}H]-L-leucine in EAT cells are regulated through the alteration of cyclic AMP level or due to changes in the activity of membranous enzymatic systems such as Na^+-K^+ATPase.

MATERIALS AND METHODS

Preparations. EAT cells were maintained in ICR male mice, harvested seven days after transplantation and treated as described previously [15]. Cell suspension in incubation medium (Buffer A) contained 50 mM sodium tricine [*N*-Tris (hydroxymethyl)-methylglycine], 4 mM sodium phosphate buffer, pH = 7.4, 100 mM NaCl, 5 mM KCl and 2 mM $MgCl_2$. The cells were incubated with or without quercetin, theophylline, N^6, $O^{2'}$-dibutyryl cyclic AMP (DBc AMP), cyclic AMP, prostaglandin-E_2 (PGE_2) and ouabain at concentrations and combinations indicated in each experiment. Since dimethyl sulfoxide (DMSO) was used as solvent for quercetin and PGE_2, this solvent was added to all the other flasks in the amount of 5 μl/ml.

Dextran sulfate treated cells (DST cells) damaged in their permeability barrier were prepared as described by McCoy *et al.* [13]. The cells (10 mg protein per ml) were incubated for 20 min at 4° with 200 μg/ml dextran sulfate in Buffer A. At the end of the incubation period the dextran sulfate was removed by ten fold dilution of the cell suspension and centrifugation (10 min 800 *g*). The DST cells were found to be permeable to the vital stain erythrosine B, their lactate production was lowered and found to be unaffected by quercetin as reported [13].

Determinations. The incorporation of [^{3}H]thymidine and [^{3}H]uridine in EAT cells was measured in an incubation medium containing of Buffer A and 3 mg cell protein in a final volume of 1 ml. Unless otherwise indicated, the cells were preincubated for 10 min at 30°, after which 0.5 μCi [^{3}H]thymidine or [^{3}H]uridine and 10 μmol D-glucose were added to the incubation medium. The incubation proceeded for 30 min under the conditions described in the legends of the figures and tables. At the end of the incubation period a volume of 0.1 ml was removed, and placed on 24 mm paper filter disc (Whatman No. 3 MM) and prepared for counting by the procedure of Bollum [16]. The paper discs were placed in scintillation vials which contained 10 ml scintillation solution (5 g of PPO and 100 mg of POPOP per liter of 33 per cent v/v Triton x-100 and 67 per cent toluene). The radioactivity in the samples was determined by using Packard Tricarb model 3380 liquid scintillation counter.

The incorporation of [^{3}H]-L-leucine in EAT cells was measured by the same procedure described above except that the preparation for the counting of the paper filter discs was proceeded as described by Mans and Novelli [17]. The determination of cyclic AMP level was performed as previously described [10, 11], and the amount of this nucleotide was determined by the procedure of Gilman [18]. Protein concentration of the cells was assayed according to Lowry *et al.* [19].

The incorporation of [^{3}]thymidine, [^{3}H]uridine and [^{3}H]-L-leucine as well as the cyclic AMP level in DST cells were determined under the same conditions as described for untreated cells.

Chemicals. Quercetin, ouabain, theophylline, cyclic AMP, DBc AMP and DMSO were obtained from Sigma Chemical Co., St. Louis, U.S.A. Prostaglandin-E_2 was kindly provided by John Pike, Upjohn Co. Kalamazoo, Mich. Dextran sulfate was purchased from Pharmacia Chemical Inc., N.J. Cyclic [^{3}H] AMP (specific activity 22 Ci/mM) was obtained from New England Nuclear, Boston, Mass. [^{3}H]thymidine (specific activity 35.2 Ci/mM), [^{3}H]uridine (specific activity 22.5 Ci/mM) and [^{3}H]-L-leucine (specific activity 1.6 Ci/mM) were purchased from the Nuclear Research Center-Negev, Israel.

RESULTS

Time and concentration dependance of the quercetin and theophylline effect on [^{3}H] thymidine, [^{3}H]uridine and [^{3}H]-L-leucine incorporation in EAT cells. The inhibitory effect of various concentrations of quercetin upon the incorporation of [^{3}H]thymidine, [^{3}H] uridine and [^{3}H]-L-leucine into EAT cells is illustrated in Fig. 1. Almost complete inhibition of [^{3}H]thymidine incorporation is reached by the presence of 100 μM quercetin. When the same concentration of this flavone is added to the incubation medium, [^{3}H]uridine incorporation and [^{3}H]-L-leucine incorporation are inhibited by only 60 and 35 per cent respectively. Similar observations are demonstrated for theophylline (Fig. 2), where 10 mM theophylline inhibits [^{3}H]thymidine incorporation by 80 per cent, [^{3}H]uridine incorporation by 62 per cent and [^{3}H]-L-leucine incorporation by 45 per cent.

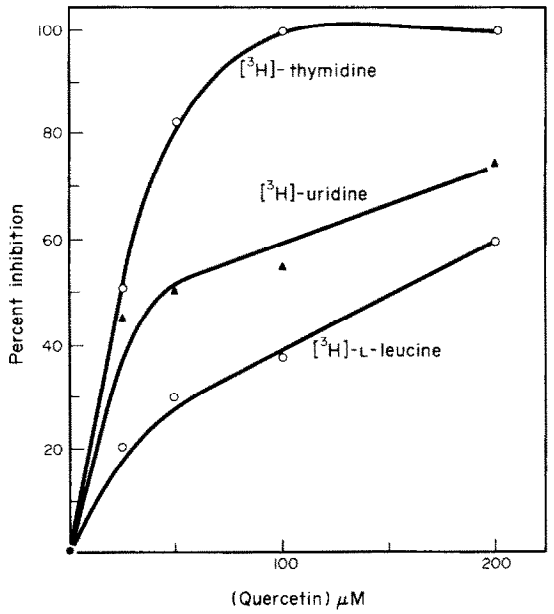

Fig. 1. The effect of increasing concentrations of quercetin on [^{3}H]thymidine, [^{3}H]uridine and [^{3}H]-L-leucine incorporation in EAT cells. The cells (3 mg protein per ml) were preincubated for 10 min at 30° in Buffer A. After the addition of the radioactive compounds with 10 μmol glucose the cells were incubated for 30 min at 30°. The incorporation of the radioactive compounds were determined as described in Materials and Methods. The data represent the mean of four experiments with S. D. of 15–23% for each point. The per cent inhibition was calculated from the results in which the flavone was absent.

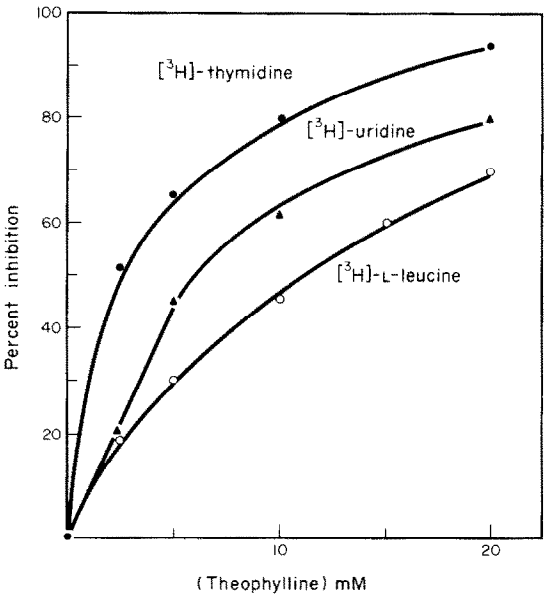

Fig. 2. The effect of increasing concentrations of theophylline on [^{3}H]thymidine [^{3}H]uridine and [^{3}H]-L-leucine incorporation in EAT cells. The incorporation of the radioactive compounds were determined as described in Fig. 1 in the presence of increasing concentrations of theophylline. The data represent the mean of four experiments with S. D. of 10–21% for each point. The per cent inhibition was calculated from the results in which the theophylline was absent.

The observations represented in these figures indicate that the inhibitory effects of quercetin and theophylline on the above mentioned macromolecular synthesis during 30 min of incubation is most efficient in the case of DNA synthesis and has the lowest influence upon [^{3}H]-L-leucine incorporation. The zero time of these experiments follows 10 min preincubation with the inhibitors. Therefore, it was interesting to estimate the length of time lasting from the addition of the inhibitor until the appearance of its effect on macromolecular synthesis. According to the experiments represented in Fig. 3 a lag period of 4–6 minutes exists until the inhibitory effect of quercetin upon the incorporation of [^{3}H]thymidine (Fig. 3a), [^{3}H]uridine (Fig. 3b) and [^{3}H]-L-leucine (Fig. 3c) into EAT cells takes place. As shown in Fig. 3d, significant increase in cyclic AMP levels in these cells starts 4 min after the addition of the flavone to the incubation medium.

Cyclic AMP levels and synthesis of macromolecules in EAT cells. The experiment described in Table 1 tends

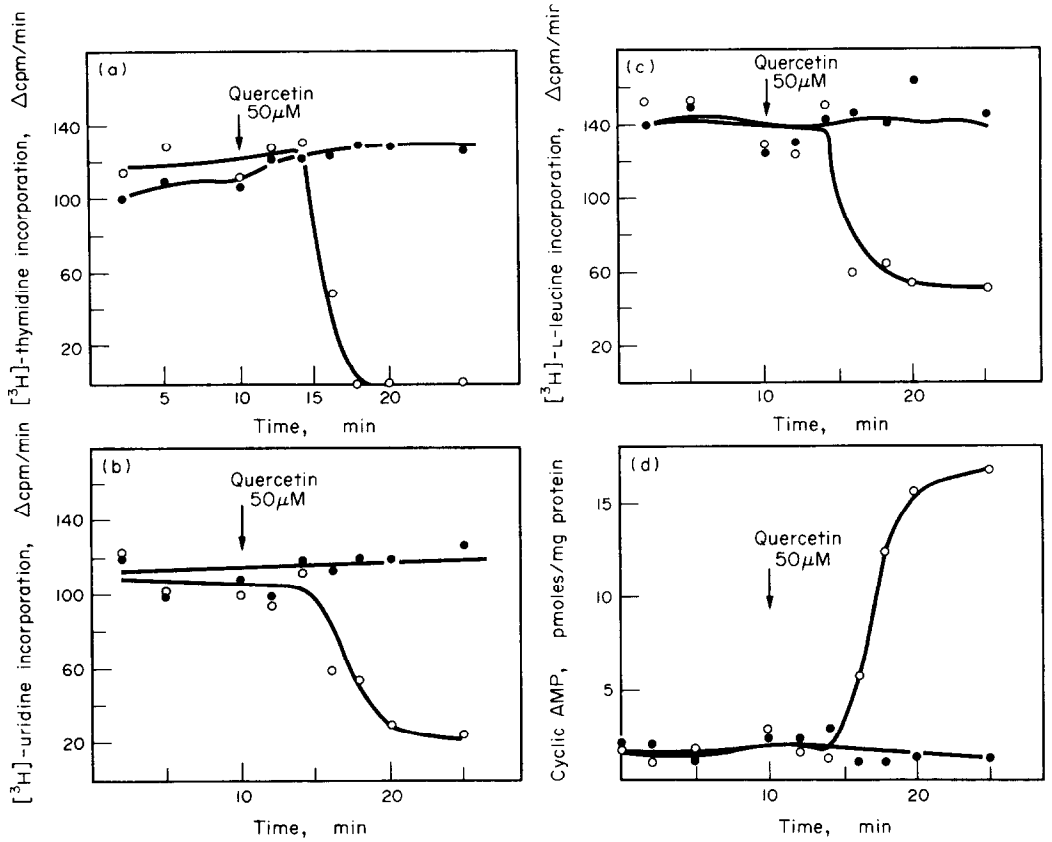

Fig. 3. Time length of EAT cells response to quercetin. Cells (3 mg protein per ml) were incubated in 1 ml Buffer A which contained 10 μmol glucose and 0.5 μCi of radioactive compound. In the experiments in which cyclic AMP was determined (d) the cells were incubated in Buffer A which contained 10 mM glucose. After 10 min incubation quercetin was added to the reaction mixture at final concentration of 50 μM. Samples for determination of [^{3}H]thymidine incorporation (a), [^{3}H]uridine incorporation (b), [^{3}H]-L-leucine incorporation (c) and cyclic AMP levels (d) were taken at the intervals presented in the figures. The data represent the rate of radioactivity change at the times indicated in the figures. The data shown in the figures are from one representative experiment out of four.

to clarify the relationship between the elevation of cyclic AMP levels and the synthesis of macromolecules. While quercetin, theophylline and PGE_2 elevate cyclic AMP levels as previously reported [11], PGE_2 unlike quercetin and theophylline does not affect RNA and protein synthesis and slightly inhibited DNA synthesis. No additional inhibitory effect is observed when combination of quercetin and theophylline, quercetin and PGE_2 and theophylline and PGE_2 are used. The critical point whether cyclic AMP is involved in regulation of macromolecules synthesis in EAT cells was examined by adding DBc AMP and cyclic AMP in the incubation medium. As represented in Table 2a 1.0 mM DBc AMP inhibits almost 70 per cent of DNA, 80 per cent of RNA and about 50 per cent of protein synthesis. Under the same conditions 1 mM cyclic AMP inhibits 25 per cent of the [^{3}H]thymidine incorporation, 45 per cent of the

Table 1. Cyclic AMP level and synthesis of macromolecules in EAT cells

	[^{3}H]-thymidine	[^{3}H]-uridine	[^{3}H]-L-leucine	Cyclic AMP
	CPM/30 min per mg protein			pmoles/mg protein
	mean ± S. D.	mean ± S. D.	mean ± S. D.	mean ± S. D.
Control	2128 ± 150	2483 ± 220	1974 ± 210	4.4 ± 1.2
+ 50 μM quercetin	46 ± 6	1310 ± 152	1192 ± 131	19.9 ± 3.1
+ 10 mM theophylline	184 ± 2	583 ± 71	1179 ± 125	30.2 ± 10.6
+ 1.5×10^{-4}M PGE_2	1614 ± 125	2241 ± 25	2175 ± 203	17.3 ± 3.9
+ quercetin + theophylline	68 ± 10	492 ± 60	1210 ± 159	39.8 ± 12.4
+ quercetin + PGE_2	103 ± 8	1361 ± 150	1084 ± 118	71.6 ± 21.9
+ theophylline + PGE_2	165 + 22	589 ± 95	1097 ± 95	100 ± 24

Cyclic AMP level and incorporation of [^{3}H]-thymidine, [^{3}H]-uridine and [^{3}H]-L-leucine in the presence of 5.10^{-5} M quercetin, 10^{-2} M theophylline and 1.5×10^{-4} M prostaglandin-E_2 were determined as described in Materials and Methods. Number of experiments: 4 Mean values ± S.D. are given.

Table 2. The effect of DBc AMP on [^{3}H]-thymidine, [^{3}H]-uridine and [^{3}H]-L-leucine incorporation in control and DST cells.

		[^{3}H]-thymidine CPM/30 min per mg protein	% from control	[^{3}H]-uridine CPM/30 min per mg protein	% from control	[^{3}H]-L-leucine CPM/30 min per mg protein	% from control
a Control cells	No additions	1847 ± 270	100	1908 ± 254	100	1274 ± 121	100
	+ 1 mM DBc AMP	531 ± 78	29	443 ± 75	23	626 ± 85	49
b DST cells	No additions	1424 ± 162	100	993 ± 143	100	430 ± 62	100
	+ 1 mM DBc AMP	284 ± 40	20	731 ± 95	74	414 ± 71	96

Incorporation of [^{3}H]-thymidine, [^{3}H]-uridine and [^{3}H]-L-leucine in the presence or absence of 1 mM DBc AMP was determined in control and DST cells (3 mg protein per ml) as described in Materials and Methods. Number of Experiments: 3. Mean values ± S.D. are given.

[^{3}H]uridine incorporation and 30 per cent of [^{3}H]-L-leucine incorporation (unpublished data). The data shown in this table are consistent with other reports claiming that DBc AMP has inhibitory effect upon synthesis of DNA [20–22] and RNA [20–22]. On the other hand, the inhibitory effect of DBc AMP on [^{3}H]-L-leucine incorporation, can be taken into consideration [23] even though it contradicts earlier reports [20].

Incorporation of [^{3}H]*thymidine,* [^{3}H]*uridine and* [^{3}H]-L-*leucine in DST and control EAT cells.* The effect of cyclic AMP on synthesis of DNA, RNA and protein was further studied by treating EAT cells with dextrane sulfate. As shown in Table 2b this treatment lowers about 20 per cent of the [^{3}H] thymidine incorporation, 60 per cent of the [^{3}H] uridine incorporation and 75 per cent [^{3}H]-L-leucine incorporation. Furthermore, DNA synthesis in DST cells is inhibited as well as in control cells by DBc AMP, while the inhibitory effect of RNA synthesis by this cyclic nucleotide is less expressed in DST cells. Complete abolishment of the DBc AMP inhibitory effect on [^{3}H]-L-leucine incorporation is found by treatment of the cells with dextrane sulfate.

In order to investigate if the inhibitory effect of quercetin on Na^+-K^+ ATPase or on phosphodiesterase is involved in the regulation of DNA, RNA and protein synthesis the effect of this bioflavonoid was compared with these of theophylline and ouabain in control and DST cells. As demonstrated in Table 3 both $5 \cdot 10^{-5}$ M quercetin and 10 mM theophylline have similar inhibitory effect on [^{3}H]thymidine incorporation in DST and untreated cells. The incorporation of [^{3}H]uridine was less affected by quercetin and theophylline in DST cells than in control cells (Table 4). Under the same conditions the above mentioned two drugs caused 35–40 per cent inhibition in the incorporation of [^{3}H]-L-leucine in control cells, while DST cells were completely unaffected (Table 5). Alternatively, ouabain—the specific inhibitor of Na^+-K^+ ATPase [24], was found to be uneffective on both [^{3}H]thymidine and [^{3}H] uridine incorporation in control and DST cells (Table 3 and 4). However, although administration of 10^{-3} M ouabain to the incubation medium was found to inhibit portein synthesis in the same manner as quercetin and theophylline in the control cells, no effect of this drug was found when DST cells were used (Table 5).

Table 3. The effect of dextran sulfate treatment on [^{3}H]-thymidine incorporation in EAT cells.

		[^{3}H]-thymidine incorporation No additions	quercetin 5×10^{-5} M	theophylline 10^{-2} M	ouabain 10^{-3} M
Control cells	CPM/30 min per mg protein	2439 ± 356	376 ± 42	463 ± 62	2364 ± 370
	Percent from control	100	15.4	19	97
DST cells	CPM/30 min per mg protein	1538 ± 215	252 ± 41	415 ± 51	1553 ± 280
	percent from control	100	16.4	27	100

[^{3}H]-Thymidine incorporation in the absence or presence of quercetin (5×10^{-5} M), theophylline (10^{-2} M) and ouabain (10^{-3} M) was determined in control and DST cells (3 mg protein per ml) as described in Materials and Methods. Number of experiments: 4. Mean values ± S. D. are given.

Table 4. The effect of dextran sulfate treatment on [^{3}H]uridine incorporation in EAT cells

		[^{3}H]uridine incorporation			
		No addition	quercetin 5×10^{-5} M	theophylline 10^{-2} M	ouabain 10^{-3} M
Control cells	CPM/30 min per mg protein	2245 ± 280	634 ± 71	622 ± 81	2574 ± 312
	Percent from control	100	28	28	114
DST cells	CPM/30 min per mg protein	1572 ± 210	976 ± 122	1108 ± 130	1976 ± 195
	Percent from control	100	62	70	107

[^{3}H]-uridine incorporation in the absence or presence of quercetin (5×10^{-5} M), theophylline (10^{-2} M) and ouabain (10^{-3} M) was determined in control and DST cells (3 mg protein per ml) as described in Materials and Methods. Number of Experiments: 4. Mean values ± S. D. are given.

Table 5. The effect of dextran sulfate treatment on [^{3}H]-L-leucine incorporation in EAT cells

		[^{3}H]-L-leucine incorporation			
		No addition	quercetin 5.10^{-5} M	theophylline 10^{-2} M	ouabain 10^{-3} M
Control cells	CPM/30 min per mg protein	1702 ± 195	1057 ± 134	1109 ± 161	1105 ± 157
	percent from	100	62	65	65
DST cells	CPM/30 min per mg protein	1125 ± 145	1093 ± 150	1135 ± 149	1123 ± 112
	percent from control	100	97	100	100

[^{3}H]-L-leucine incorporation in the absence or presence of quercetin (5×10^{-5} M), theophylline (10^{-2} M) and ouabain (10^{-3} M) was determined in control and DST cells (3 mg protein per ml) as described in Materials and Methods. Number of experiments: 4. Mean values ± S. D. are given

DISCUSSION

The involvement of ATPase and phosphodiestase in quercetin regulation of metabolic pathways in EAT cells. Recent studies suggest that the high aerobic glycolysis caused by excessive ATPase activities such as Na^+-K^+ ATPase, mitochondrial ATPase and Ca^{2+} ATPase may contribute to malignacy [9, 12, 15, 25]. In order to investigate this hypothesis, potent inhibitors of ATPase activities were used. Bioflavoids such as quercetin were found to inhibit. ATPase activity of different pump systems [26–28], aerobic glycolysis of malignant cells [9, 12] and *in vitro* growth and proliferation of several tumor cells [9]. In our studies it was demonstrated that quercetin, and to a certain extent ouabain, elevate cyclic AMP levels in EAT cells [10, 11]. These observations may indicate relationships between Na^+-K^+ ATPase activity and cyclic AMP levels as postulated in several studies [29, 30]. On the other hand, it seems more likely that the main reason for the elevation of cyclic AMP levels in EAT cells was due to the inhibition of phosphodiesterase activity by this flavone [11]. As shown in Fig. 1, quercetin inhibits macromolecular synthesis in EAT cells. Following these observations, it becomes interesting to find out whether Na^+-K^+ ATPase activity or cyclic AMP elevation caused by decreased phosphodiesterase activity are responsible for the changes in the rate of synthesis of macromolecules in EAT cells. The results shown in Fig. 2 favour the involvement of cyclic AMP in the regulation of macromolecular synthesis since theophylline, an inhibitor of phosphodiesterase, mimics the inhibitory effect of the flavone on [^{3}H]thymidine, [^{3}H]uridine and [^{3}H]-L-leucine incorporation into EAT cells. Since theophylline inhibits also the rate of glycolysis in EAT cells and as proposed by Racker *et al*, high glycolytic rate is a result of excessive ATPase activity of the Na^+-K^+ pump in these cells [9, 12, 14, 15] one can argue that his might be the reason for the inhibitory effect of theophylline upon macromolecular synthesis. However, the fact that the inhibition of lactic acid production by theophylline is very poor in comparison to that of quercetin [11], makes more likely the proposal of the responsibility of cyclic AMP increased levels for the changes in the rate of macromolecular synthesis. Moreover, the slight inhibitory effect of theophylline on the rate of glycolysis could be due to decrease of hexose transport [31].

The results shown in Fig. 3 indicate similar lag period of the effect of quercetin upon [^{3}H]thymidine, [^{3}H]uridine and [^{3}H]leucine incorporation and upon cyclic AMP levels. Such indication strengthens the hypothesis that the quercetin effect in the above mentioned systems requires the involvement of cyclic AMP.

Cyclic AMP as regulator of DNA, RNA and protein synthesis in EAT cells. Reduction in [^{3}H]thymidine, [^{3}H]uridine and [^{3}H]-L-leucine incorporation due to administration of DBc AMP in the incubation medium

are demonstrated in this study (Table 2a) As described in many other reports, the addition of cyclic AMP or its derivative DBc AMP to the incubation medium of some normal and malignant cells, growing *in vitro*, causes inhibition of cell growth and proliferation [33–38]. The inhibitory effect of these cyclic nucleotides on synthesis of DNA, RNA or proteins can be taken into consideration as one of the main contributions to this phenomenon [20–22]. Considering the fact that the quercetin changes cyclic AMP levels, it is difficult to make a clear-cut conclusion regarding the difference in the effect of quercetin on DNA, RNA and protein synthesis since DBc AMP affects similarly [^{3}H]thymidine and [^{3}H]uridine incorporation. Moreover, PGE_2, which was demonstrated to cause cyclic AMP elevation in EAT cells [11], slightly decreases DNA synthesis, while no effect of this compound is observed in the case of RNA or protein synthesis (Table 1). Unlike its effect upon cyclic AMP levels, PGE_2 does not show synergistic effect upon synthesis of macromolecules when administrated in combination with quercetin or theophylline (Table 1). Thus, the findings with PGE_2 do not fully agree with the hypothesis that increased cyclic AMP level is associated with the regulation of DNA, RNA and protein synthesis in EAT cells treated with quercetin. The involvement of cyclic AMP might be postulated only on the ground of its compartmentalization inside the cell, as emerges from various studies of cyclic AMP and the protein kinase system in human lymphocytes [32, 33].

The involvement of Na^+-K^+ *ATPase and transport properties of the plasma membrane in regulation of DNA, and protein synthesis in EAT cells.* The inhibitory effects of quercetin, theophylline or DBc AMP on the transport properties of the cell membrane may have also contribution to the incorporation of the radioactive compounds in these cells. Indeed an inhibitory effect of DBc AMP on [^{3}H]thymidine transport in Chinese hamster ovary was demonstrated [39]. Furthermore, theophylline was demonstrated to inhibit hexose transport [31] and recently quercetin was shown to be associated with the reduction of Ca^{2+} transport in mast cells [40]. On the other hand quercetin did not alter ^{86}Rb uptake [12] or hexose transport [11] in EAT cells.

The possibility that the transport of radioactive compounds are involved in the inhibitory effects of DBc AMP quercetin and theophylline on synthesis of macromolecules in EAT cells was determined by of EAT cells with dextrane sulfate the inhibitory effect of quercetin on lactate production and [^{14}C]valine treating these cells with the polyanion dextrane sulphate. It was recently demonstrated, that by treatment incorporation was completely diminished [13, 14]. Following these observations, McCoy and Racker [14] suggested, that this polyanion destroys the permeability barrier of the cells and therefore permits free ion movement of ions and other compounds in the treated cells. Table 2b shows that treatment of EAT cells with dextrane sulfate slightly lowers the incorporation of [^{3}H]thymidine, but the inhibitory effect of cyclic AMP is unchanged. These observations correspond well with the findings which demonstrate similar elevation of cyclic AMP level caused by quercetin in control and DST cells [11]. Furthermore, since quercetin and theophylline inhibit [^{3}H]thymidine incorporation in both control and DST cells (Table 3), this inhibitory effect cannot be associated with inhibition of the transport of ions or the radioactive thymidine in the cells. The integrity of the membrane is probably involved in the inhibitory effect of cyclic AMP on incorporation of [^{3}H] uridine in RNA.

The data shown in Table 4 further demonstrate the identity between the effect of quercetin and theophylline on [^{3}H]uridine incorporation. Since ouabain does not inhibit [^{3}H]uridine incorporation it seems that Na^+-K^+ ATPase is not involved in the regulation of RNA synthesis in these cells but the intact membrane which was destroyed by dextran sulfate treatment may be a regulatory target for the cyclic AMP inhibitory effect, which was directly added to the incubation medium (Table 2), or elevated by quercetin or theophylline (Table 4). On the other hand, the inhibitory effect of quercetin on incorporation of [^{3}H]-L-leucine (Table 5) which resembles the inhibitory effects of DBc AMP (Table 2), theophylline and ouabain, may postulate the involvement of the Na^+-K^+ pump system in the regulation of protein synthesis. Considering the fact that there is complete abolishment of the inhibitory effect caused by DBc, AMP, quercetin, theophylline and ouabain on [^{3}H]-L-leucine incorporation in DST cells, relationship is considered between the Na^+-K^+ ATPase system and cyclic AMP level in regulation of protein synthesis by the intact plasma membrane in EAT cells. From these experiments, it is impossible to distinguish whether this effect is because of the destruction of sites in the membrane needed for cyclic AMP regulation of protein synthesis, or due to the effect on the Na^+-K^+ ATPase system which is involved in the regulation of Pi and adenine nucleotide concentrations in these cells [14].

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