present in chicken liver. The pH optimum of this enzyme activity is 7.4 and, unlike AICAR transformylase, shows no requirement for potassium ions. The kinetic parameters of this enzyme activity have not been reported.

The putative nucleotide product of the AICAR transformylase catalyzed reaction is formyl-AICAR. There is a report of a mutant of *Salmonella typhimurium* which lacks the enzyme IMP cyclohydrolase but has measurable amounts of AICAR transformylase (Gots et al., 1969). Formyl-AICAR, however, was not isolated from these cultures but its presence was inferred indirectly by an increase in aryl amines, measured by the Bratton–Marshall test, following acid hydrolysis. Since our data demonstrates the acid-catalyzed formation of AICAR from DAR (Table II and III), the presence of a diazotizable aryl amine detected by the Bratton–Marshall test is insufficient to unambiguously demonstrate the accumulation of formyl-AICAR in this mutant.

It is possible that DAR may exist in rapid equilibrium with a very small amount of an aromatic nonpurine nucleotide (i.e., a pyrimidine or imidazole compound) which binds to the enzyme and represents the true substrate for IMP cyclohydrolase. Indeed, any number of rapid chemical equilibria preceding the binding of the true substrate by the enzyme may be postulated. With due regard for the above considerations, we hypothesize that DAR is most likely either a naturally occurring substance formed as the nucleotide product of the AICAR transformylase catalyzed reaction or merely a substrate analogue of IMP cyclohydrolase. Experiments are now underway to test these possibilities.

Acknowledgments

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Inhibition of Lactate Transport and Glycolysis in Ehrlich Ascites Tumor Cells by Bioflavonoids†

Judith A. Belt, John A. Thomas,‡ Robert N. Buchsbaum, and Efraim Racker*

ABSTRACT: Bioflavonoids are potent inhibitors of lactate transport in Ehrlich ascites tumor cells. The most effective bioflavonoids have four to five hydroxyl groups. Sugar substitution at carbon three, or reduction of the double bond between carbons two and three, decreases their inhibitory activity. Quercetin, the most extensively studied of these compounds, inhibits lactate efflux by 50% at 0.1 μg/mg of protein. On additon of quercetin to glycolyzing Ehrlich ascites tumor cells, lactate accumulates inside the cell and the intracellular pH drops. Total lactate production is also inhibited. Nigericin prevents the internal acidification that occurs in the presence of quercetin and also reduces the inhibition of glycolysis. Thus, it appears that inhibition of lactate efflux can affect glycolysis through a lowering of the intracellular pH. The inhibitory effect of quercetin on glycolysis can be explained by its effect on lactate efflux and its previously reported effect on the Na^+–K^+ ATPase [Suolinna, E.-M., et al. (1974) *J. Natl. Cancer Inst. 53*, 1515].

Cells that produce more lactic acid than they can metabolize contain a transport system for monocarboxylic acids (cf. Harold & Levin, 1974). Spencer & Lehninger (1976) have shown that lactate is rapidly excreted by Ehrlich ascites tumor cells via a lactate–proton symport mechanism. Human erythrocytes excrete lactate by the same mechanism (Dubinsky & Racker, 1978). Since the ascites tumor cells, like all other malignant tumors, produce large amounts of lactate, even under aerobic conditions, a study of effects of intracellular accumulation of lactate and protons seemed of interest. To this purpose, Thomas et al. (1979) have developed a procedure that permits continuous measurement of intracellular pH in ascites tumor cells and they have correlated pH changes with

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‡ Present address: Biochemistry Section, School of Medicine, University of South Dakota, Vermillion, SD 57069.

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lactate accumulation. In the present paper, we report on the effect of bioflavonoids on lactate–proton transport in Ehrlich ascites tumor cells and its relationship to the rate of glycolysis.

Materials and Methods

Ehrlich ascites tumor cells were maintained in male, white mice (Blu:HA(ICR) Blue Spruce Farms, Inc., Altamont, NY) as described previously (Wu & Racker, 1959). Cells were harvested 7–10 days after injection by procedures described below.

Materials. *N*-2-Hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes), 2-(*N*-morpholino)ethanesulfonic acid (Mes), *N*-tris(hydroxymethyl)methylglycine (Tricine), morpholinopropanesulfonic acid (Mops), heparin, rotenone, bovine serum albumin, and L-lactic acid were obtained from Sigma Chemical Co., St. Louis, MO. Tri-*n*-butyltin chloride was obtained from Aldrich Chemical Co., Milwaukee, WI; L-[U-^{14}C]lactate, [^{14}C]methylamine, methoxy[^{3}H]inulin, [*carboxyl*-^{14}C]inulin, $^{3}H_2O$, and [U-^{14}C]sucrose were from New England Nuclear, Boston, MA; Versilube F50 and SF96(50) were from General Electric Co., Silicone Products Dept., Waterford, NY, and aqueous counting scintillant (ACS) was from Amersham Corp., Arlington Heights, IL. All other chemicals were of reagent grade.

Lactate Efflux. Low-Temperature Loading. Cells were harvested in an equal volume of 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 10 mM Na–Hepes (pH. 7.4), and 10 U/mL heparin. Glucose (10 mM) and rotenone (5 μg/mL) were added and the cells were stored overnight at 0 °C.

Low-pH Loading. Cells were harvested in 10 volumes of 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 10 mM Na–Hepes (pH 7.4). After centrifugation (500*g* for 5 min), the cells were suspended at 10–20 mg of protein/mL in 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$ 10 mM Na–Mes (pH 6.0), 10 mM glucose, and 5 μg/mL rotenone and incubated at 20 °C for 30 min. The cells were then cooled to 0–4 °C and washed as described under Efflux Measurements.

Efflux Measurements. Lactate-loaded cells were washed twice with cold pH 6 buffer (without glucose or rotenone) and suspended at 25 mg of protein/mL in the same buffer. Efflux was initiated by diluting a sample of cells (0.1–0.2 mL) into 2.5 mL of 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 10 mM Na–Hepes (pH 7.4) at the temperature indicated. The reaction was stopped by addition of 2.5 mL of ice cold 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 10 mM Mes (pH 5). After centrifugation for 10 min at 3000*g*, the cell pellet was resuspended in 1 mL of H_2O and deproteinized with 0.4 mL of 2 M perchloric acid. The acid extract (0.8 mL) was neutralized with 0.2 mL of 2 M KOH, 0.4 M KCl, 0.4 M imidazole, and a sample assayed for lactate.

L-[^{14}*C*]*Lactate Uptake.* Cells were harvested in 110 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 50 mM Na–Tricine (pH 8.2), washed twice, and resuspended at 8 mg of protein/mL in the same buffer. Lactate uptake was initiated by adding 0.1-mL cells to 0.3 mL of a solution containing 110 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 50 mM Na–Mes (pH 6.2), and the indicated concentration of L-[^{14}C]lactate. The final pH of the reaction was 6.6. The reaction was stopped by addition of 0.1 mL of cold buffer (pH 6.2) containing 5 mM $HgCl_2$, and the tubes were placed on ice. A sample of the mixture (0.4 mL) was centrifuged for 45 s in a Coleman microfuge. The walls of the tube and top of the pellet were washed three times with buffer containing $HgCl_2$, and the pellet was suspended in 0.1 mL of H_2O and counted in 5 mL of ACS. Zero-time values were determined in the presence of $HgCl_2$ and subtracted from all values.

External pH Measurements. External pH changes during lactate uptake were measured as described previously (Spencer & Lehninger, 1976).

Internal pH Measurements. Cells washed with 105 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 4 mM NaP_i, and 50 mM Na–Mops (pH 7.4) were loaded with 6-carboxyfluorescein diacetate as described by Thomas et al. (1979). Absorbance changes were followed at 490 nm (464-nm reference) in an Aminco DW-2 spectrophotometer with a thermostated cell compartment.

In some experiments, the internal pH was determined from the distribution of [^{14}C]methylamine (Rottenberg et al., 1972) using [^{3}H]methoxyinulin to correct for the extracellular water space. The internal volume was determined with $^{3}H_3O$ using [^{14}C]inulin to correct for extracellular water. After incubation as indicated, a 0.2-mL sample of cells was centrifuged through 0.1 mL of silicone oil (Versilube F50: SF96(50), 5.2:1) into 0.1 mL of 5% trichloroacetic acid in 8% glycerol. A sample (0.05 mL) of this fraction and the supernatant (0.1 mL) were counted in 10 mL of ACS.

Glycolysis. Cells harvested in 105 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$ 4 mM NaP_i, and 50 mM Na–Hepes (pH 7.3) were depleted of intracellular lactate by incubation for 10 min at 30 °C. The cells were then washed twice and resuspended in the same buffer. After a 15-min incubation with the inhibitors at 30 °C, glycolysis was initiated by addition of 10 mM glucose. The reaction was stopped after 30 min by addition of an equal volume of ice cold buffer. Samples were centrifuged (3000*g* for 10 min), and the pellet was deproteinized as described under Efflux Measurements. Samples of the neutralized pellet extract and the supernatant were assayed for lactate. Total lactate production was calculated as the sum of lactate in the pellet and supernatant. [^{14}C]-Sucrose (0.25 μCi/mL) was used to correct for carry-over of extracellular lactate into the pellet.

In experiments where only total lactate production was determined, the reaction was stopped by the addition of perchloric acid and the neutralized extract was assayed for lactate.

Other Methods. Protein was determined by the method of Lowry et al. (1951) with bovine serum albumin as a standard. Lactic acid was determined as described by Hohorst (1962). Dextran sulfate treated cells were prepared as described by McCoy et al. (1976).

Results and Discussion

Efflux of Lactate from Ehrlich Ascites Tumor Cells. In order to study the effect of bioflavonoids on efflux of lactate from ascites tumor cells, a method was developed that allowed us to determine net efflux of lactate. When cells were incubated with glucose and rotenone at pH 7.4 and 20 °C, the internal lactate level remained constant at 20–30 nmol/mg of protein. However, if the cells were incubated at either 0 °C or an acid pH, lactate was accumulated inside the cells (Table I). The latter procedure was effective because transport takes place by the lactate–proton symport mechanism (Spencer & Lehninger, 1976), and efflux was therefore inhibited by external acid pH. On shifting from an acid to an alkaline pH, lactate efflux occurred. The procedure of metabolizing glucose at 0 °C was effective because the transport mechanism was much more dependent on temperature than was glycolysis, as was previously shown in the case of human erythrocytes (Dubinsky & Racker, 1978). The lactate-loaded cells (100 nmol/mg of protein) had an internal volume of 7 μL/mg of protein giving an estimated internal lactate concentration of 14 mM. The internal pH under these conditions,

Table I: Lactate Loading of Ascites Tumor Cells by Glycolysis at Low Temperature and at Low pH[a]

low-temp loading		low-pH loading	
time (h)	nmol of lactate/mg of protein	time (min)	nmol of lactate/mg of protein
0	24	0	37
2	33	5	91
5	44	15	100
24	106	30	106

[a] Low-temperature loading: Cells were incubated with 10 mM glucose at 0 °C as described under Materials and Methods. At the times indicated, an aliquot of cells was washed with 10 volumes of cold buffer and lactate was determined in the pellet. Low-pH loading: Cells were incubated with 10 mM glucose at pH 6.0 and 20 °C as described under Materials and Methods. At the times indicated, an aliquot of cells was washed and assayed for lactate as described above.

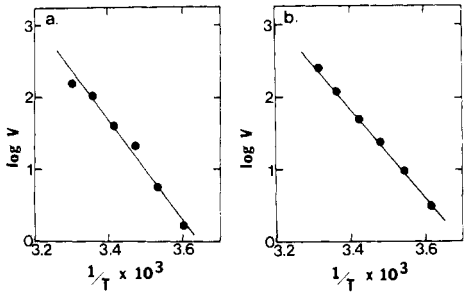


FIGURE 1: Temperature dependence of lactate efflux and uptake. (a) Arrhenius plot of lactate efflux. Cells were loaded with lactate at low pH as described under Materials and Methods. Efflux was assayed at pH 7.4 and at the temperatures indicated except that 10^{-4} M iodoacetate was added to the cells and the assay buffer to inhibit glycolysis. The initial internal lactate level was 100 nmol/mg of protein. (b) Arrhenius plot of lactate uptake. Cells were harvested and washed in 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 10 mM Na–Hepes (pH 7.4) and assayed for H^+ uptake in 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 10 mM Na–Mes(pH 6.0), and 30 mM sodium lactate (pH 6.0). The rate of lactate uptake was determined from the initial rate of H^+ uptake by using a lactate/H^+ ratio of 1 as described by Spencer & Lehninger (1976).

as determined with [^{14}C]methylamine, was about 6.3.

The effect of temperature on lactate efflux is shown in Figure 1a. In this experiment, iodoacetate (10^{-4} M) was added to prevent lactate formation from residual glucose. This concentration of iodoacetate had no effect on lactate transport. The activation energies calculated from Ahrrenius plots (Figures 1a and 1b) for both efflux and uptake are about 30 kcal/mol and are in agreement with the value of 33 kcal/mol for uptake reported by Spencer & Lehninger (1976). The effect of external pH on lactate efflux is shown in Figure 2. As extracellular pH increased, the rate of lactate efflux increased, whereas the rate of lactate influx has been shown to decrease with increasing pH (Spencer & Lehninger, 1976). These data are consistent with a lactate–proton symport mechanism.

Effect of Bioflavonoids on Lactate Transport. A comparison of the effect of various bioflavonoids revealed that these compounds vary widely in their effectiveness as inhibitors of lactate uptake (Table II). From these and other experiments, it appears that (a) compounds with four or five OH groups are most effective, (b) sugar substitutions at carbon 3 diminish the effectiveness, (c) a methoxy group at carbon 4′ decreases inhibition, but not at carbon 3 or 6, and (d) reduction of the double bond between carbons 2 and 3 lowers activity. Although some of these features resemble those observed in studies of inhibition of Na^+-K^+ ATPase activity and glycolysis

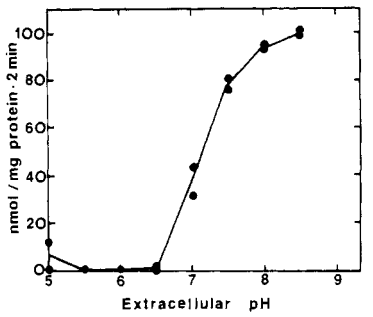
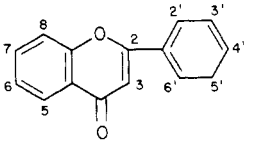


FIGURE 2: Effect of pH on lactate efflux. Cells were loaded with lactate at low temperatures as described under Materials and Methods. Efflux assays were for 2 min at 15 °C at the indicated pH in Na–Mes buffers at pH 5.0 to 7.0 and Na–Tricine buffers at pH 7.5–8.5. The initial internal lactate level was 126 nmol/mg of protein.

Table II: Effect of Bioflavonoids on Lactate Uptake[a]



flavone

common name	hydroxyl pattern and substituents	lactate uptake (nmol/(mg of protein min))	% inhibn
none		25.8	
b	3,3′,4′-OH	27.6	(+7)
apigenin[c]	5,7,4′-OH	17.8	30
fisetin[c]	3,7,3′,4′-OH	12.6	52
morin[d]	3,5,7,2′,4′-OH	11.4	56
quercetin[e]	3,5,7,3′,4′-OH	8.5	67
kaempferid[f]	3,5,7-OH, 4′-OCH_3	31.3	(+21)
K_3[f]	5,7,4′-OH, 3,6-OCH_3	13.4	48
f	5,7,3′-OH, 3,6,4′-OCH_3	21.6	16
quercetrin[b]	5,7,3′,4′-OH, 3-rhamnose	25.0	3
rutin[d]	5,7,3′,4′-OH, 3-rhamnose-glucose	30.8	(+19)
dihydro-quercetin[g]	2,3-dihydro, 3,5,7,3′,4′-OH	27.2	(+6)

[a] [^{14}C]Lactate uptake was determined as described under Materials and Methods. The protein concentration in the assay was 2 mg/mL, lactate was 10 mM, and bioflavonoids were 6 μM (equivalent to 0.9 μg of quercetin/mg of protein). A zero-time value of 9.1 nmol/mg of protein has been substracted from all values. [b] Supplied by Dr. T. A. Geissman. [c] Supplied by Aldrich Chemical Co., Milwaukee, WI. [d] Supplied by J. T. Baker Chemical Co., Phillipsburg, NJ. [e] Supplied by Sigma Chemical Co., St. Louis, MO. [f] Supplied by Dr. E. Wollenweber. [g] Supplied by Dr. J. Chopin.

(Suolinna et al., 1975), there are distinct differences both quantitatively and qualitatively. In general, lactate transport was inhibited by bioflavonoids at considerably lower concentrations than required for the inhibition of glycolysis and the Na^+-K^+ ATPase (Suolinna et al., 1975). Qualitatively, morin was less effective than quercetin as an inhibitor of glycolysis (Suolinna et al., 1975), whereas they were almost equally effective as inhibitors of lactate uptake (Table II and Figure 5). However, we cannot correlate these findings directly since, as will be shown later, uptake and efflux of lactate exhibit different susceptibility to bioflavonoids. Of particular interest is the methoxy derivative K_3 which inhibits glycolysis and lactate transport (Table II) but not the Na^+-K^+ ATPase (Racker, 1976).

Quercetin appears to be a better inhibitor of lactate efflux than of lactate uptake. As shown in Figure 3, between 0.4 and 0.9 μg quercetin per mg of protein was required for a 50%

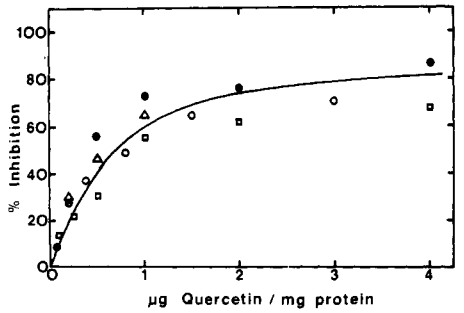


FIGURE 3: Quercetin inhibition of lactate uptake Cells were washed in pH 8.2 (●, ○, □) or pH 7.4 (△) buffer and assayed for lactate uptake in pH 6.2 buffer with 10 mM [^{14}C]lactate. Assays were for 1 min at 15 °C. The final protein concentration was 2 mg/mL (●, ○, □) and the quercetin concentration varied as indicated or the quercetin concentration was held at 1 μg/mL (△) and the protein concentration varied from 1 to 5 mg/mL. Control rates were 36 (●), 45 (○), 30 (□), and 35 nmol/(mg of protein min) (△).

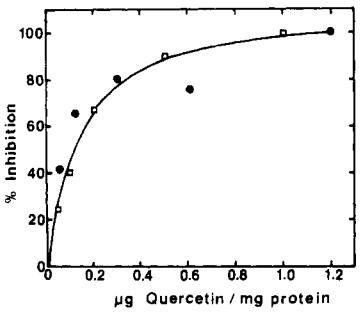


FIGURE 4: Quercetin inhibition of lactate efflux. Cells were loaded with lactate at a low pH as described under Materials and Methods and assayed for lactate efflux at pH 7.4. (●) Assays at 0.8 mg of protein/mL and 20 °C; the control rate was 37 nmol/(mg of protein min) and the initial lactate concentration was 115 nmol/mg of protein. (□) Assays at 2 mg of protein/mL and 15 °C; the control rate was 12.5 nmol/(mg of protein min) and the initial lactate concentration 62 nmol/mg of protein.

inhibition of uptake at pH 6.2 at 10 mM external lactate. Figure 4 shows that lactate efflux was inhibited 50% at about 0.1 μg of quercetin/mg of protein. Although we have not ruled out some differences in the intracellular and extracellular medium which may be responsible for these observations, the more interesting explanation is that we are dealing with an asymmetric transporter which interacts with quercetin on the outside and is partially protected by the presence of lactate in the medium. In line with this interpretation was the finding that there was competition between lactate and three bioflavonoids that were tested on lactate upake (Figure 5). It would be desirable to conduct similar experiments on lactate efflux but this is complicated by the difficulty of simultaneously controlling the two critical variables of intracellular pH and lactate concentration.

Effect of Bioflavonoids on Internal and Total Lactate Produced during Glycolysis. In view of the pronounced effect of bioflavonoids on lactate efflux, it became necessary to reexamine our previous data on the effect of bioflavonoids on glycolysis (Suolinna et al., 1974, 1975). We have always used samples of extracellular fluid for the determination of lactate production since under most circumstances the amount of lactate trapped in the cells was negligible. However, this was not the case in the presence of bioflavonoids as shown in Figure 6a. Quercetin produced a significant increase of the internal lactate concentration which was paralleled by a decrease of the intracellular pH (Figure 6b). Total lactate production was inhibited, but measurement of the external lactate resulted in an overestimation of inhibition.

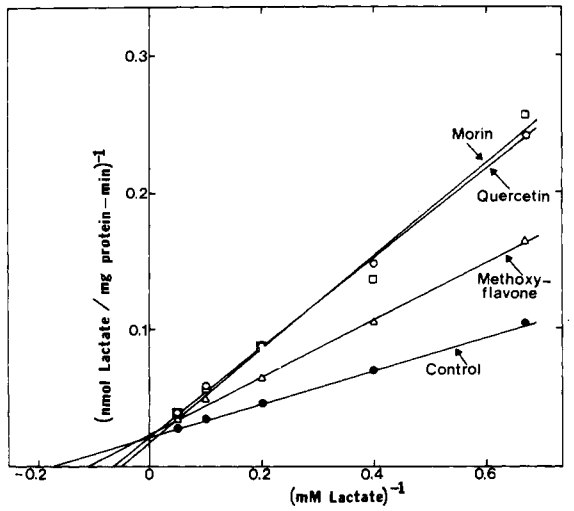


FIGURE 5: Inhibition of lactate uptake by quercetin, morin, and methoxyflavone. Cells were washed with pH 8.2 buffer and assayed for [^{14}C]lactate uptake as described under Materials and Methods. Assays were for 1 min at 15 °C and the final protein concentration was 2 mg/mL. The bioflavonoid concentration was 0.5 μg/mg of protein. Control (●); quercetin (○); morin (□); and 5,7,4′-hydroxy-3,6-methoxyflavone (K_3) (△).

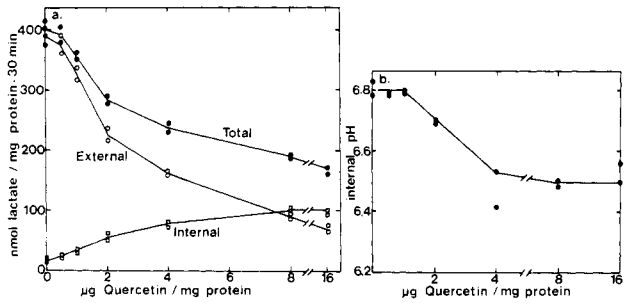


FIGURE 6: Effect of quercetin on glycolysis and internal pH. (a) Internal, external, and total lactate production was determined as described under Materials and Methods. Assays were for 30 min at 30 °C and the final protein concentration was 2 mg/mL. (b) Internal pH was determined under the same conditions with [^{14}C]methylamine.

Figure 6a shows that the internal lactate levels began to rise at about 1 μg of quercetin/mg of protein. This concentration gave better than 95% inhibition of lactate efflux in the direct measurements of efflux (Figure 4). This difference in the amount of quercetin required in the two assays is to be expected because the capacity of the transporter greatly exceeds the rate of lactate production, as was shown by Spencer & Lehninger (1976). In such cases, virtually complete inhibition of the transporter is necessary before an increase of the internal lactate levels can be observed. The experiments in Figure 6 show a good correlation between the inhibition of lactate efflux (increase of internal lactate), the decrease of intracellular pH, and the inhibition of total lactate production.

In the experiments shown in Figure 6b, the intracellular pH was determined from the distribution of [^{14}C]methylamine across the plasma membrane. The effect of quercetin on the intracellular pH was also analyzed kinetically by spectroscopic studies (Figure 7) with 6-carboxyfluorescein diacetate as the pH probe (Thomas et al., 1979). Both measurements showed a marked intracellular acidification caused by quercetin. According to the methylamine method, which is particularly useful for comparative studies of pH changes over the longer periods of glycolysis that are described in this paper, the intracellular pH of glycolyzing cells was lower than the medium (by about 0.6 unit). However, this pH differenc could be due to some internal compartmentalization of the me-

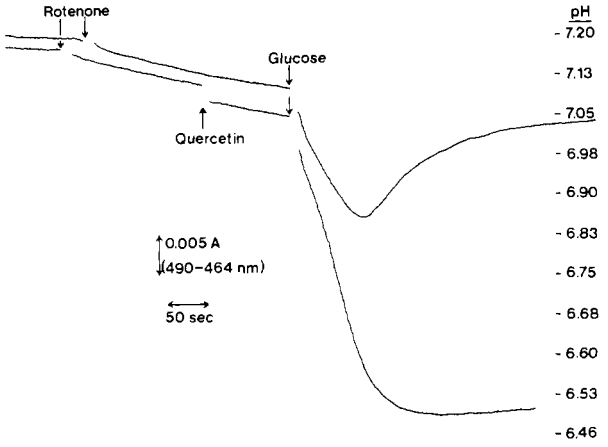


FIGURE 7: Effect of quercetin on internal pH during glycolysis. Internal pH changes were determined spectrophotometrically at 20 °C with 6-carboxyfluorescein as described under Materials and Methods. The protein concentration was 2.5 mg/mL, rotenone 15 μM, quercetin 4 μg/mg of protein, and glucose 10 mM.

Table III: Effect of Quercetin and K_3 on Internal pH during Glycolysis with and without Nigericin[a]

	internal pH	
	−nigericin	+nigericin
control	6.74	6.94
+quercetin	6.46	6.94
+K_3	6.55	7.03

[a] Internal pH was determined by using [^{14}C]methylamine as described under Materials and Methods. Cells (2.7 mg/mL) were preincubated with inhibitors and the appropriate isotope pair ([^{14}C]methylamine/[^{3}H]inulin or $^{3}H_2O$/[^{14}C]inulin) in pH 7.3 buffer for 15 min at 30 °C. Glycolysis was initiated by addition of 10 mM glucose, and incubations were stopped after 30 min by centrifugation of a 0.2-mL sample through silicone oil. The quercetin and K_3 concentrations were 12 μg/mg of protein and nigericin was 5 μg/mL. All assays were done in the presence of 2 μM antimycin A and 8 μg/mL rutamycin.

thylamine. The spectroscopic measurements, which are well suited for rapid initial kinetic measurements, showed a transient acidification followed by a rise of pH until the original value was almost reached. This biphasic kinetic pH response was confirmed by analyses of intracellular lactate levels (Thomas et al., 1979). High-resolution ^{31}P nuclear magnetic resonance studies of Ehrlich ascites tumor cells (Navon et al., 1977) indicate less than a 0.2 pH unit difference between intracellular and extracellular pH during glycolysis.

Does the Lowering of the Intracellular pH Contribute to the Inhibition of Glycolysis by Bioflavonoids? It has previously been shown that glycolysis of Ehrlich ascites tumor cells is inhibited by decreasing the extracellular pH from 7.4 to 6.4 (Wilhelm et al., 1971). Similar pH effects have also been observed in cell free extracts of leukocytes (Halperin et al., 1969). More recently, Ugurbil et al. (1978) have shown that lowering the intracellular pH inhibits glycolysis in *E. coli*. We have approached the question of the role of intracellular pH on glycolysis in two ways. We first examined the effect of nigericin which acts as a H^+ for K^+ or Na^+ exchanger (cf. Douglas & Cockrell, 1974). As shown in Table III, the internal acidification which occurred in the presence of bioflavonoids was eliminated by nigericin. As can be seen from Table IV, experiment 1, nigericin reduced, but did not eliminate, the inhibition of glycolysis by quercetin or K_3. These experiments were complicated by the fact that mitochondrial inhibitors had to be added in order to avoid an effect of nigericin on the mitochondria. Invariably, in the presence of

Table IV: Effect of Nigericin on Quercetin, K_3, and Ouabain Inhibition of Glycolysis[a]

	−nigericin		+nigericin	
	nmol of lactate/(mg of protein × 30 min)	% inhibn	nmol of lactate/(mg of protein × 30 min)	% inhibn
Expt 1 (with Antimycin A and Rutamycin)				
(a) control	619		542	
+quercetin	360	42	456	16
+K_3	432	30	478	12
(b) control	689		649	
+ouabain	672	2	568	12
Expt 2 (without Antimycin A and Rutamycin)				
control	273			
+quercetin	150	45		
+K_3	217	21		
+ouabain	151	45		

[a] Total lactate production was determined as described under Materials and Methods. The protein concentration was 2.6 mg/mL, quercetin and K_3 were 12 μg/mg of protein, ouabain was 1 mM, and nigericin was 5 μg/mL. Antimycin A (2 μM) and rutamycin (8 μg/mL) were added where indicated.

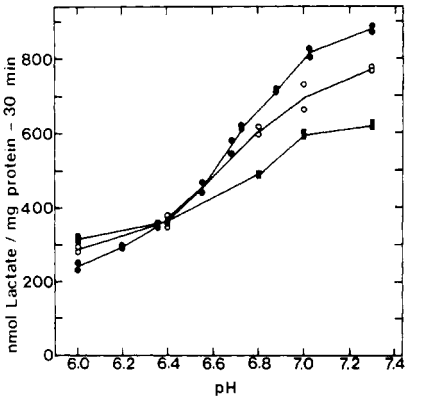


FIGURE 8: Effect of intracellular pH on total lactate production. Total lactate production was determined as described under Materials and Methods except that 5 μM tributyltin was added to equilibrate internal and external pH. Na–Mes buffers were used at pH 6.0–6.8 and Na–Hepes buffers were used at pH 6.8–7.4. The three curves represent three different experiments.

rutamycin and antimycin A, the contribution of the Na^+K^+ pump to the ADP and P_i pool required by glycolysis became less visible as documented by a loss of sensitivity of glycolysis to ouabain (Table IV, experiment 2). This is not unexpected since ADP and P_i are used by mitochondria as well as by the glycolytic enzymes. Thus, the regeneration of these cofactors is more rate limiting in the absence than in the presence of mitochondrial inhibitors. A second approach to the problem of the role of intracellular pH was taken with tributyltin chloride which acts as an OH^- for Cl^- exchanger (Selwyn et al., 1970). Experiments with methylamine and 6-carboxyfluorescein diacetate indicated that the internal and external pH are rapidly equilibrated in the presence of this ionophore (data not shown). As shown in Figure 8, a lowering of the intracellular pH did have an effect on the rate of glycolysis, a conclusion born out by similar experiments with dextran sulfate treated cells in which pH equilibration with the medium pH is readily achieved (data not shown).

It is clear from these experiments that the effect of bioflavonoids on glycolysis are more complex than originally visualized. We previously proposed that bioflavonoids inhibit glycolysis through inhibition of the plasma membrane Na^+-K^+ ATPase (Suolinna et al., 1974, 1975). The data presented here

indicate that bioflavonoids also have a major effect on lactate excretion and that the resulting internal acidification can inhibit glycolysis. Thus, it appears that bioflavonoids inhibit glycolysis through their action on both the lactate transporter and the Na^+-K^+ ATPase. This explains our previous observation (Racker, 1976) that a bioflavonoid (K_3) inhibits glycolysis in intact cells, although it has little or no effect on the Na^+-K^+ ATPase activity. Since K_3 did not inhibit glycolysis in dextran sulfate treated cells (Racker, 1976), its action could not be explained by inactivation of hexokinase (Graziani, 1977). On the other hand, one would not expect an inhibitor of lactate transport to inhibit glycolysis in extracts or in dextran sulfate treated cells. Bioflavonoids have been shown to inhibit hexose transport in some cells (Kimmich & Randles, 1978; Salter et al., 1978); however, quercetin does not inhibit hexose uptake by Ehrlich ascites tumor cells (E.-M. Suolinna, G. D. McCoy, and E. Racker, unpublished observations).

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