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Effects of Hyperthermia and L-Ascorbic Acid on Glucose, Pyruvate, and Lactate Metabolism in Ehrlich Ascites Carcinoma Cells (40604)[1]

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After several years of intense interest in hyperthermia as a potential treatment for cancer, little is known about the mechanisms of heat-dependent tumor cell destruction. Environmental or extracellular factors, particularly nutritional state and degree of oxygenation, have been shown to influence the susceptibility of tumor cells to destruction by hyperthermic treatment (1–3). Overgaard has postulated that hyperthermia inhibits respiration, but not glycolysis, resulting in increased lactate levels and a decreased pH (4, 5). An increase in cellular acidity has been shown to enhance hyperthermic lethality (6). This communication examines the effect of hyperthermia on glucose, pyruvate, and lactate metabolism in Ehrlich ascites tumor cells (EATC). The role of ascorbic acid, which has been shown to greatly increase respiration in EATC suspensions (7) was also examined.

Materials and methods. EATC were serially transplanted intraperitoneally at regular intervals in male Swiss mice.[2] Cells were harvested 6–14 days after transplantation. The protein content was determined by a biuret method using bovine serum albumin as the standard (8). Experimental hyperthermia and the effect of supplemental ascorbic acid were examined simultaneously.

Metabolic studies were performed in 25-ml stoppered siliconized glass Erlenmeyer flasks with specially constructed center wells for $^{14}CO_2$ collection. All incubations were performed in Krebs Ringers phosphate buffer without calcium (18). The $^{14}CO_2$ collection technique has been described (7, 9). Labeled [*U*-^{14}C]glucose (NEC-042) and [1-^{14}C]pyruvate (NEC-255) were obtained from New England Nuclear (Boston, Mass.). The concentration of ascorbic acid in the flasks was either 0 or 1 m*M*. A proportional temperature controller (Yellow Springs Instruments Model 72) was used to precisely control shaker bath water temperature (±0.1°).

Following the incubation period, the reactions were stopped by injecting 1 ml of 2 *N* $HClO_4$ through the serum caps. The flasks were then shaken an additional hour to recover $^{14}CO_2$. Blanks were prepared by adding PCA before addition of the cell suspension. After shaking with PCA, the paper and vial were removed and placed in a scintillation vial containing 15 ml of cocktail (7) before counting in a Packard Tri-Carb scintillation spectrometer. Quench correction was done using the channels ratio method.

All *in vitro* tumor cell experiments were in the presence of glucose as the substrate. Colorimetric analysis of lactic acid was performed enzymatically with a Gilford 240 digital spectrometer (10).

EATC metabolism of [1-^{14}C]pyruvate with supplemental combinations of ascorbic acid (1 m*M*), theophylline (1 m*M*), and dibutyryl cyclic AMP (1 m*M*) was studied *in vitro* at 37.5° and 42.5°. These experiments were conducted to study whether ascorbic acid may alter metabolism by potentiating intracellular cAMP levels.

In a separate preliminary study the effect of dietary supplementation of 5% ascorbate on tumor cell metabolism was examined.

Results. The effects of hyperthermia and of vitamin C on EATC [*U*-^{14}C]glucose metabolism are given on Table I. At 37.5° and 42.5° ascorbic acid significantly increased the metabolism of [*U*-^{14}C]glucose. There was no statistically significant effect of hyperthermia in the presence or absence of vitamin C. The effects of hyperthermia and of ascorbic acid

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[2] ICR strain, Harlan Industries, Indianapolis, Ind.

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TABLE I. EFFECTS OF HYPERTHERMIA AND ASCORBIC ACID ON THE *in Vitro* METABOLISM OF [*U*-^{14}C]GLUCOSE AND LACTATE PRODUCTION IN EATC

Treatment	Glucose metabolism[a] (dpm/mg protein)	Lactate[b] (nmol/mg protein)
37.5°	155 ± 12[1]	1181 ± 27[1]
37.5° + ascorbate	274 ± 14[2]	410 ± 62[2]
42.5°	144 ± 5[1]	1265 ± 22[1]
42.5° + ascorbate	353 ± 37[2]	482 ± 6[2]

[a] EATC incubated 1 hr with 0.25 μCi [U-^{14}C]glucose and 20 μmol cold glucose. Means ± SE with an unlike superscript differ ($P < 0.05$, LSD test) significantly from the control mean (37.5°). Seven replicates per datum. Vitamin C (+C) concentration was 1 m*M*.

[b] EATC incubated 1 hr with 20 μmol glucose. Mean ± SE with an unlike superscript differ ($P < 0.05$, LSD test) significantly from the control mean (37.5°). Five replicates per datum at 37.5° + ascorbate, all other six replicates per datum. Ascorbate was added at a final concentration of 1 m*M*.

on EATC lactate content are listed in Table I. Vitamin C reduced lactate levels at 37.5° and at 42.5°. Again, there was no statistically significant effect of hyperthermia in the presence or absence of ascorbic acid.

The effects of hyperthermia and ascorbic, theophylline, and dibutyryl cyclic AMP upon EATC pyruvate metabolism are listed in Table II. Ascorbic acid (1 m*M*) significantly increased [1-^{14}C]pyruvate metabolism at 37.5° and 42.5°. Neither cyclic AMP nor theophylline significantly altered metabolism at 37.5° or 42.5°. Theophylline plus ascorbic acid did not change metabolism at 37.5° or 42.5°. However, dibutyryl cyclic AMP plus ascorbic acid significantly increased EATC pyruvate metabolism at 37.5° and 42.5°.

Supplementation of a purified diet (11) fed to tumor-bearing mice with 5% ascorbic acid resulted in a depression in lactate content of the isolated tumor cells. Lactate content of tumor cells isolated from control mice was 352 ± 27 nmol/mg protein and 213 ± 49 nmol/mg protein in cells isolated from ascorbic acid-supplemented mice.

Discussion. Hyperthermia for a short duration does not significantly change EATC metabolism of [*U*-^{14}C]glucose. Similarly, lactate levels are not significantly altered by 1-hr hyperthermia. These data indicate that short-term hyperthermic exposure has little effect upon EATC metabolism and lactate content in agreement with other biochemical studies (11, 12). We have incubated EATC for 1, 2, and 3 hr at 37.5° and 42.5° and observed no significant change due to heating in the metabolism of [*U*-^{14}C]glucose (7). Lactic acid does not significantly change during 1 hr hyperthermic treatment (Table II). Our results obtained *in vitro* on EATC do not support Overgaard's hypothetical model of hyperthermic tumor cell destruction. However, Overgaard's hypothesis comes from studies involving solid tumor cells heated *in vivo*. More work on the effects of heating on metabolism of normal and tumor cells, both *in vitro* and *in vivo*, is needed to determine the validity of this important hypothesis.

Ascorbic acid stimulates EATC metabolism markedly and reduces the lactate content of EATC (Table I). Preliminary evidence obtained in mice fed a high ascorbic acid diet (5%) indicated that similar qualitative effects of ascorbic acid occur *in vivo* and *in vitro*. It is probable that lactate is converted to pyruvate in response to the stimulation of EATC aerobic metabolism by ascorbate. Ascorbic acid may have effected EATC by acting as a reducing agent in the electron transport chain, or by inhibiting the enzyme, phosphodiesterase, thereby potentiating cAMP concentrations (13–15). However, lactate content is unchanged with the addition of 1 m*M*

TABLE II. EFFECTS OF HYPERTHERMIA, ASCORBIC ACID, THEOPHYLLINE, AND DEBUTYRUL CYCLIC AMP ON THE *in Vitro* METABOLISM OF [1-^{14}C]PYRUVATE IN EATC[a]

Treatment	Pyruvate metabolism (dpm/mg protein)
37.5°	580 ± 18[1]
37.5° + theophylline	550 ± 58[1]
37.5° + dbcAMP	598 ± 33[1]
37.5° + ascorbate	997 ± 57[2]
37.5° + ascorbate + theophylline	621 ± 28[1]
37.5° + ascorbate + dbcAMP	904 ± 30[2]
42.5°	531 ± 44[1]
42.5° + theophylline	728 ± 70[1]
42.5° + dbcAMP	632 ± 80[1]
42.5° + ascorbate	855 ± 70[2]
42.5° + ascorbate + theophylline	627 ± 86[1]
42.5° + ascorbate + dbcAMP	1013 ± 138[2]

[a] EATC incubated 1 hr with 0.02 μCi [1-^{14}C]pyruvate and 20 μmol cold pyruvate. Means ± SE with an unlike superscript differ ($P < 0.05$, LSD test) significantly from the control mean (37.5°). Three replicates per datum. Ascorbate, theophylline, and dbcAMP were added at a final concentration of 1 m*M*.

dbcAMP (1-hr incubation, unpublished observations).

Ascorbic acid (1 m*M*) significantly increases EATC pyruvate metabolism at 37.5° and 42.5° (Table II). The physiological effects of cyclic AMP and of theophylline have been studied extensively and have been shown to effect numerous cellular processes including permeability, gluconeogenesis, and ketogenesis (16–18). If ascorbic acid alters metabolism primarily by inhibiting phosphodiesterase activity, one would expect to observe a similar change in metabolism with theophylline or dbcAMP alone. This is not observed (Table II). Theophylline inhibited the increase metabolism of pyruvate caused by ascorbic acid. Triner and Nahas (19) observed that theophylline administration in the rat causes a hyperlacticemia. Increased conversion of pyruvate to lactate may account for the effect of theophylline on ascorbic acid-treated tumor cells. Data obtained with dbcAMP and theophylline imply that ascorbic acid does not alter metabolism in EATC by potentiating intracellular cAMP concentrations.

Summary. Short exposure (1 hr) to hyperthermia (42.5°) is not accompanied by altered cellular respiration in EATC. Ascorbic acid has been shown to significantly effect metabolism in both *in vitro* and *in vivo* experiments. Ascorbic acid results in a significant reduction in lactate concentrations in EATC. The present evidence suggest that these alterations in metabolism are not due to alterations in cAMP concentrations. Alterations in the electron transport chain may account for the changes in metabolism observed with ascorbic acid supplementation. Although ascorbic acid does not inhibit or destroy EATC under our conditions, it does lead to significant metabolic changes.

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