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REPORTS

THE ROLE OF FATTY ACID OXIDATION IN THE EPIDERMIS

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Using cultured epidermal cells from guinea pig ears, we have determined that the epidermis has a consistently low respiratory quotient which is indicative of lipid metabolism. When the tissue was supplied with an exogenous source of free fatty acid, the rate of oxidation was quite large. Inhibition of fatty acid oxidation by α-bromopalmitate, an inhibitor of fatty acid oxidation in other mammalian cells, was associated with a decrease in both the rate of respiration and a decrease in the total pool of adenosine triphosphate (ATP).

These data indicate that α-bromopalmitate, through its inhibitory effect on fatty acid oxidation, decreases the amount of substrate entering the tricarboxylic acid cycle which in turn decreases the rate of both oxygen uptake and ATP production. A consequence of this lowered ATP level is a decreased rate of DNA synthesis, an effect which may be beneficial in controlling hyperproliferative diseases of the skin.

Recent experiments which have been reported in the literature suggest that respiration generates the majority of energy for the epidermis in vitro, producing adenosine triphosphate (ATP) at a rate of 0.5 μmole/hr/mg of tissue [1]. This ATP then serves as the chemical energy supply for the cell.

While glucose is considered to be the major contributor to the production of ATP in some mammalian cells, other substrates can also be employed. The heart, for example, oxidizes large amounts of free fatty acids for its energy supply. When skin is maintained in vitro in the absence of any added substrate, a considerable amount of oxygen uptake can be measured, which is presumably due to the use of some stored endogenous substrate(s). Consumption of glucose, glycogen, and protein have been shown to account for only 35% of the total oxygen uptake [2]. By exclusion, lipids have been implied to be the main source of energy in the epidermis.

Our objective in studying epidermal respiration has been to control cell division and/or cell differentiation in some abnormal skin conditions. One way to accomplish this objective may be through the regulation of ATP production. In hyperproliferative disease states, the skin might require an excess of energy for cell division to continue at an accelerated rate. For example, in the hyperproliferative skin disease state of psoriasis, there is an increased rate of respiration [3]. More recently it has also been shown that in psoriatic lesions, total epidermal lipogenesis was raised compared to matched control "uninvolved" epidermis [4]. Therefore, if one could manipulate the production of the energy supply in hyperproliferative skin diseases, one might regulate the rate of that proliferation. For this reason, we examined the relationship between fatty acid oxidation, respiration, and DNA synthesis.

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Abbreviations:
ATP: adenosine triphosphate
KRPB: Krebs-Ringer phosphate buffer
MEM: Eagle's Minimum Essential Medium
PCA: perchloric acid
RQ: respiratory quotient

MATERIALS AND METHODS

Isolation of Epidermal Cells from Guinea Pig Ears

Epidermal cells were obtained from the ear tissue of mature albino guinea pigs (Murphy Breeding Labs of Indianapolis, Indiana) using a modification of the method of Cruckshank, Cooper, and Hooper [5]. These modifications included using a dermatome to remove the ear tissue, and stirring the trypsinized epidermis for 20 minutes in culture medium. The isolated cells were either immediately used for an experiment, or they were cultured in 60 × 15 mm plastic Petri dishes. The medium used to grow these cells was Eagle's Minimum Essential Medium (MEM) supplemented with 10% fetal calf serum.

Respiration

Oxygen uptake and carbon dioxide production were measured in a Gilson Differential Respirometer. When slices of skin were used, the epidermis from hairless mouse (Jackson Labs, Bar Harbor, Maine) skin was excised with a 0.1 mm shim. When cultured cells were

used, they were released from the culture plate with 0.025% trypsin, placed in Krebs-Ringer Phosphate Buffer (KRPB) minus Ca^{++}, and then placed in 15-ml Warburg flasks on the respirometer. Each flask contained approximately 6×10^6 cells. This medium was then supplemented with the appropriate substrates. The bath temperature was maintained at 37°C, and the flasks were shaken at 98 rpm. In addition to the KRPB and cells, each flask contained 0.4 ml of NaOH (5%) in the center well and 0.5 ml of H_2SO_4 (4 N) in the sidearm. The flasks were allowed to equilibrate for 15 minutes before measurements of oxygen uptake were begun.

Measurement of Respiratory Quotient (RQ)

To measure the respiratory quotient, two flasks were used for each time point. One Warburg flask contained 0.4 ml 5% NaOH in the center well and the other Warburg flask contained 0.4 ml water in the center well. At predetermined times, the acid was dumped from the sidearm into the main chamber of the reaction vessel. Fifteen minutes after the acid was dumped, final respirometer readings were taken. The flask containing water in the center well measured the carbon dioxide released plus oxygen consumed. The flasks containing 5% NaOH in the center well measured oxygen uptake only, since the carbon dioxide was absorbed by the NaOH. Subtracting the oxygen uptake from the carbon dioxide released + oxygen uptake gave the amount of carbon dioxide released. Thus, the carbon dioxide released divided by the oxygen consumed yielded the respiratory quotient (RQ = μl CO_2/μl O_2).

Fatty Acid Oxidation

After the prescribed time in culture, the epidermal cells were released from the Petri dishes. The cell suspension was spun down in a Sorvall RC-2B at 600 × *g* for 5 minutes. The cells were then diluted (usually 3×10^6 cells/ml) with KRPB, pH 7.4.

During measurement of fatty acid oxidation, the epidermal cells were incubated with 0.2 mM 1-^{14}C-palmitic acid (New England Nuclear 12.5-17.9 mCi/mmole) in KRPB, pH 7.4. Earlier experiments in our laboratory have shown that the rate of 1-^{14}C-palmitate oxidation is constant between 0.1 mM and 0.3 mM. At predetermined times, the acid from the sidearm was dumped into the main chamber of the reaction vessel and the released $^{14}CO_2$ was collected on the NaOH wick in the center well. The wick and residual NaOH in the center well were removed and the amount of $^{14}CO_2$ was measured on a Packard Tri-Carb Scintillation Counter.

ATP Levels in Epidermal Cells

To determine the amount of ATP in the cultured epidermal cells, the growth medium was decanted from the Petri dishes. Then 2 ml of cold 6% perchloric acid (PCA) was added to the cells on the Petri dishes. The cells were washed into a centrifuge tube and placed in an ice bath for 60 minutes. After this time, the cells were centrifuged at 600 × *g* for 10 minutes. The PCA in the supernatant was titrated with 0.5 N KOH to pH 6.8. The insoluble potassium perchlorate was removed by centrifugation and an aliquot of the supernatant was used to measure the level of ATP by the method of Bucher [6].

DNA Synthesis

Cells were treated in the same manner as described for the measurements of fatty acid oxidation and respiration with the exception that the KRPB was supplemented with 25 μCi/ml of thymidine (methyl-^{3}H) (New England Nuclear, 20 Ci/mmole). At the appropriate time, the reactions were stopped by making the solution 6% PCA. The subsequent extraction, isolation, and hydrolysis of DNA were performed according to a modification of Schneider's method [7] as described below.

The tissue homogenate was adjusted to 6% PCA and placed in an ice bath for 30 minutes. After this time, the suspension was centrifuged for 15 minutes at 2500 × *g*. The sediment was washed three times with 6% PCA and resuspended in 1 ml of 6% PCA. The suspension was heated at 85°C for 15 minutes. The sediment after centrifugation was twice re-extracted with an additional ml of 6% PCA. All supernatant fractions from the hot acid hydrolysis were combined and aliquots were taken for radiochemical assay and DNA determinations.

In Vitro Treatment with α-Bromopalmitate

When the effects of α-bromopalmitate on respiration, fatty acid oxidation, ATP levels, and DNA synthesis were measured, the MEM was supplemented with 0.1 mM α-bromopalmitate (Eastman Kodak, >98%) bound to albumin (fatty acid poor, Pentex) at a molar ratio of 5:1. Cells not treated with α-bromopalmitate received only albumin. The α-bromopalmitate in the medium remained in contact with the cells for either 6 or 30 hours.

In Vivo Treatment with α-Bromopalmitate

In vivo evaluation of the effect of α-bromopalmitate on fatty acid oxidation, ATP levels, lactate production, and DNA synthesis in the skin was performed on hairless mice (Jackson Labs, Bar Harbor, Maine). Animals weighing approximately 25 gm were injected intraperitoneally at zero time, 6 hours, and 24 hours with various concentrations of α-bromopalmitate in phosphate buffer (0.1 mg, 1 mg, or 5 mg of α-bromopalmitate per animal). DNA synthesis was measured at the indicated time by the injection of 100 μCi of ^{3}H-thymidine/animal. Thirty minutes after the injection of ^{3}H-thymidine, the animals were sacrificed and 8 mm punches of skin were quickly taken and placed in liquid nitrogen. Extraction, isolation, and hydrolysis of DNA were performed according to the modification of the Schneider procedure described above.

Fatty acid oxidation was measured in vitro on 8 mm punch biopsies taken after the injection of the α-bromopalmitate in vivo. The biopsies were placed in KRPB, pH 7.4, supplemented with 0.2 mM 1-^{14}C-palmitate. $^{14}CO_2$ from the oxidation of the palmitate was collected for 2 hours and measured as previously described.

For the measurement of both lactate and ATP, 8 mm punch biopsies from the mice were placed in liquid nitrogen. These samples were crushed in liquid nitrogen and homogenized in 3 ml of 6% PCA. After extraction of the ATP and lactate with 6% PCA, the samples were centrifuged in a Sorvall RC-2B at 3500 × *g* for 10 minutes at 4°C. The supernatant was removed and used to measure ATP [6] and lactate [8]. The cell pellet was dissolved in 1 N NaOH and protein was determined by a modified Lowry procedure [9].

RESULTS

Figure 1 shows the data from a typical experiment in which dermatome slices were incubated without any added substrate. The rate of oxygen

uptake was linear for at least 180 minutes. Also presented in Fig 1 is the amount of carbon dioxide produced in relationship to the oxygen uptake. This point represents the total carbon dioxide given off over a 3-hour period. Normally, respiratory quotients of 0.71 ± 0.05 (n = 18) were obtained.

Figure 2 presents the data from a typical experiment in which skin dermatome slices were incubated with 1-^{14}C-palmitate for various periods. This experiment was done to determine the extent of fatty acid oxidation in the skin. The data in Fig 2 demonstrate that the capacity of the skin to oxidize exogenous palmitate is linear with time.

The data from an experiment in which isolated epidermal cells were incubated with an exogenous supply of 1-^{14}C-palmitate are presented in Table I. Release of $^{14}CO_2$ indicates the cells took up the fatty acid and used it as an energy source. Table I also presents the effect of α-bromopalmitate on the oxidation of 1-^{14}C-palmitate in isolated epidermal cells. Addition of 0.1 mM α-bromopalmitate caused a 70% reduction in the amount of $^{14}CO_2$ released after three hours. If the data from this Table are plotted, the lines, if extrapolated linearly, do not pass through zero. The reason for this is not known, since earlier time points were not taken.

To determine if the inhibition of $^{14}CO_2$ production from 1-^{14}C-palmitate by α-bromopalmitate was related to an inhibition in respiration, cells were incubated under conditions similar to those in Table I, and the rate of respiration was measured. The data presented in Fig 3 demonstrate that α-bromopalmitate caused not only a reduc-

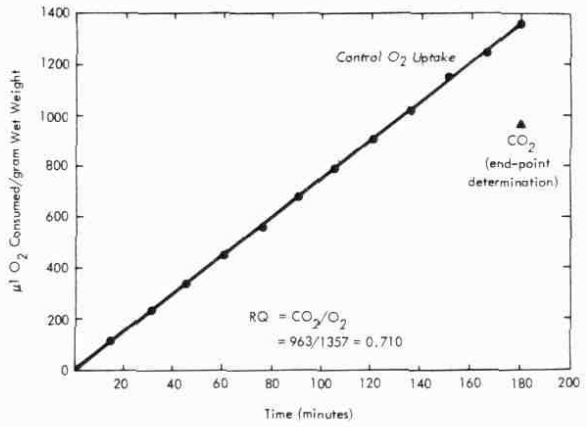

FIG 1 Endogenous respiration of hairless mouse skin. Each flask contained KRPB pH 7.4, and dermatome slices of hairless mouse skin. Reactions were carried at 37°C. Carbon dioxide was measured only at the 3-hr time point. Reactions were stopped by the addition of 0.5 ml of 4 N H_2SO_4 and the carbon dioxide liberated was collected on the NaOH in the center well. Calculations for carbon dioxide were as described in the "Materials and Methods" section.

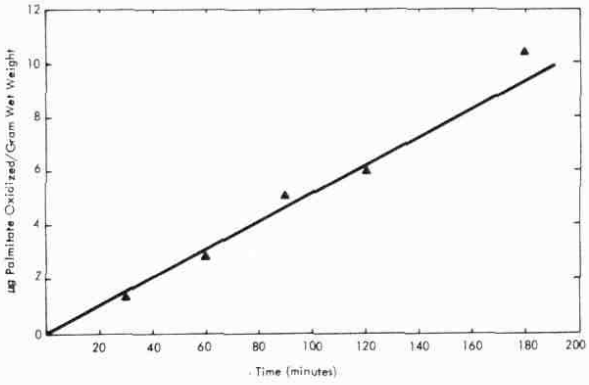

FIG 2 Capacity of hairless mouse skin to oxidize 1-^{14}C-palmitate. Conditions are identical to those in Fig 1 with the exception that the KRPB was supplemented with 0.2 mM 1-^{14}C-palmitate bound to albumin at a 5 to 1 molar ratio.

TABLE I. *Effect of α-bromopalmitate on 1-^{14}-C-palmitate oxidation on epidermal cells in vitro*

μg 1-^{14}C-Palmitate Oxidized to $^{14}CO_2$/mg Protein (X̄ ± SD, n = 5)			
Time (min)	Control	Treated[a]	% Inhibition
60	0.92 ± 0.04	0.44 ± 0.03[b]	52
120	1.52 ± 0.21	0.53 ± 0.04[b]	65
180	2.01 ± 0.28	0.60 ± 0.03[b]	70

[a] Epidermal cells were incubated in KRPB, pH = 7.4, supplemented with 0.2 mM 1-^{14}C-palmitic acid (New England Nuclear 12.5 – 17.9 mCi/mM) bound to fatty acid–poor albumin in a molar ratio of 5:1. Cells not treated with 0.1 mM α-bromopalmitate received only albumin.

[b] Significantly different from control values ($p \leq .05$).

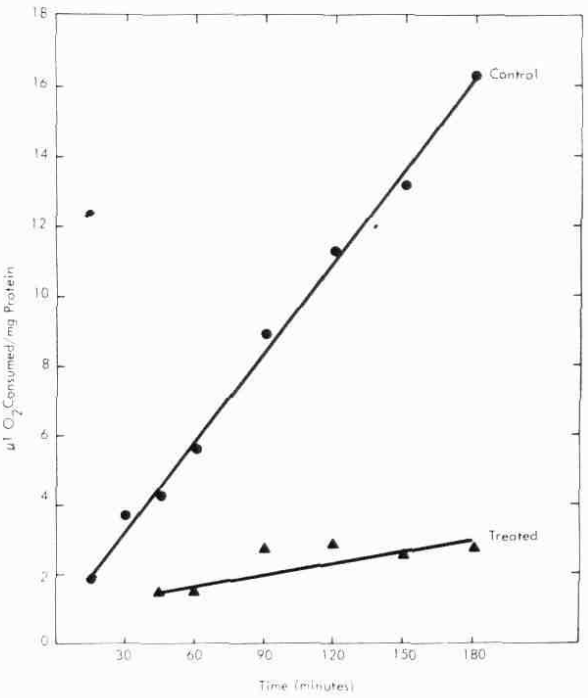

FIG 3 Effect of α-bromopalmitate on oxygen uptake in epidermal cells in culture for 30 hr. Epidermal cells were incubated in KRPB pH = 7.4 supplemented with 0.2 mM 1-^{14}C-palmitic acid (New England Nuclear 12.5–17.9 mCi/mM) bound to fatty acid poor albumin in a molar ratio of 5:1. Cells not treated with 0.1 mM α-bromopalmitate received only albumin. Oxygen uptake was measured on a Gilson Respirometer.

tion in fatty acid oxidation (Table I), but also caused an 80% reduction in the amount of oxygen taken up by the cells after incubation for 3 hours.

Since our hypothesis is that inhibition of fatty acid oxidation and respiration will cause a decrease in the energy level of the cells, the ATP levels of cultured epidermal cells were measured after treatment with α-bromopalmitate. The levels of ATP in these cells grown in culture with α-bromopalmitate showed a 30% decrease in the total ATP content of the cells when compared to nontreated cells.

Inhibition of fatty acid oxidation and subsequent depletion of ATP pools may also produce a lower rate of DNA synthesis. The data from an experiment in which epidermal cells were cultured in the presence of α-bromopalmitate and DNA synthesis was measured, are presented in Fig 4. These data suggest that DNA synthesis is also affected by α-bromopalmitate.

The effects of α-bromopalmitate on ATP levels, fatty acid oxidation, lactate production, and DNA synthesis in vitro six hours and 30 hours after a single injection of α-bromopalmitate were determined. As can be seen in Table II, treatment with α-bromopalmitate lead to a decrease in the amount of exogenous fatty acid which is oxidized. At approximately this same time, the synthesis of DNA was also inhibited. Lactate levels also decreased. Only a slight inhibition of ATP production was found at the highest dose level of α-bromopalmitate. Also presented in Table II are data for these same parameters 30 hours after treatment with α-bromopalmitate. Again, the amount of fatty acid oxidized was decreased after injection of α-bromopalmitate. At 30 hours, the total level of ATP was also affected, showing a decrease of approximately 35% when compared to the control. Inhibition of DNA synthesis was maintained during this period, also. Lactate levels, however, were increased relative to control, in contrast to the observation six hours after in vivo treatment. It also appeared that there was a slight inhibitory effect produced by the vehicle, since the control values decreased between the 6th and 30th hours.

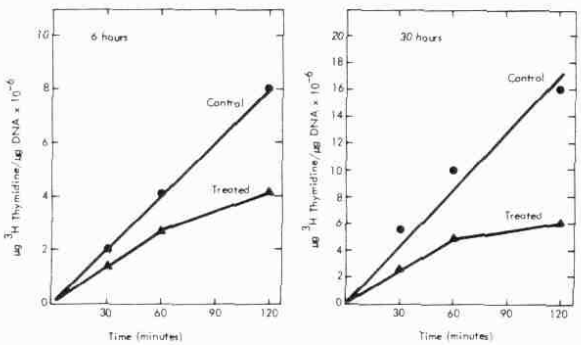

FIG 4 The effect of 6 hr and 30 hr treatment in culture with α-bromopalmitate on DNA synthesis. Cells were cultured in MEM supplemented with 0.1 mM α-bromopalmitate for either 6 or 30 hours. Cells were released from their respective Petri dishes as described earlier. Cells were then placed in Warburg flasks in KRPB pH = 7.4 supplemented with 25 μc/ml of ³H-thymidine (5 mc/60 μg). At the appropriate time, the reactions were stopped by making the solution 6% in PCA.

DISCUSSION

In this paper we attempt to draw together some of our ideas concerning the role of fatty acid oxidation in skin. Our hypothesis is that fatty acid oxidation plays an important role in the production of the necessary energy needed for the process of proliferation. If this hypothesis is true, then those compounds which affect fatty acid oxidation might also affect the proliferative capacity of the skin. Therefore, in diseases which are exemplified by abnormal proliferation, inhibitors of fatty acid oxidation might have a therapeutic effect.

Lipids have been implicated as the substrate for skin respiration [2]. In an attempt to determine if lipids were being utilized, respiratory quotients (RQ) were determined. These quotients (carbon dioxide produced/oxygen consumed) indicate the nature of the substrate being metabolized. Thus,

TABLE II. *Effect of α-bromopalmitate on various metabolic parameters of hairless mouse skin ($\bar{X} \pm SD$, $n = 6$)*

Treatment	hr After in vivo treatment	μg Palmitate oxidized to $^{14}CO_2$/mg protein/hr	% Inhibition(−) or % stimulation(+)	μg ATP/mg Protein	% Inhibition(−) or % stimulation(+)	μg ³H-thymidine/μg DNA × 10^{-7}	% Inhibition(−) or % stimulation(+)	μg Lactate/mg protein	% Inhibition(−) or % stimulation(+)
5 mg α-Bromopalmitate injected/animal	6	1.95 ± 0.24[a]	−60	1.81 ± 0.24[a]	−19	2.19 ± 0.66[a]	−74	2.23 ± 0.18[a]	−42
1 mg α-Bromopalmitate injected/animal	6	0.99 ± 0.17[a]	−80	2.32 ± 0.23	–	5.71 ± 1.30[a]	−32	2.52 ± 0.50[a]	−35
0.1 mg α-Bromopalmitate injected/animal	6	3.85 ± 0.46[a]	−22	2.50 ± 0.15	+11	8.39 ± 1.70	–	2.92 ± 0.60[a]	−24
Control (vehicle only)	6	4.91 ± 0.38	–	2.24 ± 0.33	–	8.40 ± 2.50	–	3.85 ± 0.29	–
5 mg α-Bromopalmitate injected/animal	30	1.79 ± 0.21[a]	−54	1.18 ± 0.03[a]	−34	2.19 ± 0.71[a]	−73	3.62 ± 0.26[a]	+29
1 mg α-Bromopalmitate injected/animal	30	2.27 ± 0.36[a]	−42	1.16 ± 0.30[a]	−35	4.74 ± 0.65[a]	−42	3.42 ± 0.64	+21
0.1 mg α-bromopalmitate injected/animal	30	–	–	1.47 ± 0.41	−17	9.14 ± 1.30	+11	2.98 ± 0.69	+6
Control (vehicle only)	30	3.89 ± 0.53	–	1.78 ± 0.09	–	8.17 ± 2.60	–	2.81 ± 0.42	–

[a] Significantly different from corresponding control values ($p \leq 0.05$).

an RQ of 1.0 would indicate that carbohydrates are being oxidized while RQ's of 0.85 to 0.90 would be indicative of protein metabolism. Lipids, on the other hand, have the lowest RQ of any substrate (~0.7–0.8) due to their low oxygen content relative to their carbon content, i.e., their state of oxidation. Consistent with lipid oxidation, the RQ values obtained were 0.71 ± 0.05 (n = 18).

In this paper we demonstrated that α-bromopalmitate inhibited the release of $^{14}CO_2$ from 1-^{14}C-palmitate and that there was also inhibition of O_2 uptake by epidermal cells in culture. Since both respiration and 1-^{14}C-palmitate oxidation were decreased in the presence of α-bromopalmitate, it appears likely that a large portion of the oxygen taken up by cells was the result of fatty acid oxidation.

Cells incubated in the presence of α-bromopalmitate had a decrease in their total ATP content. This indicates that reduced fatty acid oxidation leads to a reduced level of ATP. Certainly, other explanations could account for the decrease in the level of ATP (increase in anabolic process, stimulation of ATPase activity, etc.). However, from the data taken collectively, it would seem that the decrease in oxygen uptake could be directly related to a decreased synthesis in ATP, especially since Liebsohn et al [10] have demonstrated that 90% of the ATP in vivo was derived through respiration.

Since inhibition of fatty acid oxidation led to a decrease in ATP levels, epidermal cells were incubated in the presence of α-bromopalmitate for various periods of time and the effect of this inhibition on DNA synthesis was measured. After a 6-hr incubation in the presence of α-bromopalmitate, DNA synthesis was inhibited, probably through the inhibition of fatty acid oxidation and subsequent inhibition of ATP.

Cells cultured for 30 hours had a higher rate of DNA synthesis than cells cultured for only six hours. Although we do not have any direct evidence, we believe that the reduced rate of DNA synthesis observed after 6 hours of culture may be due to the reaction of the cells to the trypsin treatment which released them from the epidermis. Morphologically, cells recover from the trypsin treatment approximately 20–24 hr after culturing. Since these cells were treated with α-bromopalmitate 6 hr after culturing, the cells may not have fully recovered in the total 12 hr. Whatever the case, the inhibition of DNA synthesis in cells treated with α-bromopalmitate did occur at both 6 and 30 hours.

The concept concerning the inhibition of fatty acid oxidation and its effect on DNA synthesis was also examined in vivo in hairless mice. Six hours after a single injection of α-bromopalmitate, the rate of fatty acid oxidation was depressed. These data were similar to those obtained in vitro.

Data are also presented on the effect of α-bromopalmitate on ATP levels, DNA synthesis, and lactate levels of mouse skin in vivo and fatty acid oxidation in vitro 30 hours after in vivo treatment. Again, the reaction to α-bromopalmitate by the skin is exemplified in a decreased rate of fatty acid oxidation. After 30 hours, the reduced fatty acid oxidation lowers the overall energy state of the skin since ATP levels are decreased. Although evidence that inhibition in fatty acid oxidation is responsible for these decreased levels of ATP has not been shown, it appears logical if fatty acid oxidation were responsible for ATP production. Nevertheless, since we have shown that fatty acid oxidation and respiration are interrelated, and evidence exists in the literature that respiration is responsible for 90% of the ATP production by the skin, our conclusion would not be unreasonable.

After 30 hours of treatment with α-bromopalmitate, the skin has decreased fatty acid oxidation, decreased DNA synthesis, and decreased levels of energy (ATP). We believe that the cell's response to these perturbations is increased glucose utilization as indicated by increased lactate levels. However, it appears that the increase in glucose oxidation as measured by increased lactate levels was not able to compensate for the decreased fatty acid oxidation since the levels of ATP did not return to normal.

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