# Glycolysis inhibition by palmitate in renal cells cultured in a two-chamber system

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**Bolon, Claire, Catherine Gauthier, and Hélène Simonnet.** Glycolysis inhibition by palmitate in renal cells cultured in a two-chamber system. *Am. J. Physiol.* 273 (*Cell Physiol.* 42): C1732–C1738, 1997.—A major shortcoming of renal proximal tubular cells (RPTC) in culture is the gradual modification of their energy metabolism from the oxidative type to the glycolytic type. To test the possible reduction of glycolysis by naturally occurring long-chain fatty acids, RPTC were cultured in a two-chamber system, with albumin-bound palmitate (0.4 mM) added to the basolateral chamber after confluency. Twenty-four hours of contact with palmitate decreased glycolysis by 38% provided that carnitine was present; lactate production was decreased by 38%, and the decrease in glycolysis resulted from a similar decrease of basolateral and apical net uptake of glucose. In contrast to the previously described effect of the nonphysiological oxidative substrate heptanoate, palmitate promoted a long-term decrease in lactate production and sustained excellent cellular growth. After 4 days of contact, decreased glycolysis was maintained even in the absence of carnitine and resulted from a decrease of basolateral uptake only, suggestive of long-term regulation different from the earlier effects. Thus, although cultured RPTC lost their oxidative phenotype, they exhibited a type of regulation (Randle effect) that is found in the oxidative-type but not in the glycolytic-type tissues, therefore unmasking a regulative capacity barely detectable in fresh RPTC. Low $P_{O_2}$ (50 mmHg in the apical chamber) could be a major cause of elevated glycolysis and could hinder the effects of palmitate.

fatty acids; kidney; cell culture; differentiation

A MAJOR SHORTCOMING of renal proximal tubular cell (RPTC) culture is the gradual modification of cellular energy metabolism toward glycolysis (1, 7, 11, 12, 14, 35, 37). In situ or in fresh preparations, RPTC obtain their energy by an oxidative process using substrates other than glucose (lactate, citrate, fatty acids) (15, 23); RPTC are the site of extensive, active uptake and transepithelial transport of glucose (18), but only a small amount of the glucose, if any, that is taken up is metabolized, resulting in $CO_2$ and lactate production (7, 22, 23, 29). In addition, RPTC are gluconeogenic (23). Earlier attempts to improve the metabolic phenotype of cultured tubular proximal cells have shown that glycolysis may be reduced by shaking the medium and thus improving oxygen supply (1, 14, 25, 30); however, conflicting results (35) have shown that it would be useful to measure actual $P_{O_2}$ in this type of experiment. Other factors such as hormone supplementation or nutrient deficiency or excess could be responsible for the modification of energy metabolism in long-term RPTC culture. The widely used insulin supplement was shown to decrease neoglucogenesis, although it was not the cause of increased glycolysis (25); the same study showed that 5 mM lactate increased gluconeogenesis and decreased glycolysis. Glucose itself at high concentrations was reported to induce a loss of differentiation (35), and the excellent oxidative substrate, heptanoate (2 mM), a non-naturally occurring, medium-chain fatty acid, decreased glycolysis in short-term, 24-h cultures but was not found to be able to modify glycolysis in 7-day cultures and decreased growth (1).

In this paper, we report the use of a two-chamber system to test the effect of a long-chain fatty acid on the growth and metabolic phenotype of cultured proximal tubular cells. Indeed, epithelial cells are generally cultured in serum-free, albumin-free medium, which prevents fibroblast growth (9); however, this also prevents the use of insoluble, long-chain fatty acids carried in plasma by albumin. Because fatty acids enter the RPTC at the basolateral side, the luminal side being devoid of albumin and thus of fatty acids, we applied albumin-bound palmitate from the basolateral side of the insert only.

In fact, in mammals, the effects of long-chain fatty acids on glucose utilization vary from inhibition to activation, depending on *1*) the organ under consideration, *2*) the metabolic nature of the cell (oxidative or glycolytic), and *3*) the concentration of fatty acids. In heart (19, 28, 34), liver (13, 31), and lymphocytes (2), fatty acids decrease glycolysis; in addition, in hepatocytes, gluconeogenesis is activated during starvation by free fatty acids that are released from adipocytes (26, 34). In adipocytes, by contrast, glucose uptake and phosphofructokinase are activated by fatty acids (17, 21). In skeletal muscles, fatty acids decrease glucose utilization in oxidative-type muscles only (17, 19); moreover, low concentrations of fatty acids have been shown to inhibit glucose utilization, whereas high concentrations had an activating effect (19). During hyperinsulinemia, muscle glucose uptake is decreased by fatty acids (20). Taken as a whole, these organ specificities meet the demand of glucose exchanges during starvation or during postprandial states. In the kidney proximal tubule, where glycolysis is minimal (23), few data are available; however, it has been shown in rat in vivo that increased plasma concentrations of fatty acids during starvation have no effect on the low renal extraction rate of glucose (15). The aim of this study is to test whether a low physiological concentration of palmitate (0.4 mM) is able to decrease the high rate of glycolysis that is found in cultured RPTC.

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## METHODS

*Animals.* Female New Zealand rabbits, 1.2–1.5 kg (Elevage Scientifique des Dombes, Châtillon-sur-Chalaronne, France), were used for all studies and were killed by an intravenous injection of pentobarbital sodium (5 ml of a 0.03 g/ml solution).

*Materials.* Culture medium, serum, trypsin-EDTA, and versene were from GIBCO BRL (Inchinnan, UK); hormone supplements, *N*-2-hydroxyethylpiperazine-*N′*-2-ethanesulfonic acid (HEPES) buffer, carnitine, and lysozyme were from Sigma (St. Louis, MO); bovine fatty acid-free albumin originated from Sigma or from Boehringer (Mannheim, Germany); enzymes were from Boehringer; and palmitate and other chemicals were from Merck (Darmstadt, Germany). Albumin-bound palmitate was prepared as follows: palmitic acid (25 mg/ml) was dissolved in NaOH at 37°C for 30 min and then added (after cooling) to fatty acid-free bovine serum albumin (100 mg/ml); the final solution contained 1 g palmitate/100 g defatted albumin and 0.9 g/l NaCl.

*Culture techniques.* Cell preparation and culture have been described in detail in a previous paper (7); briefly, cells were isolated from rabbit renal cortex by mechanical dissociation followed by sequential filtration on polypropylene sieves; cells were cultured in a two-chamber system on permeable membranes made of pure collagen from rat tail tendon. The collagen film formed on a 1-mm mesh nylon gauze was mounted on 12-mm inserts made in our laboratory. The cells were seeded into the inserts in 0.5 ml of culture medium (Dulbecco's modified Eagle's medium-Ham's F-12 containing 10 mM HEPES, 25 mM bicarbonate, 4 mM glutamine, 1 g/l glucose, 5 μg/ml insulin, 10 ng/ml epidermal growth factor, $10^{-8}$ M triiodothyronine, $5 \times 10^{-8}$ M hydrocortisone, 5 μg/ml transferrin and $3 \times 10^{-8}$ M sodium selenium), and 1.75 ml of medium was added to the well outside the insert in a 12-well plate. Plates were maintained at 37°C in an air-$CO_2$ mixture (95:5) saturated with $H_2O$ vapor.

During the first 3 days only, the culture medium contained 10% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. After 3 days the inserts were transferred to six-well plates containing 0.75 ml of medium inside the insert and 7 ml outside the insert; the medium was replaced every 3 days. Bacterial contamination resulting from the lack of antibiotics was prevented by addition of 20 μg/ml egg white lysozyme throughout the culture; control studies showed no modification of the cellular characteristics resulting from the lysozyme.

*Fatty acid exposure protocol.* In two preliminary experiments, palmitate (125 mg/l, bound to 7.4 g/l fatty acid-free albumin) was introduced from the first day of culture into serum-free medium in both chambers of the system. The result in both experiments was the inability of cells to attach and spread onto the substratum; cells cultured in a similar system with fatty acid-free albumin only exhibited normal growth. Therefore, the protocol for palmitate provision to the cells was modified as follows.

After 6 days, when confluency was achieved and watertightness was carefully checked (by leaving each filled insert out of the basolateral medium for 1 min), inserts were divided into four groups. *Group 1* was cultured with the basal medium inside (i.e., on the apical pole of the cells) and outside the insert (basolateral pole). *Group 2* was cultured with 10 g/l fatty acid-free bovine serum albumin added to the medium outside only (basolateral pole). In *group 3*, albumin-bound palmitate (10 g/l fatty acid-free albumin and 100 mg/l palmitate) was added to the basolateral medium only. *Group 4* was cultured like *group 3* except that, in addition, 0.05 mM carnitine was introduced into the basolateral medium. On *day 7* or on *day 10*, 100 μl of medium were pipetted into stoppered capillaries for immediate gas determination (the end of the pipette being placed just underneath the insert for the basolateral sampling and just above the monolayer for the apical sampling), and 300 μl of medium were pipetted out into $HClO_4$-containing tubes (2% final content) and stored at −20°C for further determination of metabolites. Inserts were rinsed with phosphate-buffered saline (PBS), and the transepithelial resistance of the monolayer in PBS was occasionally measured with a Millicell-ERS ohmmeter (Millipore). The cellular contents of aminopeptidase activity, protein, and DNA were then determined as indicated below.

*Biochemical analysis.* $P_{O_2}$ was measured with a blood gas analyser (Radiometer BMS K 30). Cellular aminopeptidase M activity was determined in situ with L-alanine-nitro-4-anilide as substrate with use of the method of S. G. George and A. J. Kenny as previously described (see Ref. 7). Cellular DNA content was measured in the same cells after solubilization of the entire monolayer with the alkaline solution A + B + C of O. H. Lowry, as previously described (see Ref. 7). To eliminate protein contamination of the monolayer by serum albumin, the protein-to-DNA ratio was determined in cells detached from the collagen film by trypsin treatment as follows: after a 10-min rinsing of both sides of the inserts with versene (1:5,000), 2 ml of trypsin (3 g/l)-EDTA (0.2 g/l) were added to both sides of the inserts for 15 min at 37°C, inducing rounding but not detachment of cells. After a rinsing with PBS, most cells could be detached by thorough repeated pipettings. Proteins were determined with the Bradford reagent, and DNA was determined with the method of C. Labarca and K. Paigen, as previously described (see Ref. 7).

Glucose, lactate, and fatty acids were determined enzymatically with spectrophotometric methods, and changes in the concentration of NADH or NADPH (7, 33) were recorded after neutralization of samples with a mixture of KOH (20%, wt/vol) $H_3PO_4$ (0.15 M).

*Calculations.* Metabolite consumption or production was calculated as the difference between the total content (luminal + basolateral) of control medium from inserts incubated without cells and the total content of medium from inserts incubated with cells.

Statistical analyses were performed with the use of analysis of variance followed by protected tests of Fisher.

## RESULTS

*Effect of palmitate on renal tubular cell growth.* Twenty-four hour contact of cultured cells with palmitate induced no change in DNA content (Table 1); a slight, nonsignificant increase in protein-DNA was seen when carnitine was provided in addition to palmitate. The cellular DNA content increased from *day 7* to *day 10* in the basal medium, showing that, under these conditions, growth was not arrested at *day 7* despite monolayer confluency and watertightness; however, this late growth was suppressed by palmitate in the presence of carnitine. The protein-to-DNA ratio was increased by palmitate at *day 10*, i.e., after 4 days of supplementation, irrespective of the presence of carnitine.

*Effect of palmitate on glycolysis.* Our cultured cells under basal conditions exhibited a very high glycolysis rate with >100 μmol glucose consumed·day$^{-1}$·mg protein$^{-1}$. The lactate production-to-glucose consumption ratio was increased to ~1.4 [normal ratio in freshly

Table 1. *Effect of palmitate on DNA content and protein-to-DNA ratio in renal cultured cells*

| | Basal | BSA | Palm | Carn |
|---|---|---|---|---|
| DNA, μg/insert | | | | |
| *Day 7* | 2.91 ± 0.44 | 2.98 ± 0.64 | 2.71 ± 0.44 | 2.91 ± 0.24 |
| *Day 10* | 3.92 ± 0.93† | 3.39 ± 0.84 | 3.21 ± 0.69 | 2.96 ± 0.59* |
| Protein/DNA, μg/μg | | | | |
| *Day 7* | 48.9 ± 17.1 | 53.1 ± 19.6 | 54.4 ± 16.6 | 62.3 ± 23.5 |
| *Day 10* | 53.0 ± 15.9 | 59.0 ± 14.6 | 78.5 ± 31.2* | 76.2 ± 20.5* |
| Proteins, μg/insert | | | | |
| *Day 7* | 142 | 158 | 147 | 181 |
| *Day 10* | 211 | 216 | 252 | 226 |

DNA and protein/DNA data are means ± SD for 8–11 points from 4–5 cultures; protein data are products of mean protein/DNA and mean DNA. Basal, basal medium on both sides of insert; BSA, basal medium with 10 g/l fatty acid-free albumin outside the insert added from *day 6;* palm, basal medium with 10 g/l albumin and 0.4 mM bound palmitate outside the insert added from *day 6;* carn, identical to palm except that 0.05 mM carnitine was added to the palmitate-containing medium. *$P < 0.05$ compared with basal medium; †$P < 0.01$ compared with *day 7* data of basal medium.

isolated RPTC, 0.5 (7)]; therefore, glucose was not totally converted into lactate.

Table 2 shows that, at *day 7*, palmitate decreased glycolysis by 38% after only 1 day of contact only when carnitine, which allows fatty acids to enter mitochondria, was added to the cells; albumin per se slightly decreased glycolysis so that the change in glycolysis brought about by the addition of carnitine to the palmitate-containing medium was −33%. Fatty acid determination (Table 2) showed that, at *day 7*, very little palmitate was taken up by cells in the absence of carnitine; it should be stressed that this net uptake measurement is subject to high standard deviation, probably as a result of the high renal fatty acid turnover (23). Addition of carnitine increased the net uptake of fatty acid to 1.3 μmol·mg$^{-1}$·day$^{-1}$. Given that in vivo kidney takes up 13.2 μmol free fatty acid·100 g wet wt$^{-1}$·min$^{-1}$ (2.7 μmol·mg protein$^{-1}$·day$^{-1}$) (10), fatty acid uptake by our cultured cells in the presence of carnitine was similar to that measured in vivo. The changes in lactate production were similar to those of glucose consumption, so that the lactate production-to-glucose consumption ratio was not altered by palmitate with or without carnitine.

Table 2. *Effect of palmitate on metabolism of renal cultured cells*

| | Basal | BSA | Palm | Carn |
|---|---|---|---|---|
| Glycolysis | | | | |
| *Day 7* | 115 ± 36 | 97 ± 22 | 106 ± 23 | 71 ± 13[a,b,d] |
| *Day 10* | 83 ± 17 | 80 ± 10 | 67 ± 12[c,d] | 69 ± 12[e,h] |
| Lactate production | | | | |
| *Day 7* | 152 ± 29 | 132 ± 30 | 142 ± 25 | 96 ± 21[a,b] |
| *Day 10* | 128 ± 22 | 110 ± 19[e] | 93 ± 17[a,g] | 97 ± 18[a,h] |
| Fatty acid consumption | | | | |
| *Day 7* | | | 0.3 ± 1.00 | 1.3 ± 0.7[f] |
| *Day 10* | | | 0.4 ± 0.6 | 0.9 ± 1.0 |

Values are means ± SD in μmol metabolite subtracted or added to medium·mg protein$^{-1}$·day$^{-1}$; $n$ = 13–16 points from 6–8 cultures. $P < 0.001$ compared with cells from [a]basal or [b]palm. $P < 0.01$ compared with cells from [c]basal or [d]BSA. $P < 0.05$ compared with cells from [e]basal, [f]palm, or [g]BSA. [h]Nonsignificant vs. palm.

Table 2 shows that, at *day 10*, 4 days of palmitate supplementation induced a 20% decrease in glucose consumption even in the absence of carnitine and despite the fact that net fatty acid uptake was not significantly increased in the absence of carnitine. At this time, the modifications in lactate production paralleled that of glucose consumption so that the lactate production-to-glucose consumption ratio was not altered by palmitate. In addition, glycolysis decreased between *day 7* and *day 10* even in the absence of palmitate (Table 2, $P < 0.01$); this could be linked to quiescence of the cells after confluency because glycolysis was shown to be increased in growing cells (24).

*Transepithelial distribution of glucose and lactate.* In our system, there was a transepithelial glucose gradient of 3.43 mM/0.95 mM (basolateral content/apical content, $P < 0.001$) across the cellular monolayer 24 h after medium replacement at *day 6* in basal medium. This probably resulted from differences in glucose uptake from each chamber, although transepithelial transport cannot be excluded. Table 3 shows that the decrease in glycolysis induced by 24 h of palmitate plus carnitine provision resulted from a similar decrease in glucose net uptake from both sides of the monolayer, because basolateral uptake decreased by 41%, whereas apical uptake decreased by 28%. On the other hand, after 4 days of provision of palmitate, the 20% decrease of glycolysis induced by palmitate only resulted from a decrease of net basolateral uptake.

Table 3. *Net metabolite uptake or efflux in each chamber by renal cells*

| | Apical Chamber | | | | Basolateral Chamber | | | |
|---|---|---|---|---|---|---|---|---|
| | Basal | BSA | Palm | Carn | Basal | BSA | Palm | Carn |
| Glucose net uptake | | | | | | | | |
| *Day 7* | 20.6 ± 3.7 | 17.6 ± 2.6* | 19.6 ± 3.2 | 14.9 ± 2.3‡ | 94.0 ± 35.0 | 79.3 ± 20.8* | 85.4 ± 20.1 | 55.0 ± 11.7‡ |
| *Day 10* | 15.4 ± 2.2 | 11.6 ± 3.5† | 10.9 ± 2.4 | 11.9 ± 2.5 | 70.5 ± 15.2 | 70.9 ± 5.0 | 55.7 ± 12.3‡ | 54.0 ± 10.8 |
| Lactate net efflux | | | | | | | | |
| *Day 7* | 24.6 ± 6.3 | 20.9 ± 4.8 | 20.3 ± 5.7 | 15.1 ± 3.3† | 129.9 ± 25.1 | 110.3 ± 27.8* | 119.7 ± 23.5 | 79.9 ± 20.2‡ |
| *Day 10* | 20.7 ± 5.0 | 16.1 ± 4.2* | 14.2 ± 3.0 | 15.9 ± 3.3 | 108.2 ± 18.9 | 92.6 ± 13.7* | 76.6 ± 14.9† | 79.5 ± 13.4 |

Values are means ± SD in μmol·mg protein$^{-1}$·day$^{-1}$ of 12–16 points from 6–8 cultures. Cells were cultured as described in METHODS, and glucose and lactate were determined after 7 or 10 days in apical or basolateral medium. *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$ compared with preceding conditions (column to immediate left).

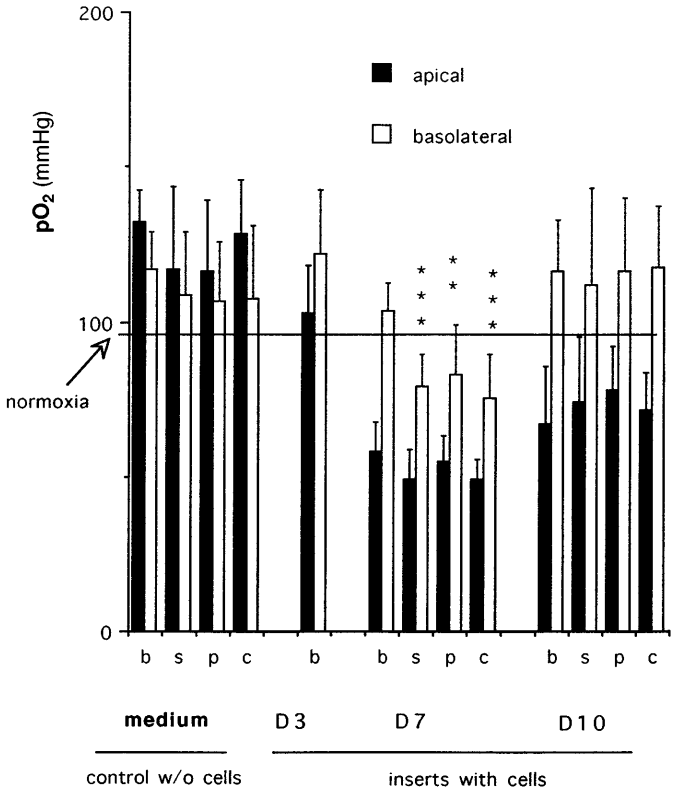


Fig. 1. Oxygenation status of the 2-chamber system. $P_{O_2}$ of apical and basolateral medium were measured as described in METHODS after 24-h incubation at 37°C in 95% air-5% $CO_2$ in control inserts without cells (medium) and in inserts with cultured cells at different stages of growth [*day* (D) *3*, D*7*, and D*10*]. b, basal medium on both sides of inserts; s, serum albumin (10 g/l) added from *day 6* outside the insert; p, palmitate (0.4 mM) bound to serum albumin (10 g/l) added from *day 6* outside the insert; c, identical to p except that carnitine (0.05 mM) was added to palmitate-containing medium. Data are means ± SD of 8–12 points from 4–5 cultures (D*7* and D*10*) or 4–8 points from 3–5 cultures (control medium and D*3*). $^{**}P < 0.01$; $^{***}P < 0.001$ compared with basal medium corresponding data.

Lactate distribution was also asymmetrical, and a gradient of 4.73 mM/2.62 mM (apical/basolateral, $P < 0.001$) was established, after 1 day of incubation, at *day 7* in basal medium. This gradient was dependent on the physical characteristics of the system because similar cells on inserts placed in similar medium in 12-well plates (0.5 ml medium apical/1.75 ml basolateral) did not demonstrate a lactate gradient (lactate 5.8 ± 1.1 mM apical/5.7 ± 1.9 mM basolateral; $n = 13$, not significantly different). It must be emphasized that a glucose gradient was always observed, even in a 12-well system, with a mean basolateral-to-apical ratio of 8.6 (7), suggesting that the lactate gradient is not of the same nature as that of glucose.

The changes in lactate efflux in each chamber paralleled those of glucose uptake; indeed, the 38% decrease in total net lactate efflux induced by palmitate plus carnitine resulted from a similar 38% decrease of lactate efflux in each chamber (Table 3).

*Oxygenation status of the system*. There was a $P_{O_2}$ gradient between the apical and basolateral sides of the inserts (apical < basolateral, error $P < 0.001$; Fig. 1) as soon as cells were present, including at *day 3*, long before confluency. In fact, at *day 7*, cell respiration induced a sharp decrease of apical $P_{O_2}$ to far below the normal plasma value, whereas basolateral $P_{O_2}$ remained within the normal range in basal conditions.

Figure 1 shows that addition of albumin to the basolateral side of cultured cells decreased basolateral $P_{O_2}$ to values below normoxia after 1 day; after 4 days, despite the cellular protein increase, basolateral $P_{O_2}$ returned to normoxia. The fact that basolateral $P_{O_2}$ decreased only in the presence of cultured cells suggested that *1*) $O_2$ was able to diffuse to a certain extent across the collagen substratum and *2*) addition of albumin to the medium limited the $O_2$ supply from the atmosphere to the medium. In an attempt to overcome this limitation, we agitated the culture plates with inserts at the maximal orbital rate allowed by this system (80 revolutions/min); however, in three cultures with albumin, palmitate, and carnitine, shaking resulted in only a slight increase of apical $P_{O_2}$ to 67 ± 14 mmHg, and there was no increase of basolateral $P_{O_2}$. It should be noted that shaking induced such friction on the collagen film that, in these experiments, we had to increase collagen thickness to prevent destruction of the matrix.

Table 4. *Effect of palmitate on membrane parameters of renal cultured cells*

| | Basal | BSA | Palm | Carn |
|---|---|---|---|---|
| Aminopeptidase, nmol·min$^{-1}$·mg protein$^{-1}$ | | | | |
| *Day 7* | 42.9 ± 5.6 | 39.5 ± 5.3 | 39.9 ± 6.2 | 33.0 ± 4.3[a,d] |
| *Day 10* | 34.7 ± 5.9 | 34.9 ± 8.6 | 27.3 ± 6.5[b] | 33.1 ± 6.6[c,e] |
| Electrical resistance, Ω·cm$^2$ | | | | |
| *Day 7* | 32 ± 11 | 38 ± 22 | 36 ± 18 | 28 ± 20 |

Values are means ± SD of 14–16 points from 7–8 cultures (aminopeptidase) or 7–8 points from 4 cultures (resistance). [a]$P <$ 0.001 compared with cells from basal. $P < 0.05$ compared with cells from [b]basal, [c]palm, or [d]BSA. [e]Nonsignificant compared with BSA. Carn value for electrical resistance is nonsignificant.

*Effect of palmitate on aminopeptidase and transepithelial resistance.* RPTC in culture gradually lose brush borders and brush-border enzyme activity (1, 7, 37). Because this could result from the absence of lipidic nutrients in the culture medium, aminopeptidase, a brush-border enzyme, was determined after palmitate provision to our cells; however, as shown in Table 4, no improvement was produced by palmitate, either in the presence or absence of carnitine. Similarly, no change in electrical resistance of the monolayer was induced by the presence of palmitate.

## DISCUSSION

*Evidence of early glycolysis regulation by fatty acids in cultured proximal tubular renal cells.* The fact that in cultured RPTC glycolysis may be decreased by 24-h exposure to palmitate is interesting for several reasons. First, it shows for the first time that cultured RPTC have the capacity to regulate glycolysis by means of the naturally occuring long-chain fatty acids (LCFA); this

capacity is a typical feature of hepatocytes, heart, and oxidative-type muscle (13, 19, 20, 27, 28, 34). In these organs, three possible sites of inhibition have been identified; the main site is phosphofructokinase, but short- and long-term inhibition of pyruvate dehydrogenase has also been described (13, 16, 27, 34). Both effects were suppressed by inhibition of fatty acid mitochondrial transport or oxidation (13, 16, 27, 31); in addition, in muscle that is insulin responsive, insulin-sensitive glucose transport was shown to be inhibited by fatty acids (17). In our study with cultured RPTC, the fact that lactate production is lowered by palmitate to the same extent as glycolysis, together with the fact that glucose uptake from both sides of the cell is lowered by palmitate, is consistent with the view that an intracellular step, such as phosphofructokinase inhibition, mediates the palmitate effect on glycolysis and that the oxidative pathway is not affected by palmitate. However, in view of the relatively long duration (24 h) of the palmitate exposure, allowing slight changes in the protein/DNA content, it is not excluded that genomic regulation of other sites might add its effects to pure enzymic-stage regulation.

The second point of interest is that RPTC in culture, despite their apparent loss of the oxidative phenotype, exhibit a type of regulation that is found in oxidative-type but not in glycolytic-type tissues (4, 13, 19, 31, 34). In contrast, in hepatoma cells, which exhibit the same glycolytic metabolism as our cells and which also originate from an oxidative tissue, glycolysis does not decrease but increases in the presence of palmitate (6); this indicates that the loss of differentiation of energy metabolism in RPTC in primary culture is not so profound as that in transformed cells and suggests that this loss is reversible.

It should also be noticed that this inhibitory effect on glycolysis has never been described in noncultured RPTC, in which glycolysis is either absent, as in the rat (23), or low, as in the rabbit (7, 8, 22); the only study dealing with the effect of palmitate on glycolysis, which used rabbit cortex (which is not pure RPTC), showed no effect on lactate production, although glucose uptake or accumulation was decreased (22). Thus our results show that the glycolysis increase resulting from culture uncovers a regulation capacity typical of oxidative tissue.

*Effect of palmitate on renal proximal cell differentiation*. Our results are in good agreement with those previously obtained by Aleo and Schnellman (1); these authors partially prevented glycolysis increase in cultured RPTC by adding 2 mM heptanoate. However, from our results, the use of the LCFA palmitate provides two advantages over heptanoate. First, palmitate added from confluency sustained excellent growth for at least 4 days although it arrested DNA increase, and, despite the presence of albumin, the carrier of palmitate, the growth of a possible fibroblastic population was not demonstrated, as shown by the RPTC marker aminopeptidase. In contrast, heptanoate added from *day 0* was inhibitory to growth (1); this inhibition could be due in part to the timing of the application of the fatty acid, perhaps as a result of a physical mechanism, because, as indicated in METHODS, when we tried to introduce palmitate at *day 0*, the cells failed to spread and grow. The role of fatty acids during initiation of growth has also been documented by experiments showing that the $C_4$ fatty acid butyrate improves attachment but is inhibitory for initial growth at high concentrations (36). A second advantage of palmitate is that it causes a long-term decrease of glycolysis, whereas heptanoate was reported to decrease glycolysis for only 1 day and to induce a paradoxical rise in lactate production after 7 days (1). These discrepancies probably result from the different metabolic fates of LCFA and medium-chain fatty acids (MCFA); indeed MCFA are mostly oxidized in a carnitine-independent process, whereas LCFA enter into the mitochondria more slowly via carnitine palmitoyl transferase, a key regulating enzyme of lipid metabolism. In parallel, LCFA are rapidly recycled into cellular lipids and lipoproteins (3). Thus differences in long-term regulation by these two fatty acid types are not to be excluded. Besides, the dicarboxylic acids formed after several hours by MCFA only have been shown to decrease mitochondrial ATP production (3); in addition, after several days of MCFA supply, adaptive changes have been found, decreasing oxidation of these fatty acids (3) and possibly causing the paradoxical rise of lactate in heptanoate-supplemented RPTC (1). The carnitine requirement for palmitate's early effect is not surprising, because this carrier is not synthesized by RPTC (5). Unexpectedly, palmitate slightly decreased glycolysis at *day 10* in the absence of carnitine, suggesting the existence of a long-term effect of palmitate independent of its oxidation. This long-term decrease of glycolysis, in contrast to that of the early effect, resulted from decreased uptake from the basolateral side only, suggesting a membrane effect restricted to this pole of the cell, which could be a lesser transport rate by the facilitated glucose transporter (GLUT). In fact, this transport in RPTC is restricted to the basolateral membrane, whereas sodium-coupled active glucose transporter is localized in the apical membrane (18); in cultured rabbit RPTC, these two types of transporters have been shown to collaborate for glucose uptake (25). Therefore, long-term effects of palmitate could possibly reflect not just a simple continuation of short-term effects but a completely different mechanism of action.

The palmitate effect, although interesting, was not sufficient to lower glycolysis to the fresh tissue level, which is $\sim$2 $\mu mol \cdot mg^{-1} \cdot day^{-1}$ (7); this shows that the lack of fatty acids was not the main cause of increased glycolysis in our cultured cells. This very high glycolytic rate deserves comment. First, it seems much higher than that observed by other authors (1, 35). However, it is difficult to compare all of these data because other authors limited the medium supply to the cells to obtain a low medium depth and good oxygen diffusion; under these conditions glycolysis was limited by glucose exhaustion and was not a measurement of glycolytic capacity. This was not the case in the present study with a two-chamber system, where 1 $cm^2$ of

cellular monolayer was faced with 7.75 ml medium, and the glucose consumption, when measured, represented only one-third of the total glucose. From these observations, it seems that glycolysis in culture may reach very high levels even with physiological glucose concentrations, and it also seems that 100 $\mu mol \cdot mg^{-1} \cdot day^{-1}$ is a maximum in our conditions.

It should be noted that the main cause of elevated glycolysis could be hypoxia; this could explain both the glycolysis increase by the Pasteur effect and the increase of the lactate-to-glucose ratio by the decreased NAD regenerative capacity of the respiratory chain. Actually, $P_{O_2}$ was very low in the apical medium of confluent cultured cells, showing active $O_2$ consumption from this side of the cells. $O_2$ could also be taken up from the basolateral side because, at least in medium containing albumin, basolateral $P_{O_2}$ decreased in the presence of cells. It is also likely that the collagen film thickness (~6 μm on electron microscopy, not shown) slows down $O_2$ diffusion from the basolateral chamber to cells because at *day 3*, i.e., before confluency, $O_2$ consumption was already too fast to allow equilibration of apical $P_{O_2}$ across the collagen film. Therefore, a reduced $O_2$ supply from apical and basolateral sides could increase glycolysis, which was shown to be sensitive to acute anoxia (22). The fact that basolateral $P_{O_2}$ returned to normoxia after 4 days of exposure to albumin in the medium suggests that, as an adaptive response to hypoxia, cultured RPTC reduce their $O_2$ consumption.

Albumin decreased basolateral $P_{O_2}$ in the presence of respiring cells, probably by slowing down $O_2$ diffusion across the culture medium, because the gas diffusion rate depends on gas solubility, which is slightly decreased in albumin solutions, and is inversely proportional to the medium viscosity, which is increased by albumin. Indeed the value of $O_2$ diffusivity in serum is one-half that in water, owing mainly to increased viscosity (38); moreover, albumin per se was shown to have no effect on mitochondrial respiration (32). This $P_{O_2}$ decrease is especially inopportune because fatty acids need $O_2$ for their oxidation and increase $O_2$ consumption (26, 31); it is possible that hypoxia might hinder the decrease of glycolysis due to palmitate because acute anoxia has been shown to suppress the effect of fatty acids on glycolysis (28). In addition, our results show that a two-chamber system, although it is especially designed for an asymmetrical supply of substrates such as fatty acids and has the advantage of an almost unlimited substrate supply, is particularly difficult to shake and thus to be efficiently oxygenated.

Improvement of membrane synthesis could have been expected upon palmitate provision to RPTC in culture because palmitate is not only oxidized, like MCFA, but is also incorporated into cellular lipids and lipoproteins (3, 23); this point could explain the shift of the protein-to-DNA ratio in our palmitate-treated cells; however, the fact that palmitate was unable to prevent the gradual decrease in aminopeptidase during culture shows that brush-border development in this cell type is determined by another unknown factor.

In conclusion, this work has allowed us to show that palmitate provision to RPTC cultured on a two-chamber system can improve the metabolic phenotype by decreasing glycolysis; this in turn unmasks a regulative capacity that is barely measurable in fresh renal tissue. In contrast to the previously described effect of the nonphysiological oxidative substrate heptanoate, palmitate promoted a long-term decrease of lactate production and sustained excellent cell growth; the long-term effect of palmitate on glycolysis is shown to be different from the short-term, carnitine-mediated effect. However, the oxygenation limitations specific to the two-chamber system are problematic; further investigations are necessary to develop a more efficient system.

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