Effects of palmitate on the metabolism of leukocytes from guinea pig exudate

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SUMMARY

The metabolism of palmitate by polymorphonuclear leukocytes (PMN) from guinea pig exudate in the presence of physiological concentrations of human serum albumin has been studied. The data indicate that with increasing concentration of palmitate in the medium (over a range of 0.54 to 5.4 μmoles/ml), there is a concomitant increase in uptake of oxygen and palmitate. The RQ decreased from 1.34 to 0.82 when palmitate was added to PMN (initial concentration: 3.8 μmoles/ml).

A comparison was made between the effect of palmitate, and the effect of phagocytosis of polystyrene particles, on leukocyte respiration. The effect of palmitate was found to be additive to the effect due to phagocytosis when the latter occurred maximally, suggesting that the effect of palmitate on oxygen uptake was probably not due to phagocytosis of a high molecular weight palmitate-albumin complex.

Palmitate uptake and the stimulation of oxygen uptake by palmitate were markedly inhibited by the glycolytic inhibitors sodium fluoride and iodoacetic acid. Cyanide and dinitrophenol had no effect on palmitate uptake or on the stimulation of oxygen uptake by palmitate. Palmitate uptake was the same in the presence and absence of glucose in the medium or under aerobic and anaerobic conditions. Glucose caused a 39% inhibition of the oxygen uptake of cells incubated in the absence of palmitate (Crabtree effect), whereas in the presence of palmitate, where there was a marked increase in oxygen uptake, no Crabtree effect was noted. Guinea pig monocytes (MN) were found to be more active metabolically than PMN. Oxygen and glucose uptake were seven times greater and palmitate uptake four times greater in MN as compared to PMN. With the addition of palmitate, only PMN showed a significant increase in oxygen uptake although the absolute increments in O_2 uptake were the same for PMN and MN. Palmitate had no significant effect on the oxygen uptake of guinea pig circulating blood leukocytes and no net palmitate uptake was observed with such cells.

As a preliminary phase of the study of leukocyte fat metabolism *in vitro*, we chose to study the effect of a naturally occurring free fatty acid (FFA), palmitic acid, on the uptake of oxygen and glucose by readily available guinea pig exudate leukocytes. Studies by Elsbach (1) on polymorphonuclear leukocytes (PMN) of rabbit exudate and by Day (2) on monocytes (MN)

of rabbit exudate indicate that these cells oxidize C^{14}-labeled FFA to $C^{14}O_2$. The effect of added FFA on oxygen and glucose consumption was not studied by these workers, however.

In the present study, the effect of increasing concentrations of albumin-bound palmitate on palmitate and oxygen uptake by guinea pig exudate PMN is reported. The possibility that phagocytosis might explain some of the metabolic effects produced by adding albumin-bound palmitate to leukocytes has been examined. This paper also contains the results of

studies on the effect of various metabolic inhibitors on the metabolism of leukocytes in the presence and absence of palmitate. The relationship between glucose and palmitate metabolism has been studied, and some observations on the comparative metabolic activities of PMN and MN in the presence and absence of palmitate are reported. Some observations on the effect of palmitate on the metabolism of guinea pig circulating blood leukocytes are also included in this paper.

METHODS AND MATERIALS

Male guinea pigs (NIH strain), weighing 300–500 g, were used. These animals were fed daily with water, cabbage, and pellets until they were sacrificed.

Exudates rich in leukocytes were obtained by the injection of 10 ml of a sterile 12% casein solution into the peritoneal cavities of the guinea pigs. This method has been described in detail previously (3, cf. 4). In order to obtain suspensions rich in PMN, the exudates were removed from the animal 16–18 hr after the injection of casein, whereas a 65-hr waiting period was used to obtain suspensions rich in MN. After removal from the peritoneum, the cells were washed once with 0.9% NaCl, resuspended in unbuffered Krebs-Ringer solution, and maintained at ice-bath temperature until ready for use. Usually about 30–60 min elapsed after the cells were removed from the animal until they were placed in the Warburg flasks.

The amount of cell material in the leukocyte suspensions was determined as the average of quadruplicate cell counts, using an AO Spencer hemacytometer. The dry weights of the cell preparations from 18- and 65-hr exudates were calculated to be 1.39 and 1.58 mg/10^7 cells, respectively, from data reported by Stahelin et al (4). Differential counts on Giemsa-stained smears made from four different PMN preparations gave the following mean percentage values ($\pm$ SE of the mean): PMN 80 $\pm$ 2.1%, MN 17 $\pm$ 0.9%, lymphocytes 3 $\pm$ 1.4%. These values are in agreement with values reported by other workers (4). Differential counts on four different MN preparations gave the following results: PMN 49 $\pm$ 6.9%, MN 49 $\pm$ 6.9%, lymphocytes 2 $\pm$ 0.3%.

Oxygen uptake was measured by the conventional Warburg technique at 37° with air as the gas phase. For anaerobic studies, oil-pumped nitrogen was used for the gas phase. Leukocytes (about 10^8 cells/flask, were incubated in Krebs-Ringer phosphate medium, pH 7.4, modified to contain one-half the usual calcium concentration (5). Glucose, when added, was present in a final concentration of 5.6 μmoles/ml. Most of the experiments involved the use of a 15% solution of

human serum albumin dissolved in the Krebs-Ringer phosphate medium and adjusted to pH 7.4 with 1 N NaOH. In each vessel, 1 ml of this albumin solution (with or without palmitate) was added from the side arm of the Warburg flasks to 2 ml of the usual medium in the main chamber. In the studies of phagocytosis, polystyrene particles (9 $\times$ 10^9 particles/flask) were added from the side arm to the cell suspension. The various metabolic inhibitors employed in these studies were placed in the main chamber. In all experiments, the incubation medium had a final volume of 3.0 ml. The center wells of the flasks contained 0.2 ml of 20% KOH and a filter paper fan. For studies involving the use of KCN, the center wells were prepared as described by Robbie (6). The oxygen uptake after 1 hr of incubation was used to calculate the respiratory rates.

Studies on the oxygen uptake of guinea pig circulating blood leukocytes were carried out according to the method of Strauss and Stetson (7). Two milliters of blood, obtained by cardiac puncture with a heparinized syringe, were added to the main chamber of the Warburg vessel and 1 ml of 15% albumin with or without palmitate was added to the side arm. The center well contained 0.2 ml of 5% KOH. The blood was equilibrated with room air by shaking in the respirometer at 37° for 90 min prior to the measurements of leukocyte oxygen uptake. The pH of the blood remained constant at 7.5 during the course of these experiments. The average leukocyte count was 5.5 $\times$ 10^6 cells/ml blood and the mean ($\pm$ SE of the mean) differential count of three different blood samples was as follows: PMN 53 $\pm$ 7%, lymphocytes 43 $\pm$ 6%, and MN 4 $\pm$ 1.

Chemical determinations were carried out on suitable aliquots of supernatant fluid obtained by centrifugation of the contents of flasks removed at the beginning and at the end of a 2-hr incubation period. The difference between the initial and final analyses represented the amount of substrate utilized by the cells. FFA were determined titrimetrically as described previously (8), and glucose by the method of Somogyi and Nelson (9, 10).

Human serum albumin essentially free of FFA was prepared according to the method of Goodman (11). Sodium palmitate solutions were prepared as follows: 460 mg palmitic acid was dissolved in 5 ml absolute ethanol, titrated with 1 N NaOH to pH 7.0, and then evaporated to dryness under nitrogen at 50°. The residue of sodium palmitate was mixed thoroughly with 100 ml of 15% FFA-free human serum albumin (dissolved in Krebs-Ringer phosphate medium) and heated to 50° on a hot-water bath for 15–20 min. After standing overnight in a refrigerator, the solution was

TABLE 1. EFFECT OF VARYING PALMITATE CONCENTRATION ON OXYGEN AND PALMITATE UPTAKE*

Medium Palmitate (μmole/ml)	Oxygen Uptake (μl/hr/mg dry wt)	Palmitate Uptake (μmole/hr/10 mg dry wt)
0	3.1	—
0.54	3.1	0
1.08	3.2	0
1.62	3.2	0.27
2.14	3.4	0.31
2.70	4.4	0.47
3.24	4.5	0.53
3.75	5.3	0.56
4.30	6.9	0.67
4.81	8.9	1.03
5.36	9.6	1.30

* PMN 80%, MN 17%, lymphocytes 3%. Initially 5.6 μmoles glucose/ml medium were present. The results are the average of three experiments on three different leukocyte preparations.
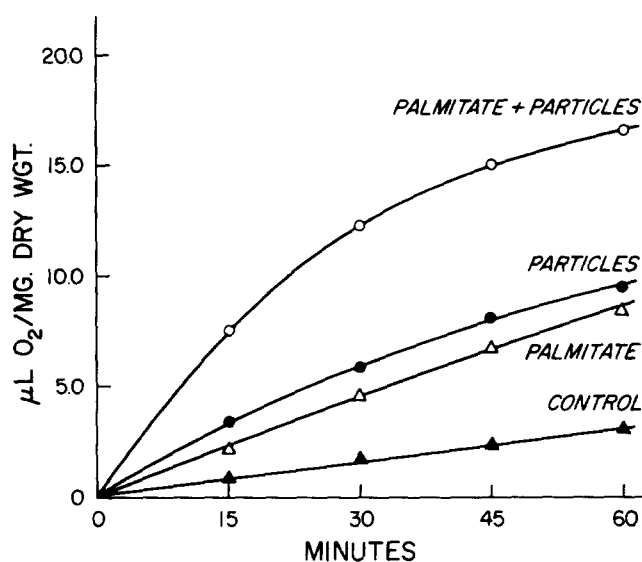

FIG. 1. Effect of palmitate, polystyrene particles, and palmitate plus polystyrene particles on oxygen uptake by leukocytes from guinea pig exudate (80% PMN, 17% MN, and 3% lymphocytes). All vessels contained 5% human albumin. Initially 4.4 μmoles palmitate and 5.6 μmoles glucose/ml medium were present. The results are expressed as the average of two experiments on two different leukocyte preparations.

filtered through Whatman No. 1 filter paper. Any microcrystals of sodium palmitate remaining in the solution after filtration were removed by centrifugation at 26,000 g for 30 min in a Servall refrigerated centrifuge. The final pH was 7.4 and the final concentration of palmitate varied between 15–18 mEq/liter.

Human serum albumin was kindly supplied by the American Red Cross. Reagent grade palmitic acid was obtained from Fisher Scientific Co., Fairlawn, N. J. Polystyrene particles (1.171 μ diam) were obtained from the Dow Chemical Co., Midland, Mich. Oil-pumped nitrogen was obtained from the Linde Co., Essington, Pa.

The significance of differences between means of paired determinations was evaluated by Student's "t" test.

RESULTS

Effect of Palmitate on Oxygen Uptake, FFA Uptake, and CO₂ Production. Table 1 shows a marked increase in the oxygen consumption of exudate leukocytes, consisting chiefly of PMN, with the addition of palmitate at concentrations above 2.14 μmoles/ml. The respiratory pathway stimulated by palmitate was not saturated at the highest concentration of palmitate employed despite the fact that it was 2–5 times greater than the highest plasma concentrations of FFA usually encountered in physiological and pathological states (8).

The rate of palmitate uptake was observed to be roughly proportional to the concentration of palmitate in the medium above a palmitate concentration of 1.08 μmoles/ml. Microscopic examination of Giemsa-

stained smears of the leukocytes 2 hr after the addition of palmitate showed a marked depletion of the heterophilic granules of the PMN.

In five experiments on five different preparations of PMN, CO₂ production was determined by the direct method (5). After a 2-hr incubation period, the mean ($\pm$SE of the mean) CO₂ production by the cells in the absence and presence of palmitate was 3.0 $\pm$ 0.4 and 4.2 $\pm$ 0.1 μmoles/10 mg cell dry weight, respectively; the mean ($\pm$SE of the mean) oxygen uptake was 2.2 $\pm$ 0.2 and 5.2 $\pm$ 0.2 μmoles/10 mg cell dry weight, respectively. The stimulatory effect of palmitate on CO₂ production and O₂ uptake was found to be highly significant (p < 0.001) on the basis of Student's "t" test. In these experiments, with an initial palmitate concentration of 3.8 μmoles/ml medium, the mean ($\pm$SE of the mean) rate of palmitate uptake was 1.06 $\pm$ 0.14 μmoles/2 hr/10 mg cell dry weight. The mean ($\pm$SE of the mean) RQ in the absence of palmitate was 1.34 $\pm$ 0.06 and in the presence of palmitate was 0.82 $\pm$ 0.03.

Comparison Between the Effect of Palmitate and Polystyrene Particles on Respiration and Glucose Uptake. As shown in Fig. 1, the oxygen uptake of guinea pig exudate leukocytes (80% PMN) was approximately tripled in the presence of albumin-bound palmitate or during phagocytosis of polystyrene particles. When such cells were exposed to palmitate and particles simultaneously,

TABLE 2. Effect of Inhibitors on Palmitate Metabolism in Leukocytes*

Conditions	Oxygen Uptake (µl/hr/mg dry wt)		Glucose Uptake (µmole/hr/10 mg dry wt)		Palmitate Uptake (µmole/hr/10 mg dry wt)
Palmitate	−	+	−	+	+
Control	2.4 ± 0.4	4.6 ± 1.1†	2.8 ± 0.2	2.9 ± 0.2	0.87 ± 0.20
KCN (10^{-3} M)	1.4 ± 0.3	4.3 ± 1.0†	3.0 ± 0.3	3.0 ± 0.2	0.85 ± 0.24
NaF (2×10^{-2} M)	6.7 ± 0.4	6.8 ± 0.3	0.2 ± 0.1	0.2 ± 0.1	0.18 ± 0.09‡
IAA (10^{-3} M)	1.5 ± 0.1	1.4 ± 0.1	0	0.1 ± 0.1	0.12 ± 0.08§

* PMN 80%, MN 17%, lymphocytes 3%. Initially 4.5 µmoles of palmitate and 5.6 µmoles glucose/ml medium were present. The results are expressed as the mean and standard error of the mean of three experiments on three different leukocyte preparations.

† $p < 0.05$, probability value for % differences between oxygen uptake in the presence and absence of palmitate.

‡ $p = 0.01$ } probability values for % decrease from control.
§ $p = 0.001$

the increment in oxygen uptake was approximately equal to the sum of the increments due to palmitate and particles when added separately. Preliminary studies on the effect of particle concentration indicated that the respiratory increment was maximal at the concentration of particles employed in this study (9×10^9 particles/flask). Either in the presence or absence of palmitate, 80–90% of the cells had phagocytized particles too numerous to count under the microscope. Glucose uptake (2.0 µmoles/hr/10 mg dry weight) was not affected by palmitate but was decreased by 21% in the presence of particles after 2 hr of incubation time. The addition of particles plus palmitate caused a 40% inhibition of glucose uptake. Palmitate uptake (0.61 µmoles/hr/10 mg dry weight) was unaffected by the presence of particles.

The Effect of Inhibitors on Palmitate Metabolism. The mean results of three studies of the effect of various metabolic inhibitors on the uptake of oxygen, glucose, and palmitate by exudate leukocytes (80% PMN) are shown in Table 2. Potassium cyanide had no significant effect on palmitate or glucose uptake. Although

cyanide depressed oxygen uptake in the control flasks as expected (12), the effect of cyanide on oxygen uptake was abolished in the presence of palmitate. Palmitate and glucose uptake were markedly inhibited by the addition of sodium fluoride or sodium iodoacetate (IAA). In the presence of NaF and IAA, no increase in oxygen uptake was observed after the addition of palmitate.

Effect of Glucose, Dinitrophenol, and Anaerobiosis on Palmitate Metabolism. Table 3 shows the mean results of three studies in which metabolism by guinea pig PMN (80%) in the presence and absence of palmitate was studied under various incubation conditions. The removal of glucose from the incubation medium had no significant effect on palmitate uptake or on oxygen uptake in the presence of palmitate. In the absence of palmitate, a 63% increase in oxygen uptake (Crabtree effect) was noted when glucose was removed from the medium.

Dinitrophenol (10^{-4} M) produced no change in palmitate or glucose uptake and no effect was observed on respiration in the presence and absence of palmitate

TABLE 3. Effect of Glucose, Dinitrophenol, and Anaerobiosis on Metabolic Activities of Leukocytes*

Conditions			O₂ Uptake (µl/hr/mg dry wt)		Glucose Uptake (µmole/hr/10 mg dry wt)		Palmitate Uptake (µmole/hr/10 mg dry wt)
Aerobic	Glucose	10^{-4}M DNP	Control	Palmitate	Control	Palmitate	
+	+	−	2.4 ± 0.3	6.1 ± 0.5†	2.9 ± 0.1	2.9 ± 0.4	0.97 ± 0.27
+	−	−	3.9 ± 0.4	6.1 ± 0.7‡	—	—	0.83 ± 0.27
+	+	+	2.3 ± 0.2	6.6 ± 0.8§	2.9 ± 0.4	2.9 ± 0.5	1.00 ± 0.23
−	+	−	—	—	4.4 ± 0.5	4.1 ± 0.5	0.92 ± 0.27

* PMN 80%, MN 17%, lymphocytes 3%. Initially 4.6 µmoles palmitate and 5.6 µmoles glucose/ml medium were present. The results are expressed as the mean and standard error of the mean of three experiments on three different leukocyte preparations.

† $p < 0.005$
‡ $p < 0.001$ } probability values for % differences between oxygen uptake in the presence and absence of palmitate.
§ $p = 0.01$

TABLE 4. COMPARISON OF GUINEA PIG EXUDATE PMN AND MN WITH RESPECT TO OXYGEN, GLUCOSE, AND PALMITATE UPTAKE*

Dry Weight (%)		O₂ Uptake (μl/hr/mg dry wt)			Glucose Uptake (μmole/hr/10 mg dry wt)			Palmitate Uptake (μmole/hr/10 mg dry wt)
PMN	MN	Control	Albumin	Palmitate	Control	Albumin	Palmitate	
77	23	2.3 ± 0.3	2.6 ± 0.3	5.2 ± 0.7†	2.9 ± 0.2	3.3 ± 0.4	3.3 ± 0.3	0.83 ± 0.04
41	59	4.6 ± 0.4	5.0 ± 0.4	7.6 ± 0.4‡	5.5 ± 0.3	5.1 ± 0.5	6.6 ± 0.3	1.41 ± 0.24
100 (calc.)		0.8 ± 0.4	1.1 ± 0.2	3.8 ± 1.2§	1.2 ± 0.3	2.2 ± 0.8	1.3 ± 0.5	0.48 ± 0.11
	100 (calc.)	7.2 ± 0.4	7.8 ± 0.6	10.1 ± 1.0	8.8 ± 1.0	6.8 ± 1.0	10.2 ± 0.7	1.93 ± 0.31‖

* The dry weight ratios of 77% PMN/23% MN and 41% PMN/59% MN correspond to differential count ratios of 80% PMN/17% MN and 49% PMN/50% MN, respectively. Initially 4.6 μmoles palmitate and 5.6 μmoles glucose/ml medium were present. The results are expressed as the mean and standard error of the mean of four experiments on four different leukocyte preparations.

† $p < 0.005$ ⎫
‡ $p < 0.01$ ⎬ probability values for % differences between albumin and palmitate.
§ $p < 0.05$ ⎭
‖ $p < 0.02$, probability value for % difference between MN and PMN.

In a preliminary study, the effect of dinitrophenol (DNP) at concentrations varying from 10^{-4} to 10^{-3} M was studied. The results obtained with 10^{-4} M DN were essentially the same as those obtained with higher concentrations except that at 4×10^{-4} M and 8×10^{-4} M DNP there was a 36 and 38% stimulation of palmitate uptake, respectively.

The glucose uptake of cells incubated under anaerobic conditions was 52% greater than that of cells incubated under aerobic conditions (Pasteur effect). No significant difference in palmitate uptake under these conditions was observed, however.

Comparison Between the Metabolic Activities of PMN and MN. By comparing the metabolic activities of leukocytes obtained from 18-hr (80% PMN, 17% MN) and 65-hr (49% PMN, 49% MN) exudates, it was possible, using simultaneous equations, to make inferences regarding the metabolic properties of the individual cell types. Similar calculations have been used by other workers in studies on human blood leukocytes (13). In order to simplify the calculations, it was assumed that the total activity of the small number of lymphocytes (2–3%) present in these preparations was negligible in comparison to that of the PMN and MN. Data reported by Antonioli (14) indicate that the various cell types do not affect the metabolic activities of each other in mixed suspensions. It was also assumed that the metabolic properties of PMN and MN were the same in 18-hr and 65-hr exudates.

The effects of the addition of albumin and of albumin-bound palmitate on the oxygen and glucose consumption of PMN and MN exudate leukocytes are estimated in Table 4. Cells incubated in ordinary Krebs-Ringer

phosphate medium containing glucose served as controls in these studies. The values shown in the first two rows represent the data obtained by experiment and the values in the last two rows represent the calculated values for the metabolic activities of "pure" PMN and MN. For the dry weights of "pure" PMN and MN, values of 1.26 and 1.88 mg/10⁷ cells, respectively, were used (4).

In agreement with reports by other workers (4), MN were much more active metabolically than PMN. Oxygen and glucose uptake were about seven times greater and palmitate uptake was about four times greater in MN than in PMN. Palmitate increased the oxygen uptake of PMN 245%. The mean increase in oxygen uptake by MN after palmitate addition was about equal to that observed with PMN but, on the basis of four experiments, this increase was not statistically significant ($p > 0.05$). Albumin had no significant effect on the oxygen uptake of PMN and MN. Neither palmitate nor albumin had any significant effect on glucose uptake by PMN and MN.

Effect of Palmitate on Guinea Pig Circulating Leukocytes. In order to determine whether or not guinea pig circulating leukocytes responded to the addition of FFA in a manner similar to exudate leukocytes, studies of the oxygen and FFA uptake by leukocytes in whole blood were carried out. In three separate experiments on blood drawn from three different animals, the mean (± SE of the mean) oxygen uptake of the leukocytes after the addition of albumin was 35 ± 5 μl/hr/10⁷ cells. In the presence of palmitate (final concentration: 5.7 μmoles/ml), the mean (± SE of the mean) oxygen uptake was 40 ± 6 μl/hr/10⁷ cells. The difference between

these two rates was not statistically significant (p > 0.05). No net uptake of added FFA could be detected in these studies.

DISCUSSION

The present study indicates that PMN and MN obtained from inflammatory exudates readily ingest palmitate in the presence of physiological concentrations of albumin. Our data show that with increasing palmitate in the medium, there is a concomitant increase in oxygen and palmitate uptake and in CO_2 production. On the basis of studies of the effect of increasing palmitate concentration on the oxidation of palmitate-1-C^{14} to $C^{14}O_2$ in the striated muscle of the rat, Eaton and Steinberg (15) have proposed that the regulation of serum concentrations of FFA may be of central importance in determining over-all rates of body metabolism.

A correlation between rate of FFA uptake and concentration of FFA in plasma or medium *in vivo* and *in vitro* has been reported. Armstrong *et al.* (16) concluded from studies of plasma FFA turnover in the dog that, under a variety of conditions, changes in FFA uptake are simple mass-action effects of changes in FFA concentration. In studies of arteriovenous differences of FFA in the dog kidney, Hohenleitner and Spitzer (17) observed that the degree of FFA removal was proportional to the arterial concentration of the metabolite. Similar observations were made with regard to hepatic FFA uptake in studies by Fine and Williams (18). The conversion of palmitate-1-C^{14} to neutral lipid of striated muscle *in vitro* has also been reported to increase with increasing concentration of palmitate in the medium (15, 19). Similar relationships between FFA concentration and FFA uptake have been reported for adipose tissue (20) and cardiac muscle (21, 22). As our data indicate, however, the generality of this relationship breaks down with respect to circulating blood leukocytes since we were unable to detect any FFA uptake by these cells *in vitro*. Whether or not circulating leukocytes take up FFA from the blood *in vivo* remains to be determined.

On the basis of the RQ value of 0.82, obtained after the addition of palmitate to PMN, it is highly probable that the increments in O_2 uptake and CO_2 production are due primarily to the oxidation of FFA. This conclusion is supported by the work of Elsbach (1) who has observed that C^{14}-labeled FFA are oxidized to $C^{14}O_2$ by rabbit exudate PMN. From the data obtained in the experiments on CO_2 production, it may be calculated that after 2 hr the total increment in CO_2 production due to palmitate addition was 1.2 μmoles/10 mg cell dry weight. This amount of CO_2 would be equivalent to

0.08 μmole palmitate. The total uptake of palmitate during this period was 1.06 μmoles/10 mg cell dry weight. If we assume that the entire increment in CO_2 production was due to the oxidation of palmitate, we calculate that 8% of the palmitate taken up was oxidized.

Elsbach (1) has reported that FFA are rapidly incorporated into the cell lipids of rabbit exudate PMN. If esterification as triglyceride is important in the uptake of palmitate, then the ATP required for the formation of triglyceride could come either from glycolysis or from oxidative phosphorylation. That the latter process is not required for palmitate uptake is indicated by the data obtained from the anaerobic and dinitrophenol studies. The maintenance of an adequate glycolytic rate appears to be required for palmitate uptake since the potent glycolytic inhibitors, sodium fluoride and iodoacetate, almost completely inhibited the uptake of palmitate. Removal of glucose from the medium had little effect, however, which suggests that the slow rate of glycolysis of glucose-6-phosphate derived from glycogen in these cells (23) is sufficient to allow palmitate uptake to occur at a rate similar to that observed in the presence of glucose.

In evaluating the effect of palmitate on leukocyte respiration, a comparison can be made with the effects of phagocytosis. It would appear that the palmitate effect is not due to phagocytosis of a high molecular weight palmitate-albumin complex since the palmitate effect on respiration was additive to the effect due to phagocytosis when the latter occurred maximally.

The cyanide studies indicate that the respiratory increment due to palmitate is not mediated by cytochrome oxidase. A cyanide-insensitive respiratory pathway has also been reported to be stimulated during phagocytosis (12). In a previous study (23, 24), the presence of an oxidase for DPNH and TPNH, which functions in the presence of high concentrations of cyanide, was demonstrated in guinea pig exudate leukocytes. This oxidase apparently is released by lysis from the specific granules of the polymorphonuclear leukocyte during phagocytosis (23, 24, cf. 25). A depletion of these specific granules was also noted in the present study following the addition of palmitate. In view of these findings, an explanation for the stimulation of oxygen uptake by palmitate might be that FFA oxidation stimulates the reduction of DPN to DPNH (26, 27), which in turn stimulates oxygen uptake via the cyanide, insensitive DPNH oxidase.

Certain findings in the present study also suggest possible differences between the effects of phagocytosis and those of palmitate. Data obtained by Sbarra and Karnovsky (12) indicate that cyanide partially inhibits

the respiratory increment during phagocytosis. Our data show, however, that the respiratory increment with palmitate was greater in the presence of cyanide than in the absence of this inhibitor. The effect of cyanide on oxygen uptake was abolished in the presence of palmitate. A further difference is the absence of a Crabtree effect in the presence of palmitate, whereas a Crabtree effect has been observed in phagocytizing cells (12).

Our finding that circulating leukocytes differ from exudate leukocytes with respect to their metabolic behavior in the presence of palmitate supports the recent work of Antonioli (14), who has found differences in glycolytic, proteolytic, and lipolytic activities between such cells. At present, however, the reason for these differences remains uncertain.

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