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Research Article

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Energy Substrate Metabolism in Fresh and Stored Human Platelets

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ABSTRACT The latent capacity of human platelets for oxidizing several important energy-yielding substrates has been revealed by hypoosmolar incubation conditions.

The data show that the human platelet has a considerable capacity to oxidize both glucose and long-chain fatty acids. Long-chain fatty acids appear to rank favorably with glucose as a potential energy substrate. In a number of mammalian tissues, (–)-carnitine serves to regulate the rate at which long-chain fatty acids are oxidized. Evidence was obtained which suggests that (–)-carnitine functions in a similar role in the platelet.

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INTRODUCTION

Human platelets are readily obtainable in large quantities as suspensions of intact, discrete cells. As such, they are eminently suitable models with which to study the control that permeability of the outer cell membrane exerts on mitochondrial function. Obviously, mitochondria which do not "see" substrates cannot utilize them. Likewise, an enormous reserve capacity of mitochondria for energy substrate utilization may only be revealed if the outer membrane and then the mitochondrial membrane are altered so as to admit larger-than-usual amounts of a given substrate.

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It has been shown that isolated liver mitochondria are relatively impermeable to certain molecules (1–4) for which there exist specific oxidative enzymes. The latter have been called latent mitochondrial enzymes (2), i.e., their function is best studied only when the intact mitochondrial membrane has been mechanically disrupted, treated with detergents, or exposed to hypotonic media. The latter would seem to us to be one of the more physiological means by which to probe for latency.

By exposing intact human platelets to hypoosmotic conditions we have revealed their latent capacities for handling certain energy-yielding substrates. Beginning at levels of hypotonicity which are clinically compatible with life, we have found a marked enhancement of the platelets' capacity to oxidize pyruvate, succinate, and glucose, and a parallel reduction in their ability to oxidize long-chain fatty acids. Added (–)-carnitine was found to correct the deficit in fatty acid oxidation and to simultaneously blunt the augmentation of glucose oxidation induced by hypotonic exposure.

Using these observations as a starting point we have shown that (–)-carnitine plays a role in regulating the rate at which long-chain fatty acids are oxidized by human platelets, and that long-chain fatty acids rank favorably with glucose as a potential energy substrate for the platelet.

Extension of these investigations on energy substrate utilization to stored platelets revealed that the oxidation of long-chain fatty acids is considerably impaired by storage of platelets at 4°C for 24 hr, a defect which cannot be corrected by added (–)-carnitine. Concomitant reductions in oxidative activity towards glucose, pyruvate, and succinate were also shown after the same storage interval. Collectively, these observations suggest that storage of platelets at 4°C results in severe impairment of their respiratory capacity.

METHODS

Preparation of platelet suspensions. For each study, 500 ml of whole blood was freshly drawn from each of two fasting donors using acid-citrate-dextrose (ACD) anticoagulant and a plastic bag system.¹ The blood was centrifuged at 250 g for 15 min after which the upper three-fourths of the platelet-rich plasma (PRP) was delivered into a dry 300 ml plastic transfer pack. Excess ACD, 1 ml for each 6 ml of PRP, was then added to prevent platelet clumping during resuspension of the platelet pellet (5).

The PRP-ACD mixture was centrifuged at 150 g for 10 min to sediment erythrocytes. The PRP was separated from the erythrocytes and the centrifugation for removal of erythrocytes was repeated until the sediment was completely white. The PRP was then centrifuged at 1000 g for 30 min to obtain a platelet pellet. The latter was washed by being resuspended in a mixture of 19 ml of 300 mOsm potassium phosphate buffer, pH 7.3, and 1 ml ACD solution, and finally resuspended in 5 ml of fresh wash medium. The entire separation procedure was carried out at 0°–4°C, and the final platelet suspension was kept in the plastic bag in a container of ice until used. All studies were started within 6 hr of the phlebotomies.

For experiments in which low glucose concentrations were required, glucose-free citric acid-citrate solution (ACD minus glucose) was substituted for the extra ACD solution added to both the PRP and the phosphate buffer.

Leukocyte and erythrocyte counts were done by standard procedures; platelet counts, by the method of Brecher and Cronkite (6); protein determinations, by the biuret method (7). The platelet suspensions contained less than 50 white blood cells or 500 red blood cells/mm³. The average platelet count was 6×10^8 /mm³. Each milligram of platelet protein was equivalent to approximately 5×10^8 platelets.

Assays. The oxidation rates of ¹⁴C-labeled substrates by platelet suspensions were assessed by the method of Snyder and Godfrey (8). Incubations were carried out in a shaking water bath at 37°C.

In assessing glucose oxidation, the initial specific activity of the glucose in the reaction mixtures was determined by measuring the glucose concentration in the platelet suspension immediately before incubation, and by adding known amounts of glucose-¹⁴C to the incubation flasks. Time course experiments were performed to determine whether the glucose specific activity changed under the various experimental conditions used. Since the reaction rates were linear for as long as 4 hr and the ratios of ¹⁴CO₂ production remained constant between the various experimental conditions, it was considered that significant glycogenolysis with pool dilution did not take place during the incubation. Glucose concentration in the reaction mixtures, as measured by the glucose oxidase method (9), was 5–6 mmoles/liter when platelets were prepared with ACD and 0.5–1.5 mmoles/liter when prepared with glucose-free citric acid-citrate solution.

Changes of the endogenous free fatty acid concentration in the platelets during storage or incubation could likewise change the specific radioactivity of the oxidizable fatty acid pools. This possibility was assessed by measuring the free fatty acid levels in fresh and stored platelets, and by carrying out time course studies. Washed heptane extracts of platelets prepared by the procedure of Trout, Estes, and Friedberg (10) were analyzed for free fatty acid content by the titrimetric procedure of Dole and Meinertz (11). In

both fresh and stored platelets, the fatty acid levels ranged from 13 to 21 μmoles/mg protein (mean 17.2 ± 1.8 SE). Calculation of the effect of small and variable increases in fatty acid levels after storage of any single batch of platelets indicated that under the assay conditions used, the maximal decrease in initial specific radioactivity was 4.1% in 24 hr and 7.5% in 48 hr. By comparison with the much greater per cent decreases in the rates of oleic acid-¹⁴C oxidation after storage of the platelets, the small changes in specific radioactivity of the fatty acid pool were considered negligible. Changes in the specific activity of the fatty acid pool during incubation periods were excluded by the linearity of the reaction rates for as long as 4 hr, in both fresh and stored platelets.

Substrate oxidation varied linearly with platelet protein concentration of up to 2.5 mg/ml of reaction mixture.

The rate of incorporation of oleic acid-¹⁴C into phospholipid was determined by taking 1-ml aliquots of the reaction mixture at the termination of the oxidation assay and extracting them by the method of Blich and Dyer (12). Lecithin was separated by thin-layer chromatography and assayed for radioactivity as reported previously (13).

When the effect of medium osmolarity on platelet metabolism was investigated, the osmolarity of the reaction mixtures was varied by using appropriate mixtures of 300 and 600 mOsm KCl-Tris, pH 7.5, and distilled water. Final osmolarities were measured by the vapor pressure method at 37°C.

When the effect of storage on platelet metabolism was studied, the platelet suspensions were stored in plastic bags at 4°C, and aliquots were removed as required.

Radioactivity was measured in a liquid scintillation spectrometer using a fluor containing 2,5-diphenyloxazole (PPO, 5 g/liter) and 1,4 bis-2-(methyl-5-phenyloxazolyl)benzene (POPOP, 0.3 g/liter) in toluene. Correction for quenching was carried out by the channel ratio method according to Bruno and Christian (14).

Substrates. Radioactive compounds, oleic acid-¹⁴C, glucose-U-¹⁴C, pyruvate-¹⁴C, and succinate-¹⁴C were purchased.² As determined by gas-liquid and thin-layer chromatography the oleic acid was at least 98% radio and chemically pure. Adenosine triphosphate (ATP),³ CoASH,⁴ and (–)-carnitine,⁵ also were purchased. (+)-carnitine was prepared by the method of Strack and Lorenz (15). All other chemicals were reagent grade.

RESULTS

Energy substrate utilization. The capacity of platelets to oxidize long-chain fatty acids and glucose, as is shown in Fig. 1, is in accord with the observations of other investigators (16–20).

Since, in nondisrupted cell systems, access of substrate to intracellular activating and oxidizing enzyme sites might be hindered by surface membrane barriers, the maximal oxidative capacities of the intact cell may not be revealed in the usual in vitro assay system. Based on the premise that changes in the osmotic pressure of extracellular fluid may unmask the activity of

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¹ Baxter-Fenwal, supplied by Europarenteral S.A., Brussels, Belgium.

latent intracellular enzymes (1-4), the platelets were exposed to varying osmolaric conditions during the oxidative assay to determine if such modification would affect the oxidation rates of oleic acid and glucose. Stepwise reduction of the medium osmolarity from 350 to 100 mOsm effected a progressive fall in the rate of oleic acid-1-¹⁴C oxidation from a level of 10.8 ± 0.5 mμmoles/4 mg protein per 3 hr at 300 mOsm to 3.3 ± 0.4 mμmoles at 200 mOsm, and even lower as the medium osmolarity was further decreased (Fig. 1). In sharp contrast, the oxidation rate of glucose-U-¹⁴C rose from 22.0 ± 1.3 to 45.0 ± 1.8 mμmoles/4 mg protein per 3 hr as the medium osmolarity was reduced from 300 to 200 mOsm. Further decrease in the osmolarity, however, resulted in a precipitous decline in glucose oxidation to less than control levels.

Pyruvate oxidation followed the pattern of glucose oxidation at various levels of osmolarity. However, suc-

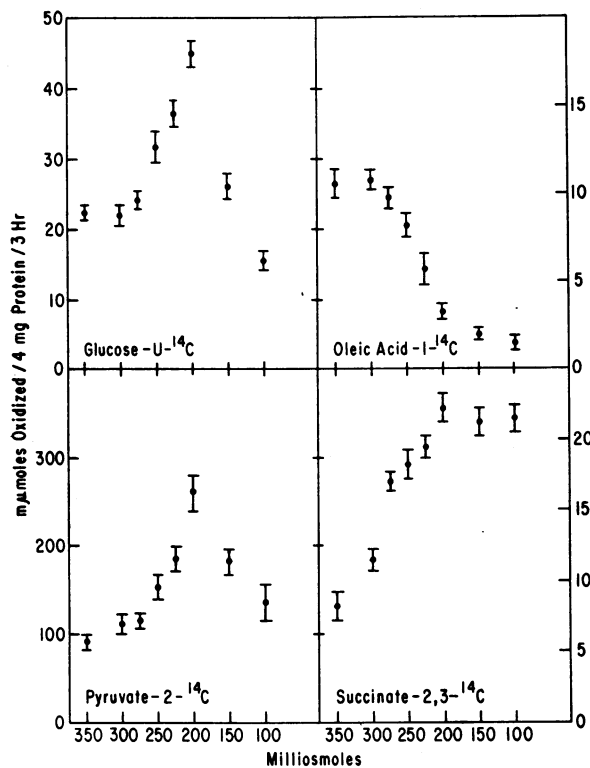


FIGURE 1 Effect of osmolarity on glucose, oleate, pyruvate, and succinate oxidation by platelets. Each reaction mixture contained 4 mg of platelet protein in potassium phosphate-ACD, glucose-U-¹⁴C, 12 μmoles (1.5 μc/mmole), oleic acid-1-¹⁴C, 40 mμmoles (3.6 μc/μmole), pyruvate-2-¹⁴C, 2 μmoles (77.5 μc/mmole), or succinate-2,3-¹⁴C, 2 μmoles (42 μc/mmole), and KCl-Tris buffer, pH 7.5, adjusted to the osmolarity indicated. Final reaction volume was 2.0 ml. Incubations were at 37°C for 3 hr. Each point represents the mean value of four separate experiments and is given with the SEM.

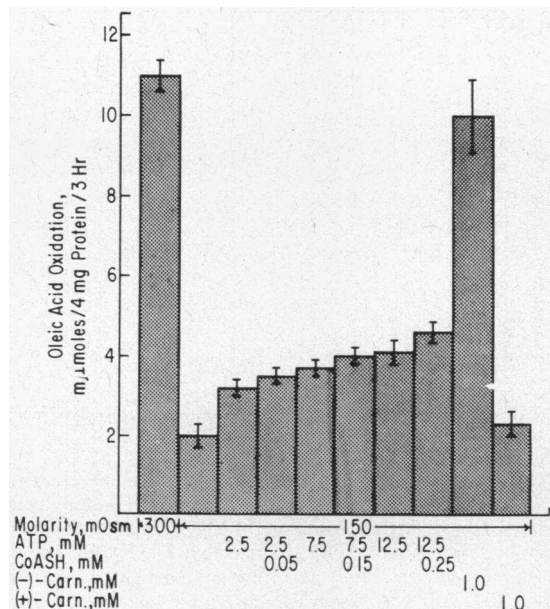


FIGURE 2 Restorative effect of carnitine, ATP, and CoASH on oleate oxidation of platelets in hyposmolar medium. Each reaction flask contained 4 mg of platelet protein in potassium phosphate-ACD, oleic acid-1-¹⁴C, 40 mμmoles, and ATP, CoA, or carnitine in the indicated concentrations. The osmolarity was adjusted with KCl-Tris buffer, pH 7.5. Final reaction volume was 2.0 ml. Incubations were at 37°C for 3 hr. Each bar represents the mean value of four separate experiments and is given with the SEM.

inate oxidation remained elevated above baseline levels even at the lowest levels of osmolarity.

For the metabolic conversion of long-chain fatty acids to CO₂, activation of the fatty acid to fatty acyl CoA, and transesterification of the latter with (-)-carnitine to form fatty acyl carnitine, appear to be prerequisite extramitochondrial events in many cells (21-24). Since the availability of the ATP, CoA, and carnitine necessary for these steps could have become suboptimal and thus rate limiting in oleate oxidation, consequent to the lowering of the medium osmolarity, experiments were conducted in which these components were supplied. As shown in Fig. 2, addition of ATP in final concentrations ranging from 2.5 to 12.5 mmoles/liter and CoA from 0.05 to 0.25 mmoles/liter effected only a small increment in the depressed oxidation rate of oleate evident at 150 mOsm. On the other hand, addition of (-)-carnitine resulted in virtually a total restoration of oleate oxidation to control levels. The specificity of the (-)-carnitine-effected restoration is supported by the complete ineffectiveness of the unnatural isomer (+)-carnitine under identical conditions.

In accord with the observations of Deykin and Desser (25), long-chain fatty acids were incorporated into platelet lecithin (Fig. 3). When the medium osmolarity

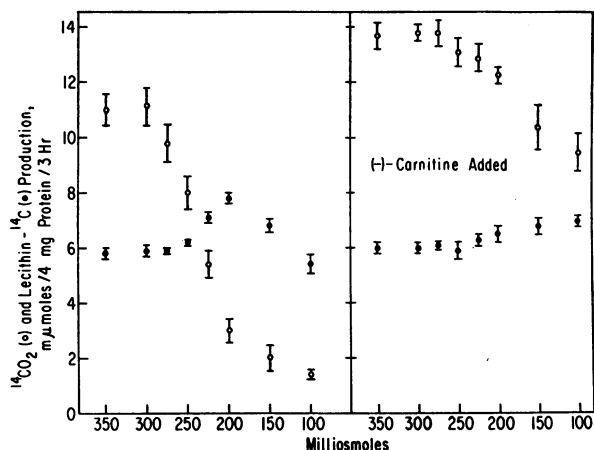


FIGURE 3 Effects of hypoosmolarity and of (-)-carnitine on the conversion of oleic acid-1-¹⁴C into CO₂ and lecithin. Each reaction mixture contained 4 mg of platelet protein in potassium phosphate-ACD, oleic acid-1-¹⁴C, 40 μmoles, and 2 μmoles of (-)-carnitine as indicated. KCl-Tris buffer, pH 7.5, was used to adjust the osmolarity of the reaction mixture. Final reaction volume was 2.0 ml. Incubation conditions were as given previously. After collection of ¹⁴CO₂, lecithin-¹⁴C was isolated and quantified as given in Methods. Each point represents the mean value of four experiments and is given with the SEM.

was decreased from 350 to 200 mOsm, the inhibition of oleate oxidation was accompanied by an increased rate of lecithin-¹⁴C formation. Thus, neither ATP nor CoA became rate limiting in long-chain fatty acid incorporation into platelet lecithin at the lowered osmolarity. Addition of (-)-carnitine to the medium, which prevented the marked inhibition of oleate oxidation at 200 mOsm, also prevented the rise in the rate of lecithin-¹⁴C synthesis. Thus, exogenous (-)-carnitine, but not ATP or CoA, possessed the capacity to maintain the metabolic functions of the platelet with respect to long-chain fatty acid oxidation and incorporation into lecithin under the adverse conditions brought about by a pronounced decrease in the medium osmolarity.

In a number of tissues, glucose and long-chain fatty acids have a sparing effect on the oxidation of each other (26). As shown in Fig. 4, under control osmolarity, each of these energy substrates had the capacity to spare conversion of the other to CO₂ by the order of 25–30%. Addition of (-)-carnitine accentuated the sparing effect on glucose by oleate, decreasing the oxidation rate of glucose to less than 50% of the control value. More strikingly, (-)-carnitine completely prevented the sparing effect of glucose on oleate oxidation.

When the medium osmolarity was decreased to 200 mOsm, glucose oxidation increased approximately 100%, and, as might have been anticipated, added oleic acid had a negligible sparing effect. Addition of (-)-carnitine, however, decreased the rate of glucose oxidation

by more than 50%. The potential of (-)-carnitine in enabling the oleic acid to spare glucose oxidation by the platelet is emphasized by the complete lack of an effect of glucose in modifying the rate of oleic acid oxidation at the lower medium osmolarity.

Metabolic activity of stored platelets. Storage has been demonstrated to have a detrimental effect on several aspects of platelet behavior: decreased survival *in vivo* (27), and decreased respiratory (28,29) and glycolytic (30) capacity. In accord with the previous investigations (28, 29) platelets kept at 4°C for 24 and 48 hr showed a decreasing capacity to oxidize oleic acid (Fig. 5).

Storage for 24 hr caused a decreased rate of oleate oxidation in 350–250 mOsm incubation media. However, in 200–100 mOsm media, its oxidation was more active after 24 hr storage than with fresh platelets, in the absence of added (-)-carnitine. Addition of (-)-carnitine to incubations of stored platelets permitted

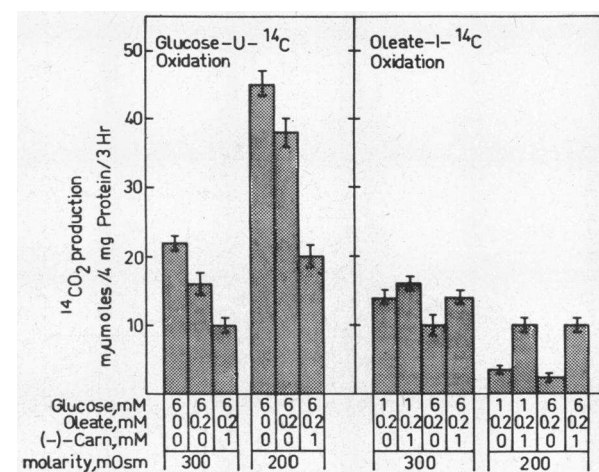


FIGURE 4 Comparative sparing effects of glucose and oleic acid oxidation in platelets. Each reaction mixture contained 4 mg of platelet protein in potassium phosphate-acid citrate, glucose-U-¹⁴C (3.9 μc/μmole), nonradioactive glucose, oleic acid-1-¹⁴C (29 μc/μmole), or nonradioactive oleic acid and (-)-carnitine (carn), all in the final concentrations indicated. The osmolarity was adjusted using KCl-Tris buffer, pH 7.5, and distilled water. Final reaction volume was 2.0 ml. Incubations were at 37°C for 3 hr. Means and standard errors were calculated from the data of four separate experiments. The *P* values were as follows (in parentheses): for the sparing effect of glucose on oleate oxidation at 300 mOsm without added (-)-carnitine (< 0.01) and with (-)-carnitine (0.02); for the stimulation of oleate oxidation by (-)-carnitine in glucose-poor media, at 300 mOsm (0.08), and in glucose-rich media (< 0.01); for the sparing effect of glucose on oleate oxidation at 200 mOsm without (-)-carnitine (0.08) and with (-)-carnitine (no significant difference); for the stimulation of oleate oxidation by (-)-carnitine in glucose-poor or in glucose-rich media (< 0.001); for all comparisons among the data for glucose oxidation (< 0.001).

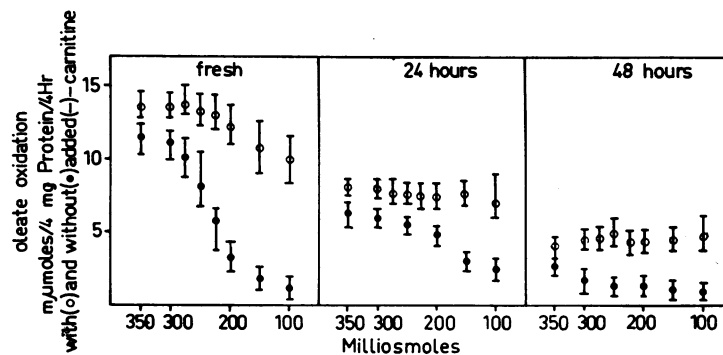


FIGURE 5 Effect of storage at 4°C on oleate oxidation by platelets. Each reaction mixture contained 4 mg of platelet protein in potassium phosphate-ACD, oleic acid-1-¹⁴C, 40 μmoles, and KCl-Tris buffer, pH 7.5, adjusted to the indicated osmolarity. Where indicated (-)-carnitine, 2 μmoles, was added. Final reaction volume was 2.0 ml. Incubations were at 37°C for 3 hr. Each point represents the mean value of four separate experiments and is given with the SEM.

the same rate of oleate oxidation to occur at all osmolarities, but did not restore the rate to that observed with fresh platelets.

However, there was a striking difference in the loss of glucose oxidation capacity at various levels of osmolarity (Fig. 6). After 24 hr storage, glucose oxidation was 80 and 49% of the control values at 300 and 200 mOsm, respectively. The larger decrement was found at 200 mOsm, where fresh platelets had shown the greatest increment in glucose oxidation. Thus, at 200 mOsm, stored platelets showed a similar loss of oxidative activity towards glucose as was observed with fatty acid at 300 mOsm. Nevertheless, after 24 hr storage, the rate of glucose oxidation in 200 mOsm media was the same as that found in fresh platelets in 300 mOsm media.

To define the basis of the apparent difference in stability between the oxidative pathways of oleate and glucose during storage, fresh and stored platelets were assessed in their capacity to oxidize pyruvate and succinate at various levels of osmolarity. Pyruvate oxidation fell to about 35%, and succinate to 50%, of the initial capacity after 24 hr; similar values were found with hypoosmolaric incubations (Fig. 6). These data suggest that pyruvate oxidative decarboxylation and Krebs cycle activity might be seriously impaired after 24 hr of storage at 4°C.

After an additional 24 hr of storage, further deterioration of oxidative capacity was evident. Only in the presence of exogenous (-)-carnitine could oleate oxidation be sustained at approximately one-third of its capacity in the fresh platelet, an oxidative level also retained by glucose.

The progressive decline in the oxidation rate of oleic acid shown by the platelets with storage (Fig. 5) is

analogous to that shown by fresh platelets exposed to increasingly hypoosmolaric conditions (Fig. 3). The analogy between these two states is also evident in the rates of lecithin-¹⁴C synthesis in that, as with hypoosmoticity (Fig. 3), storage conditions were associated with an increase in the rate of oleate incorporation into lecithin (Fig. 7). Since exogenous ATP had some restorative effect on oleic acid oxidation under marked hypoosmolarity (Fig. 2), the effect of ATP on the severely depressed long-chain fatty acid oxidation of the stored platelet was also investigated. As shown in Fig. 7, exogenous ATP failed to have a beneficial effect on the oleic acid oxidation by stored platelets. On the other hand, the exogenous ATP did add to the increment of lecithin-¹⁴C synthesis exhibited by the stored platelet.

DISCUSSION

In 1951, Lehninger showed that mitochondria which had been exposed to hypotonic media during preparation and then restored to isosmoticity were better able to oxidize added NADH than those which had been prepared entirely in isotonic media (1). He attributed this finding to enhanced permeability of the mitochondrial membrane resulting from hypotonic exposure.

In 1963, Chappell and Greville wrote of "latent" mitochondrial enzymes, the activity of which could be unmasked by cell rupture, detergents, or hypotonic incubation media (2). They quoted work by Hogeboom and Schneider (3) and Bendall and de Duve (4) which indicated that glutamate dehydrogenase was released by mechanical disruption, but remained bound to particles after detergent or hypotonic treatment.

Chappell and Greville favored the notion that latency was probably "a consequence of impermeability of the

mitochondrial membrane," and that various types of mitochondrial swelling, hypotonic exposure among them, lead to activation of certain enzymes (2). They reasoned that distention of the membrane could allow for "freer passage of substrates, particularly those of small molecular weight."

In 1968, Rothstein again emphasized that swelling and shrinking due to changes in tonicity may change the permeability of the membrane, and that osmotic gradients can be used to increase the flow of water and solutes (31).

More recently, Haldar and Freeman found that the medium osmolarity affects amino acid incorporation into mitochondrial protein, the latter being inhibited with hyperosmoticity (32). They emphasized that this effect was not necessarily a consequence of altered permeability, but rather a direct inhibition at some stage of protein synthesis. Nevertheless, they did not completely exclude the role of increased permeability as an explanation for their findings.

All of the aforementioned authors did their work with isolated mitochondria. Our studies would appear to extend the concept of latency to the whole cell.

The intact platelet is known to behave as an osmometer (33), as has also been shown with intact liver mitochondria (34). It is to be expected, therefore, that

the swelling of platelet mitochondria will follow that of the whole cell, and that both will be sensitive to medium osmoticity.

The data obtained by exposing platelets to graded decrements of osmolarity have revealed certain metabolic capacities in fresh cells and metabolic deficits in stored cells, which hitherto have been less well defined.

Oxidizing capacity of fresh platelets. The major potential energy-yielding substrates utilized by human platelets appear to be glucose and long-chain fatty acids (16-20). In most tissues, the rate of usage of these substrates is subject to some form of regulatory control (35). In the case of glucose, considerable evidence supports the thesis that an insulin-responsive transport mechanism and the sensitivity of phosphofructokinase to cellular citrate levels exert a control on the rate at which glucose is oxidized (36).

Platelet glycolysis has been shown to be increased by insulin (18). However, there is conflicting evidence regarding the effect of citrate on platelet glycolysis, with both stimulatory and apparently inhibitory effects having been shown by Karpatkin (18) and Rossi (37), respectively.

As for long-chain fatty acids, an additional form of control appears to be operative. The rate at which long-chain fatty acids are utilized for oxidation has been

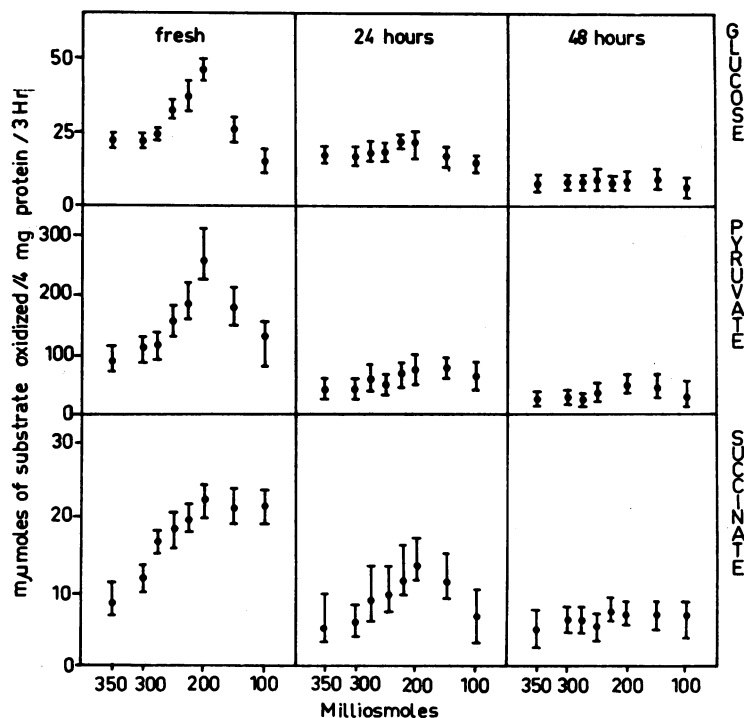


FIGURE 6 Effect of storage at 4°C on glucose, pyruvate, and succinate oxidation by platelets. The conditions were identical with those described in the legend to Fig. 1.

shown to be under the influence of (–)-carnitine (21–23), a constituent common to most, if not all, mammalian tissues (38, 39). It has been postulated that (–)-carnitine performs this regulatory function by serving as a carrier of activated fatty acyl groups across a mitochondrial barrier separating the sites of activation from those of oxidation (21–23). This carrier mechanism might also provide a means for translocating long-chain fatty acids across plasmalemmal barriers (40).

We have shown that the rate of glucose oxidation was enhanced almost 2-fold on decreasing the medium osmolarity from 300 to 200 mOsm. The oxidation rate of oleic acid, on the other hand, was markedly depressed under the same conditions, but could be fully restored to the level of the platelet oxidizing oleic acid under control osmolar conditions by the addition of (–)-carnitine to the hypoosmolaric medium. These observations suggest that whereas the total capacity of the platelet for oxidizing long-chain fatty acids is manifested under isosmotic incubation conditions, a substantial reserve for enhancing the rate of glucose oxidation may be unmasked by exposure to hypoosmoticity. A similar reserve capacity for substrate oxidation might be triggered into operation by the mechanical events of platelet agglutina-

tion and contraction, which appear to be ATP dependent, and are accompanied by an increased rate of energy substrate utilization (18).

Role of carnitine in the platelet. In view of the observations that glucose, pyruvate, and succinate oxidations were not inhibited at 200 mOsm, the component parts of the long-chain fatty acid pathway before the Krebs cycle were investigated in order to localize the metabolic basis of the impaired oleic acid oxidation under hypoosmolaric conditions. In the initial steps in long-chain fatty acid utilization, fatty acids are converted to CoA esters in the presence of ATP and CoASH (41). If the fatty acid is destined for oxidation, then the activated fatty acyl group can be transesterified in the presence of (–)-carnitine to form fatty acyl carnitine derivatives (21–23). The formation of these carnitine esters in some cells has been shown to be obligatory and rate controlling in long-chain fatty acid oxidation (24).

That ATP was rate limiting in oleate oxidation by the platelet in the hypoosmolaric medium appears unlikely for the following reasons: (a) glucose phosphorylation as indicated by oxidation was not impeded; (b) incorporation of oleate into lecithin, a reaction requiring prior activation of the fatty acid, was not depressed; and (c) ATP and CoASH, which are necessary for a carnitine-effected stimulation of long-chain fatty acid oxidation, were sufficiently abundant in these platelets to permit complete restoration of the depressed oleate oxidation on addition of (–)-carnitine to the hypoosmolaric medium. In addition, exogenous ATP in final concentrations ranging from 2.5 to 12.5×10^{-8} mole/liter with or without CoASH in final concentrations ranging from 5 to 25×10^{-5} mole/liter effected little or no stimulation of oleate oxidation. Since there is evidence, however, that nucleotides may be unable to penetrate into human platelets (42), this last observation can not be accepted as unqualified evidence related to intracellular ATP levels. It should be recalled, however, that hypotonically treated, hemolyzed erythrocytes have been shown to admit considerable amounts of ATP (43). Although we did not measure intracellular ATP levels, it is reasonable to propose that our 150 mOsm-exposed platelets allowed at least a part of the added ATP to pass through their membranes.

In striking contrast to ATP, exogenous (–)-carnitine under identical conditions resulted in a complete restoration in the oleate oxidation rate. (+)-carnitine, the unnatural isomer, was entirely without effect. Since carnitine is extremely water soluble and has a molecular weight comparable to glucose, the presence of sub-optimally effective levels of carnitine within platelets suspended in the hypoosmolaric medium might have been due to its equilibration between the intra- and

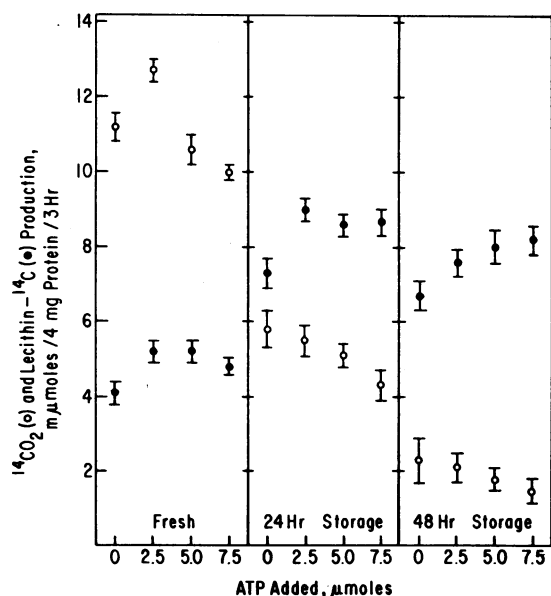


FIGURE 7 Restorative effect of ATP on oleic acid oxidation and incorporation into lecithin after storage at 4°C. Each reaction flask contained 4 mg platelet protein in potassium phosphate-ACD, oleic acid- ^{14}C , 40 μmoles , and ATP at the indicated amounts. KCl-Tris buffer, pH 7.5, was used to obtain an osmolarity of 300 mOsm. Final reaction volume was 2.0 ml and standard conditions of incubation were used. After collection of $^{14}\text{CO}_2$, lecithin- ^{14}C was isolated and quantified as given in Methods. Each point represents the mean value of four separate experiments and is given with the SEM.

extraplatelet space. These data are consonant with the proposal that (—)-carnitine participates in long-chain fatty acid oxidation in the human platelet as it has been demonstrated to do in a number of other mammalian tissues.

Metabolism of stored platelets. The effect of storage on oxygen consumption and glucose utilization by the platelet has been the subject of a number of investigations directed towards defining optimal conditions for platelet preservation. Thus, Estes, McGovern, Goldstein, and Rota (44) reported maintenance of normal levels of oxygen consumption, glucose utilization, and lactate production by platelets stored in whole blood at 4°C for as long as 3 wk. Campbell, Small, and Dameshek (28) and Yoshimura and Djerassi (29), on the other hand, maintained that oxygen consumption of stored platelets is severely depressed after storage. Under the preservation conditions used in the current study, glucose and oleate oxidation in isosmotic incubation media were reduced by 23 and 45%, respectively, after 24 hr of storage, and by 65 and 83%, after 48 hr. The concurrent reductions in the capacity of the stored platelet to oxidize pyruvate and succinate suggest that the defective oxidation of glucose and oleic acid could be attributed to lesions beyond glycolysis for the former, and in the Krebs cycle for the latter. A defect in the Krebs cycle is likewise consistent with the minimal effectiveness of (—)-carnitine in restoring the oleate oxidation rate. Likewise, exogenous ATP was completely ineffective in raising the depressed rate of oleate oxidation in the stored platelets.

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