Functional Utilization of Palmitate, Octanoate, and Glucose by the Isolated Rat Heart[1] (36993)

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The functional utilization of glucose to support cardiac contractility has been demonstrated in two ways: (a) the addition of glucose to isolated heart preparations, made hypodynamic by exposure to solutions deficient in energy-yielding substrates, resulted in a restoration of developed tension (1–3). (b) Such restoration of developed tension by glucose is prevented with known inhibitors of glucose metabolism (1, 3–7). However, it has not been established that fatty acids are functionally utilized by the heart although they are certainly metabolized by this tissue (8–12). Therefore, one purpose of this study was to determine the functional utilization of the fatty acids, palmitate and octanoate, by observing their effects on isolated perfused rat hearts made hypodynamic by exposure to substrate-free medium in the presence and absence of the metabolic inhibitor, delta-4-pentenoic acid.

Soloff and Wiedeman (13) infused palmitate intra-arterially into the wing of a bat and produced microscopically observable vasoconstriction. Severeid, Connor and Long (14) observed that stearic acid infusion into the perfused rabbit heart resulted in a marked increase in coronary resistance which was eliminated when the fatty acid was complexed with albumin. A further purpose of our investigation was to determine if such vasomotor changes could be elicited in the perfused rat heart upon the infusion of palmitate.

Methods. Male, Sprague-Dawley rats weighing 225–325 g were killed by decapitation. The hearts were rapidly excised and mounted on a modified nonrecirculating Langendorff perfusion apparatus (15). A silk suture was passed through the apex of the heart and the suture was attached to the lever arm of a Grass strain gauge to monitor developed tension. Perfusion pressure was measured by means of a Grass pressure transducer. The force of contraction and the perfusion pressure were recorded on a Beckman Offner recorder. The heart was subjected to a resting tension of 10 g throughout the experiment. Nichrome wire electrodes were placed in the atria and the heart was driven electrically at a rate of 180 beats/min with a Grass stimulator. The pacing voltage of 10 msec duration was set at 1.5 times the threshold voltage.

The perfusing medium was a modified Krebs-Henseleit solution (15), pH 7.4, containing (m*M*): NaCl, 120; KCl, 6; $MgSO_4 \cdot 7H_2O$, 1.34; $NaH_2PO_4 \cdot H_2O$, 1.21; $CaCl_2 \cdot 2H_2O$, 1.22; *d*-glucose, 5.56; and $NaHCO_3$, 25.3. The perfusate was filtered through a Millipore filter of 0.45 μm pore diameter prior to its use and remained clear. The perfusate was maintained at 30° and was bubbled with 95% O_2–5% CO_2. A continuous perfusion rate of 10 ml/min was achieved by means of a Harvard peristaltic pump.

The hearts were perfused for 60 min with the glucose-containing medium. During this time the hearts were equilibrated and the force of contraction was maximized. From 60 to 90 min the hearts were perfused with one of three solutions: (a) normal (glucose-containing) medium; (b) substrate-free (nonglucose-con-

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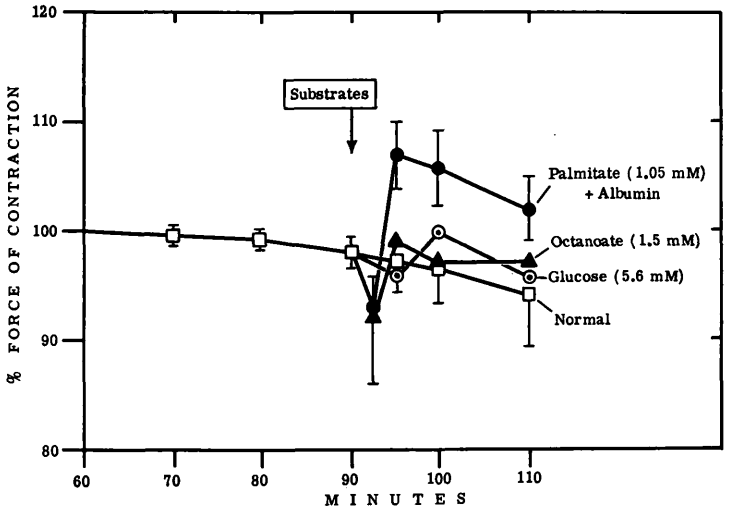

FIG. 1. The effects of substrates on the force of contraction of hearts perfused with normal medium. Normal refers to Krebs–Ringer bicarbonate medium containing 5.56 m*M* glucose. Substrates added at 90 min. Points shown represent mean ± SE of 4 experiments except for additional glucose curve which represents one experiment. In this and subsequent figures, points beyond 90 min are normalized to the 90 min value.

taining) medium; (c) substrate-free medium containing 0.25 m*M* delta-4-pentenoic acid. A two-way stopcock permitted easy switching from the normal medium to either of the other media. Then from 90 to 110 min the following substrates were infused: (a) 0.66–1.05 m*M* palmitate complexed to 1.0% bovine serum albumin (Sigma Fraction V); (b) 1.5 m*M* octanoate; or (c) 5.56 m*M* glucose. The palmitate was emulsified with the albumin and this emulsion was filtered through a Millipore filter prior to infusion. The titratable fatty acid concentration was determined by the method of Dole (16). The octanoate was dissolved in 2 *M* NaOH in a molar ratio of 1:1. The substrates were infused into the perfusion stream by means of a Harvard infusion pump at a rate of 1.0 ml/min. The perfusion rate was adjusted to 10 ml/min.

Results. In an effort to determine whether palmitate, octanoate, or additional glucose had any effect on the force of contraction of control hearts, these substrates were infused into hearts perfused with normal medium (Fig. 1). Neither glucose nor octanoate produced any appreciable change in the force of contraction from the force generated by those hearts perfused with normal medium. Palmitate produced a small (approx 10%) increase.

The effects of these same three substrates in hearts perfused with substrate-free medium are illustrated in Fig. 2. The force of contraction decreased during the 60–90 min time interval, presumably because the hearts were dependent solely upon endogenous rather than exogenous energy sources for supporting contractility. Palmitate, octanoate, and glucose produced similar positive inotropic effects in the hearts perfused only with substrate-free medium. The increase in the force of contraction observed upon the infusion of albumin may be the result of the 0.60 m*M* titratable fatty acid impurity which was found to be present in the 1% albumin solution. The enhancement of force by palmitate was then partially the result of this impurity, but the magnitude of the palmitate response indicates that the positive inotropic effect was largely the result of the palmitate.

Only octanoate effected an increase in the force of contraction in the hearts perfused with substrate-free medium containing the metabolic inhibitor, delta-4-pentenoic acid (Fig. 3). This octanoate response was smaller than that observed in the substrate-free experiments. One might surmise then that delta-

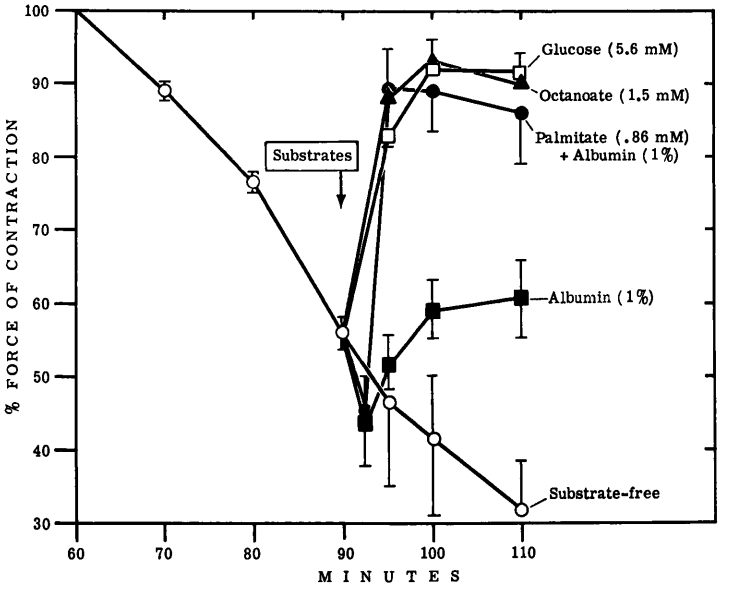

FIG. 2. The effects of substrates on the force of contraction of hearts perfused with substrate-free medium. Substrate-free refers to the normal Krebs-Ringer bicarbonate medium without glucose. At 60 min medium changed from normal to substrate-free. Substrates added at 90 min. Points shown represent mean ± SE of 4 experiments.

4-pentenoic acid had partially inhibited the positive inotropic action of octanoate. Neither palmitate nor glucose could overcome the effects of this metabolic block.

Perfusion pressure, as a measure of coronary resistance, was also monitored in these experi-

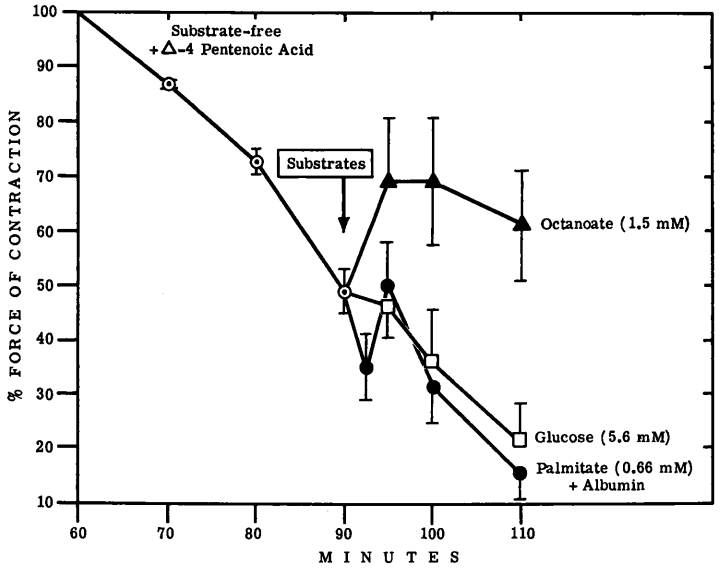

FIG. 3. The effects of substrates on the force of contraction of hearts perfused with substrate-free medium containing delta-4-pentenoic acid. At 60 min medium changed from normal to substrate-free containing 0.25 m*M* delta-4-pentenoic acid. Substrates added at 90 min. Points shown represent means ± SE of 4 experiments.

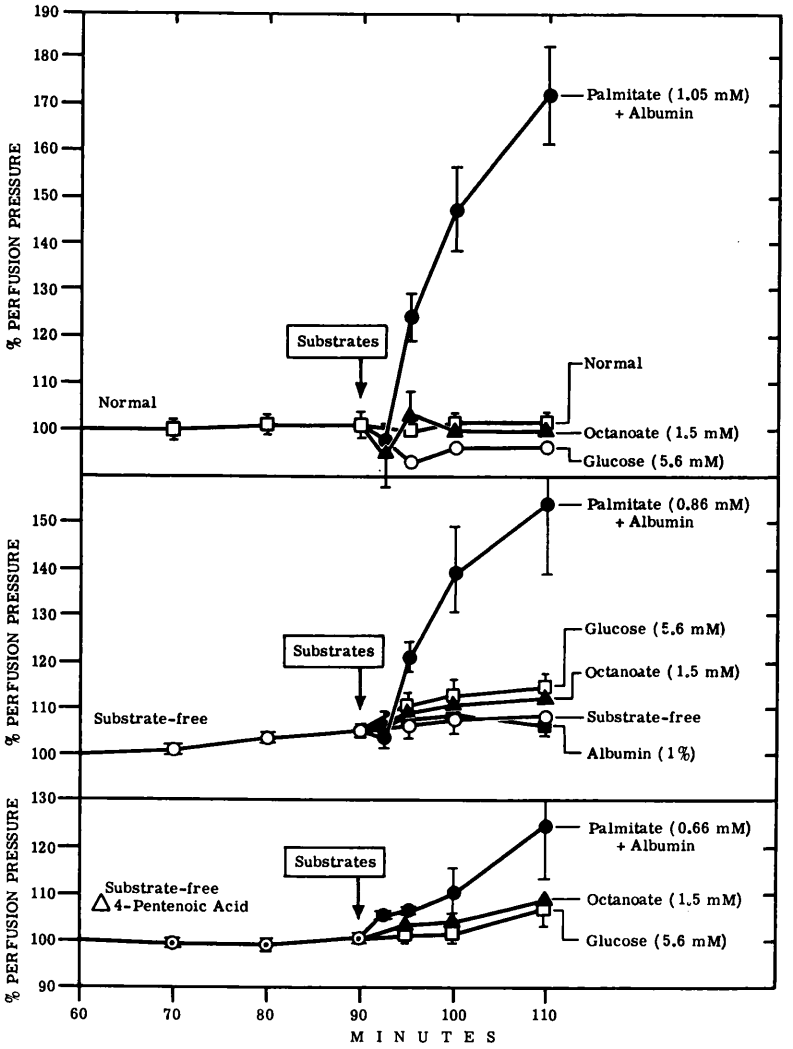

FIG. 4. The effects of substrates on the perfusion pressure of hearts perfused with normal, substrate-free, or substrate-free plus delta-4-pentenoic acid-containing medium. See Figs. 1, 2, and 3 for legends.

ments. In Fig. 4, the effects of the substrates on the perfusion pressure are represented. Palmitate (1.05 m*M*) infusion increased the perfusion pressure 72% over the 60 min control value. The infusion of 0.86 m*M* palmitate in the hearts perfused with substrate-free medium resulted in a 53% increase in the perfusion presure. Palmitate (0.66 m*M*) infusion to the hearts perfused with delta-4-pentenoic acid-containing medium produced a smaller but still definite effect on coronary resistance. The perfusion pressure expressed as a function of the measured palmitate concentration is depicted in Fig. 5. There is an apparent rise in perfusion pressure as the palmitate concentration increased. Neither octanoate nor glucose had any significant effect on perfusion pressure in any of the experiments.

Discussion. Palmitate or octanoate had little or no effect on the force of contraction in medium containing 5.56 m*M* glucose. Shipp, Opie, and Challoner (17) demonstrated that palmitate can inhibit the metabolism of glu-

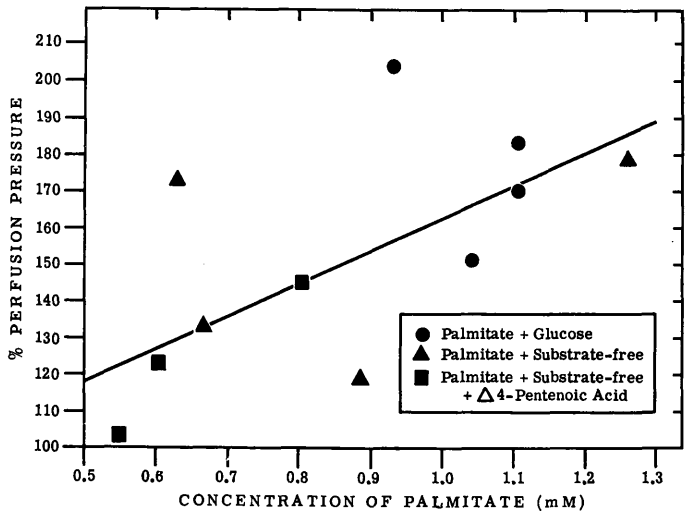

FIG. 5. Correlation between the concentration of palmitate and the perfusion pressure. Correlation coefficient is 0.62.

cose in rat heart. Our results suggest that if indeed glucose metabolism is inhibited, palmitate metabolism is increased sufficiently so that the force of contraction is not compromised. Opie (18) observed the effects of octanoate in perfused rat hearts and concluded that this fatty acid, in high concentration, was toxic to normal rat heart and that it gave rise to a decrease in contractility as well as to disturbances in rhythm. Our results indicate that 1.5 m*M* octanoate had no deleterious effect on the force of contraction.

The positive inotropic action of glucose, octanoate, and palmitate in hearts made hypodynamic by perfusion with substrate-free medium may be interpreted as indicating that these substrates are used to provide fuel for the contractile process. Such an interpretation is strengthened by the experiments with the metabolic inhibitor, delta-4-pentenoic acid (19–21). Its presumed mechanism of action is based on the ability to tie up extramitochondrial coenzyme A and carnitine (19).

Palmitate and octanoate, the representative long and medium chain saturated fatty acids used in these experiments, enter the mitochondria through different processes. Palmitate is activated by extramitochondrial coenzyme A and the resultant complex, palmityl coenzyme A, moves through the outer mitochondrial membrane. Palmityl coenzyme A is then converted into a palmityl carnitine ester which then passes through the intramitochondrial membrane. Once within the mitochondrial matrix, palmityl coenzyme A is reformed and the various steps of beta oxidation take place (12, 22). Octanoate, on the other hand, is not dependent upon either activation in the extramitochondrial space by coenzyme A or transport into the matrix by carnitine (23). This intermediate chain fatty acid crosses both mitochondrial membranes and is subsequently activated to octanyl coenzyme A in the matrix. Thus, delta-4-pentenoic acid, by decreasing levels of extramitochondrial coenzyme A and carnitine, prevents palmitate from producing its characteristic positive inotropic effect in substrate-free medium (Fig. 3). Since beta oxidation of octanoate is unrelated to metabolic steps blocked by delta-4-pentenoic acid, this fatty acid is still capable of augmenting the force of contraction in substrate-free medium which contains the blocker. The increase in force seen with octanoate is somewhat reduced from that observed in hearts perfused with substrate-free medium not containing delta-4-pentenoic acid receiving octanoate (Fig. 2). Ruderman *et al.* (20) state that if incubation with pentenois acid is sustained for a long

enough period of time, intramitochondrial levels of coenzyme A (necessary for activation of octantoate prior to beta oxidation) may also be decreased. The amount of time required to produce this effect has not been established. The hearts in these experiments have presumably been exposed to the metabolic blocker long enough to at least partially tie up the intramitochondrial coenzyme A, thereby partially blocking the octanoate effect on the force.

Williamson, Rostend and Peterson (21) suggested that the disturbance of extramitochondrial coenzyme A metabolism by delta-4-pentenoic acid affects several coenzyme A dependent enzyme systems. One of the most sensitive of these enzyme systems is the pyruvate dehydrogenase enzyme complex, which, subsequent to its activation, allows the end product of glucose metabolism via glycolysis, namely pyruvate, to enter the mitochondrion and be metabolized by the citric acid cycle. This block of pyruvate dehydrogenase would explain why glucose did not produce a force of contraction increase in the hearts perfused with delta-4-pentenoic acid. Thus the presence of an agent known to inhibit the metabolism of exogenous substrates prevents these substrates from producing their typical positive inotropic responses. This information suggests that glucose, octanoate, and palmitate exert their effects on the force of contraction as a result of their metabolism and the subsequent energy production.

The largest increases in perfusion pressure were observed upon infusion of the higher palmitate concentrations (Figs. 4 and 5). From Severeid, Connor and Long's observations (14) one might speculate that the large perfusion pressure increases recorded with the higher concentrations of palmitate may have resulted from the saturation of the albumin binding sites by palmitate (24) and the consequent "spilling over" of unbound free fatty acids. This unbound fatty acid may have then had a vasoconstricor action, thereby increasing the perfusion pressure. No increases in coronary resistance were observed with octanoate or glucose. From our observations, we conclude that the amount of palmitate-induced vasoconstriction in the coronary vessels of the perfused heart is a function of the concentration of this long chain fatty acid which is present in the medium in the unbound form.

Summary. The addition of palmitate, octanoate, or glucose to hearts perfused with Krebs–Henseleit bicarbonate medium containing 5.56 m*M* glucose resulted in little, if any, change in developed tension. The same substrates, however, produced marked increases in developed tension when administered to hearts made hypodynamic with substrate-free medium. The addition of delta-4-pentenoic acid to the substrate-free medium prevented the positive inotropic actions of palmitate and glucose and partially reduced that of octanoate. These data suggest that these three substrates exert their actions on the force of contraction as a consequence of their metabolism.

Palmitate produced an increase in coronary resistance in the perfused rat heart. This effect is apparently a function of the amount of palmitate which is not bound to albumin.

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