PREFERENTIAL OXIDATION OF ACETOACETATE BY THE PERFUSED HEART*

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It has been well established that heart muscle can oxidize ketone bodies and fatty acids (Barnes, et al, 1938; Bing, et al, 1954; Ballard, et al, 1960; Shipp, et al, 1961). We wish to report results of experiments which provide direct evidence that acetoacetate is oxidized in preference to glucose by the myocardium.

MATERIALS AND METHODS

The perfusion techniques used in these studies were modifications of the apparatus and methods described by Morgan, et al, (1961). To permit measurement of the formation of $C^{14}O_2$ resulting from the oxidation of a labelled substrate, a closed system was developed which included a small volume of Krebs-Henseleit bicarbonate buffer, and 130 ml of a gas mixture of O_2-CO_2 (95:5%). Provision was made in the apparatus for sampling both the perfusion fluid and the gas phase, and for introducing substrates into the closed system during a perfusion.

Sodium acetoacetate and sodium acetoacetate-3-C^{14} were prepared via the crystalline lithium salts (Hall, 1961). Paper chromatograms of sodium acetoacetate-3-C^{14} in three different solvents revealed no radioactive contaminants. The purity of the preparation based on the absorption of the Al^{+++} complex at 254 mμ (Hall, 1961) was 99.8%. Glucose was determined by the anthrone method (Mokrasch, 1954); acetoacetate according to Walker (1954); and lactate according to Hohorst, et al (1959). $C^{14}O_2$ from perfusate and gas samples was precipitated (with carrier) as $BaCO_3$ and the radioactivity of suitably prepared mounts was determined.

RESULTS AND DISCUSSION

The addition of acetoacetate to a perfusate containing glucose-U-C^{14} as the substrate resulted in an immediate and nearly complete cessation of glucose oxi-

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dation to $C^{14}O_2$. (Fig. 1). When acetoacetate was apparently consumed, glucose oxidation resumed and the rate of $C^{14}O_2$ production approximated the initial rate.

To determine whether acetoacetate was oxidized, experiments were carried out measuring the production of $C^{14}O_2$ from acetoacetate-3-C^{14} which was added to a perfusate containing unlabelled glucose. Even at the relatively low concentration of acetoacetate employed, oxidation to CO_2 was rapid and complete. (Fig. 2). As in the previous experiments, glucose consumption decreased and lactate accumulated after the addition of acetoacetate.

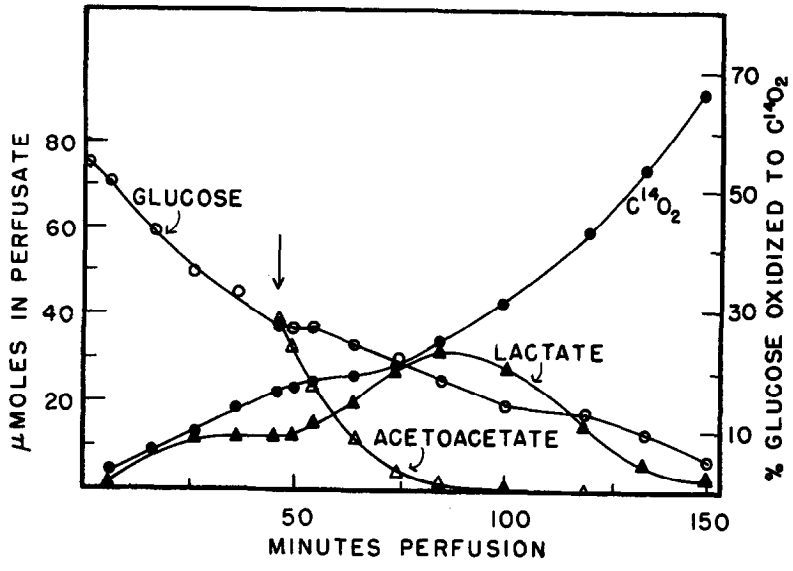

Figure 1. The effect of the addition of acetoacetate on the rate of glucose-U-C^{14} oxidation by the perfused heart. The male rat heart (wet wt., 1.76 g) was perfused with 15 ml of Krebs-Henseleit bicarbonate buffer containing 5.0×10^{-3} M glucose-U-C^{14}. At the time indicated by the vertical arrow 38.5 u moles of sodium acetoacetate was added to the perfusate.

The data presented provide direct evidence that acetoacetate is preferentially oxidized by the myocardium in the presence of an adequate supply of glucose. Shipp, et al (1961) have shown that fatty acids are also oxidized in preference to glucose by the perfused heart. An apparently direct correlation between cardiac glycogen deposition and the severity of induced ketosis in the rat has been found (Lackey, et al (1946). The preferential oxidation of fatty acids and of acetoacetate by the myocardium would spare the oxidation of glucose and provides a probable explanation for increase in cardiac glycogen which occurs during fasting and diabetes.

Of interest is the accumulation of lactate in the perfusate after the addition of acetoacetate (Ottaway and Sarkar, (1958). Whether this accumulation is

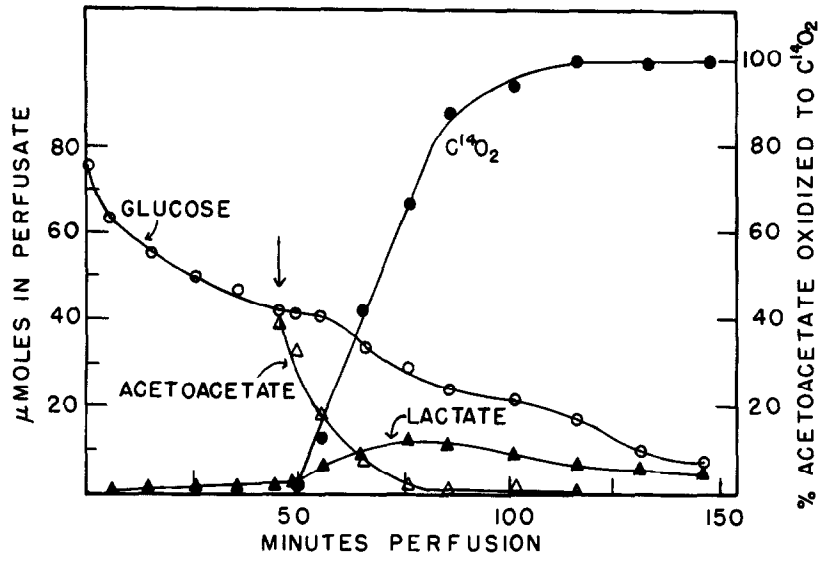

Figure 2. Oxidation of acetoacetate-3-C^{14} in the presence of glucose by the perfused heart. The male rat heart (wet wt., 1.68 g) was perfused with 15 ml of Krebs-Henseleit bicarbonate buffer containing 5.0 x 10^{-3} M glucose. At the time indicated by the vertical arrow, 38.5 u moles of sodium acetoacetate-3-C^{14} was added to the perfusate.

indicative of a cytoplasmic demand for ATP which is not supplied by oxidative phosphorylation in the mitochondria is under study.

While this manuscript was in preparation, Williamson and Krebs (1961) reported that acetoacetate is oxidized in preference to glucose and endogenous substrates by the perfused heart. These conclusions were based on indirect measurements of O_2 consumption, and changes in the perfusate concentrations of glucose, acetoacetate, β-hydroxybutyrate and lactate. Our studies are in agreement with these conclusions.

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