The increased metabolic demand produced by epinephrine in the perfused rat heart is accompanied by a twofold increase in oxygen consumption (6) and glucose oxidation (25), indicating that the relative contribution of glucose to the fuel of respiration is unchanged and that lipid remains the main energy source. The effect of epinephrine on the utilization of circulating lipid by the myocardium has not been clearly defined, although increased utilization of circulating triglyceride has been suggested from studies of myocardial lipoprotein lipase (LPL) in rats treated chronically with epinephrine (1). Recently Gousios and Felts (11) and Gold et al. (10) have reported that epinephrine and norepinephrine increased myocardial extraction and oxidation of palmitate-1-C\textsuperscript{14}.

These studies and those in the preceding paper were designed to study the utilization of circulating triglyceride and fatty acids (FFA) in conditions in which myocardial LPL is known to be elevated. In addition, the effect of lipid on the epinephrine-induced stimulation of glucose oxidation was also investigated.

METHODS

The perfusion apparatus, techniques, and analytical methods have been previously described (13, 14). Male albino Sprague-Dawley rats (Charles River Laboratories), weighing 190-270 g, allowed food and water ad lib., were used in all experiments. For all perfusions, sodium ethylenediaminetetraacetate (5 x 10\textsuperscript{-5} M) was added to the Krebs bicarbonate-Ringer buffer. This has been demonstrated to potentiate the effects of epinephrine, presumably by preventing its degradation by heavy metals, without altering basal glucose uptake and oxidation (25) or oxidation of palmitate-1-C\textsuperscript{14} (unreported observations). Glucose U-C\textsuperscript{14} was obtained from New England Nuclear Corporation, Boston, Massachusetts. Control data recorded in this paper are the same as those presented in the preceding paper.

RESULTS

Chylomicron tripalmitin-C\textsuperscript{14} metabolism. Epinephrine (0.2 \textmu g/ml) did not significantly alter the conversion of chylomicron tripalmitin-C\textsuperscript{14} (TP-C\textsuperscript{14}) to C\textsuperscript{14}O\textsubscript{2} (Table 1). The recovery of TP-C\textsuperscript{14} in perfusate FFA decreased from 21 \pm 1.5 to 17 \pm 0.6 \textmu moles/g heart, dry wt. The total TP-C\textsuperscript{14} disappearing from the perfusate was not altered. TP-C\textsuperscript{14} disappearance is differentiated from myocardial uptake since a large proportion of that which
disappears as triglyceride is recovered in the perfusate FFA fraction. Myocardial TP-C14 uptake (the sum of C14 recovered in CO2, myocardial lipids, and intermediates) increased by 30% from 2.19 ± 0.14 to 2.97 ± 0.08 μmoles/g heart, dry wt, most of which was recovered in myocardial triglyceride (P < .01), although recovery in phospholipid also increased significantly (P < .01). The difference in the recovery of C14 between the two groups cannot account for the differences in TP-C14 uptake.

Palmitate-1-C14 metabolism. The effect of epinephrine on palmitate-1-C14 metabolism is shown in Table 2. FFA uptake, determined by titration, decreased from 32 ± 1 to 27 ± 1 μmoles/g heart, dry wt, in the presence of epinephrine, whereas palmitate-1-C14 uptake determined by the decrease in perfusate C14 counts remained unchanged. The perfusate FFA specific activity decreased by 10.2 ± 0.32.246 on May 20, 2017 http://ajplegacy.physiology.org/ Downloaded from

Glycerol release. In the presence of glucose and palmitate-1-C14, 2.50 ± 0.10 μmoles glycerol/g heart, dry wt per hour were released by the perfused heart (Table 2). Epinephrine produced a two- to threefold increase in glycerol release in the presence of these substrates.

Glucose metabolism. The effect of chylomicron triglyceride and palmitate on glucose uptake and lactate production is shown in Table 3. Hearts of control rats removed glucose at a rate of 80 ± 5 μmoles/g dry wt per hr. Lactate production during this interval was 10 ± 4 μmoles/g dry wt, and represented approximately 15% of the glucose uptake. Epinephrine produced a threefold increase in glucose uptake and markedly elevated lactate production. Both chylomicron triglyceride and palmitate suppressed glucose uptake 50% while producing a moderate elevation of lactate production. With palmitate, C14O2 production from glucose-U-C14 decreased 90-95% from 43 ± 5 to 2.97 ± 0.25 μmoles/g
lipid utilization

levels of cytoplasmic DPNH due to predominance of inhibition of pyruvate decarboxylation (I T rate formation was similar to that observed by William-son (25). There are several interesting features of its cose in the presence of free fatty acids. The addition of effect on the metabolism of circulating lipid and on glu-

DISCUSSION

Elevation of citrate levels within the cell and inhibition of phosphofructokinase (9, 10). Son has demonstrated that epinephrine increases the concent-
rations of all intracellular intermediates of the anaerobic glycolytic pathway (25), the results of the present study, the contributions of both palmitate-14 and chylomicron tripalmitin-14 to the fuel of respiration of the perfused rat heart is calculated to have decreased from 36% to 0.2% and palmitate-1-14 and chylomicron tripalmitin-14 to the fuel of respiration of the perfused rat heart is calculated to have decreased from 36% to 0.2% and palmitate-1-14 and chylomicron tripalmitin-14 to the fuel of respiration of the perfused rat heart is calculated to have decreased from 36% to 0.2% and palmitate-1-14 and chylomicron tripalmitin-14 to the fuel of respiration of the perfused rat heart is calculated to have decreased from 36% to 0.2% and palmitate-1-14 and chylomicron tripalmitin-14 to the fuel of respiration of the perfused rat heart is calculated to have decreased from 36% to 0.2% and palmitate-1-14 and chylomicron tripalmitin-C4. The studies of Williamson have demonstrated that 0.2 pg/ml epinephrine produced near maximal changes in oxygen consumption (6), glucose uptake, and oxidation (25). If Williamson’s values for oxygen consumption can be applied to the present study, the contributions of both palmitate-14 and positive effect of epinephrine on glucose production was observed. Although epinephrine had a relatively greater effect on glucose-U-C4 oxidation in the presence of palmitate, C4O2 production was only 25% of that observed when glucose alone was the substrate.

Chylomicron triglyceride and fatty acids were equally effective in decreasing glucose uptake by the perfused rat heart although not to levels observed in diabetes (13). The inhibitory effects of fatty acid on glucose uptake and glycolysis are thought to be mediated partially by elevation of citrate levels within the cell and inhibition of phosphofructokinase (9, 17), and partially from the inhibition of pyruvate decarboxylation (16). Elevated levels of cytoplasmic DPNH due to predominance of lipid utilization (16) may contribute to the moderate elevation in lactate production.

The effect of epinephrine on glucose uptake and lactate formation was similar to that observed by William-son (25). There are several interesting features of its effect on the metabolism of circulating lipid and on glu-

FIG. 1. Effect of epinephrine on C4O2 production from glucose-U-C14 in the presence and absence of palmitate. Hearts were perfused with buffer containing 5 mM glucose, 5 X 10^{-3} M EDTA, 1% albumin, 0.5 mm palmitate, and 0.1 pg/ml glucose-U-C4. Epinephrine was added after 15 or 30 min of perfusion to give a circulating concentration of 0.2 pg/ml. Values are the means of 12 hearts until the addition of epi-

nephrine; thereafter, the means of 6 hearts. O Represents control hearts, ○ represents epinephrine-
treated hearts.

dry wt, thus confirming the observations of Shipp et al. (22). Epinephrine elevated glucose uptake and lactate production to rates that were equal to or greater than those observed in the absence of circulating lipid. The elevation of glucose uptake in the absence of palmitate suggested that epinephrine might have specifically stimu-
lated glucose utilization. In Fig. 1, the effect of epi-

nephrine on glucose-U-C14 oxidation in the presence and absence of palmitate is shown. Thirty minutes after the addition of epinephrine, C4O2 production had increased six- to sevenfold over control rates in the presence of palmitate but only twofold in its absence. Although epinephrine had a relatively greater effect on glucose-U-C4 oxidation in the presence of palmitate, C4O2 production was only 25% of that observed when glucose alone was the substrate.

epinephrine in vitro did not increase C4O2 production from either chylomicron tripalmitin-C4 or palmitate-1-

C4. The studies of Williamson have demonstrated that 0.2 pg/ml epinephrine produced near maximal changes in oxygen consumption (6), glucose uptake, and oxidation (25). If Williamson’s values for oxygen consumption can be applied to the present study, the contributions of both palmitate-1-C4 and chylomicron tripalmitin-C4 to the fuel of respiration of the perfused rat heart is calculated to have decreased from 36% to 18% on addition of epinephrine. This was unexpected since, under similar experimental conditions, Williamson had demonstrated that epinephrine increased glucose oxidation and oxygen consumption twofold and it might have been assumed that the relative contribution of all substrates to the fuel of respiration would remain unchanged. Epinephrine preferentially stimulated glucose-U-C4 oxidation in the presence of palmitate, although C4O2 production was still significantly less than that observed when glucose alone was the substrate. The effect of epinephrine on myocardial glucose transport and phosphorylation has been thought to be secondary to changes in contractility (25), since epinephrine decreased glucose uptake and phosphorylation by rat diaphragm. Although William-

son has demonstrated that epinephrine increases the concentrations of all intracellular intermediates of the an-

aerobic glycolytic pathway (25), the results of the present studies would indicate that this stimulatory effect was not sufficient to completely reverse the inhibition of gly-

colysis produced by palmitate. The data, however, do suggest that epinephrine may have an effect on glucose transport that is distinct from its inotropic action. Despite enhanced oxidation, the theoretical contribution of glucose to the fuel of myocardial respiration is calculated to be less than 15%. Glycogen may have made
up part of the deficit, yet the bulk of myocardial fuel must still have been derived from endogenous lipid (23, 26). Endogenous lipid utilization was enhanced as indicated by increased glycerol release. Since the myocardium utilized glycerol to a limited extent, glycerol release can only qualitatively reflect endogenous lipid utilization (13) and therefore cannot be used to calculate the quantitative contribution of endogenous lipid to the fuel of respiration as proposed by Randle et al. (18).

The increased recovery of palmitate-1-C\(^14\) in myocardial lipid without increased uptake is difficult to explain. However, since epinephrine stimulates endogenous lipid turnover and elevation of tissue FFA in rat diaphragm and heart (8), it is possible that the exchange between fatty acids derived from endogenous triglyceride and those in the medium was enhanced. This phenomenon occurs normally to a limited extent in the perfused rat heart (4, 22). The exaggerated decrease in palmitate-1-C\(^14\) specific activity is compatible with such an exchange and would offer an explanation for the increased recovery of palmitate-1-C\(^14\) in myocardial lipid without involving a net increase in FFA transport. If such were the case, a decreased specific activity of tissue FFA and their CoA derivatives would be expected and C\(^14\)O\(_2\) from labeled perfusate FFA would be decreased even though the actual CO\(_2\) production may have remained unchanged or increased. The diminished C\(^14\)O\(_2\) produced by epinephrine and also observed with hearts of alloxan-diabetic and starved rats in the preceding paper may be partially due to this phenomenon. However, this does not reconcile the difference between these data and those of Gousios and Felts (11) and Gold et al. (10). A 30% increase in C\(^14\)O\(_2\) production from palmitate-1-C\(^14\) has been demonstrated with both epinephrine and norepinephrine in the perfused rabbit heart (11). Since a recirculatory apparatus was not used, changes in fatty acid concentration were determined by \(^{14}\)C differences of lipid-bound radioactivity and changes in specific activity may not have been appreciated. Comparison with the results obtained from the in situ perfusion of dog hearts (10) is more difficult since the action of norepinephrine on peripheral FFA release also has to be considered. Despite a fall in palmitate-1-C\(^14\) specific activity due to the peripheral lipolytic action of norepinephrine and in C\(^14\)O\(_2\) production, the fraction of arterial palmitate-C\(^14\) appearing as C\(^14\)O\(_2\) increased, leading to the conclusion that norepinephrine increased free fatty acid oxidation (10). Also, because of methodology, a norepinephrine-induced exchange between myocardial and perfusate fatty acids would not be appreciated. It is of interest to note that these observations were made in the absence of any increase in oxygen consumption.

As with the hearts of diabetic rats, the uptake of chylomicron triglyceride did not correlate with that which might be expected from the levels of LPL observed following chronic administration of epinephrine to rats in vivo (1). The discrepancy may be due to the relatively brief in vivo nature of the present experiments since administration of a single dose of epinephrine in vivo also did not alter LPL content (1). Yet LPL content can be influenced acutely as demonstrated by the twofold increase following 60 min of exercise in rats (15). Rat heart has been shown to contain more than one lipase (2) and, since glycerol release was increased acutely, it is possible that epinephrine's effect on myocardial lipolytic activity was mediated through activation of a specific lipase, similar to its mechanism of action on adipose tissue (24). A role for lipoprotein lipase in endogenous lipid turnover cannot be excluded, as it has been demonstrated that 70% of myocardial LPL is microsomal bound (1). Not only was there no increase in circulating triglyceride uptake, but hydrolysis was also unchanged. This may be due to the relatively low concentration of chylomicron triglyceride (equivalent to 50 mg/100 ml available in the perfusate), the high lipolytic activity of rat heart (5), or the release of lytic enzyme into the perfusate (3). In this regard, it would be of interest to determine whether hydrolysis of perfusate chylomicron triglyceride would continue after removal of the hearts from the perfusion apparatus.

In conclusion, the data suggest that epinephrine specifically increases glucose oxidation and endogenous lipid turnover in the isolated perfused rat heart but does not stimulate oxidation of exogenous triglyceride or fatty acid. Since fatty acid utilization by liver (21), skeletal muscle (7), and heart (4) is related to concentration, it is likely that epinephrine increases myocardial FFA uptake by virtue of its ability to elevate circulating FFA, rather than by a direct effect on myocardial lipid extraction. A similar conclusion has been reached by Gold and his co-workers (10).

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