THE EFFECT OF OCTANOATE ON THE CARBOHYDRATE METABOLISM OF MYOMETRIUM FROM THE PREGNANT RHESUS MONKEY*

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Summary. The intermediary metabolism of myometrial slices from pregnant rhesus monkeys (*Macaca mulatta*) was investigated in an effort to evaluate the relative importance of lipid and carbohydrate as substrates for this tissue. The effects of a high level of free fatty acid on glucose uptake and utilization, such as are known to occur in diabetes mellitus and starvation, were also studied.

Myometrial slices from pregnant rhesus monkeys were incubated in medium containing [U- ^{14}C]glucose or [1- ^{14}C]octanoate in the presence and in the absence of 0.4 to 4.0 mM-octanoate. The uptake of [^{14}C]octanoate increased from 50 ± 3 S.E.M. to 187 ± 10 S.E.M. $\mu\text{mol/g}$ non-collagenous protein nitrogen/hr when the octanoate concentration in the medium was raised from 0.4 to 4.0 mM. About 30% of the total CO_2 was derived from this labelled substrate at both octanoate concentrations. Three to 5% of the label from the octanoate uptake appeared in the lipid fraction at 0.4 and 4.0 mM-octanoate; 35 and 6% of the label appeared in the CO_2 fraction at these respective concentrations. In spite of the extensive utilization of octanoate, this free fatty acid (1.0 and 4.0 mM) had no effect on the [^{14}C]glucose uptake and [^{14}C]lactate production although there was a marked decrease in [^{14}C]glucose conversion to $^{14}\text{CO}_2$. Both octanoate concentrations increased glycogen breakdown and total lactate production. No change in O_2 consumption occurred with 1 mM-octanoate, but a 4.0 mM concentration caused a small decrease in QO_2 .

INTRODUCTION

Relatively little work has been done on the intermediary metabolism of smooth muscle (Carsten, 1968), though a recent report indicated that glycolysis, or the conversion of glucose to lactate, appears to be a more important metabolic pathway in pregnant rhesus myometrium than in voluntary skeletal muscle,

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even though the oxygen consumption for the two different tissues is similar (Beatty, Basinger & Bocek, 1969).

Of late, there has been considerable interest in the effect of free fatty acids (FFA) on carbohydrate metabolism. Some workers have suggested that increased FFA metabolism might be the cause of the abnormalities of carbohydrate metabolism in diabetes, starvation, carbohydrate deprivation and other pathological states involving an increase in plasma FFA (Randle, Garland, Hales & Newsholme, 1963; Jenkins, 1967; Williamson, Browning, Scholz, Kreisberg & Fritz, 1968). This hypothesis, commonly called the glucose-fatty acid cycle theory, implies that increased availability of FFA and ketone bodies for oxidation is responsible for the decrease in glucose utilization by muscle (diaphragm and cardiac) and the increase in hepatic gluconeogenesis. However, conflicting experimental results *in vivo* were obtained in both dogs and humans when FFA levels were increased by oral or intravenous administration of fat (Ruderman, Toews & Shafir, 1969). Ruderman *et al.* (1969) reviewed a considerable body of evidence and concluded that FFA metabolism does influence glucose homeostasis in the rat, although the data for other species, including man, are less conclusive. Both *in vitro* and *in vivo*, a high concentration of FFA in plasma, perfusion fluid or incubation medium produces a pattern of metabolism which resembles that found in corresponding tissues (cardiac muscle, diaphragm and liver) from diabetic and fasted rats. The question then arises, does the increased plasma FFA level in these pathological conditions have any effect on the carbohydrate metabolism of smooth muscle, specifically uterine smooth muscle? In the experiments reported here, the effects of varying concentrations of octanoate (0.4 mM to 4.0 mM) on [^{14}C]-glucose uptake and conversion to lactate, CO_2 and glycogen by myometrial slices from pregnant rhesus monkeys were investigated in order to examine the interrelationship between carbohydrate and FFA utilization in the rhesus myometrium. The uptake and metabolism of [^{14}C]octanoate were also studied to evaluate the importance of exogenous lipid as an energy source for the myometrium.

MATERIALS AND METHODS

Tissues and incubation

Uterine biopsies were obtained at the beginning of Caesarean sections from 126- to 154-day pregnant rhesus monkeys. The average gestational period for rhesus monkeys in our colony is about 166 days. The mother was fasted overnight and anaesthetized in the morning with halothane (1% or less) in a mixture of 75% oxygen and 25% nitrous oxide. Biopsies of the same area of the fundus away from the site of placental attachment were obtained within 5 min after the peritoneal cavity was opened. A strip of uterine tissue about 50 mm long and 10 mm wide was carefully stripped of endometrium and cut by hand into slices not more than 1 mm thick. Histological examination of the stripped myometrium indicated that the samples were free of endometrial tissue. Preparation of the tissues usually took less than 5 min, during which time the slices were soaked at room temperature in oxygenated Krebs glycyglycine

medium containing 5.55 μmol of glucose/ml (NaCl, 123 mM; KCl, 1.8 mM; CaCl_2 , 1.7 mM; MgSO_4 , 0.6 mM; and KH_2PO_4 , 0.6 mM). Glycylglycine (24 mM) was preferred to phosphate-buffered medium for measurement of oxygen consumption because of the pronounced effect of inorganic phosphate on carbohydrate metabolism. Aliquots of muscle, about 200 to 250 mg each, were drained on filter paper and placed in weighed Warburg flasks containing 3 ml of medium, pH 7.4 at 37° C, with 5.55 μmol of glucose/ml in the main chamber and 0.4 ml of 9.5 N-NaOH in one side-arm. Medium for appropriate flasks also contained 0.4, 1.0 or 4.0 μmol of octanoate/ml. The flasks were re-weighed, flushed for 5 min with 100% O_2 , and incubated for 135 min at 37° C. At the end of a 15-min equilibration period, [^{14}C]glucose (3.9 μC) or [^{14}C]octanoate (0.2 or 2.0 μC) was added to each flask from a second side-arm. The experiment was terminated after the 135-min incubation by the addition of 0.1 ml of 18 N- H_2SO_4 through a rubber vial closure on a side-arm. The flasks were shaken for an additional 40 min to ensure complete recovery of CO_2 in the NaOH.

Analyses

Glucose, lactate, and glycogen were determined as previously described (Beatty, Peterson, Basinger & Bocek, 1966). Lactate productions were calculated from the concentration in the medium after 135 min of incubation minus the concentration after 15 min (equilibration period). It was assumed that the lactate level in the tissues was reflected by that in the medium since, after 15 min equilibration, the ratio of the concentration of lactate in the tissue water to the concentration of lactate in the medium was not significantly different from unity. Octanoate was determined on a chloroform extract of the medium to which a phosphate buffer (0.1 M, pH 6.2) had been added with the AutoAnalyzer according to the method of Dalton & Kowalski (1967). Purity of the octanoate was checked chromatographically. For determination of total lipid, muscle slices were homogenized in $\text{CHCl}_3:\text{CH}_3\text{OH}$ (2:1) and washed according to the method of Folch, Lees & Stanley (1957), and an aliquot was evaporated and weighed. At the end of 15 min equilibration and 2 hr incubation, 0.1 ml of 18 N- H_2SO_4 was added to the main chamber through a vial closure on a side-arm with a syringe and needle. The flask was shaken for 40 min to absorb the CO_2 . The NaOH was removed from the side-arm under nitrogen and the CO_2 values were determined by a modification of the Technicon AutoAnalyzer (N-methodology) micromethod for CO_2 . Carbon dioxide production was calculated from the value after 135 min incubation minus the value after 15 min incubation. The radioactivity of the medium was determined by counting a 0.025-ml aliquot of the medium in 10 ml of Omnifluor scintillation fluid (toluene plus absolute alcohol) in a scintillation counter. For calculating glucose uptake, the ^{14}C activity in the medium after incubation was subtracted from the original activity; the ^{14}C activity of the lactate produced during incubation was added to this difference to correct for the production of [^{14}C]lactate from [^{14}C]glucose. Uptake of [^{14}C]octanoate was measured by subtracting the activity in the medium after incubation from the original activity. Aliquots of the medium, or aliquots of a CHCl_3 extract of the medium in a

phosphate buffer, were counted in a scintillation counter. This extraction procedure gave a recovery of $99 \pm 0.7\%$ S.E., $n = 10$. The radioactivity of lactate, CO_2 and glycogen was measured with a Tracerlab low background counter as previously described (Beatty *et al.*, 1966). For determination of the ^{14}C activity in the lipid fraction, a 0.025-ml aliquot of the $\text{CHCl}_3:\text{CH}_3\text{OH}$ extract of muscle washed to constant activity was counted in a liquid scintillation counter.

Hydroxyproline was estimated according to the method of Woessner (1961), except that the HCl-digest of the muscle was passed through a Dowex-50 column (Moore & Stein, 1951) to separate hydroxyproline from the rest of the amino acids before colorimetric analysis. The presence in plasma of a non-hydroxyproline chromophore, which interferes with the existing assays for hydroxyproline, has been reported (LeRoy, Kaplan, Udenfriend & Sjoerdsma, 1964). However, after passage through the Dowex-50 column, no interfering chromophore was present in the hydroxyproline fraction. Bentley & Hanson (1969) have demonstrated the presence of hydroxyproline in elastin, but the amount is small compared to that in collagen (1/10); the amount of elastin compared to the amount of collagen in myometrium is also small (Beatty *et al.*, 1969). Therefore, we assumed that all of the hydroxyproline is in the collagen and that hydroxyproline represents 13.4% of collagenous protein (Newman & Logan, 1950), and calculated the collagen content from the hydroxyproline values. Harding & Wesley (1968) have recently demonstrated that the amino acid composition of human uterus is similar to that of bone and tendon collagen. The data in this paper are reported in terms of non-collagenous protein nitrogen (NCN) to correct for the variations in the amounts of collagen, the metabolically inactive protein in uterine tissues. Nitrogen was determined with the Technicon AutoAnalyzer, after Kjeldahl digestion. Differences in data were not considered significant when the P value was >0.05 .

RESULTS

Calculation of data

In metabolic comparisons of individual tissues or organs, the more generally accepted bases of reference are the dry weight, total nitrogen, non-collagenous protein nitrogen (NCN), or deoxyribose nucleic acid (DNA) concentration rather than the wet weight (McCaman, 1963). Since uterine growth during pregnancy is associated with nuclear proliferation as well as hypertrophy, DNA cannot be used as a reference standard. The data in this paper were calculated on the basis of NCN, because we wished to compare the overall metabolism of the sartorius and uterine muscles and because the pregnant uterus contains 3 to 3.6 times more collagen than the sartorius.

Effect of octanoate on glucose uptake, glycolysis and glycogenolysis

Neither 1 nor 4 mM-octanoate had any effect on glucose uptake or production of $[^{14}\text{C}]$ lactate from $[^{14}\text{C}]$ glucose by myometrial slices from pregnant rhesus monkeys (Table 1), though both concentrations of octanoate increased the production of unlabelled lactate. Presumably, this lactate arose from glyco-

genolysis, since there was no change in glucose uptake or [^{14}C]lactate production. Both 1 and 4 mM-octanoate caused sufficient glycogenolysis, about 165 $\mu\text{mol/g NCN/hr}$ and 290 $\mu\text{mol/g NCN/hr}$ (mmol glucose equivalents) respectively, to account for the increase in lactate production. There was no change in the incorporation of label from [^{14}C]glucose into glycogen at 1 mM-octanoate. Marked glycogenolysis occurred at 4 mM-octanoate, and there was also a concomitant decrease in incorporation of label into glycogen to one-third the control value. The incorporation of label from [^{14}C]glucose into $^{14}\text{CO}_2$ was decreased equally by both concentrations of octanoate.

TABLE 1

EFFECT OF OCTANOATE ON CARBOHYDRATE METABOLISM OF UTERINE MUSCLE SLICES INCUBATED IN MEDIUM CONTAINING [$\text{U-}^{14}\text{C}$]GLUCOSE (130- TO 145-DAY PREGNANT RHESUS MONKEYS)

	Control	Octanoate			
		1 mM	P*	4 mM	P*
Glucose uptake/hr $\mu\text{mol/g NCN}\dagger$	375 $\pm$ 43	386 $\pm$ 28	NS	422 $\pm$ 23	NS
Lactate prod./hr $\mu\text{mol/g NCN}$	420 $\pm$ 32	608 $\pm$ 34	<0.05	751 $\pm$ 73	<0.01
$\text{d/min/mg NCN} \times 10^3$	103 $\pm$ 24	105 $\pm$ 19	NS	95 $\pm$ 20	NS
CO_2 prod./hr $\text{d/min/mg NCN} \times 10^2$	84 $\pm$ 7	34 $\pm$ 2	<0.005	30 $\pm$ 4	<0.005
Glycogen $\ddagger$ mmol/g NCN	1.51 $\pm$ 0.11	1.33 $\pm$ 0.24	NS	1.08 $\pm$ 0.16	<0.005
$\text{d/min/mg NCN} \times 10^2$	98 $\pm$ 6	110 $\pm$ 12	NS	30 $\pm$ 4§	<0.005
$\text{QO}_2/2 \text{ hr}$ mmol/g NCN	1.90 $\pm$ 0.07	2.06 $\pm$ 0.11	NS	1.66 $\pm$ 0.10	<0.05

Values are means $\pm$ S.E.M., duplicate flasks, five animals. Myometrial slices were incubated for 135 min in Krebs glycyglycine-buffered medium with 5.55 $\mu\text{mol glucose/ml}$. [$\text{U-}^{14}\text{C}$]glucose (1.3 $\mu\text{C/ml}$) was added at the end of a 15-min equilibration period. Specific activity of medium glucose $286 \times 10^4 \text{ d/min/mg}$.

* Statistical analysis on basis of paired observations. Control versus octanoate.

$\dagger$ NCN = Non-collagenous protein nitrogen.

$\ddagger$ Calculated as mmol glucose equivalents. End of incubation.

§ N = 4.

Effect of octanoate on O_2 consumption and total CO_2 production

One mM-octanoate had no effect on the QO_2 when compared to the control series; however, 4 mM decreased the oxygen consumption about 10% (Table 1). Total CO_2 productions were not measured in the first series of experiments (Table 1), but in the second series, when the octanoate level in the medium was increased from 0.4 to 4.0 mM (Table 2), both O_2 consumption and CO_2 production were decreased; the respiratory quotient (RQ) was not significantly different from 0.70 at both concentrations. These RQ values are the same as those previously reported (Beatty *et al.*, 1969) for myometrial slices incubated in medium containing glucose without added octanoate. We have no explanation for the fact that myometrial slices have an RQ typical of fatty acid oxidation while voluntary skeletal muscle under similar experimental conditions has

an RQ of about 0.8, which is typical for the utilization of a mixture of substrates.

Utilization of [^{14}C]octanoate by myometrium

The metabolic products of FFA utilization are thought to influence glucose utilization (Williamson *et al.*, 1968; Ruderman *et al.*, 1969). In view of the lack of effect of FFA on glucose uptake, it seemed necessary to prove that octanoate was being metabolized by this myometrial preparation. As can be seen in Table 2, octanoate is well utilized by the myometrium. Increasing the octanoate

TABLE 2
METABOLISM OF [^{14}C]OCTANOATE AND THE EFFECT OF OCTANOATE ON METABOLISM BY MYOMETRIAL SLICES FROM 126- TO 154-DAY PREGNANT RHESUS MONKEYS

	Octanoate			
	No.*	0.4 mM	4.0 mM	P
Octanoate uptake/hr $\mu\text{mol/g}$ NCN†	7	50 $\pm$ 3	187 $\pm$ 10	<0.005
% of label from uptake appearing in lipid	4	3.4 $\pm$ 0.6	4.9 $\pm$ 0.9	NS
% of label from uptake appearing in CO_2	5	35 $\pm$ 3	6.4 $\pm$ 1	<0.005
% total CO_2 derived from octanoate‡	6	31 $\pm$ 2	30 $\pm$ 1	NS
Lactate prod./hr $\mu\text{mol/g}$ NCN	7	453 $\pm$ 82	899 $\pm$ 118	<0.02
Glycogen§ mmol/g NCN	7	1.18 $\pm$ 0.11	0.66 $\pm$ 0.10	<0.005
QO_2 /2 hr mmol/g NCN	7	1.66 $\pm$ 0.06	1.28 $\pm$ 0.13	<0.005
CO_2 prod./2 hr mmol/g NCN	6	1.18 $\pm$ 0.07	0.92 $\pm$ 0.12	<0.05
RQ	5	0.72 $\pm$ 0.02	0.70 $\pm$ 0.02	NS

Values are means $\pm$ S.E.M. Statistical analyses are calculated on the basis of paired observations. Myometrial slices were incubated for 135 min in 3 ml Krebs glycyglycine-buffered medium, pH 7.4, at 37° C with 5.55 μmol glucose/ml medium. [^{14}C] Octanoate (0.2 or 2.0 μC) was added at the end of a 15-min equilibration period to flasks containing 0.4 or 4.0 mM-octanoate, respectively.

* Number of monkeys, duplicate flasks.

† NCN = Non-collagenous protein nitrogen.

‡ Specific activity of CO_2 (d/min/mmol carbon)/specific activity of octanoate (d/min/mmol carbon).

§ End of incubation, calculated as mmol glucose equivalents.

concentration ten-fold, increased octanoate uptake about four-fold. The effect of the 4-mM-octanoate on lactate production, glycogen breakdown and QO_2 in Tables 1 and 2 is similar. About the same amount of [^{14}C]octanoate was oxidized by the citric acid cycle (CAC) of both 0.4 and 4.0-mM concentrations, since the total CO_2 production and the percentage of total CO_2 derived from [^{14}C]octanoate remained the same. The percentage of label from the octanoate uptake appearing in CO_2 , however, decreased at the higher concentration. It is assumed that the oxidation rate of the myometrium did not vary with time since the QO_2 of this preparation was the same during the 1st and 2nd

hour of incubation. Apparently at 0.4 mM-octanoate, a maximum value for the percentage of total CO_2 that could be derived from octanoate had been reached. Thirty per cent is also the value for the percentage of total CO_2 derived from octanoate by rhesus sartorius muscle at 4.0-mM concentrations (Cassens, Bocek & Beatty, 1969); Buse & Buse (1967) have reported that 30% of the octanoate uptake was oxidized to $^{14}\text{CO}_2$ by rat diaphragm (1.0 mM-octanoate). The fact that as much as 30% of the total CO_2 can be derived from octanoate (Table 2) without any effect on $[^{14}\text{C}]$ glucose uptake or $[^{14}\text{C}]$ lactate production from $[^{14}\text{C}]$ glucose (Table 1) indicates that the metabolic products of FFA utilization, such as acetyl CoA and citrate, do not affect glucose uptake and glycolysis in our myometrial preparation. Less than 1% of the activity from octanoate uptake was found in the glycogen at both concentrations, a value that was considered to be insignificant. The percentage of uptake appearing in the lipid fraction remained the same when the octanoate uptake increased 3.7 times, which reflects an absolute increase in the incorporation into the lipid fraction. Some of this incorporation may well be direct. Knittle & Hirsch (1965) have reported that a significant amount of direct esterification occurs with octanoate, at least in the epididymal fat pad of the rat.

DISCUSSION

Despite considerable interest in functional disorders of the human uterus, very few observations have been made on the intermediary metabolism of the myometrium, especially from pregnant animals, and even fewer on primate myometrium. It has previously been demonstrated that, when calculated on the basis of non-stromal or non-collagenous protein nitrogen, the O_2 consumption of myometrium from pregnant rhesus monkeys is about $0.7 \mu\text{mol/g NCN/hr}$ (Beatty *et al.*, 1969), a value similar to the QO_2 of rhesus voluntary skeletal muscle (Beatty, Basinger & Bocek, 1968). The utilization of carbohydrate appears to be more important in the pregnant myometrium than in the voluntary skeletal muscle of the rhesus monkey, since both the glucose uptake and lactate production of the myometrium are twice as great as the respective values for skeletal muscle fibre groups when these values are calculated on the basis of NCN (Beatty *et al.*, 1969). However, because of the low RQ, 0.70, demonstrated for resting myometrium and the fact that only 5% of the total CO_2 was produced from $[^{14}\text{C}]$ glucose, the major fuel for this tissue, as well as for voluntary skeletal muscle, is probably lipid rather than carbohydrate. This conclusion is supported by the fact that the octanoate uptake of the myometrium in the experiments reported in this paper was even higher, $187 \pm 10 \mu\text{mol/g NCN/hr}$, than the octanoate uptake of voluntary skeletal muscle, $117 \pm 3 \mu\text{mol/g NCN/hr}$ (Cassens *et al.*, 1969).

Although octanoate was extensively oxidized by the myometrium, this FFA had no effect on the glucose uptake of this tissue. Fatty acid inhibition of glucose uptake has been demonstrated repeatedly in perfused rat hearts (Shipp, Opie & Challoner, 1961; Randle, Newsholme & Garland, 1964; Vahouny, Liljenquist, Wilson, Lioa & Rodis, 1968). However, Cassens *et al.* (1969) were unable to show any effect of octanoate on glucose uptake by voluntary skeletal

muscle from the rhesus monkey. Hashimoto & Dayton (1968) were also unable to show an effect of octanoate or palmitate on glucose uptake by rat aorta. Using an isolated rat diaphragm preparation, Schonfeld & Kipnis (1968) reported that the FFA (palmitic and oleic) had no effect on glucose uptake over a wide range of concentrations. Since the inhibiting effect of FFA on enzyme activity is modified by the intracellular protein level (Shafir, Lauris & Cahill, 1968), one might postulate that cardiac muscle has a different protein concentration at the location of the key glycolytic enzymes than does smooth or voluntary skeletal muscle. Although Ruderman *et al.* (1969) concluded that FFA do influence glucose metabolism in the rat, Exton, Corbin & Park (1969) measured glucose metabolism by perfused rat livers and found that, while ketogenesis was markedly increased, FFA did not alter gluconeogenesis.

Even though octanoate had no effect on glucose uptake and [^{14}C]lactate production from [^{14}C]glucose, the incorporation of label from [^{14}C]glucose into CO_2 by myometrium was decreased by both concentrations of octanoate. This decrease in [^{14}C]glucose oxidation to $^{14}\text{CO}_2$ in the presence of FFA may be interpreted as evidence for the production of unlabelled lactate and acetyl CoA from glycogen, with subsequent dilution of the label from [^{14}C]glucose entering the citric acid cycle, since octanoate did increase glycogenolysis in myometrial slices. However, the maximum increase in unlabelled lactate production in the production of octanoate was 80% compared to the control value, and the production of $^{14}\text{CO}_2$ in the control series was 180% higher than in the 4 mM-octanoate series. Thus, it appears that additional factors must be involved in the decreased incorporation into CO_2 . There could also be inhibition of one or more enzymes, such as pyruvate kinase or pyruvate dehydrogenase, involved in glucose oxidation by effector molecules produced from FFA utilization, or preferential oxidation of FFA with glucose sparing (Williamson *et al.*, 1968). Several groups of workers have reported that FFA decreases the conversion of [^{14}C]glucose to $^{14}\text{CO}_2$ both in rat heart (Shipp *et al.*, 1961; Shipp, 1964) and in rat aorta (Hashimoto & Dayton, 1968).

The fact that octanoate had no effect on myometrial O_2 consumption at a 0.4 mM concentration and only slightly decreased O_2 consumption of 4 mM concentration is not in agreement with other published results in which octanoate and other FFA stimulated the O_2 consumption of perfused rat heart and voluntary striated muscle (Challoner & Steinberg, 1966; Cassens *et al.*, 1969). In view of the increase in O_2 consumption in these tissues, several workers (Challoner & Steinberg, 1966; Ruderman *et al.*, 1969) have suggested that FFA may act as an uncoupling agent. Davis & Gibson (1969) have recently presented evidence that FFA may facilitate hepatic gluconeogenesis by uncoupling mitochondrial respiration. However, no evidence for an uncoupling effect of octanoate was observed in myometrium; the only influence octanoate had on O_2 consumption was a depressant effect.

Octanoate had a glycogenolytic effect in the myometrial preparation, and the same effect has been demonstrated in other tissues. Cassens *et al.* (1969) demonstrated that muscle fibre groups incubated in medium containing octanoate had lower glycogen levels than control groups at the end of the experimental period; Exton *et al.* (1969) reported data suggesting that octanoate

caused marked glycogenolysis in perfused rat liver. Net glycogen synthesis in perfused rat heart was either unchanged or increased (Randle *et al.*, 1964).

Octanoate was used in the present experiments because it is a water-soluble FFA and can be studied without added albumin. The addition of albumin to the incubation medium has been shown to influence carbohydrate metabolism and can cause both increases and decreases in glucose uptake by the isolated rat diaphragm under different experimental conditions (Mahler & Szabo, 1967; Hashimoto & Dayton, 1968). The use of octanoate has been questioned because it is not a constituent of the mammalian FFA pool. However, Randle *et al.* (1964) reported that the effects of octanoate and palmitate on the carbohydrate metabolism of perfused rat heart were similar; both octanoate and palmitate decreased the incorporation of ^{14}C from leucine into the protein of isolated rat diaphragm (Buse & Buse, 1967) and perfused rat heart (Willingham, Biggars & Buse, 1969). Both octanoate and oleate stimulated ketogenesis but not gluconeogenesis in perfused livers from rats (Exton & Park, 1967).

Marked species differences are apparent in the responses of a number of enzymes to diet and hormones (Scrutton & Utter, 1968). There appear to be marked species differences *in vivo* in the response to an increase in plasma FFA. In rats, intraperitoneal octanoate raised the blood glucose level (Friedman, Goodman & Weinhouse, 1967). In dogs, intravenous octanoate (Sanbar, Evans, Boniface & Hetenyi, 1967) or oleate (Greenough, Crespin & Steinberg, 1967) or infusion of cottonseed oil followed by heparin to induce intravascular lipolysis (Seyffert & Madison, 1967) resulted in hypoglycaemia. In man, both an increase and a decrease in tolerance to intravenous glucose have been reported after plasma FFA levels were artificially raised (Ruderman *et al.*, 1969). However, it is difficult to assess whether these results are due to changes in peripheral glucose utilization, to changes in hepatic glucogenesis, or to an increase in plasma insulin concentrations. An investigation of the individual tissues and organs that might be involved in the glucose-fatty acid cycle would appear to be essential. In view of these species differences, tissue from an animal as closely related to man as the rhesus monkey is an experimental tool of particular importance.

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