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Effects of medium fatty acid concentration, epinephrine, and glucose on palmitate-1-C^{14} oxidation and incorporation into neutral lipids by skeletal muscle *in vitro*

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SUMMARY

The rate of oxidation of palmitate-1-C^{14} to $C^{14}O_2$ by rat skeletal muscle *in vitro* was shown to increase markedly as a function of the concentrations of free fatty acids (FFA) in the medium. Neither epinephrine nor glucose had any important effect. The rate of incorporation of palmitate-1-C^{14} into tissue neutral lipids was also increased by higher levels of FFA in the medium. At any given concentration of FFA this incorporation was also increased by glucose but was not altered by epinephrine. It is concluded that the deposition of triglyceride reported to occur in muscle after epinephrine or norepinephrine administration is due to the effects of these hormones on serum FFA levels and not to a direct effect on muscle metabolism. The profound effect of FFA concentration on the rate of FFA oxidation suggests that regulation of the serum concentrations of this labile lipid fraction may be of central importance in determining over-all rates of body metabolism. In particular, it is proposed that the elevated metabolic rates after epinephrine administration and in hyperthyroidism may, in part, be directly attributable to the elevations of serum FFA in these conditions.

Evidence accumulating over the past several years, both from studies *in vivo* and *in vitro*, indicates the important role of lipids in muscle metabolism. From studies of arteriovenous differences across the human forearm, Andres *et al.* (1) showed that the major substrate could not be carbohydrate and, from the fact that the respiratory quotient found was 0.80, concluded that oxidation of lipid must account for the larger part of the metabolic activity. Over short time intervals the muscle can apparently utilize its endogenous supplies of lipid (2, 3), but over any extended period of time it must ultimately depend upon substrate transported from adipose tissue depots. The work of Gordon and Cherkes (4) and of Dole (5) led to the elucidation of the central role of the serum free (or unesterified) fatty acids (FFA) in the transport of lipid from depots to sites of utilization. Direct studies of arteriovenous differences have established this lipid fraction as a primary source of fat calories in cardiac muscle, and probably also in skeletal muscle (6).

The question then arises as to the site at which homeostatic control of the rate of lipid oxidation is exercised. Since the muscle mass probably accounts for the largest fraction of the fat calories utilized as an energy source, the question of the mechanism of control is especially pertinent in that tissue. Is control of the amount of fat oxidized exercised predominantly within the muscle tissue, for example, by hormonal regulation of metabolic rates or by substrate-concentration feedback? Or is the control exercised by adjustments in the concentration of extracellular FFA made available to the tissue? Both general types of control may, of course, be involved. One purpose of the present studies was to test the relative importance of FFA concentration in determining the rate of its oxidation by muscle.

A second purpose was to explore the relation between FFA concentration and the rate of triglyceride synthesis. Studies by Feigelson *et al.* (7) in this laboratory have shown that when serum FFA concentration is maintained at a high level by constant intravenous

infusion of epinephrine or norepinephrine, there is a rapid and marked increase in liver triglyceride concentration and, although to a lesser extent, in skeletal muscle triglyceride concentration. Maling and Highman (8) have observed deposition of neutral fat in the myocardium after intravenous infusion of large doses of either epinephrine or norepinephrine. It was of interest to determine whether triglyceride deposition induced by catecholamines might be due to some direct effect on muscle metabolism or whether it could be adequately explained on the basis of the elevated serum FFA concentration secondary to stimulation of FFA release from adipose tissue (4, 5, 9).

Finally, in view of the inverse relationship between the relative rates of lipid oxidation and the availability of glucose in the intact animal, as indicated by changes in respiratory quotient, it was of interest to determine whether glucose would, in an isolated system, influence the rate of oxidation of FFA. The alternative possibilities are, again, that control is exercised exclusively by adjustments of the rate of release of FFA from adipose tissue, which has been shown to occur (6), or that both mechanisms operate.

METHODS AND MATERIALS

Male Sprague-Dawley rats fed ad libitum on a standard laboratory chow diet were used. An extensor muscle lying beneath the gastrocnemius muscle was dissected free *in toto*. The paired muscle from each rat was used as a control. These muscles weighed approximately 50 mg and measured approximately 13 × 1 × 3 mm.

The tissues were incubated in 3 ml of Krebs-Ringer phosphate buffer (10), pH 7.4, containing 3% bovine serum albumin (Armour Pharmaceutical Company, list 2293, fraction V from bovine plasma). The Q_{0_2} of these muscle strips measured in the absence of added substrate was 4.1, 4.2, and 5.2 μl/mg (dry)/hour in preparations from three different rats. Fritz *et al.* (11) have reported Q_{0_2} values of 5.79 for diaphragm, and 2.34 for *L. dorsi* muscle.

The unlabeled palmitic acid was Fisher Reagent Grade, and the palmitic acid-1-C^{14} was obtained from Nuclear Chicago Corporation, Desplaines, Illinois. The unlabeled palmitic acid was dissolved in hot 0.154 M NaCl by neutralization with equivalent amounts of 2 N KOH and was then added to the appropriately concentrated medium. Twenty-five microliters of palmitic acid-1-C^{14}, representing approximately 25,000 cpm/ml of final medium, was added to each incubation flask.

The basic albumin-containing medium contained 0.23 μeq/ml of titratable FFA, which was 53.5% palmitic acid, as shown by gas-liquid chromatography. The unlabeled palmitic acid added to raise the medium FFA concentration was found actually to contain only 85% palmitic acid. These values were used to calculate the true dilution of precursor palmitate-1-C^{14} when unlabeled palmitate was added at different concentrations to the basic medium. In the results reported below, the amount of palmitate metabolized is, in every case, expressed as the minimum number of μeq of medium palmitate oxidized or incorporated, based on the total recovered radioactivity in each fraction studied, divided by the calculated specific radioactivity of the medium palmitate.

The addition of internal standards showed that the added unlabeled palmitic acid did not exert a quenching effect in the determination of the radioactivity of the medium. It was also shown that the specific activity of the medium palmitic acid-1-C^{14} was not affected by the order of addition of labeled and unlabeled palmitic acid.

In the experiments using epinephrine, the final concentration used was 3 μg/ml. The glucose concentrations were determined enzymatically, using "Glucostat" reagents obtained from the Worthington Biochemical Corporation, Freehold, New Jersey.

Incubation Procedure. The rats were killed by swift decapitation, and the paired muscles were immediately dissected out, weighed, and dropped into 3 ml of medium in 25-ml Erlenmeyer flasks. The flasks were then flushed with oxygen for 5 minutes in an incubator at 38°, shaking at 60 cycles per minute. The flasks were then recapped with a rubber plug containing a glass cup holding 0.3 cc of 30% KOH and a 2 × 1 cm piece of folded Whatman #2 filter paper. At the end of a 2-hour incubation, 0.3 cc of 4 N H_2SO_4 was injected, and the flasks were shaken for an additional 20 minutes to ensure complete CO_2 collection. The contents of the glass cup, including the filter paper, were then washed into a graduated cylinder with distilled water to a final volume of 10 cc. After the addition of 0.1 cc of 1:10 Triton GR-5 detergent (Rohm and Haas, Inc., Philadelphia, Pa.), 3 ml of the aqueous solution containing collected $C^{14}O_2$ was pipetted onto 1 g of anthracene crystals (Distillation Products Industries, Rochester, N.Y.; blue-violet fluorescent grade) in counting vials. The $C^{14}O_2$ was then counted in a Packard Tri-carb liquid scintillation spectrometer according to the method of Steinberg (12).

The muscle was removed at the end of the incubation, rinsed twice in distilled water, blotted, and homogenized in 3 ml of distilled water. The homogenate was washed into a 60-ml separatory funnel with three

10-ml aliquots of the extraction mixture described by Dole (5), modified only by the use of isooctane in place of heptane (isopropanol-isooctane-1 N H_2SO_4 40:10:1) (v/v). After 2 hours the phases were broken by adding 18 ml of isooctane and 12 ml of H_2O. The phases were separated and the isooctane phase was washed with 15 ml of Borgström's alkaline ethanol (ethanol-H_2O-1 N NaOH 50:44:5) (v/v) (13). The alkaline aqueous phase from this wash was backwashed with 10 ml of isooctane and the latter was combined with the isooctane phase from the initial extraction. The pooled isooctane extracts were washed with alkaline ethanol, and the alkaline-ethanol washes were combined.

An aliquot of the pooled isooctane extracts was taken to dryness in a glass counting vial, diphenyloxazole in toluene (5 mg/ml) was added, and radioactivity was assayed using a Packard Tri-carb liquid scintillation spectrometer. The lipids of this fraction are designated neutral lipid.

The pooled alkaline-ethanol washes were acidified and the FFA were re-extracted into 10 ml of isooctane. One aliquot of this was titrated (5), and a second aliquot was taken to dryness for radioassay as described above. This fraction is designated tissue free fatty acids.

The extent to which phospholipid contributed to the radioactivity in the neutral lipid fraction was directly determined using column chromatography on silicic acid (14). Two grams of 325-mesh silicic acid (Bio-Rad Laboratories, Richmond, Calif.) was slurried into a glass column with chloroform. The material was activated by successive washes with 4 ml of diethyl ether, 12 ml of acetone-ether 1:1 (v/v), and 8 ml of diethyl ether. An aliquot of the neutral lipid fraction was added to the top of the column in 1 ml of chloroform, and elution was carried out with 80 ml of chloroform followed by 80 ml of methanol. In several experiments, at different levels of medium FFA, some with glucose in the medium and some without, the radioactivity in the chloroform eluate was found to account for 96.8% to 99.7% of the total radioactivity in the neutral lipid fraction.

FFA concentrations of the medium were measured by the method of Dole (5).

RESULTS

Effect of FFA Concentration. As shown in Table 1, the conversion of palmitate-1-C^{14} to neutral lipid increased with increasing concentrations of palmitate in the medium. The relation was not a regular one, perhaps because of the small number of experiments at each concentration, but the trend is clear. There was an eightfold increase in calculated incorporation as the medium concentration was raised from 0.12 up to 1.64 μeq/ml. The increases in oxidation of palmitate to $C^{14}O_2$ over this range of medium palmitate concentrations were of a similar magnitude. The total amounts of C^{14} appearing in $C^{14}O_2$ were similar to the amounts converted to neutral lipid.

TABLE 1. METABOLISM OF PALMITATE-1-C^{14} AT VARIOUS CONCENTRATIONS OF PALMITATE IN THE MEDIUM

Total Medium FFA	Medium Palmitate	Conversion to Neutral Lipid*	Conversion to $C^{14}O_2$*
μeq/ml	*μeq/ml*	*μeq/g/2 hrs*	*μeq/g/2 hrs*
0.23	0.12 (6)†	0.13 ± 0.02	0.07 ± 0.01
0.46	0.31 (5)	0.21 ± 0.01	0.33 ± 0.04
0.90	0.69 (3)	0.83 ± 0.20	0.52 ± 0.04
1.05	0.82 (6)	0.57 ± 0.01	0.39 ± 0.02
1.18	0.93 (3)	0.49 ± 0.06	0.54 ± 0.06
1.38	1.09 (3)	0.83 ± 0.06	0.71 ± 0.04
1.66	1.33 (5)	0.71 ± 0.08	0.56 ± 0.07
1.82	1.46 (3)	1.26 ± 0.08	0.81 ± 0.05
2.02	1.64 (3)	1.07 ± 0.03	0.87 ± 0.06

* Based on specific activity of medium palmitate determined as described under Methods. $\frac{\text{cpm in neutral lipid (or in } CO_2)}{\text{S. A. of medium palmitate}}$

† Numbers in parentheses indicate the numbers of pairs of tissues studied. The difference between the values at a medium concentration of 0.12 μeq/ml and the values at all higher concentrations are highly significant ($p < 0.001$).

The relative specific activity of the tissue FFA fraction at the end of the incubation at different initial concentrations of medium FFA is shown in Table 2, expressed as a percentage of the zero time specific activity of the total medium FFA (without regard to composition of the fatty acids). The specific activity of the tissue FFA was in all cases well below that of the medium FFA, but approached it more closely at

TABLE 2. CHANGES IN RELATIVE SPECIFIC ACTIVITY OF TISSUE FFA WITH VARYING FFA CONCENTRATIONS IN THE MEDIUM

Concentration of Medium Palmitate	Relative Specific Activity of Tissue FFA*
μeq/ml	*per cent*
0.12	3.3
0.68	4.0
0.92	9.8
1.09	18.8
1.46	22.6
1.64	18.2

* $\frac{\text{Final specific activity of tissue FFA}}{\text{Initial specific activity of medium FFA}} \times 100$

Specific activities are expressed in terms of total radioactivity per total titratable FFA without reference to fatty acid composition of the fractions.

TABLE 3. EFFECT OF EPINEPHRINE (1 μG/ML MEDIUM) ON PALMITATE UTILIZATION IN MUSCLE

Concentration of Medium Palmitate	Concentration of Medium Glucose	Number of Pairs of Muscles Studied	Conversion to Neutral Lipid*		Conversion to CO_2*		Relative Specific Activity of Tissue FFA†	
			Control	Epinephrine	Control	Epinephrine	Control	Epinephrine
μeq/ml	*mg/100 ml*		*μeq/g/2 hrs*		*μeq/g/2 hrs*		*per cent*	*per cent*
0.22	None	3			0.57 ± 0.08	0.46 ± 0.07	9 ± 1	8 ± 0.4
					($p < 0.5$)			
0.43	None	5	0.42 ± 0.02	0.43 ± 0.03	0.19 ± 0.01	0.14 ± 0.01	12 ± 1	12 ± 1
					($p < 0.005$)			
1.47	None	5	0.82 ± 0.05	0.78 ± 0.06	0.58 ± 0.11	0.51 ± 0.10		
					($p < 0.5$)			
0.14	77	3	0.20 ± 0.02	0.20 ± 0.03	0.12 ± 0.01	0.13 ± 0.01	4.2	4.2
0.89	77	3	0.95 ± 0.02	0.82 ± 0.13	0.47 ± 0.01	0.62 ± 0.06	29.7	29.5
0.89	71	6	0.91 ± 0.02	0.84 ± 0.03	0.26 ± 0.01	0.28 ± 0.02	21.7	18.6

The probability of differences between means occurring by chance has been evaluated by the (t) test in each series of paired muscles.
* Calculated as in Table 1 and described under Methods.
† As in Table 2.

higher initial medium concentrations. The important bearing of these findings on interpretation of the results shown in Table 1 is discussed below. It should be noted that the FFA in the medium at concentrations of 0.68 μeq/ml and above was 70% to 80% palmitic acid, whereas the FFA in the tissue almost certainly contained a lesser percentage of palmitic acid. It is likely that the specific activity of palmitic acid in the tissue FFA pool approached that of the medium more closely than suggested by the data in Table 2.

Effects of Epinephrine. The effect of epinephrine (1 μg/ml) on neutral lipid synthesis and on $C^{14}O_2$ production from palmitate-1-C^{14} was tested at low and at high concentrations of FFA in the medium. As shown in Table 3, there was no demonstrable effect at any concentration of FFA tested, either in the presence or in the absence of glucose.

The experiments measuring oxidation to CO_2 again showed no epinephrine effect in most cases. At an FFA concentration of 0.43 μeq/ml in the medium without glucose present, there was a 26% inhibition of CO_2 production that appeared statistically significant, but at the concentrations tested above and below this there was no significant effect.

Effects of Glucose. As shown in Table 4, glucose added to the medium markedly enhanced palmitate-1-C^{14} incorporation into neutral lipids at four different FFA concentrations. This glucose stimulation was of

TABLE 4. EFFECT OF GLUCOSE ON PALMITATE UTILIZATION IN MUSCLE

Concentration of Medium Palmitate	Concentration of Medium Glucose	Number of Pairs of Muscles Studied	Conversion to Neutral Lipid*		Conversion to CO_2*		Relative Specific Activity of Tissue FFA†	
			Control	Glucose	Control	Glucose	Control	Glucose
μeq/ml	*mg/100 ml*		*μeq/g/2 hrs*		*μeq/g/2 hrs*		*per cent*	*per cent*
0.14	82	3	0.07 ± 0.009	0.15 ± 0.009	0.07 ± 0.008	0.08 ± 0.02	3	3.4
			($p < 0.05$)					
0.93	73	3	0.49 ± 0.06	1.04 ± 0.06	0.54 ± 0.05	0.52 ± 0.04	9.6	10.7
			($p < 0.005$)					
1.11	82	3	0.80 ± 0.16	1.46 ± 0.22	1.63 ± 0.36	1.35 ± 0.32	17.3	13.4
			($p < 0.05$)				18.2	11.2
1.64	73	3	1.07 ± 0.18	2.06 ± 0.72	0.87 ± 0.06	0.76 ± 0.05		16.9
	43			0.70 ± 0.02		0.32 ± 0.02		
0.95		6‡		($p < 0.001$)				11.4
	104			0.96 ± 0.40		0.37 ± 0.02		

The probability of differences between means occurring by chance has been evaluated by the (t) test in each series of paired muscles.
* Calculated as in Table 1 and described under Methods.
† As in Table 2.
‡ Six pairs of muscles, one of each pair incubated at 43 mg/100 ml, the other at 104 mg/100 ml of glucose.

a similar magnitude (80% to 112% increase) and statistically significant ($p < 0.05$) at all four concentrations of FFA tested. On the other hand, the conversion of palmitate to CO_2 was either unchanged or, at higher concentrations of FFA, slightly decreased. This dissociation of effects indicates that the increase in neutral lipid synthesis is not attributable simply to an increased rate of penetration of labeled palmitate into the tissue but represents a true stimulation of glyceride deposition. This interpretation is further supported by the measurements of the tissue FFA specific activities which, as shown in Table 4, were not higher in the presence of glucose at any medium FFA concentration, and were actually lower at FFA concentrations of 1.11 and 1.64 μeq/ml.

Possible Effects of Interfibrillar Adipose Tissue. Since muscle is interlarded with adipose tissue, it is necessary to consider whether any of the effects observed in the intact muscle strips might be the result of changes in the metabolic patterns of such adipose tissue. The total triglyceride content of rat muscle was determined and found to be 17.5 mg/g wet weight of tissue; the triglyceride content of adipose tissue (epididymal fat pad) was 915 mg/g wet weight of tissue. If it is assumed that all of the triglyceride in the muscle represents interfibrillar adipose tissue, the amount of FFA that would be converted to CO_2 by that amount of adipose tissue can be estimated using the data of Milstein and Driscoll (15) on FFA oxidation by isolated adipose tissue. On this basis, an oxidation of 0.00015 μeq of medium palmitate per gram per hour is calculated; the observed rate of oxidation was 0.16 μeq/g/hour. It is clear that the adipose tissue present could not have contributed significantly to the observed palmitate oxidation. A similar calculation was made of the rate of triglyceride synthesis expected on the basis of the adipose tissue content, using data obtained with the epididymal fat pad incubated in glucose (16). The calculated rate was found to be one-third the observed rate. It is possible that the results obtained for effects on triglyceride synthesis are quantitatively distorted due to adipose tissue in the muscle, but they are not likely to be qualitatively altered. Furthermore, the fact that epinephrine, which has been shown to inhibit FFA incorporation into adipose tissue triglycerides (16), was without effect on incorporation into muscle triglycerides is against any major contribution of interlarded adipose tissue to the observed results.

DISCUSSION

The results of the present study suggest that a major factor governing the rate of lipid utilization in skeletal muscle is the concentration of available FFA in the serum. The rate of fatty acid oxidation to CO_2 and the rate of deposition of fatty acids in the form of triglycerides were both markedly increased as the FFA concentration in the medium was increased. Fritz and co-workers (11), using FFA concentrations up to approximately 0.6 μeq/ml, have previously reported similar findings with respect to palmitate oxidation by rat skeletal muscle. These workers, however, utilized very low concentrations of serum albumin and encountered variations in rates of oxidation with changes in fatty acid to albumin ratios. Reichl and Felt (17) have recently shown that at albumin concentrations of 1% or below, the uptake of fatty acids from the medium into adipose tissue is more rapid than it is at higher concentrations, and that glucose stimulation is less marked or not demonstrable at all. The present studies, utilizing physiological concentrations of serum albumin and covering a broader range of FFA concentrations, similar to those encountered in physiological and pathological states, supplement and extend the work of Fritz and co-workers.

The rate of conversion of labeled palmitate to $C^{14}O_2$ at the highest concentration studied here (1.64 μeq/ml) was more than ten times that at the lowest concentration (0.12 μeq/ml). These concentrations of palmitate corresponded to total FFA concentrations of 2.02 and 0.23 μeq/ml, respectively. If FFA utilization by the tissue *in vivo* is at all similarly sensitive to serum FFA concentration, it is clear that fluctuations in the concentration of this lipid component must strongly influence rates of lipid oxidation in muscle. Since muscle metabolism represents such a large fraction of the body's total metabolism, it would follow that overall metabolic rate may well be determined in large part by serum FFA levels, which in turn depend upon rates of mobilization from adipose tissue depots. For example, it is well established that serum FFA levels are, for reasons not clearly understood, consistently and strikingly elevated in hyperthyroidism (18). While this may only reflect accelerated mobilization, and thus be secondary to the prime cause of the hypermetabolism, an equally plausible hypothesis that we wish to suggest here is that the hypermetabolism is actually the direct result of the sustained high serum FFA levels, which in turn are caused by some more immediate metabolic effect of the thyroid hormone.

In a similar manner, the hypermetabolism observed after epinephrine injection may again be the direct result of the excessive mobilization of depot fat with consequent elevation of serum FFA levels. The conflicting findings regarding the calorigenic action of

epinephrine have been reviewed by Ellis (19), who concludes that the weight of evidence is against the notion that it is attributable to the increased cardiac work or to the hyperglycemia caused by epinephrine. Lundholm (20) has correlated the epinephrine-induced increases in oxygen consumption with hyperlacticacidemia. These earlier studies were done before the striking effect of catecholamines on FFA mobilization was recognized. The possibility, suggested by the present studies, that this FFA mobilization may be the immediate cause of the calorigenic action of epinephrine, deserves further investigation.

What we are proposing, in essence, is that the rate of utilization of lipid (FFA) is, to a large extent, simply controlled by the concentration of substrate available (blood FFA level) just as the rate of utilization of carbohydrate is controlled by blood glucose levels (21). In the case of glucose utilization, it is clear that hormonal control of uptake and utilization in the periphery strongly modulates the effects of substrate concentrations per se. Similar hormonal control of peripheral utilization of FFA may be demonstrated by further work, but at the moment the weight of evidence is that hormonal control is exercised primarily over the rate of mobilization, i.e., at the adipose tissue depots. Further efforts to establish *in vivo* the quantitative importance of serum FFA concentrations per se, as proposed here, appear to be justified.

The rates of incorporation of labeled palmitate into triglycerides rose with increasing medium FFA concentration in a manner similar to that observed for palmitate oxidation. This implies the possibility of net triglyceride deposition if the blood FFA are maintained at high levels over prolonged periods. The deposition of triglycerides in cardiac muscle observed by Maling and Highman (8), and in skeletal muscle by Feigelson *et al.* (7) after norepinephrine infusions, could be explained on this basis. In the present studies it has been shown that epinephrine added *in vitro* does not itself affect the rate of triglyceride synthesis from palmitate. Although other indirect effects of the catecholamines may contribute, it seems reasonable to attribute the lipid deposition observed in these *in vivo* studies mentioned above directly to the elevated FFA levels. In other words, the FFA mobilizing action of epinephrine acting on depot fat may be a sufficient explanation for the triglyceride deposition in muscle observed following its administration in large amounts. The possibility that the more marked accumulation of triglyceride in the liver during norepinephrine infusion (7) is also due to a direct effect of the elevated serum FFA levels is under study.

Oxidation of medium palmitate to CO_2 was not importantly influenced by the presence of physiological concentrations of glucose in the medium. These findings are in agreement with the earlier results of Fritz *et al.* (11). They supplement the conclusions of Allen *et al.* (22) and of Neptune *et al.* (3), based on studies of oxidation of endogenous tissue lipids, that glucose *in vitro* does not significantly alter the rate of oxidation of lipid. Recently Fritz and Kaplan (23) have shown that in diaphragm muscle taken from rats previously starved for 4 days, or from rats with chronic alloxan diabetes, the rate of palmitate oxidation is much higher than normal, and that addition of glucose to the medium returns it toward normal. Schwartzman and Brown (24), using diaphragms taken from rats starved for 24 hours, found no effect of glucose at a concentration of 150 mg/100 ml, but at 500 mg/100 ml there was a statistically significant inhibition of palmitate oxidation (approximately 25%). Under certain abnormal conditions, then, glucose can apparently influence the rate of fatty acid oxidation in muscle. More significant, however, is the agreement among the various groups that have studied the problem that in muscle tissue taken from normal, fed animals, and at physiological concentrations, glucose does not appear to alter the rate of oxidation of added long-chain fatty acids.

The physiologic effects of glucose on the relative rates of carbohydrate and lipid metabolism may then be limited to its effect on the rate of mobilization of depot fats, as suggested by Dole (5) and Gordon (6). Again, the serum FFA level appears to be a key controlling factor.

On the other hand, the rate of conversion of palmitate to neutral lipid was markedly enhanced. The interesting implication of this finding is that the endogenous stores of muscle lipid may be built up during periods when extra glucose is available (e.g., postprandially), to be drawn upon during periods when carbohydrate is less available. The studies of Volk *et al.* (2) first demonstrated the major role of endogenous lipid in oxidative metabolism of peripheral tissues. Later studies by Neptune *et al.* (3) indicate that diaphragm from fed rats contains sufficient lipid to support normal oxygen consumption *in vitro* for 4 hours, whereas the diaphragm from fasted rats has a very limited reserve of tissue lipid on which to draw. It should be noted, however, that the latter studies also indicate that much of the endogenous lipid available for oxidation is exhausted in 4 hours, even in the unstimulated diaphragm. Over any extended period of time, and certainly during exercise, the exogenous lipid brought to the tissue, primarily in the form of FFA, must be the major source of lipid substrate. Moreover, it remains to be

determined to what extent endogenous lipid would be utilized if FFA were simultaneously available.

Finally, it is important to call attention to a difficult question of interpretation arising in the present studies, as well as in the work of others using similar methods (11). The fraction of added C^{14}-palmitate appearing in the $C^{14}O_2$ or in labeled triglycerides depends not only on the rates of the reactions involved, but also on the specific activity of the immediate tissue precursors and intermediates. In the present studies the specific activity of the tissue FFA pool was measured at the end of the incubation. As shown in Table 2, the terminal specific activity was higher relative to that in the medium as the total initial medium FFA concentration was increased. This result would be anticipated as the result of a greater net transfer of labeled fatty acid into the tissue and through the tissue FFA pool en route either to CO_2 or neutral lipid. On the other hand, an effect of FFA concentration on the rate of equilibration between medium and tissue FFA cannot be ruled out. Further studies on this important point are needed before the observed isotope results can be considered conclusive.

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