Turnover of Palmitate C-14 in Diabetics and Normals.* (28311)

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Elevated levels of plasma free fatty acids (FFA) have been observed in the diabetic state(1,2) reflecting impaired glucose metabolism. The level of FFA is, however, in itself not a measure of rate of turnover (U) of fatty acids, since this is also dependent on the disappearance constant (K) and the space of distribution (S) of FFA.

The following studies were designed to study the effect of diabetes on the above parameters using carboxyl labeled palmitic acid C-14 injected intravenously.

Methods. The patients were studied in the fasting state. After one hour of rest, baseline heparinized blood samples were obtained and the patients received intravenously 2 μc of carboxyl labeled palmitate-C-14-albumen complex prepared by the method of Friedberg, *et al.*(3). Blood samples were drawn at 2 minute intervals for 10 minutes. When effect of exercise was studied subjects were placed on the treadmill at ½ mile per hour for 20 minutes.

The plasma free fatty acids were titrated by the method of Gordon and Cherkes(4) and extracted by the method of Shafrir and Steinberg(5). After titration, extracts were prepared for assay of radioactivity as outlined by Friedberg *et al.*,(3) and counted in the liquid scintillation counter. Blood glucose was determined in the auto-analyzer.

Results. Plasma Disappearance Curves. The labeled palmitic acid disappeared according to a first order (logarithmic) curve during the first 4 minutes (Table I). Significant deviations from this occurred in later parts of the curves, particularly in diabetics. Thus, the disappearance constant (K) was calculated from values obtained from the second to the fourth minutes after injection. Mean values for K were .295 ± .096. There was no significant difference between normals and diabetics. The average half life was 2.4 minutes. The effect of exercise was studied in 8 normal subjects (Table II) where K was .300 ± .084 before exercise and .496 ± .158 during exercise. The effects of exercise on the plasma FFA are shown in Fig. 1 where it is noted that elevations following exercise last as long as one hour after the exercise. Since a constant level of FFA is necessary to satisfy the criteria for a steady state in subsequent calculation of constant of disappearance, a resting period of one hour was used before all determinations in the resting state.

Space of Distribution (S) was calculated dividing the extrapolated value of plasma labeled palmitate at time 0 into the dose of labeled palmitate administered. The space of distribution (S) 3.10 ± 1.06 liters, approximated the estimated plasma volume in both normals and diabetics. The values for S were greatly altered by exercise, thus invalidating this parameter during exercise.

Turnover of FFA. Rate of turnover (U) of FFA can be estimated as follows:

$U = S \times C \times K$

where S = space of distribution in liters
C = concentration of FFA in μ moles/liter
K = disappearance constant

Turnover was 6.5 ± 1.9 μ moles per kg per min for 8 normals and 8.7 ± 4.1 for the 28 diabetics. The differences are not significant. Certainly there is no impairment of utilization

TABLE I. Parameters of Plasma Palmitate Turnover in Diabetics.

Case	FBS	FFA	K	S	U
M	167	0.74	.295	3.10	8.73
σ	49	0.32	.096	1.06	4.04

FBS = Fasting blood sugar (mg %).
FFA = Plasma free fatty acids (millimoles/l).
K = Disappearance constant.
S = C^{14} palmitate space (l).
U = Palmitate turnover (μmoles/kg/min).

$$\sigma = \sqrt{\frac{\Sigma x^2}{N}}.$$

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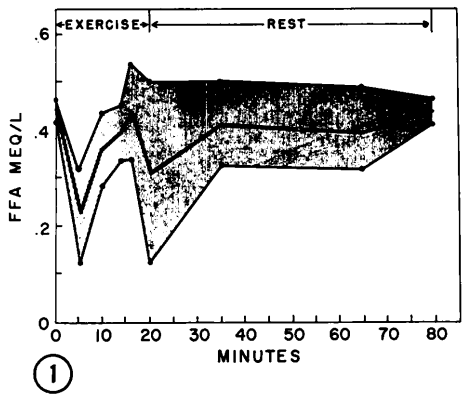

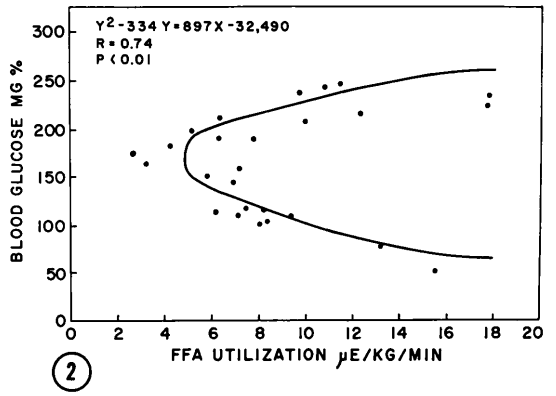

FIG. 1. Effect of exercise and rest on plasma FFA.
FIG. 2. Blood glucose *vs* FFA utilization.

TABLE II. Effect of Exercise on Plasma Disappearance of C-14-Palmitate in Normals.

Case	K Resting	K Exercise	U Resting
M	.300	.496	6.54
σ	.084	.158	1.92

$$\sigma = \sqrt{\frac{\Sigma x^2}{N}}.$$

of FFA in the diabetics. When U was compared with fasting blood sugar a parabolic distribution was noted (Fig. 2) such that at a glucose level of 167 mg % mean FFA turnover was minimal. At levels of blood sugar both above and below this, there was a tendency to increased FFA turnover. Assuming the relationship shown in Fig. 2, the correlation between fasting blood sugar and FFA turnover (U) is 0.72 ($P < .01$).

Discussion. These data show that prolonged resting periods up to one hour are necessary before the steady state conditions are suitable for estimation of turnover of FFA. It is doubtful that estimations of U during exercise are valid because of rapid changes in FFA and the error noted in the estimation S. The increase in K during exercise undoubtedly reflects an increased rate of turnover (U) of FFA, but no conclusions should be drawn as to magnitude.

The data show that U is not significantly different in diabetics and normals. Thus, the elevated FFA found in some diabetics does not reflect any impairment in metabolism of FFA, but merely shows an increased plasma pool of FFA. When the state of diabetic regulation is considered using fasting blood glucose, the data show that U is minimum at blood glucose level of 167 mg %. As the blood glucose increases U increases, reflecting an increased utilization of FFA as glucose utilization is impaired. At lower levels of glucose there is also a tendency for U to increase. This occurred in patients under treatment with insulin and probably is a result of sympathetic nervous system activity brought on by lower levels of blood sugar(6,7,8). Epinephrine is known to impair uptake of FFA by adipose tissue (9,10) and to elevate plasma FFA(1,2).

Summary. Turnover rate of free fatty acids was studied during rest and exercise in 8 normals and 28 diabetics using carboxyl labeled palmitate C-14. Conditions during exercise do not satisfy the criteria for steady state dynamics. During rest the turnover rate of free fatty acids does not differ in normals and diabetics. At both higher and lower levels of blood glucose the turnover rate is increased.

1. Dole, V. P., *J. Clin. Invest.*, 1956, v35, 150.
2. Gordon, R. S., Cherkes, A., *ibid.*, 1956, v35, 206.
3. Friedberg, S. J., Harlan, W. R., Jr., Trout, D. L., Estes, E. H., Jr., *ibid.*, 1960, v39, 215.
4. Gordon, R. S., Cherkes, A., Gates, H., *ibid.*, 1957, v36, 810.
5. Shafrir, E., Steinberg, D., *ibid.*, 1960, v39, 310.
6. Sussman, *Proc. of Am. Diabetic Assn.*, 1962, v22, 15.
7. Bolinger, R. E., Shane, S. R., Kirkpatrick, C. H., *J. Clin. Endoc. and Metab.*, 1962, v22, 873.

8. Von Euler, U. S., Luft, R., *Metabolism,* 1952, v1, 528.

9. Jeanrenaud, B., *ibid.,* 1961, v10, 535.

10. White, J. E., Engel, F. L., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 375.

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Observations on Mechanism of Action of the Antifungal Peptide, Ro2-7758.* (28312)

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The antifungal sulfur-containing peptide, Ro2-7758 (formerly X-5079C), described recently by Grunberg *et al.*(1), has certain properties not shared by other antibiotics. Besides being the first systemically useful antifungal peptide to be described, the wide discrepancy in its apparent *in vitro* and *in vivo* activities and its limited antimicrobial spectrum make an investigation of its *in vitro* activity somewhat difficult. Its apparently enhanced activity *in vivo* has not been explained on the basis of conversion to a more active form by mammalian tissues(2). A previous report from this laboratory(3) showed a markedly thickened cell wall of *Mucor corymbifera* when grown on Sabouraud agar in the presence of the drug. The present report deals with further studies attempting to demonstrate the biochemical mechanism of action.

Materials and methods. Mucor corymbifera (Duke Fungus Registry no. 2902) was used as test organism in reversal experiments. Of several organisms tested, this one had been found previously to give the most marked inhibitory pattern(3). Fleischmann's wet yeast was obtained from Standard Brands, Inc. Starch-free baker's yeast from Anheuser-Busch was used for preparation of the cell-free sulfate reductase system(4). All chemicals used were reagent grade and were used without further purification. The Ro2-7758 was obtained from Hoffmann-LaRoche.

Attempts to overcome inhibition of *M. corymbifera* with various substances were done on Sabouraud agar plates using a crossed filter paper strip technique similar to the one described by Weinberg *et al.*(5). In this procedure, a filter paper strip impregnated with a solution of the compound being tested for possible reversal of inhibition was placed on a Sabouraud agar plate uniformly streaked with a spore suspension of the test organism. Molar concentrations of these compounds were 1 × and 10 × that of the antibiotic, which has a molecular weight of about 3000. After 10 minutes, another filter paper strip soaked in a solution of 1.0 mg/ml Ro2-7758 was placed on the plate at an angle of 90° to the first strip. Plates were observed at 48 hours for presence or absence of continued growth at the intersection of the two strips.

Measurements of uptake of $S^{35}O_4$ by intact yeast cells were done in 0.1 M potassium phosphate buffer at pH 7.0, containing 1% glucose. Aliquots were removed periodically, clarified by centrifugation, and the activity remaining in the supernatant solution was measured in a Tri-Carb liquid scintillation spectrometer (Packard Instrument Co.). Calculations of sulfate uptake were made after determining packed cell volume by centrifugation of an aliquot in a Wintrobe hematocrit tube at 2000 × g for 15 minutes. Protein content of each sulfate reductase preparation was estimated by absorption of the solution at 280 λ and 260 λ.

Results. Gross appearance of cultures of *M. corymbifera* on Sabouraud agar plates inhibited by Ro2-7758 was as described previously(3). When a filter paper strip impregnated with the drug was placed on a plate

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