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Research Article

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A COMPARTMENTAL MODEL

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ABSTRACT Palmitate, glucose, and glycerol oxidation to CO_2 have been investigated in the fasted state in ten normal subjects and nine patients (six hyperlipoproteinemias, one xanthomatosis, and two glycogenosis) after intravenous injection of [1-^{14}C]palmitate, [1-^{14}C]-glucose, or [1-^{14}C]glycerol in tracer amounts. The specific activities and concentrations of plasma palmitate, glycerol, or glucose and expired CO_2 were measured at various intervals after the injection for a period of 24 h. All the studies were analyzed in terms of a multicompartment model describing the structure for each of the subsystems, the transfer of carbon label between subsystems, and the oxidation to CO_2. A bicarbonate subsystem was also included in the model to account for its role in shaping the CO_2 curves.

All the CO_2 activity following a palmitate injection could be accounted for by a direct oxidative pathway from plasm FFA with the addition of a 20-min delay compartment. The same also applied to glucose, except that the delay compartment had a mean time of about 150 min. Only about a third of the injected glycerol was directly oxidized to CO_2 from plasma; the delay time was about 4 min. Most of the remainder was converted to glucose.

In normals about 45% of the FFA is oxidized to CO_2 directly. This constitutes about 30% of the total CO_2 output. In hyperlipemia the CO_2 output is nearly unchanged and the contribution from FFA is nearly the same. There is a considerable increase (factor of 2), however, in FFA mobilization, most of which is probably diverted to triglyceride synthesis.

The glucose and glycerol subsystems are roughly the same in normals and hyperlipemics. About 50% of glucose is oxidized by the direct pathways which accounts for about 35% of the CO_2 output. Glycerol accounts for only 1.5% of the CO_2 produced.

Major changes occurred in the glycerol and glucose subsystems in glycogenosis. The changes are consistent with the known deficiency in glucose-6-phosphatase in this disorder. There is a considerable reduction (factor of 2 or more) in the release of glucose to plasma (gluconeogenesis) and in the conversion of glycerol to glucose.

Despite the integration of the kinetics of the glucose, glycerol, and FFA subsystems over a 24-h period, 36% of the CO_2 production was still unaccounted for in normals and 50% in hyperlipemics. Thus, some of the carbon must wind up in very slowly turning-over pools which supply CO_2 through subsystems not covered in these studies (triglycerides, glycogen, amino acids, etc.).

All the modeling was carried out with the aid of the SAAM25 computer program.

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INTRODUCTION

Glucose and fatty acids in plasma follow different metabolic routes in the synthesis of glycerides, phospholipids, and in oxidation to CO_2. The roles and relative magnitudes of these pathways are important in understanding normal and pathologic metabolism. Studies have been carried out on the contribution of glucose, FFA, and glycerol to glyceride synthesis in hyperlipemia (1–3). Some workers have also studied the oxidation of FFA (4–6), glucose (7–10), and glycerol (11, 12) in normals. The present study is concerned with the oxidation and inter-

TABLE I

General Description of Patients Studied

Subject	Condition	Sex	Age	Weight	Plasma volume	Total lipids	Total cholesterol	Phospholipids	Triglycerides	Glucose	Glycerol	FFA	CO_2 excretion*	Tracer used‡
			yr	*kg*	*ml*			*mg/100 ml*			*mmol/liter*		*mmol/min*	
H. J.	Normal	F	39	54	2,230	576	190	210	104	86	0.041	0.84	6.9	P
F. E.	Normal	M	44	68	3,056	515	175	228	95	102	—	0.81	8.2	P
C. J. L.	Normal	M	20	58	2,400	460	155	159	60	90	0.040	0.46	9.6	P
B. M.	Normal	F	37	55	2,275	585	185	257	45	88	0.066	0.85	6.0	P
C. J.	Normal	M	22	72	2,980	500	166	210	60	100	0.040	0.63	9.9	P
B. J.	Normal	M	20	61	2,520	489	152	170	73	106	0.047	0.75	8.8	P
N. M.	Normal	M	24	65	2,685	450	168	160	79	100	0.066	—	7.0	GL
M. M.	Normal	M	20	67	2,746	805	260	288	163	99	0.063	—	8.9	GL
L. M.	Normal	F	21	57	2,354	484	169	221	66	113	—	0.53	6.5	G
D. P.	Normal	M	22	74	3,056	573	184	234	94	101	—	0.48	11.9	G
S. M.	Type IV	M	39	89	3,675	3,320	588	638	1,736	149	—	0.82	9.0	P
J. I.	Type IV	F	34	54	2,230	3,700	298	1,010	2,150	110	—	1.32	8.3	P
L. A.	Type IV	M	45	66	2,730	2,776	534	800	1,260	115	0.052	0.83	7.9	P
D. V.	Type IV	M	44	72	2,970	1,500	354	490	605	94	0.040	0.55	7.7	GL
H. I.	Type V	M	42	95	3,925	1,150	284	335	352	95	0.040	1.02	7.7	GL
L. R.	Type V	M	50	80	3,300	1,685	270	420	900	114	0.057	0.72	10.5	GL
B. D.	Glycogen.	F	20	55	2,250	1,990	347	475	1,040	69	0.094	1.01	8.5	GL, G
G. E.	Glycogen.	M	15	26	1,066	3,260	374	772	2,360	74	0.080	1.34	7.0	GL, G
M. E.	Xanthomatosis	M	38	65	2,685	610	188	221	103	76	—	0.44	7.8	P

* Average of 21 samples collected over an 8-h period. No significant differences were observed between the CO_2 excretion rates at the beginning and at the end of the tests.

‡ G, glucose; GL, glycerol; P, palmitate.

conversion of these metabolic fuels in normals, in essential hyperlipemia (types IV and V–Fredrickson classification), and in secondary hyperlipemia (glycogenosis). Aside from the obvious physiological interest, such studies also serve to develop a universal model for the kinetics of these metabolites, to help understand the mechanism of hyperlipemia, and to identify sites or parameters affected by changes in the system. The present studies were carried out for a period of 24 h (36 h of fasting)–considerably longer than most other reported experiments of this type.

METHODS

Subjects and procedures

Experimental subjects[1] (*Table I*). Seven male and three female patients ranging in age from 20 to 44 yr served as "normals" for these studies. Eight of the patients were "normal control volunteers" and had no evidence of any organic disease. The two remaining patients were admitted for study of unrelated disorders and had no clinical or laboratory evidence of abnormal lipid and carbohydrate metabolism.

Ten abnormal subjects were studied. Patients S. M., J. I., L. A., P. A., and D. V. were type IV hyperlipoproteinemias, and L. R. and H. I. type V hyperlipoproteinemias—according to the Fredrickson classification scheme. The experiments were performed before any special diet or drug treatment. Patient M. E. had diffuse papular lesions of disseminated xanthomatosis (type histiocytosis X), ischemic heart disease, arterial lesions of lower legs, and diabetes insipidus but no hyperlipemia. Patients B. D. and G. E. were diagnosed as glycogenosis type I with a deficiency of the enzyme glucose-6-phosphatase.

Materials. [1-^{14}C]glycerol (15.3 mCi/mmol, Amersham, England, batch 54) or D-[1-^{14}C]glucose (58.8 mCi/mmol, Amersham, batch 14) were dissolved in 0.15 M sodium chloride. [1-^{14}C]Palmitic acid (55.2 mCi/mmol, Amersham, batch 44) was complexed to human serum albumim (fraction V, Institut Meyrieux, France) as follows: benzene was evaporated at room temperature and replaced by ethanol and 5 drops of a 1 N NaOH solution. Solvent was evaporated and the sodium salt was taken up in moderately warm distilled water and mixed immediately with 3 ml of previously iced human albumin 20% solution and kept overnight in the refrigerator.

In presenting the results, the quantity of ^{14}C injected was normalized to 200×10^6 dpm.

Procedure. All subjects were maintained on a standard diet for 2 wk before the test, having a caloric intake of 45% from carbohydrate,[2] 40% from fat, and 15% from protein. The subjects were fasted overnight (14 h) before and for 500 min after the beginning of the study. They then ate a normal meal and fasted again (14 h) until the following morning. A Cournand needle was placed in the brachial artery of one arm and 100 μCi of [1-^{14}C]palmitate-albumin complex, 100 μCi of [1-^{14}C]glycerol or 100 μCi of D-[1-^{14}C]-glucose (in 9–12 ml of solution) were injected within 10 s

[1] Informed written consent was obtained from the volunteers and patients.

[2] Carbohydrates consisted of potatoes, bread, vegetables, and sucrose added to cream, jelly, and pastries. The amount of starch slightly exceeded the amount of sucrose + fructose in the diet.

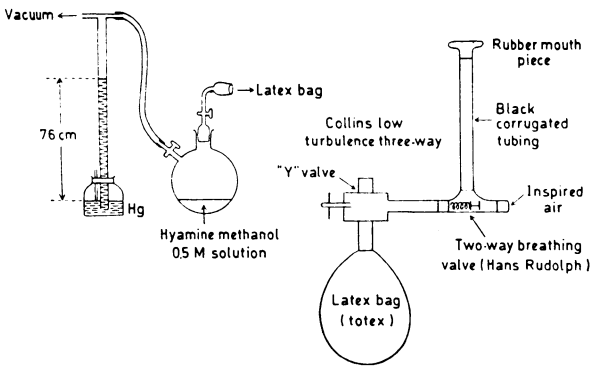

FIGURE 1 Apparatus used for CO_2 collection (see Methods). The Collins three-way valve was connected to the two-way breathing valve on one side and to two 100-liter latex bags on the other side. The rapid change of position of the Y valve (1 s) allowed the uninterrupted collection of expired air during the first hour, by 5-min collection periods.

into one of the antecubital veins of the opposite arm. Starting with the instant of injection, expired air was collected in 100-liter latex bags for 5-min periods without interruption for the 1st h, at 30-min and later 60-min intervals during the subsequent 8 h, and finally at 24 h.

Blood samples were obtained from the artery at 1-min intervals for the first 10 min, at 5-min intervals for the next 20 min, at 15-min and 30-min intervals to 180 min, and then from the vein at 60-min intervals to 480 min; one sample was taken at 720 and one at 1,440 min.

Separation of radioactivity of glycerol, glucose, and palmitate. Blood was collected in dry syringes, transferred to tubes containing heparin, mixed thoroughly, and immediately placed in ice until analytic procedures began. Plasma was separated from cells at 5°C in a refrigerated International PR-2 centrifuge (International Equipment Co., Needham Heights, Mass.) at 2,000 rpm for 20 min. Aliquots of plasma were extracted immediately for FFA, glucose, and glycerol determinations. 1-ml aliquots were extracted in 25 ml chloroform-methanol (CM)[3] 2:1 and 5 ml of 0.2% NaCl solution. Chloroform from the lower phase was removed by evaporation at 40°C under nitrogen and then fractionated by silicic acid column chromatography to separate neutral lipids and phospholipids. The chloroform phase containing neutral lipids was evaporated under nitrogen and redissolved in 10 ml of 2,2,4-trimethylpentane. FFA were separated from triglycerides and cholesterol esters by the method of Borgström (13). The purity of the FFA fraction was controlled by thin-layer chromatography (TLC) in a system of hexane-diethylether-acetic acid 80:20:1 (vol/vol/vol). FFA isolated in isooctane were evaporated in counting vials to which 15 ml of a 0.5% 2,5-diphenyloxazole solution in toluene (DPO) was added. The vials were counted in a Tracerlab Mark II liquid scintillation counter (LFE Electronics, Richmond, Calif.).

[1-^{14}C]glucose in 1 ml of plasma was obtained from the yield of $^{14}CO_2$ measured by the fermentation method of Bernstein, Lentz, Malm, Schambye, and Wood (14). $^{14}CO_2$ was trapped in 1 ml of molar Hyamine hydroxide and 15 ml of DPO solution was added for counting.

After a labeled glycerol injection, total plasma radioactivity was counted by using 200 μl of plasma in 10 ml of Instagel. 0.5 ml of plasma was deproteinized with 0.5 ml of a 6% perchloric acid solution and neutralized by 25 μl of 10 N KOH solution. 400 μl of the neutralized protein-free filtrate (supernate) was counted in 10 ml of Instagel. Radioactivity was also counted in the neutralized protein-free filtrate after passing it through an Amberlite MB3 resin (Rohm and Haas Co., Philadelphia, Pa.) column. A mean correction was made in all samples for glucose radioactivity contamination not bound to the resin particles. It has been estimated by Winkler, Rathgeb, Steele, and Altszuler (15) that infusion of [1-^{14}C]glycerol gives rise to plasma glucose ^{14}C in which 50% of the ^{14}C is in the carbon-1 and 50% in the carbon-6 position. Thus to obtain the total [^{14}C] glucose activity in each sample, the activities obtained by the fermentation method described above must be multiplied by a factor of 2.

The protein-free filtrate of all plasma samples was also placed on a ground Amberlite IRA 410 resin (Rohm and Haas Co.) column. The radioactivity retained was assumed to be due essentially to bicarbonate and lactate. Labeled bicarbonate, lactate, or acetoacetate added to a cold serum protein-free filtrate are retained at 96, 94, and 95%, respectively. The eluate was incubated for 1 h at 37°C with Glucostat reagent buffered at pH 7.0 with 0.1 M phosphate (Worthington Biochemical Corp., Freehold, N. J.) to convert glucose to gluconic acid. 2 g of a mixture consisting of zeolite, Lloyd reagent, $CuSO_4$, and $Ca(OH)_2$ (16) was added, and the sample was thoroughly shaken for 1 h. At least 98% of the gluconic acid was bound to the zeolite mixture. No glycerol was bound to the zeolite mixture. After centrifugation at 3,000 rpm the supernate was neutralized with 0.5 N HCl and counted in vials after adding 10 ml of Instagel. This radioactivity, assumed to represent exclusively glycerol, was compared to the corrected radioactivity obtained after passing of the protein-free filtrate through the Amberlite-MB-3 column (see above). To exclude a possible contamination of glycerol with lipids, an aliquot of the final supernate was extracted with CM 2:1. No radioactivity was recovered in the chloroform phase. To eliminate contamination with amino acids, aliquots of the protein-free filtrate or of the neutralized supernate after treatment with Glucostat and zeolite were placed on Zeokarb 325 (14–52 mesh, Taylor Chemicals, Inc., Baltimore, Md.) resin column previously treated with 10% HCl and distilled water. The eluate was counted in 10 ml of Instagel. Resin columns were eluted twice with 10 ml of 1 N NH_4OH (17). The extract was lyophilized and the residue redissolved in 1 ml 0.1 N HCl, adding 10 ml of Instagel in counting vials. A very small amount of radioactivity was recovered in this fraction.

Gas analysis. 10 ml of a 0.5 M Hyamine-methanol solution (hydroxide of Hyamine 10-X in methanol, Packard Instrument Co., Inc., Downers Grove, Ill.) were pipetted in round bottom flasks (2.21±0.02 liters). With the aid of an oil-vacuum pump the flask was put under controlled vacuum in such a way that the partial pressure of Hyamine-methanol equaled 35–40 mm of Hg and the volume of air collected was always the same (2.1 liters), according to the formula:

$$2.21 \text{ liters} \times \frac{P_{atm} - P_{methanol}}{P_{atm}} = 2.1 \text{ liters}$$

[3] *Abbreviations used in this paper:* CM, chloroform-methanol; DPO, 2,5-diphenyloxazole in toluene; TLC, thin-layer chromatography.

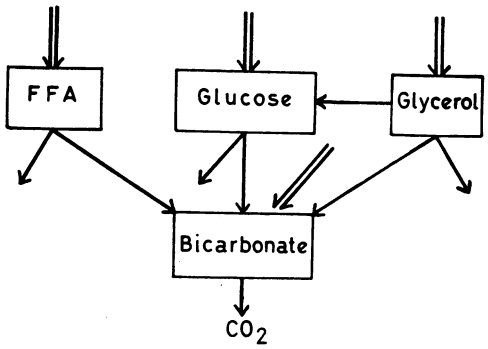

FIGURE 2 Block diagram describing the conversion of FFA, glucose, and glycerol to CO_2. Doubly lined input arrows indicate steady state tracee inputs.

Immediately after closing the connection to the vacuum pump and attaching the bag to the flask, a portion of the breath sample was allowed to enter the flask containing Hyamine. Pressure equilibrium was reached within 10 s (Fig. 1). After a ½-h absorption time, Hyamine-methanol was transferred with three methanol washings to glass-stoppered 25-ml volumetric flasks. A blank was prepared using 10 ml of 0.5 M Hyamine-methanol in 25-ml flasks, adjusted to the mark with methanol. Control measurements in Conway cells showed that CO_2 was completely and quantitatively absorbed by Hyamine (mole/mole). Three 1-ml aliquots of blank and analyses were titrated in duplicate against 0.1 N HCl to an end point with phenolphthalein, adding 5 ml of a 3% barium chloride solution (weight/volume). In each experiment, 2.1 liters of expired air were taken in duplicate for three different periods in order to test the reproducibility of the method. The coefficients of variation of the duplicate analyses were 1.1% for CO_2 and 1.5% for $^{14}CO_2$. Two 5-ml aliquots were pipetted in vials containing 10 ml for Bray's (18) mixture and counted for ^{14}C in a Nuclear Chicago liquid per millimole scintillation counter Mark II (Nuclear-Chicago Corp., Des Plaines, Ill.). Radioactivity was expressed as disintegrations per minute of CO_2 and cumulative excretion as percent of the dose of ^{14}C injected. The remaining content of each bag was measured in a wet gas meter (type M 809 n, A. Wright & Co., Ltd., Westminster, England) with a 0.1-liter precision. This permits the calculation of the total volume and, thus, the total radioactivity expired.

Chemical determinations. Glycerol concentration of plasma was analyzed after a perchloric deproteination with the enzymatic method of Wieland (19). FFA were extracted and titrated by the method of Dole (20), with 2,2,4-trimethylpentane in place of heptane. Total lipids were determined according to the method of Bragdon (21), glyceride glycerol as described by Carlson and Wadström (22), and lipid phosphorus according to Stewart and Hendry (23). Total cholesterol was determined with the Technicon AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N. Y.) by the method of Pearson modified by Boy (24), glucose by a modification of the method of Hoffman (25), and bicarbonate by the micro CO_2 proved N19A procedure. Lactate was analyzed with the UV test kit Boehringer (Boehringer Mannheim Corp., New York) with NADH (TLAA-15972). Electrophoresis of lipoproteins was performed on cellulose acetate strips by a modification of the method of Colfs and Verheyden (26), using Fat Red 7B Gurr. The quantitative determination was made by densitometry. Plasma lipoprotein lipase was assayed by using the method of Fredrickson, Ono, and Davis (27).

Definitions of symbols (28)

- L_{ij} Fraction of compartment j activity or mass transported to compartment i per unit time (min^{-1}).
- L_{0j} Fraction of compartment j activity or mass transported irreversibly out of the system per unit time (min^{-1}).
- L_{jj} Fraction of irreversible and reversible transport of activity or mass out of compartment j per unit time (min^{-1}).
- U_j Steady state transport of mass into compartment j from outside of system ($mmol \cdot min^{-1}$).
- M_j Steady state mass of compartment j (mmol).
- R_{ij} Steady state transport through the L_{ij} path: $R_{ij} = L_{ij} \cdot M_j$ ($mmol \cdot min^{-1}$).
- V_p Plasma volume (liter).
- V_j Plasma equivalent volume of compartment j (liter).
- BW Body weight.

Model and data analysis

To account fully for the observed oxidation kinetics of glycerol, glucose, and FFA, it was necessary to consider the conversion of glycerol to glucose, the oxidation kinetics of glycerol, glucose, and FFA, and the kinetics of bicarbonate. A block diagram of these subsystems and their interconnecting pathways is shown in Fig. 2. The detailed structure of each subsystem was determined from a combination of our experimental data and published literature information. The entire model was tested against all the information for consistency and uniqueness in defining the parameter values. All the modeling and data fitting was performed on a digital computer using the SAAM25 program of Berman and Weiss (29).

In calculating steady-state masses and transports all quantities were expressed in carbon-equivalent units, by using a factor of 3 for glycerol, a factor of 6 for glucose, and a factor of 17 for FFA. From the steady-state calculations it is possible to determine the fraction of each moiety converted to the others and the contribution of each moiety to the total CO_2 output.

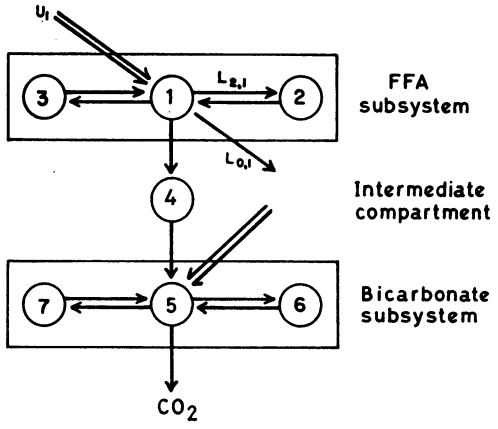

FIGURE 3 Compartmental model describing the conversion of [1-^{14}C]palmitate to $^{14}CO_2$. Compartment 4 is referred to as a coupling compartment between the two subsystems. Doubly lined arrows indicate steady-state tracee inputs.

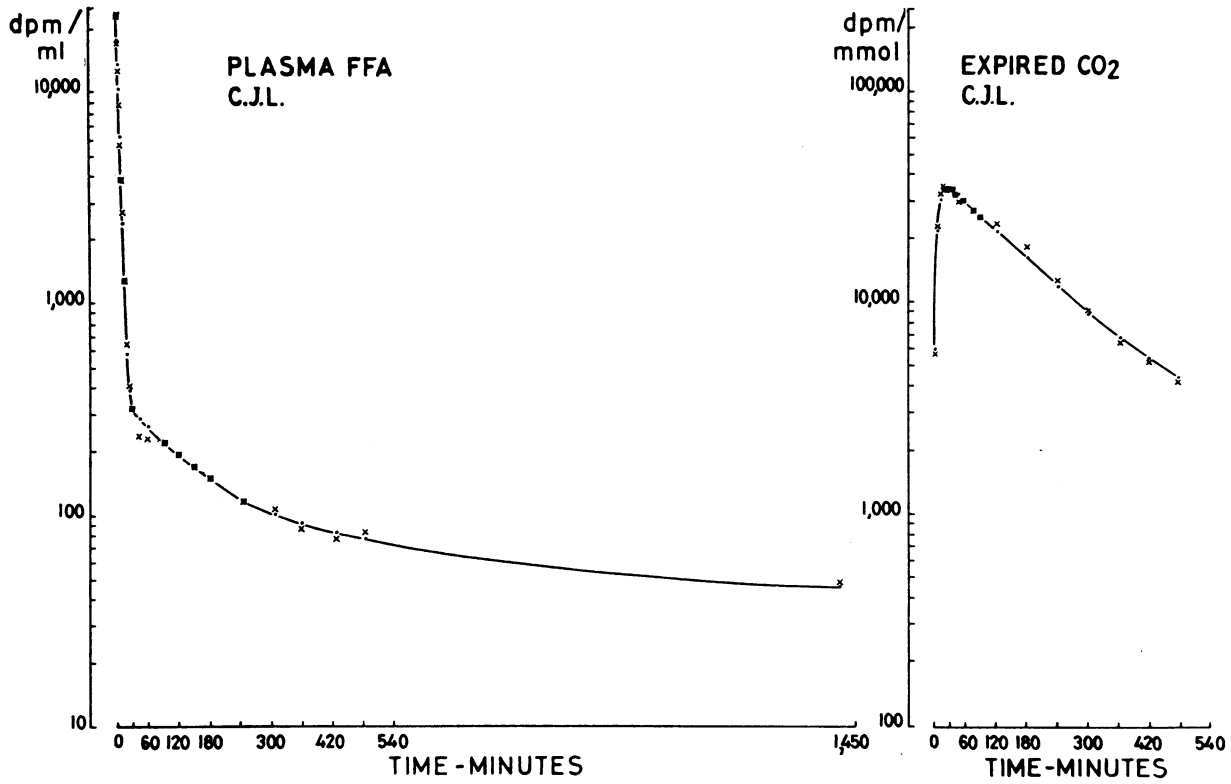

FIGURE 4 Typical kinetic data for FFA labeled experiment in normal control patient. Dots and solid lines connecting them are model predictions. ×'s are observed values. The superposition of a dot and × appears as a square.

Bicarbonate. No experiments were carried out to study specifically the bicarbonate subsystems in our subjects and a three-compartment structure proposed by Waterhouse, Baker, and Rostami (6) was adopted (Fig. 3). Estimates of parameter values and their uncertainties based on the population of Waterhouse et al. were initially entered in the model for fitting our CO_2 data. When our data contained some information about the bicarbonate subsystem, the parameter values were free to readjust, subject to the imposed a priori statistics of the population. The bicarbonate subsystem derived from the FFA-CO_2 studies was also used to analyze the glucose-CO_2 and glycerol-CO_2 subsystems.

Free fatty acids. The model for the FFA subsystem is shown in Fig. 3. Plasma FFA was simulated by a three-compartment model. The two-compartment model previously proposed by Eaton, Berman, and Steinberg (2) and Shames et al. (43) was based on at most 2-h data and was inadequate to fit the 24-h data in the present experiments.

To start with, it was assumed that the oxidation of FFA to CO_2 took place via a rapid pathway, from plasma FFA to plasma bicarbonate (5). To test this, plasma FFA data were used as a direct precursor to the three-compartmental bicarbonate model. It was observed that such a path did not satisfy the early CO_2 data; a short delay between plasma FFA and bicarbonate was needed. This was accomplished by the insertion of a compartment between the two as shown in Fig. 3. A very good fit of the data was obtained with this model as shown in Fig. 4. The rate constants were estimated with satisfactory precision as shown in Tables II and III. In fitting the data it was assumed that CO_2 specific activity approximates that of plasma bicarbonate (30, 31) and that plasma is the site of entry of newly released CO_2 into the bicarbonate subsystem (32, 33).

Glucose. The two-compartment glucose model published by Shames et al. (7) and its oxidation to bicarbonate was initially adopted. The Shames model, however, was based on 5-h studies, and did not satisfy our plasma glucose data collected over a 24-h period. We found it necessary to add a third compartment to the glucose subsystem as shown in Fig. 5. The parallel arrangement of the compartments is arbitrary since our data cannot determine the exact connectivity. This choice, however, is not critical with respect to the major conclusions of the paper.

The bicarbonate subsystem used with the glucose subsystem is the same as that used with the FFA.

The intermediate subsystem which couples glucose to bicarbonate is structurally the same as that published by Shames et al. (7), although some of the parameter values were somewhat different, as discussed later.

As shown in Fig. 6, a very good fit was obtained with the chosen model. The parameter values and their uncertainties, derived from a least square fit of the data, are shown in Table V.

Glycerol. The plasma glycerol data required a three-compartment model. By using the glucose subsystem determined previously and plasma glycerol as its precursor it was possible to generate a plasma glucose curve due to an injection of glycerol. A comparison of this curve to that

TABLE II

Parameter Values of FFA Subsystem and Its Coupling to the Bicarbonate Subsystem (Fig. 3)*

Subject	$L_{1,2}$	$L_{2,1}$	$L_{1,3}$	$L_{3,1}$	$L_{0,1}$	$L_{4,1}$	$L_{5,4}$
Normals							
H. J.	0.027 ±0.002	0.050 ±0.003	0.0017 ±0.0001	0.089 ±0.004	0.065 ±0.003	0.042 ±0.003	0.036 ±0.003
F. E.	0.070 ±0.006	0.049 ±0.005	0.0013 ±0.0001	0.114 ±0.009	0.062 ±0.004	0.040 ±0.004	0.038 ±0.002
C. J. L.	0.009 ±0.001	0.043 ±0.003	0.0009 ±0.0001	0.099 ±0.005	0.050 ±0.004	0.073 ±0.004	0.048 ±0.003
B. M.	0.024 ±0.002	0.044 ±0.003	0.0008 ±0.0001	0.082 ±0.005	0.038 ±0.003	0.034 ±0.003	0.070 ±0.005
Mean	0.033 ±0.026	0.047 ±0.004	0.0012 ±0.0004	0.096 ±0.014	0.054 ±0.012	0.047 ±0.017	0.048 ±0.015
Abnormals							
S. M. (IV)‡	0.052 ±0.004	0.059 ±0.004	0.0044 ±0.0002	0.190 ±0.007	0.071 ±0.003	0.037 ±0.003	0.071 ±0.002
J. I. (IV)‡	0.129 ±0.009	0.095 ±0.007	0.0055 ±0.0003	0.058 ±0.003	0.146 ±0.003	0.056 ±0.003	0.053 ±0.002
L. A. (IV)‡	0.100 ±0.006	0.061 ±0.004	0.0017 ±0.0001	0.090 ±0.005	0.138 ±0.004	0.054 ±0.004	0.039 ±0.003
M. E. (X)‡	0.033 ±0.002	0.049 ±0.002	0.0025 ±0.0002	0.067 ±0.002	0.119 ±0.005	0.106 ±0.005	0.037 ±0.002

* min^{-1}. Value ±SD.

‡ X, xanthomatosis; IV, type IV.

TABLE III

Parameter Values of Bicarbonate Subsystem (Fig. 3)*

Subject	$L_{6,6}$	$L_{6,5}$	$L_{5,7}$	$L_{7,5}$	$L_{0,5}$
Normals					
H. J.	0.15 ±0.02	0.10 ±0.01	0.045 ±0.004	0.23 ±0.02	0.075 ±0.006
F. E.	0.30 ±0.02	0.21 ±0.02	0.028 ±0.001	0.17 ±0.01	0.068 ±0.004
C. J. L.	0.22 ±0.02	0.16 ±0.02	0.047 ±0.004	0.19 ±0.02	0.052 ±0.004
B. M.	0.23 ±0.02	0.18 ±0.02	0.070 ±0.006	0.18 ±0.02	0.032 ±0.002
Mean	0.23 ±0.06	0.16 ±0.05	0.048 ±0.017	0.19 ±0.03	0.057 ±0.019
Abnormals					
S. M. (IV)‡	0.10 ±0.01	0.26 ±0.03	0.056 ±0.002	0.34 ±0.03	0.087 ±0.003
J. I. (IV)‡	0.30 ±0.03	0.22 ±0.02	0.027 ±0.002	0.18 ±0.02	0.073 ±0.005
L. A. (IV)‡	0.22 ±0.02	0.15 ±0.02	0.036 ±0.003	0.20 ±0.02	0.079 ±0.006
M. E. (X)‡	0.31 ±0.03	0.22 ±0.02	0.026 ±0.002	0.17 ±0.01	0.071 ±0.004

* min^{-1}. Value ±SD.

‡ X, xanthomatosis; IV, type IV.

observed showed a basic inconsistency: the tail of the calculated glucose curve was governed by the slow glycerol component and was slower than that observed. This inconsistency occurred in all the patients studied. Elimination of the slow (final) glycerol component produced an excellent fit of the glucose data. A similar inconsistency also

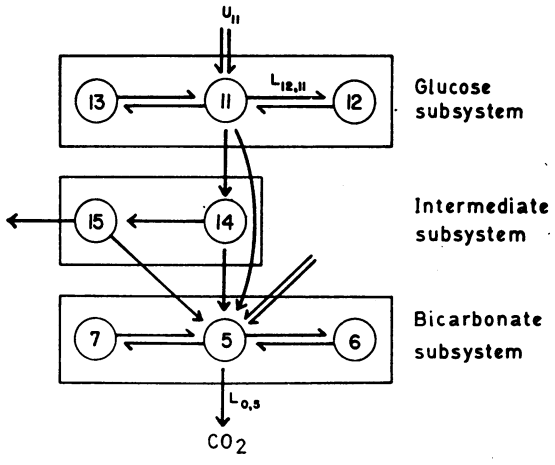

FIGURE 5 Compartmental model describing the conversion of [1-^{14}C]glucose to $^{14}CO_2$. It is a simplification of the model described by Shames, Berman, and Segel (4). Doubly lined arrows indicate steady-state inputs.

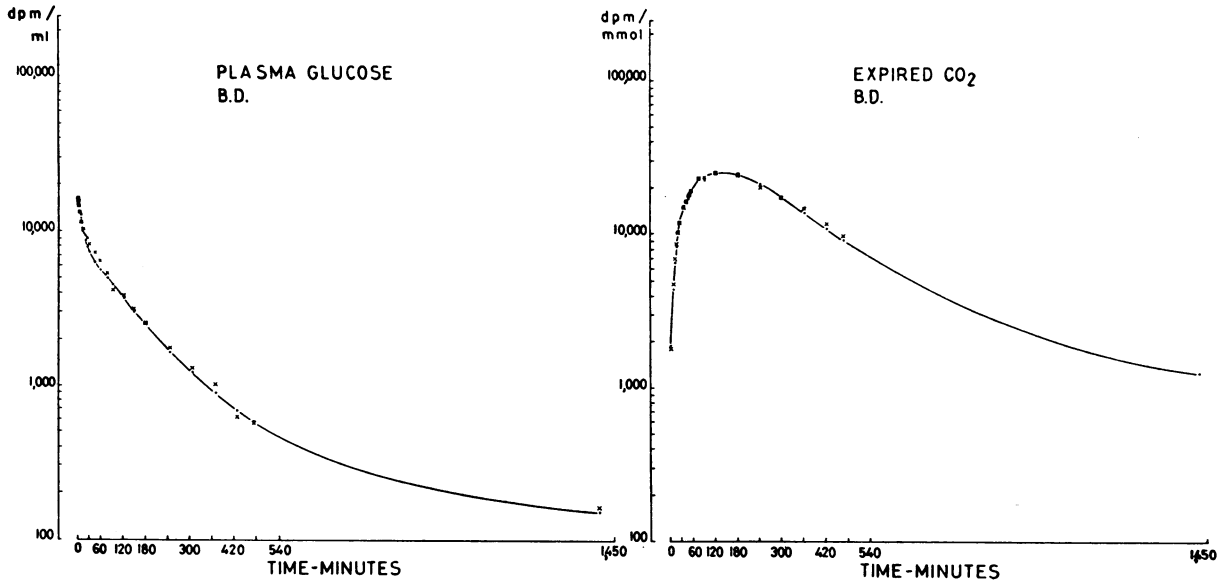

FIGURE 6 Typical kinetic data for glucose labeled experiment in a patient with glycogenosis. Dots and solid lines connecting them are model predictions. ×'s are observed values. The superposition of a dot and × appears as a square.

arose in fitting the CO_2 data. This too was eliminated by using only the two fast components of the glycerol curve. It was, therefore, concluded that the final glycerol component may not be due to glycerol,[4] and a two-compartment model was postulated for the glycerol subsystem (Fig. 7). The measured plasma glycerol curve and the one actually used in the analysis of the data and the corresponding plasma glucose and CO_2 activties are shown in Figs. 8*a*, 8*b*, and 8*c*.

The combined glycerol-glucose model, however, was not adequate to account for the observed early CO_2 activity and a more direct pathway from glycerol to bicarbonate was necessary. Such a pathway, however, still required a short delay, and a coupling compartment was introduced between plasma glycerol and bicarbonate. This delay could be due to a lactate intermediate. The contribution from this pathway is shown in Fig. 8*c*.

RESULTS

The results are presented in Tables I through VIII.

All patients were investigated under comparable nutritional conditions (i.e., a standard diet for 15 days and a 14-h fast just before the tracer injection). However, during fasting plasma FFA concentration increases and glucose concentration decreases; whereas, glycerol, glyceride, and bicarbonate concentrations do not change. For the calculation of steady state transports, plasma concentration at the time of tracer injection was chosen. Table II gives the calculated values of the rate constants for the FFA subsystem and its coupling to bicarbonate, as shown in Fig. 3. Table III gives the rate constants for the bicarbonate subsystem derived from the fatty acid experiments. The steady-state tracee values derived from the parameters of Tables II and III and plasma tracee levels are given in Table IV. The FFA subsystem was studied in the normals and hyperlipemics but not in glycogenosis. The plasma equivalent volume of distribution of compartment 1 for palmitate (V_1) is systematically larger than estimated plasma volume (V_P). (V_P was estimated as the volume of distribution of ^{131}I-labeled albumin 10 min after intravenous injection (34), which equals 41.3 ml/kg body weight [BW].).

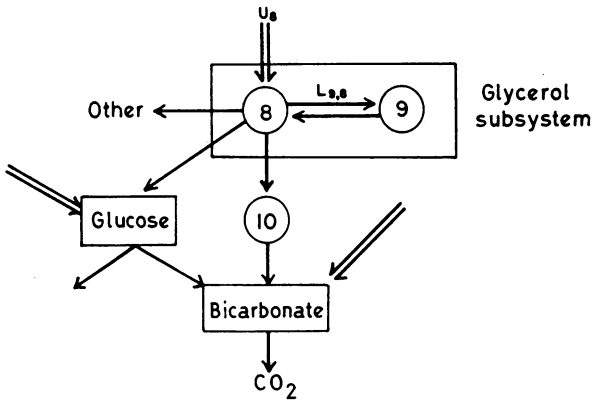

FIGURE 7 Compartmental model describing the conversion of [1-^{14}C]glycerol to $^{14}CO_2$ by the rapid pathway (through compartment 10) and through the glucose subsystem (slow pathway). Doubly lined arrows indicate steady-state tracee inputs.

[4] More recent studies, to be published, confirm this conclusion.

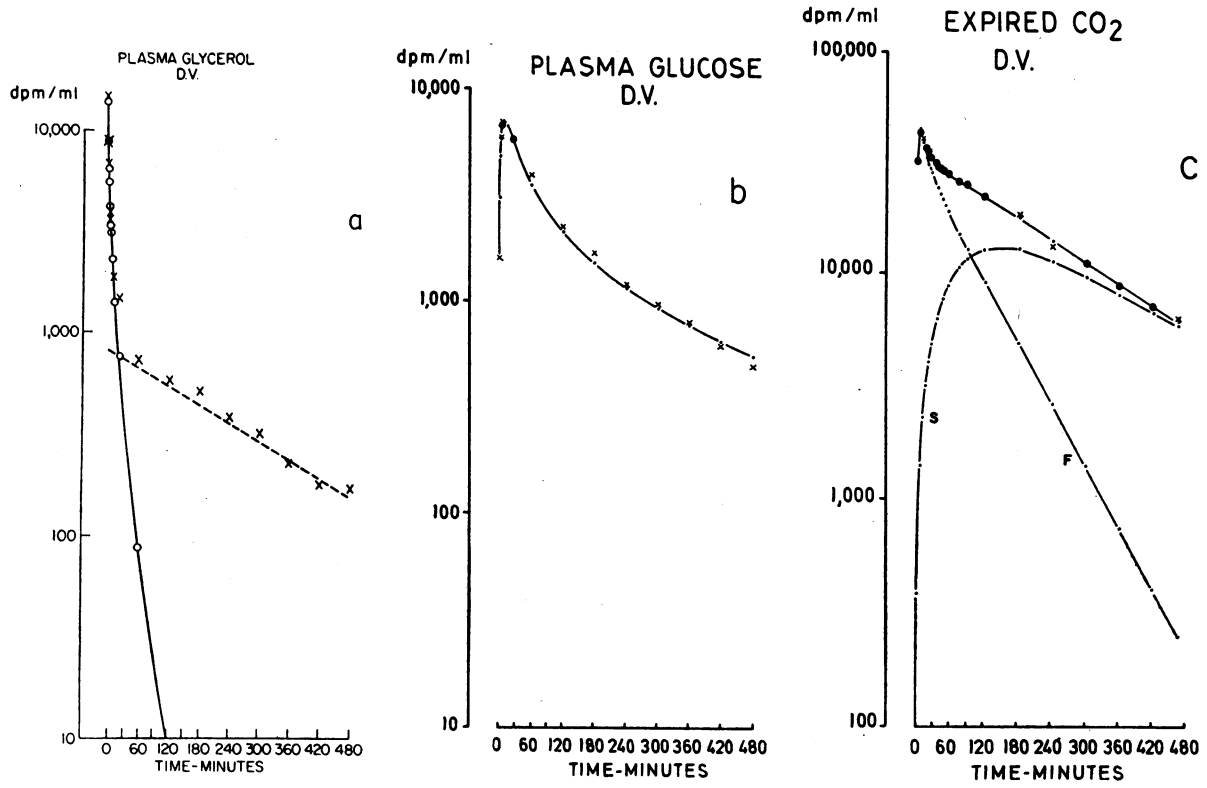

FIGURE 8 Typical kinetic data for glycerol labeled experiment in a type IV hyperlipemic patient. ×'s are observed values. (*a*) Circles indicate values calculated with the two-compartment model subsystem shown in Fig. 7, neglecting the slowest (3rd) component shown by dotted line. (See Methods, model and data analysis.) (*b*) Solid line is model prediction. (*c*) Specific activities of expired CO_2. Circles indicate calculated values. Lines between dots marked *F* and *S* indicate contributions from the rapid (lactate) and slow (glucose) pathways respectively.

The ratio V_1/V_2 was 1.80±0.10 in our four normal controls, somewhat higher than in pathological cases (1.33±0.20) and in normals (1.30) observed by Fredrickson and Gordon (4). The masses of the exchange compartments (M_2 and M_3) in the FFA subsystem are systematically greater in the normals than in the hyperlipemics, and so is U_1, the input into the FFA subsystem. No systematic differences between populations were observed in the intermediate compartment 4 or in the bicarbonate subsystem.

The parameter values for the glucose model (Fig. 5) are given in Table V. Steady-state tracee values derived by using these parameters are in Table VI. This subsystem is about the same for normals and hyperlipemics. In glycogenosis, however, there is a reduction in the inflow of glucose into the plasma (U_{11} in Fig. 5) and in the mass M_{12}.

Parameter and steady-state values of the glycerol subsystem (Fig. 7) and its coupling to bicarbonate are given in Tables VII and VIII. Plasma equivalent volumes of distribution for compartment 8 of glycerol (V_8) represent about 14% of *BW*, and is similar to plasma equivalent volumes of distribution of glucose (V_{11}). The total glycerol carbon pool (6.18 mmol or 2.06 glycerol) is greater in the two patients with glycogenosis compared to the normals or hyperlipemics on a per unit *BW* basis.

The relative amounts of carbon converted from each of the subsystems to the others are given in Table IX. Similarly, the relative contributions to bicarbonate from each of the subsystems are given in Table X. In normals about 46.5% of FFA is oxidized directly; whereas, 53.5% are incorporated into other pathways (mainly triglycerides). In hyperlipemics only about 30% of the FFA is directly oxidized. Because of the higher FFA levels in plasma, however, the actual amount of FFA oxidized directly is higher in hyperlipemics. In the xanthoma patient this pathway is comparable to normal.

In normals after a 14-h fast, about 24% of glycerol is directly oxidized; whereas, 69% is converted to glucose (Table IX). The fraction oxidized directly is lower in the three hyperlipemics (13%); whereas, in the two glycogenotics the fraction converted to glucose is lower

TABLE IV

Steady-State Values for Palmitate and Bicarbonate Subsystems*

Subject	V_1/BW	M_1	M_2	M_3	M_4	M_5	M_6	M_7	U_1	U_5	$R_{4,1}$	$R_{0,1}$	$R_{0,5}$
	%		*mmol carbon*			*mmol carbon*				*mmol carbon/min*			
Normals													
H. J.	7.6	58.8 ±1.8	109 ±11	3,078 ±216	65.3 ±6.0	91 ±6	59 ±9	465 ±37	6.29 ±0.58	4.47 ±0.41	2.47 ±0.21	3.82 ±0.33	6.94 ±2.02
F. E.	8.2	76.8 ±2.8	54 ±8	6,960 ±1,010	80.0 ±7.2	121 ±8	84 ±10	744 ±30	7.83 ±0.65	5.13 ±0.48	3.07 ±0.18	4.76 ±0.68	8.20 ±2.03
C. J. L.	6.9	31.2 ±11.0	146 ±17	3,610 ±480	47.5 ±4.0	185 ±13	150 ±20	818 ±52	3.84 ±0.23	7.32 ±0.68	2.28 ±0.18	1.56 ±0.12	9.60 ±0.08
B. M.	7.8	62.0 ±1.6	111 ±10	6,430 ±800	31.7 ±2.7	188 ±13	149 ±21	483 ±40	4.44 ±0.38	3.78 ±0.30	2.11 ±0.12	2.35 ±0.32	5.92 ±1.31
Mean	7.6 ±0.5	57.2 ±19.0	105 ±38	5,020 ±1,958	56.1 ±21.0	146 ±48	111 ±46	628 ±180	5.60 ±1.81	5.18 ±1.53	2.48 ±0.42	3.12 ±1.43	7.67 ±1.59
Abnormals													
S. M. (IV)‡	5.3	65.3 ±1.7	74 ±9	2,819 ±202	34.0 ±2.1	104 ±4	257 ±34	631 ±42	7.05 ±0.62	6.55 ±0.57	2.42 ±0.12	4.63 ±0.38	8.97 ±0.12
J. I. (IV)‡	5.8	69.7 ±1.0	51 ±4	735 ±42	73.6 ±3.0	114 ±8	84 ±12	760 ±47	14.10 ±2.10	4.40 ±0.41	3.90 ±0.15	10.20 ±0.99	8.30 ±0.15
L. A. (IV)‡	4.5	42.0 ±1.0	26 ±2	2,218 ±278	58.1 ±5.8	100 ±7	70 ±10	556 ±35	8.06 ±0.71	5.66 ±0.52	2.27 ±0.14	5.79 ±0.62	7.93 ±0.07
M. E. (X)‡	6.6	32.0 ±0.8	48 ±4	862 ±85	91.9 ±6.5	109 ±7	78 ±11	717 ±25	7.20 ±0.65	4.37 ±0.38	3.40 ±0.09	3.80 ±0.31	7.77 ±0.92

* Value ±SD.

‡ X, xanthomatosis; IV, type IV.

(16–35%). The fraction of glycerol accounted for as CO_2 via both pathways is only about 59% for normals, 43% for hyperlipemics, and 50% in glycogenosis, since some of it is lost irreversibly in the glucose subsystem.

The fraction of glucose oxidized to CO_2 in normals is about 50%, a value somewhat lower than that obtained by Baker, Shreeve, Shipley, Incefy, and Miller (8). Similar values were obtained for the hyperlipemic and glycogenotic patients.

The percentage of glucose that is derived from glycerol may be calculated from the ratio $R_{11,8}/(R_{11,8} + U_{11})$. This yields about 5.5% for the four normals, from 3 to 5.5% for the three hyperlipemics, and 1.5 and 2.7% in glycogenosis.

The relative contribution of glycerol to bicarbonate (Table X) is small (1.4%) in normals and even smaller in hyperlipemics (less than 0.7%). The contribution from glucose varies from 20 to 40% in normals and is at the low range of normals in hyperlipemics and glycogenosis. The contribution from FFA is about 33% in normals and about 23% in hyperlipemics. In the one xanthoma patient this contribution was 44%.

DISCUSSION

The quantification of exchanges and conversion pathways from one moiety to another in in vivo systems is difficult. There usually exist numerous transition pathways with widely varying transit times, and even along a single pathway there can be a distribution of transit times.

A metabolite, characterized by a set of exchangeable states, may be considered a subsystem. When there is a conversion from one metabolite to another and kinetics are followed for extended periods of time, compartments in one subsystem may appear as exchange compartments of another. On the other hand, when experiments are of short duration, only the rapid transit pathways show up, and calculated interconversion rates tend to be low (7). To be able to determine interconversion rates correctly all subsystems and pathways within a system must be properly identified, and experiments must be carried out over a long enough period to observe even the slowest of pathways. The present study of the glucose, FFA, glycerol, and bicarbonate subsystems and their integration into a single system under the constraints of the conservation laws is a step to this end. But, as will be seen, this as yet has not been fully achieved.

Glucose. The present data are derived from experiments over a 24-h period–considerably longer than most other reported kinetic experiments. Because of this some differences both in the nature of the models and magnitudes of metabolic pathways may be expected. This is particularly noticeable for the glucose subsystem which contains a total exchangeable mass considerably greater than for previously published models (7, 35). Compartments 11 and 12 of the glucose subsystem are comparable

TABLE V

Parameter Values of Glucose Subsystem*

Subject	$L_{11,12}$	$L_{12,11}$	$L_{11,13}$	$L_{13,11}$	$L_{14,11}$	$L_{5,11}$	$L_{5,14}$	$L_{15,14}$	$L_{5,15}$	$L_{0,15}$
Normals										
C. J.	0.013 ±0.003	0.027 ±0.003	0.0013 ±0.0003	0.0036 ±0.0003	0.0149 ±0.0010	0.00085 ±0.00015	0.0059 ±0.0003	0.0089 ±0.0015	0.00004 ±0.00050	0.0062 ±0.0002
B. J.	0.024 ±0.005	0.053 ±0.009	0.0017 ±0.0009	0.0061 ±0.0012	0.0175 ±0.0017	0.00082 ±0.00015	0.0057 ±0.0003	0.0109 ±0.0014	0.00032 ±0.00018	0.0062 ±0.0002
N. M.	0.027 ±0.001	0.092 ±0.007	0.0022 ±0.0003	0.0071 ±0.0005	0.0220 ±0.0010	0.00082 ±0.00014	0.0057 ±0.0003	0.0100 ±0.0013	0.00036 ±0.00030	0.0062 ±0.0002
M. M.	0.049 ±0.002	0.087 ±0.008	0.0022 ±0.0002	0.0061 ±0.0007	0.0150 ±0.0008	0.00082 ±0.00015	0.0180 ±0.0003	0.0180 ±0.0003	0.00001 ±0.00010	0.0070 ±0.0002
L. M.	0.106 ±0.005	0.120 ±0.005	0.0026 ±0.0003	0.0057 ±0.0005	0.0130 ±0.0004	0.00074 ±0.00007	0.0150 ±0.0012	0.0130 ±0.0010	0.00009 ±0.00010	0.0062 ±0.0002
D. P.	0.066 ±0.007	0.055 ±0.009	0.0019 ±0.0002	0.0060 ±0.0004	0.0110 ±0.0007	0.00052 ±0.00010	0.0090 ±0.0003	0.0057 ±0.0006	0.00042 ±0.00020	0.0062 ±0.0010
Mean	0.048 ±0.034	0.072 ±0.033	0.0020 ±0.0004	0.0058 ±0.0011	0.0156 ±0.0038	0.00076 ±0.00012	0.0099 ±0.0053	0.0110 ±0.0041	0.00020 ±0.00018	0.0063 ±0.0003
Abnormals										
D. V. (IV)‡	0.044 ±0.008	0.058 ±0.006	0.0058 ±0.0020	0.0081 ±0.0006	0.0149 ±0.0020	0.00082 ±0.00015	0.0057 ±0.0003	0.0108 ±0.0014	0.00036 ±0.00030	0.0062 ±0.0002
H. I. (V)‡	0.013 ±0.001	0.029 ±0.003	0.0012 ±0.0003	0.0037 ±0.0003	0.0131 ±0.0001	0.00073 ±0.00015	0.0054 ±0.0003	0.0150 ±0.0010	0.00028 ±0.00020	0.0062 ±0.0002
L. R. (V)‡	0.025 ±0.003	0.083 ±0.008	0.0014 ±0.0006	0.0080 ±0.0003	0.0220 ±0.0014	0.00082 ±0.00015	0.0057 ±0.0003	0.0108 ±0.0014	0.00036 ±0.00050	0.0062 ±0.0002
B. D. (G)‡	0.052 ±0.007	0.033 ±0.005	0.0013 ±0.0002	0.0032 ±0.0002	0.0087 ±0.0015	0.00083 ±0.00015	0.0058 ±0.0003	0.0075 ±0.0016	0.00140 ±0.00060	0.0062 ±0.0002
G. E. (G)‡	0.067 ±0.009	0.040 ±0.005	0.0012 ±0.0001	0.0092 ±0.0004	0.0165 ±0.0040	0.00082 ±0.00015	0.0057 ±0.0003	0.0042 ±0.0012	0.00190 ±0.00070	0.0062 ±0.0002

* min^{-1}. Value ±SD.

‡ G, glycogenosis; IV, type IV; V, type V.

to the compartments normally "seen" in experiments of shorter duration (3–5 h); whereas, compartment 13 is a carbon pool with which glucose exchanges slowly and probably includes metabolites such as glycogen, triglycerides, amino acids, etc. The exchange pathways with compartment 13, therefore, could include glycolysis and the recovery of some carbon labels through gluconeogenesis. For this reason we do not consider compartment 13 as a "glucose" compartment, even though it is part of the glucose subsystem. Even with compartment 13, however, as much as 50% of the injected glucose label leaves the subsystem ($R_{0,15}$, Table VI) and does not recycle during the experiment. This represents very slowly turning-over carbon pools such as amino acids or triglycerides. It is also of interest that with compartment 13 there seems hardly a need for the slow intermediate compartment 15 of the original Shames et al. (7) model. In view of this, compartment 13 could probably have been replaced by compartment 15 with the addition of a return path to plasma (C_{11}). At this time, we are not in a position to distinguish between these pathways of the glucose subsystem. Fortunately, this is not critical for the evaluation of the oxidative pathways as seen over a 24-h period.

The plasma equivalent space of distribution of compartment 11 (V_{11}) is about 16% of *BW*, somewhat smaller than the extracellular space, even though it includes the red cell pool. This is explained in part by the fact that in some tissues extracellular glucose concentrations are lower than in plasma. With its rapidly exchanging compartment 12 (V_{12}) the plasma equivalent space of distribution is numerically equal to 30–50% of the *BW*, comparable to previously reported glucose spaces (35). This must include some intracellular glucose exchange compartments (probably liver and kidney).

It is conceivable that the slow component in the glucose curve was artificially generated by the meal given the patient 500 min after the start of the study (i.e., after a 22-h fast). However, since glycerol and glucose carbon was found in significant amounts in proteins, the slow release of label from amino acids as a result of gluconeogenesis seems reasonable. Furthermore, examination of the data over the first 500 min (i.e., before the meal) already strongly suggests a need for a slow

TABLE VI

Steady-State Values for Glucose and Bicarbonate Subsystems*

Subject	$(V_{11}+V_{12})/BW$	M_{11}	M_{12}	M_{13}	M_{14}	M_{15}	M_8	M_6	M_7	U_{11}‡	U_5	$R_{13,11}$	$R_{12,11}$	$R_{8,11}$	$R_{14,11}$	$R_{0,15}$
	%	*mmol carbon*					*mmol carbon*			*mmol carbon/min*				*mmol carbon/min*		
Normals																
C. J.	42.7	333	691	922	335	478	146	103	757	5.03	7.49	1.19	9.0	0.283	4.96	2.97
		±14	±282	±238	±34	±48	±13	±14	±92	±0.49	±0.73	±0.10	±1.1	±0.050	±0.05	±0.28
B. J.	53.3	358	791	1,285	375	633	154	77	592	6.34	6.20	2.18	19.0	0.294	6.26	3.92
		±18	±160	±321	±49	±69	±5	±11	±49	±0.59	±0.82	±0.33	±3.4	±0.053	±0.60	±0.43
N. M.	58.4	299	965	1,019	419	639	123	86	487	6.44	4.00	2.12	27.5	0.245	6.58	3.96
		±15	±125	±92	±51	±61	±5	±10	±54	±0.46	±0.69	±0.18	±2.2	±0.020	±0.30	±0.40
M. M.	29.9	240	426	665	100	257	133	98	414	3.36	7.06	1.46	20.9	0.197	3.60	1.80
		±12	±38	±100	±6	±15	±6	±11	±38	±0.20	±0.95	±0.17	±2.0	±0.020	±0.20	±0.10
L. M.	33.7	339	384	743	123	254	118	92	408	4.66	3.86	1.93	40.7	0.251	4.41	1.57
		±16	±23	±104	±9	±15	±6	±14	±40	±0.14	±0.19	±0.17	±2.0	±0.020	±0.13	±0.08
D. P.	27.5	411	343	1,298	321	247	224	152	353	4.73	8.70	2.47	22.6	0.214	4.52	1.53
		±12	±34	±181	±25	±31	±19	±22	±56	±0.28	±0.43	±0.17	±3.6	±0.038	±0.27	±0.15
Mean	40.9	330	600	989	279	418	149	101	502	5.09	6.22	1.89	23.3	0.247	5.06	2.62
	±12.8	±57	±253	±266	±134	±190	±39	±26	±153	±1.15	±1.94	±0.48	±10.5	±0.037	±1.15	±1.15
Abnormals																
D. V. (IV)§	29.6	288	380	402	260	428	88	100	401	4.28	5.77	2.33	16.7	0.236	4.29	2.65
		±14	±84	±110	±37	±55	±4	±14	±53	±0.47	±0.52	±0.50	±2.5	±0.040	±0.47	±0.32
H. I. (V)§	42.5	396	883	1,221	250	579	79	83	343	5.32	5.74	1.47	11.5	0.289	5.19	3.59
		±4	±100	±320	±18	±25	±5	±12	±40	±0.42	±0.48	±0.12	±1.0	±0.060	±0.06	±0.11
L. R. (V)§	52.4	369	1,225	1,713	492	805	126	144	540	8.10	7.07	2.40	30.6	0.303	8.12	5.02
		±19	±136	±375	±50	±65	±6	±19	±71	±0.49	±0.88	±0.40	±3.6	±0.020	±0.05	±0.38
B. D. (G)§	33.0	255	162	628	167	165	116	174	653	2.36	5.65	0.82	8.4	0.212	2.22	1.02
		±22	±20	±86	±21	±24	±13	±31	±107	±0.35	±0.51	±0.01	±0.1	±0.040	±0.05	±0.15
G. E. (G)§	37.9	152	91	1,165	253	131	70	58	263	2.59	5.15	1.40	6.08	0.125	2.51	0.81
		±1	±13	±47	±26	±19	±5	±9	±34	±0.21	±0.51	±0.02	±0.10	±0.018	±0.02	±0.10

* Value ±SD.

‡ U_{11} includes the contribution from glycerol for patients in whom the glycerol subsystem was not studied.

§ G, glycogenosis; IV, type IV; V, type V.

component, which, when extrapolated to 24 h, leads to a value comparable to that actually observed. This lends further support for the validity of a slowly exchanging carbon compartment.

No significant differences were observed in the metabolism of glucose in hyperlipemics as compared to normals.

In the glycogenotic patients several differences were observed in the metabolism of glucose that are compatible with the known cause of the disease, namely, a reduction of the levels of glucose-6-phosphatase in liver and kidney. There was a decrease in $L_{11,12}$ compared to normals. This decrease, however, is theoretically equivalent to a decrease in $L_{11,13}$ if an irreversible glucose loss were permitted in compartment 12 (36), and, in fact, argues for the existince of such a loss. There is also a reduction in the value of U_{11} which, again, is consistent with reduced gluconeogenesis in these patients.

Glycerol. The calculated plasma equivalent volume of distribution for the central glycerol compartment 8 (V_8) is about 13.3% of BW–comparable to 16% for the central glucose compartment 11 (V_{11}). Compartment 8 exchanges with a 3–4 times larger compartment 9. The total exchangeable glycerol carbon pool (M_8+M_9) in normals is 6.90 mmol or 15 times larger than plasma glycerol (0.14 mmol glycerol). It is known (37) that most plasma glycerol enters the liver and some enters the kidneys. Presumably glycerol does not enter adipose tissue due to a lack of glycerokinase. Glycerol may enter skeletal muscle but is released back to blood as lactate and would not appear as an exchange compartment. Thus, liver and kidney most likely comprise compartment 9 of the model.

A large fraction of glycerol is rapidly converted to plasma glucose. This further supports the notion that liver and kidney account for compartment 9. In normals, after a 14-h fast about 69% of the glycerol is converted to glucose. This fraction is known to be lower in the nonfasting state (38).

In hyperlipemics the glycerol subsystem is similar to that in normals, although the fraction converted to glucose is slightly higher.

TABLE VII

Parameter Values of Glycerol Subsystem (Fig. 7) and Its Coupling to the Bicarbonate Subsystem*

Subject	$L_{8,8}$	$L_{9,8}$	$L_{0,8}$	$L_{10,8}$	$L_{11,8}$	$L_{5,10}$
Normals						
C. J.	0.059 ±0.009	0.230 ±0.018	0.012 ±0.002	0.087 ±0.007	0.162 ±0.005	0.21 ±0.02
B. J.	0.055 ±0.005	0.230 ±0.015	0.006 ±0.001	0.061 ±0.005	0.164 ±0.040	0.27 ±0.03
N. M.	0.090 ±0.008	0.210 ±0.020	0.009 ±0.003	0.075 ±0.005	0.214 ±0.040	0.35 ±0.03
M. M.	0.138 ±0.008	0.360 ±0.020	0.083 ±0.030	0.052 ±0.005	0.320 ±0.050	0.29 ±0.03
Mean	0.085 ±0.038	0.260 ±0.069	0.027 ±0.097	0.069 ±0.015	0.215 ±0.074	0.28 ±0.06
Abnormals						
D. V. (IV) ‡	0.156 ±0.013	0.280 ±0.025	0.014 ±0.001	0.048 ±0.002	0.210 ±0.020	0.37 ±0.05
H. I. (V)‡	0.037 ±0.003	0.190 ±0.010	0.021 ±0.004	0.019 ±0.001	0.110 ±0.003	0.43 ±0.05
L. R. (V)‡	0.060 ±0.005	0.230 ±0.014	0.015 ±0.001	0.017 ±0.002	0.175 ±0.040	0.34 ±0.06
B. D. (G)‡	0.078 ±0.006	0.110 ±0.007	0.059 ±0.004	0.044 ±0.002	0.021 ±0.006	0.21 ±0.01
G. E. (G)‡	0.200 ±0.016	0.230 ±0.020	0.024 ±0.002	0.023 ±0.001	0.026 ±0.002	0.26 ±0.03

* min^{-1}. Value ±SD.

‡ G, glycogenosis; IV, type IV; V, type V.

The most striking change in the glycerol subsystem in glycogenosis is the reduction in the value of $L_{11,8}$, and the fraction of glycerol converted to plasma glucose. As liver and kidney are the tissues from which glucose derived from glycerol can be released, the reduced flux is most likely due to a deficiency in glucose-6-phosphatase. Levels of glucose-6-phosphatase in the liver of our patients were, in fact, found to be dramatically low. That glucose was released to the extent found implies that the liver may hydrolyze glucose-6-phosphate by nonspecific phosphatases, as suggested by Howell, Ashton, and Wyngaarden (39). A small amount (~8%) of glucose may also be released from glycogen by action of amylo-1,6-glucosidase (40). Alternatively, there may be a less striking reduction in glucose-6-phosphatase in the kidney of glycogenotic patients (41), and the kidneys may account for most of the observed glucose release into plasma.

In the absence of glucose-6-phosphatase, glucose-6-phosphate is metabolized by alternate pathways as lactate or glycerophosphate. This may account for the increased level of lactate observed and calculated for these patients. If the kidneys have a small deficiency in glucose-6-phosphatase compared to the liver, the increased lactate levels in plasma could account for a higher gluconeogenesis in the kidneys, compensating in part for the hypoglycemia.

FFA. Some suggestions about the identity of compartments 2 and 3 of the palmitate subsystem come from a comparison of the results on hyperlipemics to normals. In the hyperlipemics there is an increase of fatty acid mobilization, U_1, and a decrease in the masses of the exchange compartments 2 and 3 (M_2 and M_3) due to increased recycling to compartment 1. If compartments 2 and 3 are part of adipose tissue, then a single factor, increased fatty acid mobilization, could explain all the changes. The same reasoning could also be invoked to explain the observed plasma equivalent volume of compartment 1 (V_1) and its decrease in hyperlipemia. A V_1 greater than plasma volume implies that part of the palmitate is freed from its complex with albumin and exchanges with a rapidly turning-over compartment. If such a compartment were also part of adipose tissue

TABLE VIII

Steady-State Values for Glycerol Subsystem*

Subject	V_8/BW	M_8	M_9	M_{10}	U_8	$R_{8,9}$	$R_{10,8}$	$R_{11,8}$
	%	*mmol carbon*				*mmol carbon/min*		
Normals								
C. J.	13.9	1.20 ±0.06	4.68 ±0.53	0.497 ±0.046	0.313 ±0.025	0.276 ±0.025	0.104 ±0.009	0.194 ±0.006
B. J.	14.6	1.26 ±0.06	5.27 ±0.58	0.278 ±0.030	0.291 ±0.030	0.286 ±0.020	0.077 ±0.007	0.207 ±0.057
N. M.	13.9	1.79 ±0.09	4.18 ±0.40	0.384 ±0.030	0.530 ±0.010	0.376 ±0.030	0.134 ±0.006	0.383 ±0.027
M. M.	10.9	1.38 ±0.07	3.60 ±0.25	0.247 ±0.026	0.630 ±0.050	0.497 ±0.025	0.071 ±0.007	0.442 ±0.073
Mean	13.3 ±1.6	1.41 ±0.26	4.43 ±0.71	0.352 ±0.113	0.441 ±0.166	0.359 ±0.102	0.097 ±0.029	0.307 ±0.125
Abnormals								
D. V. (IV)‡	13.5	1.17 ±0.06	2.70 ±0.20	0.150 ±0.030	0.320 ±0.030	0.328 ±0.020	0.056 ±0.005	0.246 ±0.054
H. I. (V)‡	13.2	1.50 ±0.07	7.70 ±0.72	0.066 ±0.008	0.230 ±0.020	0.285 ±0.018	0.028 ±0.002	0.165 ±0.005
L. R. (V)‡	13.2	1.81 ±0.09	6.94 ±0.56	0.090 ±0.010	0.375 ±0.027	0.416 ±0.003	0.031 ±0.003	0.316 ±0.070
B. D. (G)‡	20.2	3.13 ±0.15	4.41 ±0.54	0.655 ±0.113	0.388 ±0.031	0.344 ±0.027	0.138 ±0.013	0.066 ±0.027
G. E. (G)‡	23.8	1.48 ±0.07	1.70 ±0.19	0.131 ±0.021	0.108 ±0.009	0.340 ±0.030	0.034 ±0.002	0.038 ±0.004

* Value ±SD.

‡ G, glycogenosis; IV, type IV; V, type V.

(membranes, or cells in rapid exchange) then increased fatty acid mobilization would decrease the mass M_1 (as for compartments 2 and 3) and appear as a reduced plasma equivalent space.

The results also show that in hyperlipemia there is an increase in the "irreversible" loss of FFA (L_{01}), but no change in its direct rate of oxidation. This is consistent with an increase in FFA utilization for very low density lipoprotein production in hyperlipemia which is included as part of L_{01} in the model.

Insulin is known to inhibit the enzymatic hydrolysis of triglycerides and to facilitate triglyceride synthesis in adipose tissue. This is contrary to the results of our studies which show an increased FFA mobilization in hyperlipemics. We cannot explain this. It is conceivable that not all the observed insulin is biologically active and that the increased FFA conversion to triglycerides is due to mass action. It is also possible that additional factors not considered here are involved in one or more of the processes.

In normals the calculated ratio in carbon fluxes of FFA (U_1) and glycerol (U_8) is about 17, close to the theoretically expected value if all the FFA and glycerol were supplied by triglycerides in adipose tissue. This flux ratio is increased in hyperlipemics, and suggests that in these patients some FFA may come from non-adipose tissues independently of glycerol.

FFA kinetics were not performed on the glycogenotic patients. It is expected, however, that in glycogenosis hypoglycemia stimulates (through a reduction in insulin) the release of FFA by adipose tissue and inhibits the disposal of triglycerides from plasma, causing secondary hyperlipemia. A high plasma level of FFA has been observed in these patients.

CO_2 production. The model accounts (Table X) for nearly 64% of CO_2 production in normals, about half of which is furnished by FFA and half by glucose. The contribution from glycerol is small (1.4%). The remaining 36% of CO_2 production is probably due to contributions from the oxidation of amino acids and other stored substrates (triglycerides, glycogen, etc.). In view of the reduced fraction of CO_2 production accounted

TABLE IX

Fractional Conversions (in %)

Subject	From FFA to		From Glycerol to			From Glucose to	
	Bicarbonate	Other	Bicarbonate	Glucose	Other	Bicarbonate	Other
Normals							
H. J.	40	60					
F. E.	40	60					
C. J. L.	59	41					
B. M.	47	53					
C. J.			33.3	62.1	4.6	43.2	56.8
B. J.			26.4	71.0	2.6	40.2	59.8
N. M.			25.2	71.8	3.0	42.0	58.0
M. M.			11.4	70.4	18.2	52.6	47.4
L. M.						56.7	43.3
D. P.						67.7	32.3
Mean	46.5	53.5	24.1	68.8	7.1	50.4	49.6
	±9.0	±9.0	±9.2	±4.5	±7.4	±10.7	±10.7
Abnormals							
S. M. (IV)*	34	66					
J. I. (IV)*	28	72					
L. A. (IV)*	28	72					
D. V. (IV)*			17.5	77.5	5.0	41.4	58.6
H. I. (V)*			12.7	73.3	14.0	33.4	66.6
L. R. (V)*			8.4	84.3	7.2	40.4	59.6
Mean	30.0	70.0	12.9	78.4	8.7	38.4	61.6
	±3.5	±3.5	±4.5	±5.5	±4.7	±4.4	±4.4
B. D. (G)*			35.5	16.9	47.6	58.0	42.0
G. E. (G)*			31.5	35.6	32.9	69.1	30.9
M. E. (X)*	47.1	52.9					

* G, glycogenosis; X, xanthomatosis; IV, type IV; V, type V.

for in hyperlipemics (51%), it would seem that these patients may have greater amounts of stored substrates.

It is also of interest to note that in the model for normals (Fig. 9) the total inputs to the four subsystems ($U_1 + U_5 + U_8 + U_{11}$) is 14.17 mmol C/min as compared to 8.39 mmols C/min released as CO_2 This is due to the fact that the irreversible pathways shown in the various subsystems recycle to the system after considerable delays (longer than the time span of the experiments).

It was found that for each of the subsystems (FFA, glycerol, glucose) some "coupling" compartments had to be introduced between it and the bicarbonate subsystem, to account for a delay between the observed activity in plasma and its appearance in CO_2. The delay was in addition to that provided by the bicarbonate subsystem. In some studies the desired delay could also be produced by assuming that oxidation takes place in a peripheral compartment of a subsystem. This could be rationalized for the FFA subsystem. For glycerol, however, the coupling compartment is probably real, in that lactate is an intermediate between glycerol and bicarbonate. In fact, in patients B. D. and G. E. considerable activity was observed in lactate. For the glucose subsystem the coupling compartments are also probably real, and represent various intermediates in the glycolytic pathways. Another possible explanation for a delay is that CO_2 first appears in a peripheral compartment of the bicarbonate subsystem, although arguments against it were presented by Segal, Berman, and Blair (42).

The oxidation of glycerol takes place through two pathways: lactate and glucose. Fig. 8*c* shows the CO_2 contributions from each of these pathways. The lactate pathway contributes most of the early position of the CO_2 curve; whereas, the glucose component contributes most to the terminal portion. The $^{14}CO_2$ excretion curve following a single glycerol injection often shows a bump at around 40 min. This bump is not due to the superposition of the two pathways but is the result of the convolution of two subsystems, both characterized by monotone decreasing functions.

In a recent paper Bortz, Paul, Haff, and Holmes (12)

studied the oxidation and turnover of plasma glycerol in lean and obese individuals under fed and starving conditions. They employed long-term infusions of [U-^{14}C]glycerol and determined contributions from glycerol to glucose. In their calculations the authors had to assume that the sp act of CO_2 at 450 min reached at least 80% of its asymptotic value. We tested this assumption by simulating their long-term infusion on three of our patients—a normal, hyperlipemic, and glycogenotic. The simulation showed that at 8 h the CO_2 sp act derived from glycerol represents 90 (C. J.), 89 (D. V.), and 94% (B. D.) of its theoretical asymptote. Similarly, simulations showed that glucose sp act derived from glycerol at 480 min reached 84 (C. J.), 88 (D. V.), and 82% (B. D.) of their asymptotic values. Thus, an 8-h primed infusion experiment seems adequate for the quantification of the oxidation pathways for glucose and glycerol, provided the rate constants remain in the tested range. For slower equilibrating processes, however, this technique may require reevaluation as pointed out by Shames, Frank, Steinberg, and Berman (43).

TABLE X

Direct Contributions to Expired CO_2 (in %)

Subject	From FFA	From glycerol	From glucose	Total*
Normals				
H. J.	34			
F. E.	37			
C. J. L.	24			
B. M.	37			
C. J.		1.9	22.8	
B. J.		0.9	28.9	
N. M.		1.9	40.9	
M. M.		0.8	20.0	
L. M.			40.6	
D. P.			26.9	
Mean	33.0	1.4	30.0	64.4
	±6.2	±0.6	±8.9	
Abnormals				
S. M. (IV)‡	26			
J. I. (IV)‡	16			
L. A. (IV)‡	28			
D. V. (IV)‡		0.7	24.3	
H. I. (V)‡		0.4	24.1	
L. R. (V)‡		0.3	32.4	
Mean	23.3	0.47	26.9	50.7
	±6.4	±0.21	±4.7	
B. D. (G)‡		1.9	19.4	
G. E. (G)‡		0.5	26.0	
M. E. (X)‡	44			

* This value is the sum of column values which are not single population.

‡ G, glycogenosis; X, xanthomatosis; IV, type IV; V, type V.

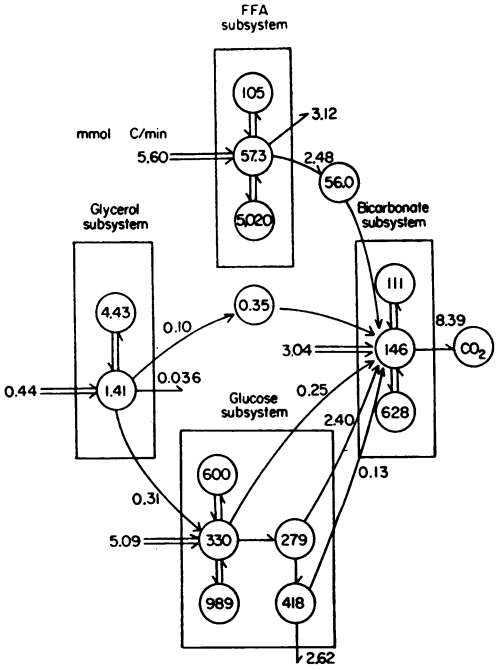

FIGURE 9 General model describing conversion of metabolites to CO_2. All values are means for normals and expressed in millimoles of carbon or millimoles of carbon per minute. (For conversion divide by 17 for palmitate, by 3 for glycerol, and by 6 for glucose.) Mass (M) of each compartment is contained in numbered circles. Doubly lined arrow indicates steady-state tracee input (U). Value on single-lined arrow indicates steady-state transport value (R).

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