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Tissue Uptake and Oxidation of Disodium Sebacate in Man

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ABSTRACT. In order to better ascertain its possible use as an alternative fuel substrate in total parenteral nutrition, sebacate (Sb) metabolism was studied in seven overnight-fasting healthy male volunteers, who received a constant iv infusion (99 mmoles over 8 hours) of disodium sebacate. Sb oxidation rate was determined using an isotopic sebacate (disodic salt of (1–10)^{14}C-sebacic acid) infusion (100 μCi from the fourth to the eighth hour of the cold sebacate infusion). Blood samples were collected during and after sebacate infusion at intervals of 30 minutes and Sb serum concentrations were determined by high performance liquid chromatography. Excreted radioactivity (μCi/min) was measured by bubbling the expired air into an apparatus containing 3 mEq hyamine to trap CO_2 from a 20-L Douglas-bag. CO_2 production and O_2 consumption were measured before and at 4 and 8 hours after starting the infusion. Twenty-four hour nitrogen excretion with urine was obtained. The RQ and the percent of calories derived from lipid oxidation were calculated by indirect calorimetry. The Sb serum level at the plateau phase was (mean ± SD) 4.54 ± 0.71 μmole/mL, the overall rate of tissue uptake was 180.89 ± 4.50 μmole/min, and the percent oxidation was 6.14 ± 0.44%. At the end of Sb infusion the RQ dropped to 0.839 ± 0.043, the percent of calories due to sebacate oxidation was 1.59 ± 0.52%, and the calories derived from lipids increased to 37.77 ± 12.90%. These data show that a definite amount of the sebacate infused is oxidized in human tissues. (*Journal of Parenteral and Enteral Nutrition* **15**:454–459, 1991)

The introduction of intravenous fat emulsions[1–3] has represented an important advance in the total parenteral nutrition (TPN) field. In fact, both long chain triglycerides (LCT) and medium chain triglycerides (MCT) provide a mean of increasing net oxidation of nutritional fuel substrates without a proportional increase of carbon dioxide production and with possible benefits regarding respiratory distress, as is the case with hypertonic glucose solutions.[4–6]

Moreover, both experimental and clinical studies have shown that sepsis is associated with a preferential utilization of lipids as a fuel source.[7,8] However, in some stages of the septic process, fat metabolism can be impaired, leading to a low clearance of plasma triglycerides. Thus, we have recently proposed the use of medium chain dicarboxylic acids (DAs), azelaic and sebacic acid, in TPN[9–11] as an energy substrate. In fact, DAs have neither toxic nor teratogenic effects[12,13] on laboratory animals, and being hydrosoluble under salt form, they can be suitable for peripheral infusion. Finally, since they do not require hydrolysis prior to intracellular utilization, and are thus readily available to cells, they may have a metabolic advantage over LCT and MCT.

When administered to humans or animals DAs are shortened by units of two carbon atoms during β-oxidation and are only partially excreted in the urine as shorter chain DAs.[14–16] Indirect calorimetric analysis has shown that azelaic acid (Az) oxidation in man is associated with a low cost of adenosine 5′-triphosphate synthesis in terms of CO_2 production.[9] However, since more than 50% of the administered dose of Az is excreted in the urine, the use of Az in TPN appears to be of doubtful advantage in terms of energetic yield. On the contrary, sebacic acid (Sb) and its products of β-oxidation, suberic acid (C8) and adipic acid (C6), are excreted in the urine at a significantly lower level than azelaic acid and pimelic acid (C7) (15% *vs* 75%) and thus the amount of Sb available as cellular fuel is very high. In addition, the energy production from sebacic acid is higher than that of azelaic acid (1.34 *vs* 1.00 Kcal/mmole), since the former is totally oxidized in the cells, whereas the latter gives malonic acid as the terminal product of β-oxidation, which cannot be oxidized any further.[11]

In order to explore more extensively the capacity of the human organism to metabolize and excrete sebacic acid, we performed studies on continuous intravenous infusion of sebacate and used as tracer (1,10)^{14}C-labeled sebacic acid. Serum levels of the diacid and excretion of radioactivity in the expired CO_2 were measured, and the uptake and oxidation of the diacid were consequently analyzed.

MATERIALS AND METHODS

Materials

(1,10)^{14}C-labeled sebacic acid (specific activity 102 mCi/mmole) was purchased from Amersham (Buckinghamshire, England). Sebacic and azelaic acids were from Sigma (St. Louis, MO). All other chemical were purity-available quality or of the highest purity available. Ten

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grams of sebacic acid salified with NaOH were dissolved in 250 mL of double distilled water. The infusions were sterilized by Millipore filters (Molsheim, France) (0.25 μm diameter) before administration.

Experimental Procedure

A continuous steady infusion of 20 g unlabeled sebacate over 480 minutes at a constant rate (206.29 μmole/min) was given by means of an electric syringe pump in seven overnight-fasting healthy, nonobese, male volunteers (mean ± SD body weight: 70.0 ± 1.5 kg) ranging from 24 to 38 years (31.5 ± 6.8). After the first 240 minutes of cold sebacate infusion, radioactively labeled Sb was infused in tracer amounts (0.416 μCi/min) simultaneously with unlabeled sebacate from 240 to 480 minutes.

Blood samples (4 mL) were collected without an anticoagulant and were centrifuged. Serum samples were frozen until analysis. Blood samples were taken every 30 minutes for a period of 600 minutes after the beginning of the unlabeled sebacate infusion. Each patient voided before starting the sebacate infusion and the 24-hour urine was collected in a container with 0.1% sodium azide to prevent bacterial growth.

The protocol was approved by the Ethical Committee of the Institutional Health Review Board of the Science Center of the Catholic University School of Medicine in Rome. Written informed consent was obtained in all cases.

Dicarboxylic Acid Analysis

Serum samples. 50 μg of azelaic acid were added to 1 mL of each serum sample as internal standard. Proteins were precipitated with ethanol kept at −20°C overnight and removed by centrifugation at 5000 rpm for 15 minutes. The pellet was washed three times with 0.6 mL of ethanol and the supernatants collected together. The supernatants were acidified to pH 1 to 2 with 1 N HCl and extracted three times with eight volumes of ethylacetate. The combined extracts were dried over anhydrous Na_2SO_4 and evaporated under nitrogen stream.

Urine samples. Urine samples (0.5 mL) were treated with cation-exchange resin (Dowex 50 W-X4, 100–200 μm mesh, H^+) to remove salts, concentrated under reduced pressure and filtered through a Millipore HV (0.45 μm) filter. The samples were extracted three times with eight volumes of ethylacetate and evaporated under N_2.

HPLC analysis. The extracted solutes from both sera and urine were dissolved in 2 mL acetonitrile-methanol (4:1, v/v) and added to 6 mg of *p*-bromophenacylbromide dissolved in CH_3CN and 14 μl of *N,N*-diisopropylethylamine as catalyst. The mixture was heated to 60°C for 15 minutes. The derivatives were purified by thin-layer chromatography (TLC) on standard thin-layer plates (20 × 20 cm) (Stratocrom SI-AP, Carlo Erba, Italy) coated with 0.25 mm of silica gel and activated by heating at 130°C for 15 minutes. The plates were developed in pentane/ether/acetic acid (92:7:0.5, v/v/v) and dicarboxylic *p*-bromophenacylesters were scraped off and extracted with three volumes of acetonitrile. After evaporation of the solvent up to a final volume of 0.5 to 1 mL, aliquots from 20 to 40 μL were injected into a liquid chromatograph (Perkin Elmer series 250) with an integrator and a scanning spectrophotometer operating in the 195 to 365 nm wavelength range (LC 235 detector diode array).

DAs derivatives were separated on a RP-18.5 μm-reversed phase column (20 cm × 4 mm i.d.), Hewlett-Packard (Palo Alto, CA) according to Passi et al.[16] Briefly, after 5 minutes isocratic elution with 60% CH_3CN in water adjusted to pH 3.10 with H_3PO_4, a gradient was performed to 100% CH_3CN in 60 minutes. The conditions were the following: flow rate 1 mL/min; range of absorbance from −0.300 to 1.000 absorbance units (AU); absorbance noise 2.5×10^{-5} AU at 245 to 255 nm; chart speed 0.25 cm/min; UV detector 255 nm.

Dicarboxylic acids as *p*-bromophenacyl diesters were fractionated by RP-HPLC, individually collected, placed in counting vials in the presence of standard scintillation solution and counted. A mixture of derivatized dicarboxylic acids from adipic (C6) to dodecandioic (C12) acids was used as a reference standard.

Other Methods

Serum lipids were extracted from another 1 mL of serum according to the method of Folch et al.[17] The lipid-containing chloroform extracts were evaporated under N_2 up to a final volume of 150 μL. The extracts were chromatographed on silica gel thin-layer plates (20 × 20 cm, 0.25 mm, Carlo Erba, Italy) in *n*-exanediethyl ether-acetic acid (60:40:4, v/v/v) and visualized with iodine vapors. The spots corresponding to the standard phospholipids, free fatty acids (FFA), triglycerides, and cholesterol esters were scraped off the plates into the vials. After that iodine was evaporated, silica gel was added with 0.5 mL of water and 10 mL of scintillation fluid (Instagel, Packard, Downers Grove, IL).

$^{14}CO_2$ Collection

Expired air was collected over 2-minute periods at regular intervals of 30 minutes for a period of 600 minutes after starting the labeled sebacate infusion, by using a 20 L Douglas-bag. The ventilation rate was assessed by a pneumotachometer. A solution of methylbenzethionium hydroxide (MH) or hyamine 1 M in methanol was prepared by adding 20 ml MH to 36 mL ethanol; 4 mL 0.1% phenophthaleine were then added as pH indicator. Aliquots of 3 mL each of this solution were put into graduated tubes and titrated with 0.15 N HCl. The next 9 mL of the solution was transferred into a bubbling apparatus to trap CO_2 from the 20 L Douglas-bag. Following the above procedure, solutions containing 3 mEq of MH were obtained: these solutions are capable of trapping exactly 3 mmol of CO_2.[18] Thereafter, the MH solution trapping $^{14}CO_2$ was added with 10 mL of 0.4% 2,5-diphenyloxazole (in PPO) toluene in scintillation fluid and counted.

Radioactivity Detection

The radioactivity was detected by a β-scintillation counter Packard TRI-CARB 460 C. Quenching was

checked by the internal standard method. We assumed that the specific activity of the shortened DAs was half that of sebacic acid, since β-oxidation from one end will remove one labeled carboxylic group.

Indirect Calorimetry

Respiratory and metabolic parameters were assessed according to published methods[19,20] using a computerized monitoring system equipped with a mass spectrometer for rapid analysis of O_2, CO_2, and N_2; a pneumotachometer for inspiratory and expiratory flow measurement; an analog/digital converter and a microcomputer which analyzes signals and computes derived parameters. The computer performed a complete analysis of inspiratory and expiratory gas, displayed the tidal volume, O_2-end and CO_2-end tidal values, O_2 consumption ($\dot{V}O_2$), CO_2 production ($\dot{V}CO_2$) and respiratory quotient (RQ) at each measurement time. $\dot{V}O_2$ (mL/min·m^2) and $\dot{V}CO_2$ (mL/min·m^2) were calculated according to the conventional formulas and RQ represented the $\dot{V}CO_2/\dot{V}O_2$ ratio. Calorimetric analysis was made basally and at 4 hours and 8 hours from the start of the infusion.

Determination of Sb Uptake and Oxidation Rates

Equations for the kinetics of labeled and unlabeled sebacate were written, assuming a single compartment, as follows[18]

$$V\frac{dC_A}{dt} = F_A - ER_d \quad (1)$$

$$V\frac{dC_B}{dt} = F_B - R_d \quad (2)$$

where C_A (μCi/mL) and C_B (μmole/mL) are the concentrations of labeled and, respectively, unlabeled sebacate; F_A (μCi/min) and F_B (μmole/min) are the infusion rates of labeled and, respectively, unlabeled sebacate; E (μCi/μmole) is the specific activity; R_d (μmole/min) is the rate of disappearance of cold sebacate; V (mL) is the apparent distribution volume. At the plateau concentration of unlabeled sebacate, C_{Bp}, the rate of Sb tissue uptake R_t (μmole/min) is equal to the Sb infusion rate minus the urinary losses. Assuming that Sb urinary excretion rate is a linear function of serum concentration, the urinary excretion rate was evaluated as renal clearance times serum concentration, so that:

$$R_t = F_B - Cl_r \cdot C_{Bp} \quad (3)$$

where Cl_r (mL/min) is the renal clearance. Cl_r was calculated as the ratio between the Sb excreted in 24-hour urine and the area under the curve (AUC_s) of Sb serum concentration *vs* time. The AUC_s was found using the trapezoidal approximation from 0 to 600 minutes, and extrapolating the concentration profile after 600 minutes with an exponential decay whose time constant was determined from the data at 570 and 600 minutes.

In the plateau phase of cold sebacate it is $C_A = EC_{Bp}$ and $R_d = F_B$, so that equation (1) can be rewritten as

$$\frac{dC_A}{dt} = \frac{1}{V}F_A - \frac{F_B}{VC_{Bp}}C_A \quad (4)$$

The distribution volume V was estimated as $V = D/\beta \cdot AUC_s$, where D (μmole) is the infused dose and β (min^{-1}) is the elimination rate constant of sebacate.[21] β was evaluated as the terminal slope of the plot of the logarithm of labeled Sb concentration *vs* time. The time constant of the rise of labeled sebacate concentration was then obtained as $\tau_A = VC_{Bp}/F_B$ (min).

The amount of Sb oxidized was calculated, according to Nordenström et al,[22] as the ratio between the total radioactivity excreted with expired air and the amount of labeled sebacate infused (percent oxidation, PO). The total radioactivity excreted was determined as the area under the curve of the rate of expired radioactivity *vs* time (AUC_e). The AUC_e was calculated as described above for serum concentration data.

Analysis of Indirect Calorimetric Data

Statistical analysis of indirect calorimetric data was performed by the analysis of variance.[23]

Since the infusion of a fuel substrate as Sb causes a definite amount of O_2 consumption and CO_2 production, the following equations were written for the interpretation of indirect calorimetry data[20]:

$$\dot{V}O_2 = 0.746\dot{G} + 2.029\dot{L} + 6.04\dot{N} + 1.386\dot{S} \quad (5)$$

$$\dot{V}CO_2 = 0.746\dot{G} + 1.430\dot{L} + 4.89\dot{N} + 1.109\dot{S} \quad (6)$$

where $\dot{V}O_2$ and $\dot{V}CO_2$ are in mL/min; $\dot{G}$, $\dot{L}$, and $\dot{S}$ are, respectively, the rates of glucose, lipid (except sebacate), and sebacate oxidation (mg/min); $\dot{N}$ is the urinary nitrogen excretion rate (mg/min). The rate of Sb oxidation was calculated as $\dot{S}$ = (PO/100) × Sb infusion rate: the numerical coefficients of $\dot{S}$ were calculated from the stoichiometric oxidative reaction of sebacate in the hypothesis of complete oxidation. The fraction of expired CO_2 due to Sb oxidation was calculated as $1.109\dot{S}/\dot{V}CO_2$. Equations 5 and 6 gave the rates of glucose and lipid oxidation, and allowed us to estimate the percentages of calories due to lipid oxidation and to sebacate oxidation.

RESULTS

The average 24-hour urinary excretion of Sb in the seven subjects under study was 11.38 ± 1.99 mmole (mean ± SD) and accounted for about 12% of the total dose infused. Over the same time, urinary losses of suberic acid (C8) and adipic acid (C6) were 2.04 ± 0.38 mmole and 1.11 ± 0.31 mmole, respectively. Overwhole, the amount of both Sb and its products of β-oxidation C8 and C6 eliminated in the urine was less than 15% of the sebacate administered.

The serum concentration (mean ± SD) of unlabeled sebacate *vs* time is represented in Figure 1. The plateau was reached after about 270 minutes of infusion at a value (mean ± SD) of 4.54 ± 0.71 μmole/mL. The renal clearance was 5.67 ± 0.97 mL/min. The overall rate of tissue uptake of cold sebacate, calculated by Equation 3, was 180.89 ± 4.50 μmole/min. The apparent distribution volume was 12.46 ± 2.02 L.

The labeled Sb concentration in serum *vs* time is

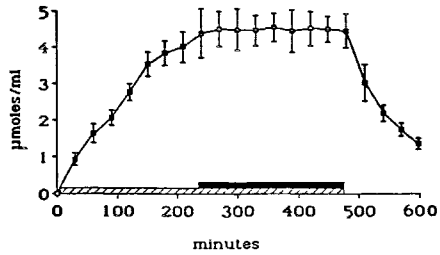

FIG. 1. Serum concentration (μmole/mL, mean ± SD) of unlabeled sebacic acid *vs* time. The *shadowed bar* indicates the time of infusion of unlabeled sebacate. The *solid bar* indicates the time of infusion of labeled sebacate.

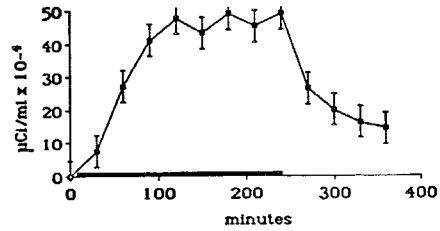

FIG. 2. Serum concentration (μCi/mL, mean ± SD) of labeled sebacic acid *vs* time. The *solid bar* indicates the time of infusion of labeled sebacate. Note that the time zero of this figure corresponds to time 240 minutes of Figure 1.

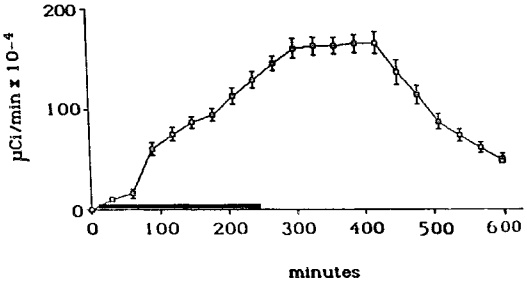

FIG. 3. Rate of $^{14}CO_2$ excretion (μCi/min, mean ± SD) *vs* time. The *solid bar* indicates the time of infusion of labeled sebacate. Same time scale as Figure 2.

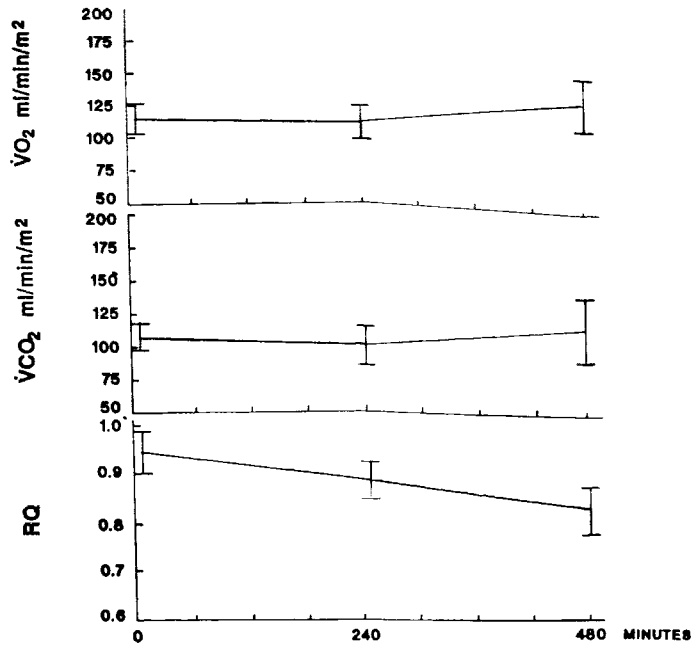

FIG. 4. Rates of O_2 consumption and CO_2 production (mL/min·m², mean ± SD) and RQ (mean ± SD) at three times during unlabeled sebacate infusion.

TABLE I
Sebacate kinetic and oxidation parameters (mean ± SD)

Serum Sb plateau concentration (μmole/mL)	4.54 ± 0.71
Distribution volume (L)	12.46 ± 2.02
Renal clearance (mL/min)	5.67 ± 0.97
Rate of tissue uptake (μmole/min)	180.89 ± 4.50
Percent oxidation (%)	6.14 ± 0.44
RQ	0.839 ± 0.043
Percent CO_2 due to Sb oxidation (%)	1.49 ± 0.49
Percent calories due to Sb oxidation (%)	1.59 ± 0.52
Percent calories due to lipid oxidation (%)	37.77 ± 12.90

plotted in Figure 2. The time constant τ_A for the rise of labeled Sb concentration (and of specific activity) in serum was estimated to be 270 minutes (mean over the seven subjects), so that a steady state could not be reached for the specific activity during the isotopic infusion. No recycled radiolabeled carbon was found in serum FFA, triglycerides, or cholesterol esters. On the contrary, 10% to 15% of the serum radioactivity was present in the aqueous fraction of serum extracts.

In all the subjects under study a detectable amount of radioactivity appeared in expired CO_2 at 30 minutes after the start of the labeled sebacate infusion (Fig. 3). There was a gradual increase in $^{14}CO_2$ excretion during the first 300 minutes of the observation period. The value of the excreted radioactivity remained elevated for other 120 minutes and then gradually declined. At 600 minutes the excretion of $^{14}CO_2$ had decreased to about 31% of the peak value. The amount of Sb oxidized, expressed as PO, was equal to 6.14 ± 0.44%.

The indirect calorimetric analysis (Fig. 4) showed that oxygen consumption significantly increased during sebacate infusion over the basal value of 113.38 ± 11.63 ml/min·m² (assuming an average body surface of 1.7 m²) up to 133.79 ± 21.20 mL/min·m² at 480 min. The values of CO_2 production slowly and gradually increased during the experiment from 105.59 ± 7.91 mL/min·m² to 112.04 ± 16.95 mL/min·m² at the end of sebacate administration. The RQ dropped significantly during infusion from 0.934 ± 0.034 to 0.839 ± 0.043, a value that is similar to the calculated RQ of sebacate.[11] The fraction of CO_2 due to Sb oxidation was 1.49 ± 0.49% at the end of sebacate infusion. The percent of calories derived from lipids increased from 7.12 ± 8.76% to 37.77 ± 12.90% at 480 minutes, the percent due to Sb oxidation being 1.59 ± 0.52%. No change in urea nitrogen excretion in the urine was observed during the study.[24] In fact, the value of urinary urea nitrogen loss over 24 hours (13.64 ± 2.38 g) was within a normal range.

Table I reports the main parameters that characterize kinetics and oxidation of sebacate (mean ± SD in the seven subjects under study).

DISCUSSION

It is well known that dicarboxylic acids given to humans and animals can be β-oxidized and excreted as

short-chain dicarboxylic acids in urine.[16] For DAs from dodecandioic to sebacic the mitochondrial transport system has been indicated to be identical to the system used by the corresponding monocarboxylic acids.[25] Peroxisomes, in turn, seem to be able to readily β-oxidize dodecandioic, sebacic and suberic acids at least, as far as adipic acid.[26] Although β-oxidation was largely demonstrated in the *in vitro* studies,[25,26] the only data available in literature on metabolism of dicarboxylic acids concern the rats.[27,28] Bergseth et al.[28] performed a study on the metabolism of suberic (C8) and dodecandioic (C12) acids administered in rats by intraperitoneal injection of both low and high doses. These authors found that a significant fraction (28–39%) of C12 was completely oxidized and that the ability of the organism to retain and oxidize DAs improves with the chain's length.

Since we recently proposed the use of Sb in man as an energy substrate in total parenteral nutrition,[11] a study of its uptake and oxidation became very important to show whether effective rate of oxidation occurs in human tissues. The mean rate of Sb tissue uptake was 180.89 μmole/min (ie, 2.58 μmole/Kg·min assuming 70 Kg of body weight) in the present experiments. This value is possibly overestimated because of underestimation of urinary excretion rate at the plateau serum Sb concentration as computed by Equation 3. The rather low mean value found for Cl_r (5.672 mL/min) could in fact be due to back-diffusion or active reabsorption of sebacate in renal tubules. Thus, the urinary excretion rate would depend on serum concentration in a nonlinear manner

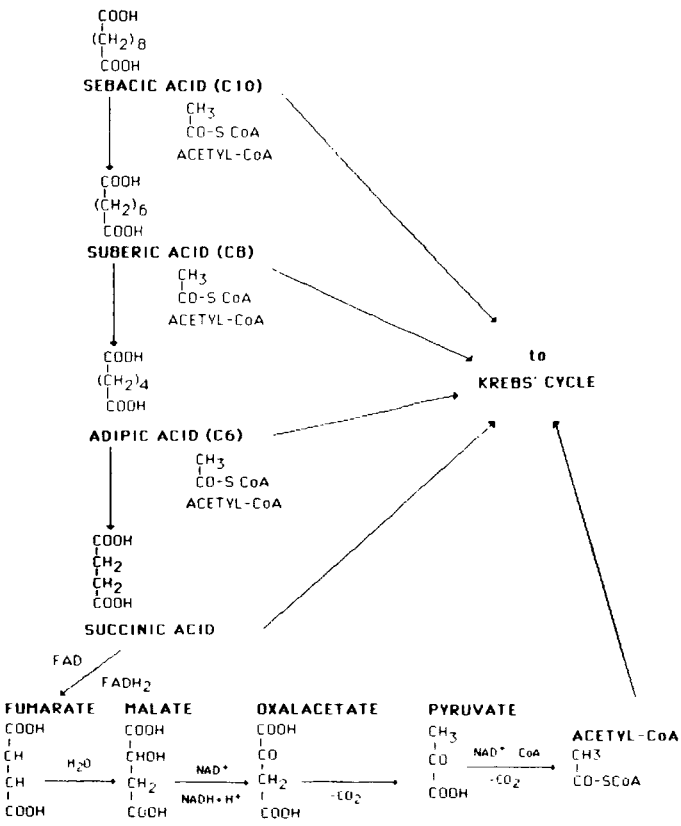

FIG. 5. Oxidation pathway of sebacic acid.

(the estimated Cl_r being an average clearance in this case), leading to underestimation of R_t. The Sb distribution volume slightly exceeds the volume of plasma plus the rapidly equilibrating interstitial water.[21]

A two-compartment model (plasma and interstitial fluids) would describe the kinetics of labeled Sb concentration in serum more accurately than the model of Equation 4. In fact, Figure 2 shows a fast rise in the plot of labeled Sb concentration *vs* time, followed by a slow rise. The calculated time constant τ_A of the monocompartmental model of Equation 4 roughly gives the time constant of the slow rise: its large value agrees with the fact that steady state was not reached during the 4 hours of infusion. The biphasic pattern is not apparent in the kinetics of cold sebacate, possibly because of a nonlinear behavior (due to active tissue uptake of sebacate) of R_d *vs* serum Sb concentration.

The appearance of $^{14}CO_2$ in expired air after only a few minutes of infusion of the (1–10)^{14}C-sebacate indicates that this compound is readily used as an energy substrate. The CO_2 created by oxidation of sebacate ranged from 4.36 to 7.31% of the total radiocarbon administered: these percentages are likely to be underestimated in view of the limited time period in which the excreted CO_2 was collected. The fact that the excretion rate of $^{14}CO_2$ did not decrease for about 3 hours after the end of labeled Sb infusion (Fig. 3) suggests that Sb was partly stored in the tissues during the observation period. Moreover, the appreciable amount of radioactivity detected in the aqueous fraction of serum indicates a partially uncomplete oxidation of sebacate in the cells, with the production of water-soluble substrates starting from succynil-CoA. The calorimetric analysis showed that the percent calories due to lipid oxidation was largely increased at the end of sebacate infusion (37.77%), whereas only a small fraction of the total calories was due to Sb oxidation (estimated to be 1.59% in the hypothesis of complete oxidation). Thus the oxidation of nonisotopic fat stores, especially liver and muscle triglycerides,[29] is probably activated since the hepatic glycogen sources are beginning to finish after an overnight fast.

In conclusion, the present data show that a substantial amount of the administered sebacate was oxidized. Sb can be considered as a more suitable energy substrate in TPN with respect to azelate since: (1) low levels of Sb and its oxidation products are excreted in the urine, and (2) contrary to odd chain DAs, Sb can be completely oxidized to CO_2 and H_2O (Fig. 5), as suggested by stoichiometric analysis.[11] In particular clinical conditions as ketosis in diabetic patients and sepsis, in which conventional substrates are scarcely utilized, sebacate could be useful. In fact, Wada and Usami[30] found that even numbered DAs reduced the diabetic ketosis, the antiketogenic properties being compatible with the hypothesis that DAs represent a possibility of gluconeogenesis *via* a synthesis of succynil-CoA. It is also known that besides glucose itself, a number of glucogenic metabolites, as aminoacids or DAs, have also antiketogenic properties towards ketoses caused by short-chain and long-chain monocarboxylic acids.[31] Further interesting studies could be undertaken to elucidate to what extent DAs could

compete in health and disease with both long and medium chain monocarboxylic acids in the β-oxidation machinery.

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