Properties of the carnitine palmitoyltransferase (EC 2.3.1.21) (CPT) enzyme system were compared in isolated mitochondria from a range of tissues in rodents, monkey, and man. Common features were as follows: (a) while membrane-bound, CPT I, but not CPT II, was inhibited reversibly by malonyl-coenzyme A (CoA) and irreversibly by CoA esters of certain oxirane carboxyllic acids; (b) the detergent, Tween-20, readily solubilized CPT II in active form while leaving CPT I membrane associated and catalytically functional; (c) octyl glucoside and Triton X-100 released active CPT II but caused essentially complete loss of CPT I activity.

Use of [14C]tetradecylglycidyl-CoA, a covalent ligand for CPT I, yielded estimates of the enzyme's monomeric molecular size: ~86 kDa in non-hepatic tissues and ~90–94 kDa in liver, depending upon species. A polyclonal antibody to purified rat liver CPT II recognized a single protein in each tissue; its apparent molecular mass was ~70 kDa in all rat tissues and ~68 kDa in all mouse tissues as well as monkey and human liver. On Northern blot analysis a rat liver CPT II cDNA probe detected a single ~2.5-kilobase mRNA in all rat and mouse tissues examined.

The following points are emphasized. First, CPT I and II are different proteins. Second, within a species CPT II, but not CPT I, is probably conserved across tissue lines. Third, slight variations in size of both enzymes were found in different species, although, at least in the case of CPT II, significant amino acid identity exists among the various isoforms. Fourth, CPT I, unlike CPT II, requires membrane integrity for catalytic function. Finally, the strategic use of detergents provides a simple means of discriminating between the two enzyme activities.

The mitochondrial β-oxidation of fatty acids, a process operative in most mammalian tissues, is initiated by the sequential action of two membrane-bound enzyme activities, carnitine palmitoyltransferase I (CPT I) and carnitine palmitoyltransferase II (CPT II). The pivotal regulatory role of the CPT system in hepatic fatty acid oxidation (and possibly in non-hepatic tissues also), its potential importance as a site for pharmacological intervention in the treatment of diabetes mellitus and the rapidly growing list of inherited defects in one or other of these enzymes have been discussed (1–5). However, understanding of structure/function/regulatory relationships surrounding the CPT I/CPT II enzymes in different body sites has lagged behind.

Two questions, for which there have been conflicting answers, must be resolved before the molecular basis of genetic defects in the CPT system can be elucidated. The first is whether in a given tissue CPT I and II represent the same enzyme protein (exhibiting different regulatory properties because of differences in their local membrane environments) or are distinct entities. The second is whether CPT I and II are structurally and functionally identical across tissue and species lines. Evidence from recent studies with two rat tissues, liver and skeletal muscle (6–12), would support the following formulation. In liver, CPT I and II are different proteins, the former being of larger monomeric size and uniquely sensitive to inhibitors such as malonyl-CoA (reversible) and CoA esters of oxirane carboxylic acids such as TG-CoA and B827-33-CoA (irreversible). CPT II is readily released in active form from the mitochondrial inner membrane by a variety of detergents. By contrast, CPT I is more deeply anchored in its (outer) membrane environment and, of particular importance, loses activity upon exposure of mitochondria to strong detergents. In skeletal muscle, CPT I and II are also distinct proteins, and again the former is the larger isozyme. However, muscle CPT I is smaller than its counterpart in liver while CPT II is a very similar if not identical protein in both sites. The responses of the muscle CPT isoforms to detergents generally parallel those of the liver enzymes.

This revised construct of the CPT system represents a significant departure from prevailing views (reviewed in Refs. 13–15). Accordingly, we wished to confirm the validity of the above conclusions by additional studies in a wider range of tissues and species, including man. The experiments outlined below establish that earlier interpretations were correct. They also provide further insight into the physical, biochemical and immunological properties of the mammalian CPT isozymes, and introduce our initial attempts to explore the system at the cDNA/mRNA level. The latter aspect is developed more fully in the accompanying paper (16) which describes the first cloning and sequencing of a full-length cDNA for a mitochondrial CPT protein.
RESULTS

All of the experiments to be described have been repeated on several occasions with identical results. Representative examples are shown.

Biochemical Studies—When used with intact mitochondria the CPT assay employed here monitors only the outer enzyme, i.e. CPT I (6). The data of Table I illustrate two points. First, in all tissues studied inclusion of malonyl-CoA in the assay subsequently resuspended in inhibitor-free medium prior to when mitochondria were first exposed to B827-33-CoA and mixture largely abolished CPT I activity, consistent with effect of B827-33-CoA was irreversible over the time frame of these studies, in keeping with previous observations using the related compound, TG-CoA (6, 7, 21). The non-reversibility was further illustrated in experiments not shown where rats were fed or injected with the sodium salt of B827-33; CPT I activity in mitochondria isolated from liver, heart, and skeletal muscle was still found to be blocked despite the multiple washing steps involved.

Also evident from Table I is that even after treatment of the mitochondria with both inhibitors a variable amount of residual CPT activity could be detected. That this was probably due to a small contribution of CPT II activity as a result of mitochondrial damage was supported by additional experiments using freeze-thawed mitochondria (to cause greater exposure of CPT II). The inhibitor insensitive fraction now rose to the region of 50–60%, as noted in earlier studies with mitochondrial membranes from rat liver and skeletal muscle (6, 22). Because agents such as TG-CoA and B827-33-CoA appear to interact covalently and selectively with CPT I in intact mitochondria they can be used in radioactive form to tag the protein and to identify it on SDS-gels (7, 21). We used this methodology to assess molecular weights of the CPT I enzymes in different species. Using [3H]TG-CoA, we previously obtained M₄ values of ~94,000 for rat liver CPT I (close to that of ~90,000 suggested by Kiorpes et al. (21)) and ~86,000 for the enzyme from rat skeletal muscle (7). The fluorograms shown in Figs. 1 and 2 indicate that the M₄ of ~86,000 for CPT I of rat skeletal muscle also holds for rat heart, mouse heart, and mouse skeletal muscle (Fig. 1). The mouse liver enzyme, while clearly of larger size, was found to be somewhat smaller than its counterpart in rat liver (~90 versus 94 kDa, Fig. 1). CPT I from human and monkey liver comigrated with the mouse liver enzyme (Fig. 2, the faintness of the band in lane 4 (though readily seen on the x-ray film) was due to limited sample availability.

We next tested the effects of detergents on CPT function in different tissues. The original clue that CPT I was a labile enzyme came from the observation that when intact mitochondria from rat liver or skeletal muscle were first preincubated in the absence of TG-CoA (CPT I active) or in its presence (CPT I irreversibly inactivated) enzyme activity measured after subsequent addition of octylglucoside or dig-

\[ \text{1 Portions of this paper (including "Experimental Procedures and Tables I–V)} \]

\[ \text{are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.} \]

\[ \text{2 The concentrations of B827-33-CoA used here were arbitrary and vastly in excess of those needed to cause complete inhibition of CPT I.} \]

\[ \text{3 Caution must be exercised, however, when comparing absolute CPT activities in intact and detergent-treated mitochondria. This is} \]

\[ \text{because the kinetic constants of the enzymes for palmitoyl-CoA and carnitine may differ substantially in the two situations and among} \]

\[ \text{the tissues examined (6, 7). Also, mitochondria from different sources may show differential resistance to damage during preparation (with} \]

\[ \text{varying exposure of CPT II in the control state).} \]
by a 10-fold dilution of the mixture in the CPT assay had no effect on enzyme activity. However, significant inhibition was observed when B827-33-CoA was included with the assay components at a concentration of 1 μM and was even more marked when the concentration of the CoA ester was raised to 2.5 μM. Results were similar in the rat, monkey, and human liver systems. It thus appears that B827-33-CoA, an irreversible inhibitor of CPT I at concentrations as low as 0.01 μM (7), acts as a reversible inhibitor of CPT II at 100–1000-fold higher concentrations. Presumably, the latter effect stems from a competitive interaction between palmitoyl-CoA and B827-33-CoA at the active site of the enzyme.

Immunological Studies—We previously reported that a rabbit polyclonal antibody raised against purified rat liver CPT II (RK40) recognized a protein of the same molecular weight on Western blots prepared from rat liver and skeletal muscle mitochondria (8). Fig. 3 shows the results of a similar experiment carried out with a larger series of tissues. In all cases a discrete band of antibody interaction was seen. The molecular mass of this protein was the same (~70 kDa) in rat liver, skeletal muscle, heart, and islets of Langerhans. However, in all three mouse tissues and in monkey liver the immunoreactive protein appeared to be slightly smaller (~68 kDa). To rule out the possibility that this difference in migration pattern was an artifact stemming from differences in sample consistency, the rat liver and mouse heart preparations were run separately and as a mixture on a second gel. The smaller size of the immunoreactive band in mouse heart compared with that in rat liver was readily visualized by the appearance of a doublet when the two samples were mixed (data not shown). Mitochondria from human liver also gave a positive signal with the rat anti-CPT II antibody; again, the molecular weight of this protein proved to be slightly smaller than its counterpart in rat tissues (Fig. 4). In all of these experiments preimmune IgG gave negative results.

The data of Table V established that detergent-solubilized CPT II from rat liver mitochondria is essentially completely removed from solution after addition of antibody RK40 followed by protein A. Preimmune antibody RK39 was ineffective. In the parallel experiment RK40 plus protein A precipitated ~73% of solubilized CPT II from human liver. In both systems, however, the immunoprecipitate displayed significant catalytic activity, suggesting that the active site of the enzyme was only partially masked by the polyclonal antibody. Similar results were obtained when the experiment was repeated with mouse and monkey liver (data not shown). In no case did antibody RK40 affect CPT I activity in mitochondrial membranes, in keeping with the fact that it failed to recognize CPT I in any of the samples subjected to Western blot analysis (Figs. 3 and 4).

Northern Blot Analyses—A 1.4-kb fragment derived from our cDNA for rat liver CPT II (16) was labeled with 32P and used to probe Northern blots of poly(A)+ RNA from various tissues. A single band of mRNA containing ~2600 nucleotides was detected in rat liver, heart, skeletal muscle and, accordingly, the presence of a similar size CPT II mRNA in normal mouse liver (Fig. 5). Figure 5 shows the presence of a similar size CPT II mRNA in normal mouse liver. Interestingly, this was obtained using only 1 μg of poly(A)+ RNA, indicating that the CPT II message is either much more abundant or more stable in mouse versus rat liver. We suspect the latter since the specific activity of CPT II was found to be similar in livers from both species (data not shown).

Discussion

The main objectives of this study were 2-fold. First, we sought further support for a newly emerging construct of the
mitochondrial CPT enzyme system in rat tissues (7, 8, 15) that differs substantially from previously held views. Second, we wished to determine whether the general characteristics of this system are applicable across a wider range of tissues and species, including man. A primary goal was to begin to explore the system at the molecular level.

Central to the discussion that follows are three basic premises. One is that CPT I inhibitors such as malonyl-CoA, TG-CoA, and the related compound, B827-33-CoA, interact at the catalytic center of the enzyme, rather than with an associated regulatory component as had once seemed possible (6–8). Although recently questioned on the basis of indirect radiolabel inactivation experiments (27), we believe this to be a reasonable supposition based on extensive competitive binding studies showing that the above mentioned ligands and 2-bromopalmitoyl-CoA, an active site-directed inhibitor of CPT I, all bind to a common locus on the enzyme (7, 15). A corollary is that the single labeled protein seen on SDS gels after treatment of intact mitochondria with radioactive TG-CoA represents CPT I (7, 21). Second, the species of CPT that is exposed upon breakage of the mitochondria, readily released from the membrane in active form by detergents, and insensitive to concentrations of inhibitors effective against CPT I, is CPT II (7, 9). Third, contaminating organelles, especially peroxisomes, sometimes present with mitochondria prepared by conventional methods of differential centrifugation and known to contain carnitine acyltransferase activity (14, 28), do not account for our results. This conclusion is based on control experiments with rat liver where mitochondria prepared using methods designed to minimize peroxisomal contamination (17) gave identical results to those obtained by standard methods. Furthermore, significant peroxisomal contamination would not be expected in mitochondrial preparations from non-hepatic tissues.

The data indicate that several features of the CPT system have been conserved in four mammalian species. In all cases CPT I was inhibited reversibly by malonyl-CoA and irreversibly by the TG-CoA analogue, B827-33-CoA. CPT II showed no sensitivity to malonyl-CoA but was suppressed by B827-33-CoA at relatively high concentrations, an effect that was readily reversible.

The differential response of CPT I and II to detergents, first noted in rat liver and skeletal muscle (7, 8), also appears to be a general phenomenon. Common features to all tissues studied included the following. Tween-20 readily solubilized CPT II in active form while leaving CPT I membrane bound and catalytically functional. By contrast, agents such as octyl glucoside and Triton X-100, which also released active CPT I from the membrane, caused essentially complete loss of CPT I activity. In the case of rat liver and skeletal muscle, the latter effect was previously shown to be independent of the salt concentration used, whereas efficient solubilization of CPT I required the presence of salt (8); whether this is true with mitochondria from all tissues remains to be established. In any event, it is evident that CPT I, unlike CPT II, is critically dependent upon membrane integrity for catalytic competence. Presumably, this reflects the enzyme’s requirement for a specific phospholipid milieu, in keeping with the findings of others (29–31).

Despite these functional similarities of the CPT enzymes across tissue and species lines some differences were revealed at other levels. In all four non-hepatic tissues (heart and skeletal muscle from the rat and mouse) CPT I migrated on SDS gels as a protein of molecular mass ~86 kDa; liver CPT I was larger, displaying M values of ~90,000 in the mouse, monkey, and man and ~94,000 in the rat. Antigenic properties of CPT I could not be assessed since it has not yet been possible to raise antibodies to the enzyme from any source. However, availability of a polyclonal antibody to purified liver CPT II (8) allowed us to conduct Western blot analyses. An immunoreacting protein was readily detected in every tissue, but its molecular size varied. We estimated values of ~70 kDa in rat liver, heart, skeletal muscle, and islets of Langerhans, and ~68 kDa in mouse liver, heart, and skeletal muscle. As was true for CPT I, CPT II from monkey and human liver comigrated with the enzyme from mouse rather than rat tissues. It is likely that within a given species the same CPT II molecule is synthesized in diverse tissues but that the protein varies slightly in size (while retaining some epitope identity) from species to species. The failure of antibody to recognize CPT I suggests that any homology in amino acid sequence between CPT I and II is not extensive or not in the critical antigenic site. A cDNA probe corresponding to rat liver CPT II detected only one species of mRNA (~2.5 kb) in four different rat tissues as well as in mouse liver, in accord with the interpretation of CPT II conservation.

We conclude that the mitochondrial CPT system is broadly similar in rodents, monkeys, and humans. Differences relate primarily to the molecular sizes of the individual proteins and to the susceptibility of CPT I to inhibition by malonyl-CoA. Two points deserve emphasis since, taken together, they could explain much of the confusion surrounding this area of investigation. First, CPT I and II are distinct enzymes. The former (the larger of the two proteins in all cases examined) is presumably endowed with the unique capacity to bind agents such as malonyl-CoA and TG-CoA, thereby undergoing reversible and irreversible inhibition, respectively (potential mechanisms have been discussed elsewhere (7, 15)). Second, the differential response of CPT I to detergents in earlier investigations of the CPT system led to erroneous conclusions, but strategic use of these agents now provides a powerful research tool. For example, they should be particularly helpful in discriminating between CPT I and II as potential sites of inherited defects in the still poorly understood CPT deficiency syndromes pending knowledge of the genetic basis for such mutations. In fact, while this work was in progress two reports on the use of detergents specifically for this purpose have appeared (32, 33).

In the accompanying paper we have taken the preliminary step toward the goal of identifying specific mutations by working out the sequence of rat liver CPT II.

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**EXPERIMENTAL PROCEDURES**

**Animals and Tissues** - Liver, heart and skeletal muscle were taken from male Sprague-Dawley rats (150g) and 100 white mice (25g) maintained on a starch diet and killed in the fasted state. In some cases IRBP was added to the red blood cells (0.3-0.5 ml) for two hours prior to sacrifice. Fresh, normal liver samples were also obtained from 6-8 year old male Cynomolgus monkeys (courtesy of Dr. John D. Dickey) and from two patients undergoing hepatic surgery (62 year old male and a 33 year old female). A batch of rat livers of Lanthanum was kindly provided by Dr. Kay McGarvey in the laboratory of Dr. Roger Granger.

**Preparation of Mitochondria** - Intact liver mitochondria from the various tissues were isolated as described previously (17) and suspended in 0.25 M sucrose. 2.5 M sucrose, pH 7.2 (Buffer A) was used and centrifuged at 10,000 rpm for 10 min. After resuspension of the mitochondria in 5 mM potassium phosphate, pH 7.5 (Buffer B) followed by centrifugation in buffer (B), the mixture was centrifuged at 100,000 g for 30 min.

**Treatment of Mitochondria**

(a) Exposure to TG-Te-CoA - One milliliter of intact mitochondria was mixed with 1 mM of mixing conditions (Buffer A, pH 7.2), 2.5 M sucrose, 0.5 M and 1.25% (v/v) fatty acid-free bovine serum albumin (FABSA) in a plastic ultracentrifuge tube. Ten microliters of 1.5 M [14C] Te-CoA (500 counts of the 5 minute) was added to give a final concentration of 1.25 M and, after standing at room temperature for 20 min, the mixture was centrifuged at 100,000 g for 10 min. The mitochondrial pellet was resuspended in 3 ml of Buffer B and frozen in liquid N2. After thawing, the mitochondrial membranes were sedimented by centrifugation at 100,000 g for 30 min, stabilized in 10 mM sodium butyrate (Buffer C), and processed for SDS-PAGE as described previously (7). The mixture was fixed, stained with commassie blue, destained, and scanned to fluorography to detect the [14C] Te-CoA labeled proteins (7).

(b) Exposure to 8532-13-CoA - One milliliter of intact mitochondria (10-12 mg of total protein) in buffer A or, in mitochondrial membranes derived thereof, was mixed with 2 ml of "staining solution". Ten to fifty microliters of a solution of 8532-13-CoA (500 counts of the 5 minute) was added to give the desired concentration of inhibitor and the mixture was maintained at room temperature for 20 min. The tubes were then centrifuged at 14,000 g for 10 min (Mitochondria) or at 100,000 g for 30 min (Membranes). Pellets were resuspended in 3 ml of Buffer A. One hundred microliters was assayed for CPT activity.

The same experiments were repeated in buffer A (without starch) and then resuspended into separate supernatant and pellet fractions (B). The latter were further treated with acetylcholinesterase or Triton X-100/0.1% (w/v) solution. Exposure of all fractions (3-5 ml) was as described above, but in each case 120 l of the entire binding mixture was assayed for CPT activity.

**Chromatographic Materials** - Thin-layer chromatography and gel electrophoresis were performed on silica gel plates. The source of other materials is given elsewhere (1-9,10).

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**[c] Western Blotting and Immunoblotting of Protein** - Mitochondrial membranes prepared from different tissues were subjected to SDS-PAGE followed by Western blotting as described previously (8). CPT-I was detected using the rabbit polyclonal antitbodies, 98Kv, directed against the purified rat liver isoform (8). The procedure was also carried out on a sample of isolated rat thiol of mitochondria.

**[d] Immunoprecipitation of CPT-I** - Mitochondrial membranes were suspended in buffer A to a density of 25-30 mg protein/ml and washed with Tween-20 (0.1%), as described earlier (8). The mixture was centrifuged at 100,000 g for 30 min and the pellet containing CPT-I was dissolved. To 0.5 ml of the supernatant (containing CPT-I) was added 0.17 ml of a solution containing ammonium (NH4) citrate (100 mM) (pH 8) (0.1 g of sodium citrate buffer, pH 7.0, 0.05 ml, and 1E, respectively). Reaction mixtures were fractioned into the time period studied (usually 4 min). In the tablets these are expressed as a percent of the control value which, for intact mitochondria and mitochondrial membranes, was generally in the range of 2-5 and 4-10 measuring protein, respectively.

**[e] Preparation of Mitochondrial Subcellular Fractions** - Liver homogenates were isolated by homogenization in a 1:3 osmotic balance followed by centrifugation through a 1:4 homogenate (18). Northern blot hybridization was carried out on nylon membranes (MS1) as described (19). DNA probes were labeled with [32P] by random nucleotide transfer of the phosphorylase C (PH-10). The sources of other reagents are given elsewhere (1-9,10).
### Table I

**Effect of 3B27-33-CaO and melatonin-CaO on CPT I activity in intact mitochondria.**

Intact mitochondria were preincubated without or with 3B27-33-CaO as described in "Experimental Procedures." After incubation in fresh medium they were exposed to CPT II activity in the absence or presence of melatonin-CaO (25 μM for liver and heart; 15 μM for skeletal muscle). Values are expressed as percent of those seen when no inhibitors were used.

| Source of mitochondria | 3B27-33-CaO in
|------------------------|------------------|
|                        | Presence (μM)    | CPT activity (% of control) |
| Rat liver              | 0               | 100                          |
|                        | 1.0             | 15                           | 16    |
| Rat skeletal muscle    | 0               | 100                          |
|                        | 2.0             | 10                           | 10    |
| Rat heart              | 0               | 100                          |
|                        | 2.0             | 10                           | 10    |
| Mouse liver            | 0               | 100                          |
|                        | 5.0             | 3                            | 4     |
| Mouse skeletal muscle  | 0               | 100                          |
|                        | 5.0             | 19                           | 15    |
| Mouse heart            | 0               | 100                          |
|                        | 5.0             | 3                            | 2     |
| Monkey liver           | 0               | 100                          |
|                        | 5.0             | 9                            | -     |
| Human liver            | 0               | 100                          |
|                        | 2.5             | 22                           | 20    |

### Table II

**Effect of 3B27-33-CaO followed by melatonin-CaO on CPT II activity in mitochondrial fraction.**

Intact mitochondria were preincubated with or without 3B27-33-CaO as described in "Experimental Procedures." After preincubation to fresh medium an aliquot of each preparation was exposed to melatonin-CaO (15 μM). All samples were then assayed for CPT activity in the absence or presence of 25 μM melatonin-CaO. Values are expressed as percent of those seen when no inhibitors were used.

| Source of
| 3B27-33-CaO in
| Presence (μM) |
|------------------|------------------|
| Rat liver         | 0               | 100                          |
|                        | 1               | 10                           | 1.5   |
| Rat skeletal muscle| 0               | 100                          |
|                        | 2               | 17                           | 12    |
| Rat heart          | 0               | 100                          |
|                        | 2.5             | 11                           | 14    |
| Human liver        | 0               | 100                          |
|                        | 2.5             | 21                           | 3.1   |
| Monkey liver       | 0               | 100                          |
|                        | 2               | 48                           | 4.6   |
| Human liver        | 0               | 100                          |
|                        | 5               | 475                          | 4.3   |

*Values from activity on the membrane of mitochondria before (CPT II) and after (CPT II) melatonin-CaO (see sequence 4).

### Table III

**Effect of 3B27-33-CaO, melatonin-CaO and Tricon 1-200 on CPT activity in cytosol and supernatant fractions.**

Mitochondrial membranes were treated with 3B27-33-CaO followed by fractionation into pellets and supernatant components. Each was assayed for CPT activity after incubation in the absence or presence of 3B27-33-CaO. pellets from each were also assayed for enzyme activity after further treatment with melatonin-CaO (15 μM). Other conditions (see "Experimental Procedures") are as follows for each experiment. Values are expressed as percent of those seen when the use of 3B27-33-CaO was discontinued.

| Source of
| 3B27-33-CaO in
| Presence (μM) |
|------------------|------------------|
| Rat liver         | Pellet           | 0               |
|                        | 1.5             | 12              |
|                   | Tricon 1-200     | 1               |
|                   | Supernatant      | 0               |
|                   | 1.5             | 100             |
|                   | Tricon 1-200     | 0.2             |
| Rat skeletal muscle| Pellet           | 0               |
|                        | 1.5             | 100             |
|                   | Tricon 1-200     | 0.2             |
|                   | Supernatant      | 0               |
|                   | 1.5             | 100             |
| Human liver        | Pellet           | 0               |
|                        | 1.5             | 100             |
|                   | Tricon 1-200     | 0.2             |
|                   | Supernatant      | 0               |
|                   | 1.5             | 100             |

### Table IV

**Effect of 3B27-33-CaO on CPT activity in cytoplasma.**

Intact mitochondria were first exposed to melatonin-CaO followed by pretreatment with 3B27-33-CaO. Each was then assayed for CPT activity after incubation in the absence or presence of 25 μM melatonin-CaO. All samples were then assayed for CPT activity in the absence or presence of 25 μM melatonin-CaO. Values are expressed as percent of those seen when no inhibitors were used.

| Source of
| 3B27-33-CaO in
| Presence (μM) |
|------------------|------------------|
| Rat liver         | Pellet           | 0               |
|                        | 1.5             | 100             |
|                   | Tricon 1-200     | 1               |
|                   | Supernatant      | 0               |
|                   | 1.5             | 100             |
| Rabbit liver       | Pellet           | 0               |
|                        | 1.5             | 100             |
|                   | Tricon 1-200     | 0.2             |
|                   | Supernatant      | 0               |
|                   | 1.5             | 100             |
| Human liver        | Pellet           | 0               |
|                        | 1.5             | 100             |
|                   | Tricon 1-200     | 0.2             |
|                   | Supernatant      | 0               |
|                   | 1.5             | 100             |
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