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NDA: 18-948 SPONSOR: SIGMA-TAU 1 OF 1

TRADE: CARNITOR GENERIC: L-CARNITINE

NDA: 18-948

TRADE: CARNITOR

SPONSOR: SIGMA-TAU

GENERIC: L-CARNITINE

APPROVAL LETTER: Y

STATISTICIAN'S REVIEW: N

SBA: Y

BIO/DISSOLUTION REVIEW: N

FINAL PRINTED LABEL: Y

MICROBIOLOGIST'S REVIEW: N

MEDICAL OFFICER'S REVIEW: Y

NAS/NRC REVIEW: N

CHEMIST'S REVIEW: Y

FEDERAL REGISTER NOTICE: N

PHARMACOLOGIST'S REVIEW: Y

DATE: 07/03/89

APPRVL

LTR



NDA 18-948

Sigma Tau, Inc.  
Attention: Milton Kletzkin, Ph.D.  
723 North Beers Street  
Holmdel, New Jersey 07733

Dear Dr. Kletzkin:

Reference is made to your new drug application dated February 8, 1983 submitted pursuant to section 505(b) of the Federal Food, Drug and Cosmetic Act for L-Carnitine Tablets.

We also acknowledge receipt of your additional communications dated June 14, July 25 and 31, September 13, October 17 and 23, and November 20, 1985.

We have completed the review of this application including the submitted draft labeling and the application is approved. However, as agreed to in the telephone conversation between Dr. Kletzkin of your firm and Dr. Troendle of FDA the drug should not be marketed until a trade name is agreed upon. Further, the following change must be made in the labeling:

In the DESCRIPTION section of the package insert, the chemical name should read "L-beta-hydroxy-gamma-trimethylamino butyric acid".

The labeling should be revised exactly as we have requested and twelve copies of the final printed version of the revised labeling must be submitted to FDA prior to marketing. Marketing of the drug before the change specified above is made and submitted to FDA renders the product misbranded under 21 U. S. C. 352.

Should additional information relating to the safety and effectiveness of this product become available prior to our receipt of the final printed labeling, revision of that labeling may be required.

Please submit one market package of the drug when it is available.

We remind you that you must comply with the requirements set forth under 21 CFR 314.80 and 314.81 for an approved NDA.

Sincerely yours,

*for Elaine C. Esber*

Elaine C. Esber, M.D.  
Director  
Office of Biologics Research and Review  
Center for Drugs and Biologics

S B B A

Summary Basis of Approval

NDA 18-948

Drug Generic Name:  
L-Carnitine Tablets

Applicant:  
Sigma-Tau, Inc.  
Holmdel, New Jersey 07733

Drug Trade Name:  
Carnitor

I. Indication for Use:

L-carnitine is indicated in the treatment of primary systemic carnitine deficiency.

II. Dosage form, route of administration and recommended dosage:

Tablets; oral.

Adults: 990 mg two or three times a day depending on clinical response.

Infants and Children: 50 to 100 mg/kg/day in divided doses with a maximum of 3 g/day.

III. Manufacturing and Controls:

A. Manufacturing and Controls

1. The synthesis of the new drug substance involves the reaction of known materials under well-defined and acceptable conditions and purified by use of standard methods.
2. Specifications and testing by appropriate methodology insure its compliance with the required standards of identity, strength, quality and purity.

B. Stability

Studies are ongoing and based on the data submitted at this time an expiration dating of 5 years at room temperature is justified.

C. Methods Validation

The methods validation is now being processed.

D. Labeling

The technical aspects of the labeling and labels are satisfactory.

F. Establishment Inspection

The facilities, equipment, manufacturing controls are in compliance with current good manufacturing practices and with conditions, commitments and requirements contained in the new drug application as indicated by appropriate reports of inspections from the Manufacturing Review Branch in the Division of Compliance.

F. Environmental Impact Analysis Report (EIAR)

EIAR indicates that the product does not have adverse environmental effects.

G. Bioavailability Requirements

Deferred by Division of Biopharmaceutics until after approval.

IV. Pharmacology:

L-Carnitine is the synthetic l-isomer of d,l-carnitine discovered 80 years ago as a constituent of muscle tissue that at first was used as a nutrient agent. It was sixty years later that its essential action in the metabolism of fatty acids was recognized. D-L-Carnitine is synthesized in liver, kidney and testis, from two essential amino acids, lysine and methionine. The l-isomer is the biologically active form.

Its biological function is to activate the transport of long-chain fatty acids such as palmitic acid across the inner mitochondrial membrane into the mitochondrial matrix where they undergo beta-oxidation resulting in the production of energy. For example, palmitic acid released from adipose tissue or derived from diet is activated by outer membrane ATP-dependent palmitoyl-CoA synthetase to form palmitoyl-CoA. Such long-chain fatty acyl-CoA esters have only a limited ability to cross the mitochondrial membrane barrier and their entry is facilitated by outer carnitine palmitoyltransferase which catalyzes a transesterification reaction in which the palmitoyl moiety from CoA is transferred to carnitine forming palmitoylcarnitine. This ester then crosses the inner mitochondrial membrane through action of a translocase. A second transesterification reaction now takes place wherein "inner" palmitoyltransferase, located in the inner mitochondrial membrane, regenerates palmitoyl-CoA for subsequent beta-oxidation and releases carnitine for a repetition of its catalytic role in overall fatty acid transport (After: Broquist, H.P. and P.R. Borum: "Carnitine Synthesis Nutritional Implications." Adv. Nutrit. Research 4:181-204, 1982).

The biophysiological functions of L carnitine have been studied by numerous investigators in several animal species during the last decade. A recent discovery is the essential role played by carnitine (and regulated by androgen) in epididymal mitochondria, in the maturation of spermatozoa, and in the ovary in the maturation and ovulation of follicles in rats, rabbits and monkeys. Evidence is also accumulating for a role of carnitine in gluconeogenesis.

Publications submitted in the NDA describing pharmacological parameters of L-carnitine in the animal model were used for the evaluation of the efficacy of L-carnitine. Some of these were performed by the staff of Sigma-Tau in their laboratories in Italy.

Toxicology: Tests were performed in rats and dogs, both of 52 weeks duration, with doses of 150, 450 and 1350 mg/kg/day in the diet in the rat study and 300, 600 and 1200 mg/kg/day in capsules in the dog study. (The clinical dose is 990 mg one to 3 times per day.)

Adverse actions from L-carnitine were limited to a slight depression of body weight gains of rats on the high dose, in contrast to a growth promoting action of the low- and mid-dose. There was also a slight reduction of body weight gains in dogs by the mid- and high-dose. In both species the reduction of body weight gains was not accompanied by any clinical signs of affected health conditions. Liquid feces were observed in dogs. There was no associated structural damage to the gastrointestinal mucosa, and the mechanism of action was not established.

Serum triglycerides were elevated in rats given the low- and mid-doses of L-carnitine. There were no related changes in the livers and kidneys.

Teratogenicity tests performed in rats and rabbits did not reveal any effects on the dams and their offspring.

Four sets of mutagenicity tests were negative.

V. Medical:

A. Introduction:

Utilization of lipid for energy requires the presence of l-carnitine for optimum transfer of long-chain fatty acids into the mitochondrial matrix, where beta-oxidation takes place. Generally, the requirements for carnitine are met by endogenous synthesis and/or dietary intake. In normal adults diet and endogenous synthesis are adequate to meet the requirements for carnitine. Systemic (blood and tissue) carnitine deficiency is manifested clinically as muscle weakness, cardiac failure, hypoglycemia and/or liver insufficiency. The clinical manifestations may resemble cardiac fibroelastosis, Reyes Syndrome, or muscular dystrophy. The enzymopathy has not been defined, and the variety of manifestations suggest that there may be several enzymopathies that fall into the category of primary systemic carnitine deficiency. The reported cases have in common low serum and/or tissue levels of carnitine. Some of the reported cases responded very dramatically to administration of exogenous l-carnitine.

Secondary carnitine deficiency occurs in organic acidurias, chronic hemodialysis, severe malnutrition, Fanconi's syndrome and valproic acid therapy. These conditions might benefit from carnitine therapy, but data have not been submitted. Diagnosis of primary systemic carnitine deficiency requires accurate quantitation of both free and acylcarnitine in serum, urine and tissue. Patients with high urine ratios of acylcarnitine to free carnitine may have carnitine deficiency secondary to defective organic acid metabolism. Because of incomplete biochemical characterization, it is not clear whether carnitine deficiency is primary or secondary in some of the submitted cases.

B. studies to provide evidence of safety and effectiveness.

There are 16 cases identified as systemic carnitine deficiency which were treated with carnitine, 11 of which provide evidence of effectiveness. An additional 50 cases not identified as systemic carnitine deficiency were treated with carnitine and provide evidence of safety only.

- a. Cases identified as systemic carnitine deficiency are listed below, whether or not they were evaluated as showing effectiveness. Five of the ten investigators had more than one case.

- S. Cederbaum, M.D., NEJMed 1980, 303:1329.
1. A 7.5 year old male patient with low carnitine levels in liver, muscle and blood was treated first with d,l-carnitine 1500-4000 mg/d p.o. and then with l-carnitine 1980 mg/d. He improved in affect, frequency of infections and in listlessness, and had diminished cardiomegaly. Liver and serum carnitine levels increased, but muscle carnitine remained low. Carnitine therapy evaluation: life-saving. He had transient diarrhea which resolved with decreased dosage. This patient has been continued on l-carnitine, 75 mg/kg/d for four years with continued benefit. Total plasma carnitine is low normal (30 micromolar). S. Cederbaum, et al., NEJMed 1984, 310, 1395.

- R.R. Chun, M.D., et al., NEJMed 1981, 305:385. Two patients with systemic carnitine deficiency were treated with l-carnitine 990 mg + 1650 mg/d.
2. A 14 month old male patient died of respiratory arrest after about 2 weeks of treatment. L-carnitine evaluation: did not alter this patient's condition.
  3. A 9 year old female had a deteriorating cardiomyopathy, familial in nature with mitral insufficiency and syncope. She was considered a candidate for a cardiac transplant, but after treatment with l-carnitine, cardiac function normalized, digitalis was discontinued and heart size returned to normal. Carnitine treatment was evaluated: produced dramatic improvement.

- A. Slonim, J. Peds. 1981, 99:551. Three patients with systemic carnitine deficiency were treated with 330 to 1320 mg/d l-carnitine (100 mg/kg/d).
4. A 2 year old male had nonketotic hypoglycemia before treatment with carnitine. After treatment he had no hypoglycemic episodes, his height, weight and appetite increased, and he appeared to be more normal. Plasma carnitine increased to normal, and on fasting he produced ketones. Carnitine treatment evaluation: produced dramatic improvement.
  5. A 3 year old female was walking and speaking better after treatment with carnitine, and had no episodes of hypoglycemia. Carnitine treatment evaluation: life-saving. She had a viral infection during treatment.
  6. A 4 1/2 year old male was treated too short a time to evaluate response.

D. Valle, M.D., J. Peds. 1982, 101:700.

7. A 5 1/2 year old male with low blood and muscle levels of carnitine was treated with 3960 mg one day and then 2980 mg/d. He had moderate cardiomegaly and minimal heart failure despite treatment with digoxin and diuretics. Within a week of starting carnitine, heart size decreased and failure disappeared. After 5 months of treatment, he was normal. Carnitine therapy evaluation: life-saving. The patient had moderate diarrhea which was dose related, and developed body odor.

C. Sansaricq, NYU Med. Center.

8. A 1 year old male with systemic carnitine deficiency was treated with 165 to 330 mg l-carnitine orally. He died of respiratory failure after 10 days of therapy. Carnitine therapy evaluation: too short a time to evaluate.

B.O. Stands, M.D., Richland Medical Park, Columbia, SC.

9. A 2 1/2 year old female with systemic carnitine deficiency was treated with 990 mg l-carnitine/d. She had several episodes of severe coma and was seriously ill before treatment but has had no further problems and is developing normally. Carnitine therapy evaluation: produced dramatic improvement. She had cough, runny nose and diarrhea of moderate severity.

R. Cruse, D.O. and S.K. Young, M.D., Hershey Med. School. Two patients with systemic carnitine deficiency were treated with 1320 to 1980 mg l-carnitine.

10. A 3 1/2 year old female had two attacks of coma before and none in the year after initiating treatment. She is stronger. Carnitine therapy evaluation: produced improvement. She had loose stools which ceased after one week. Fishy odor was eliminated by decreasing the dose of l-carnitine. She also had urinary tract infection and virus.
11. A 6 year old female remained "asymptomatic," and carnitine therapy evaluation: produced improvement. She also had loose stools and fishy odor.

P. Hartlage, M.D., Medical College of Georgia.

12. A 3 year old female with probable systemic carnitine deficiency was treated with 1980 mg l-carnitine/d orally. She did not benefit from carnitine therapy.

- J. DiLiberto, M.D. and J.E. Schumacher, M.D. of Portland, Ore. Two patients with systemic carnitine deficiency received 1-carnitine 990 or 1980 mg/d.
13. An 11 month old female had two episodes of hypoglycemia with hepatic, cerebral and muscle dysfunction. No subsequent attacks despite infections. Carnitine therapy evaluation: produced improvement. She had flu with vomiting, rash on trunk and back, fever and emesis, lethargy.
  14. A 7 year old female after carnitine treatment had increased attention span, began following sequential commands and was more compliant and affectionate. She had better leg control and became toilet trained. Carnitine therapy evaluation: produced dramatic improvement.

C.L. Hoppel, Peds. Res. 1981, 15:633. Two patients with systemic carnitine deficiency were treated with 1-carnitine.

15. A 14 year old female showed greatly improved skeletal muscle function, hepatic function, and cardiac function. Growth and development are normal. Carnitine therapy evaluation: life-saving.
16. A 9 month old male had no recurrence of acute hypotonia, weakness, hepatomegaly and hyperglycemia (sic), but duration of therapy was not sufficient to determine long-term effect on muscle and cardiac function. He had moderate diarrhea, which resolved in 2-3 days, and severe pneumonia.

Carnitine therapy was rated life-saving for 4 patients, dramatic for 4, and producing improvement for 3. The other 5 did not respond or were not treated long enough to evaluate. Six had diarrhea, and four had body odor. The dose of carnitine was decreased because of the adverse effects in some patients, but it was not discontinued for adverse effects in any patients.

B. Cases providing supportive evidence of safety of carnitine administration.

The additional 50 patients were frequently very ill, and several deaths occurred in this group, but were attributable to the underlying disease (Leigh's encephalopathy, mitochondrial myopathy, Hirschprung's Disease with short bowel syndrome and liver failure). There were reports of 7 cases of fishy body odor, 3 cases of tremulousness, sweating, faintness, hyperventilation and dizziness, one case each of mild gastrointestinal symptoms, "intestinal flu", vomiting and increased seizures, respiratory distress and hepatic failure,

transient diarrhea, hypercarotemia and apneic spells, severe diarrhea which subsided with lower dose, fever and vomiting with draining ears, stiffness and headaches, numbness of toes, and moderate burning and pain in heels and left hip. There is little reason to think any of the symptoms except gastrointestinal symptoms and body odor are related to carnitine therapy.

VI. Approved Package Insert:

A copy of the package insert is attached.

FPL

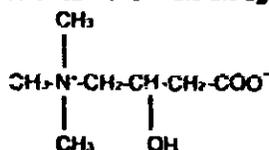
# CARNITOR\*

(L-carnitine)

Tablets

## DESCRIPTION

L-carnitine is L-beta-hydroxy-gamma-trimethylamino butyric acid (inner salt). It is a white powder with a melting point of 196-197°C and is readily soluble in water. The L-isomer of carnitine is the biologically active form.



## CLINICAL PHARMACOLOGY

L-carnitine is essential for the transport of long chain fatty acids across the inner mitochondrial membrane into the mitochondrial matrix where they undergo  $\beta$ -oxidation.

## INDICATIONS AND USAGE

L-carnitine is indicated in the treatment of primary systemic carnitine deficiency.

## CONTRAINDICATIONS

None known.

## WARNINGS

None.

## PRECAUTIONS

Mutagenicity tests have been performed in *Salmonella typhi* murium, *Saccharomyces cerevisiae*, and *Schizosaccharomyces pombe* that do not indicate that L-carnitine is mutagenic.

Long-term animal studies have not been conducted to evaluate the carcinogenicity of the compound.

Pregnancy Category B. Reproductive studies have been performed in rats and rabbits using parenteral administration at doses equivalent to 1 mg/kg.

basis to the suggested oral adult dosage and have revealed no harm to the fetus due to L-carnitine. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

#### **ADVERSE REACTIONS**

The most frequent type of adverse reaction occurring during treatment with L-carnitine is gastrointestinal.

In clinical studies, 41% of patients reported one or more gastrointestinal complaints which tended to be transient.

Typical complaints included nausea, diarrhea, and abdominal distress. Patient odor was the next most frequent adverse effect which had an incidence of 11%. Decreasing the dosage often diminishes or eliminates drug-related patient body odor or gastrointestinal symptoms when present.

#### **DOSAGE AND ADMINISTRATION**

**Adults:** The recommended oral dosage for adults is 950 mg two or three times a day, depending on clinical response.

**Infants and children:** The recommended oral dosage for infants and children is between 50 and 100 mg/kg/day in divided doses, with a maximum of 3 grams/day. The exact dosage will depend on clinical response.

#### **BIOAVAILABILITY**

The bioavailability/pharmacokinetics of L-carnitine tablets have not been determined in well controlled studies.

#### **HOW SUPPLIED**

L-carnitine is supplied as 330 mg, individually foil wrapped tablets in boxes of 90. Store at room temperature.

#### **CAUTION**

Federal (USA) law prohibits dispensing without a prescription.

\* TM application pending

 **sigma-tau**, Inc. 723 North Beers Street  
Holmdel, New Jersey 07733

MED

REV

NDA 18,948  
Carnitine  
Sigma-Tau

Submitted: 2/8/83  
Rec'd by MO: 3/3/83

MOR of NDA

I. GENERAL INFORMATION

- A. Name of drug: L-carnitine
- B. Pharm. category: naturally-occurring cofactor necessary for fatty acid oxidation in mitochondria
- C. Proposed indications: carnitine deficiency syndromes
- D. Dosage form: tablets, each containing 330 mg L-carnitine
- E. Source and method of preparation: see chem review

II. MANUFACTURING CONTROLS

See chemistry review

III. PHARMACOLOGY

See pharmacology review

IV. CLINICAL BACKGROUND

CDS, carnitine-deficiency syndrome, has been recognized for about ten years; its etiology(ies) are still not well established. CDS is considered by many to be a specific disorder of fat metabolism. Patients have been divided into two groups: (1) those with normal serum concentrations and low carnitine content in muscle, possibly resulting from a deficit in carnitine uptake [myopathic carnitine deficiency syndrome], and (2) those with systemic deficiency with decreased content of carnitine in blood and one or more tissues - a generalized metabolic disorder that may be due to a defect in carnitine synthesis [systemic carnitine deficiency syndrome]. It is apparent that any deficiencies of carnitine metabolism may result in similar signs and symptoms as impaired lipid metabolism. A diet low in fat and high in carbohydrate may thus be of use in CDS of any kind. Similarly the treatment of acute metabolic crisis in CDS requires correction of acidosis and administration of substantial amounts of IV glucose. Also, the frequent cardiac involvement in CDS may be understandable on the basis of impaired fatty acid influx into heart muscle mitochondria.

JUN 29 1983

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While the syndrome is rare, there are a number of patients documented in the literature with various types of CDS who have shown dramatic improvement - both objective and subjective - after carnitine therapy.

Carnitine is present in meat and fish and, except for gastrointestinal symptoms, primarily diarrhea, and fish-like body odor, adverse reactions have been virtually unknown.

## V. CLINICAL STUDIES

The studies reported are prototypic for an orphan drug product. The numbers of patients in the studies are small and the data available for each study are limited and largely subjective.

In general, what is known for each patient is: (1) diagnosis; (2) dosage and length of time of drug administration; (3) "Physician's Global Evaluation" of whether (a) carnitine was life-saving to the patient, (b) carnitine dramatically improved the patient's condition, (c) carnitine improved the patient's condition, (d) carnitine did not alter the patient's condition, or (e) carnitine worsened the patient's condition; (4) subjective response with regard to muscle strength, general activity, and neurological response; (5) cardiac size; and (6) tissue levels of L-carnitine. The studies will be described and evaluated in as much detail as possible on the basis of the data presented.

All patients were monitored for any adverse reaction, and all possible such reactions were recorded on the case report form. For each adverse reaction the investigator was asked to rate the severity of the reaction as mild, moderate, or severe, and to estimate the causal relationship of the effect and the drug as remote, possible, probable or definite.

### A. Study #10.

Investigator: S. Cederbaum, M.D.  
Div. of Medical Genetics  
Neuropsychiatric Inst.  
Center of Health Sciences  
760 Westwood Plaza  
L.A., CA

Study Design. Open study to assess the efficacy and safety of carnitine in the treatment of systemic CDS of a single patient, 3.5 yo M. Dx was based on determination of the level of L-carnitine in liver, muscle, and blood: all levels were low. Patient began on D,L-carnitine, at dosages ranging from 1500-4000 mg po qd; currently on 1980 mg qd L-carnitine. [Reported in NEJM 303, 1389, 1980]

Results.

Global evaluation - according to Dr. Cederbaum, carnitine was life-saving to the patient and dramatically improved the patient's condition.

Subjective response - marked improvement in affect, frequency of infections and in listlessness.

Objective response - diminished cardiomegaly; markedly increased liver and serum carnitine levels (to or above nl), muscle carnitine levels low and virtually unchanged with therapy (vol. 1, p. 102).

Adverse reactions - diarrhea, transient, resolved with decreased dosage.

Sponsor's conclusions. "Carnitine provided a successful treatment to the one patient with systemic CDS who participated in the study. The quality of this patient's life has been greatly improved by the administration of the drug. . . "

Reviewer's conclusions. I agree that there appears to be a marked subjective and objective improvement in this patient. Moreover, there is the laboratory correlation of increased liver and serum carnitine levels. Thus, the drug appears safe and effective in this patient.

B. Study #11. [Neurology 32, 1106, 1982]

Investigator: T. Snyder, M.D.  
Dept. of Neurology  
Med. Ctr. of Vt.  
Burlington, VT 05401

Study Design. Open study to assess the efficacy and safety of L-carnitine in the treatment of lipid storage myopathy.

Two patients with lipid storage myopathy entered the study. The dx of lipid storage myopathy was based on histochemistry of muscle biopsies. The concentration of L-carnitine in the muscle of one patient was 16.8 nmoles/mg protein, 7.8 nmoles/mg protein in the other (nl 11-21). For the first week, the first patient received an oral, total daily dosage of 14.85 gm of drug in 3 divided doses. The dose was then reduced to 4.95 gm/d divided into 3 doses.

For the first four days the second patient received an oral, total daily dosage of 14.85 gm of L-carnitine divided into 3 equal doses. The dose was then reduced to 4.95 gm/day.

Results.

Global evaluation - both patients showed dramatic improvement in symptomatology.

Subjective - first patient had marked improvement in his functional abilities with markedly increased muscle bulk and strength; second patient had marked improvement in muscle strength but not in bulk, with improved sense of well-being and "functional abilities."

Adverse reactions - none.

Sponsor's conclusions - "L-carnitine provided a successful treatment to two patients with lipid storage myopathy."

Reviewer's conclusions. Once again, the safety and efficacy of L-carnitine appears to have been demonstrated in 2/2 patients. Note, however, that in only one of these was true carnitine deficiency demonstrated.

C. Study #12. [NEJM 305, 385, 1981]

Investigators:

R.R. Chun, M.D.  
H.A. Peters, M.D.  
M.L. Katcher, M.D.  
Univ. of Wisconsin Hosp. and Clinics  
Madison, WI 53792  
H.E. Tripp, M.D.  
Univ. of Chicago  
Chicago, IL

Study Design. Open study in 8 patients with different syndromes to examine the safety and efficacy of L-carnitine.

Patients had following diagnoses:

Patient #1	systemic CDS
#2	systemic CDS
#3	motor neuron disease
#4	Duchenne Muscular Dystrophy
#5	myopathic CDS; Duchenne muscular dystrophy; palmitoyl transferase deficiency
#6	Duchenne Muscular Dystrophy
#7	Duchenne Muscular Dystrophy
#8	Duchenne Muscular Dystrophy

Patients received total daily doses of 990 to 1650 mg.

Results.

Global evaluation. Patient 1 - treatment with drug did not alter the patient's condition but too early to adequately evaluate. Patient 2 - treatment dramatically improved the patient's condition. Patients 3,5,6,7 - treatment did not alter their condition. Patient 4 - too early to evaluate. Patient 8 - not evaluated.

Subjective evaluation. Patient #2 had deteriorating familial cardiomyopathy, with MI and syncope, and was reportedly considered a candidate for a cardiac transplant. After treatment with drug, cardiac function has normalized, digitalis has been discontinued, and heart size has returned to normal.

Adverse reactions. Patient #1 died of respiratory arrest after being treated with drug for approximately 2 weeks; felt only remote possibility of relationship to drug. Patient #2 had mild GI sx felt possibly related to treatment with drug; in addition, this patient had odd body odor, probably related to drug. Patient #4 noted stiffness and headaches.

Sponsor's conclusions. Treatment with L-carnitine dramatically improved the condition of one patient with systemic CDS.

Reviewer's conclusions. Agree with sponsor's conclusions, as far as they go. In addition, 0/3 evaluable patients with Duchenne Muscular Dystrophy and 0/1 with "motor neuron disease" responded.

D. Study #13.

Investigator:

W.K. Engel, M.D.  
Hospital of the Good Samaritan  
L.A. CA 90017

Study Design. Open study of 22 patients with following dx:

Patient #1	myopathic CDS
#2	carnitine responsive muscle fatigue and cramps
#3	probable lipid myopathy and neuropathy
#4	myopathic carnitine deficiency
#5	myopathic carnitine deficiency
#6	myopathic carnitine deficiency
#7	increased lipid in muscle at birth
#8	lipid myopathy
#9	mitochondrial myopathy
#10	Duchenne M.D.
#11	Duchenne M.D.
#12	Duchenne M.D.
#13	Oculopharyngeal M.D.
#14	Ragged Red Fibers-increased lipid on bx
#15	myopathic carnitine deficiency
#16	Ragged red fiber disease
#17	vacuolar myopathy
#18	phosphorylase deficiency
#19	myophosphorylase deficiency
#20	Ragged red fiber disease
#21	Ragged red fiber myopathy with increased lipid
#22	vacuolar myopathy with lipid droplets

Patient: received total daily po doses of up to 15 g of drug.

### Results.

Global evaluation. In the five patients with myopathic CDS (#s 1, 4, 5, 6, and 15), one patient experienced dramatic improvement and four patients experienced improvement. In the other patients, there was apparently no effect of treatment.

Adverse reactions. Some 31 adverse reactions were reported in 10 patients. All but three in three patients were mild-moderate GI effects or fishy body odor. The remaining three were tremulousness/sweating/faintness/hyperventilation and dizziness (2).

Sponsor's conclusions. All five patients with myopathic carnitine deficiency benefited by treatment with drug; in one patient the response was dramatic.

Reviewer's conclusions. On the basis of such meager data, I cannot dispute the sponsor's conclusions. It would have been of interest to know whether laboratory values correlated with the reported clinical response.

It should also be noted that in this pivotal study (5 of the 7 patients overall in the NDA with myopathic CDS showing improvement-are from this study) that 4 of the 5 patients were on glucocorticoids during at least part of the time of administration of carnitine. This makes difficult interpretation of the effect of carnitine.

E. Study #14. [J. Peds. 99, 551, 1981]

Investigator:

A. Slonim, M.D.  
Dept. of Peds. Endocrinology  
Vanderbilt Univ. Hosp.  
Nashville, TN 37232

Study Design. Open study to assess efficacy and safety of L-carnitine in CDS in 3 patients with systemic CDS and one with myopathic CDS.

Patients received between 330 and 1320 mg of po drug qd or 100 mg/kg/d.

Adverse reactions. None reported.

Results.

Global impression and other data. Two patients with systemic CDS responded to treatment with drug (walking, speaking improved; without further episodes of hypoglycemia). The one patient with myopathic CDS did not respond to treatment. It was felt to be too early to evaluate the other patient with systemic CDS.

Adverse reactions. Single episode of a "viral illness."

Sponsor's conclusions. Treatment with drug was beneficial to two patients with systemic CDS. One patient with myopathic carnitine deficiency did not respond to treatment.

Reviewer's conclusions. Marked efficacy appears to have been demonstrated in 2/2 patients with systemic CDS. Ineffective in the one patient with myopathic CDS. Again, no safety problems.

F. Study #15 - only patient in study unevaluable.

G. Study #16. [J. Peds. 90, 700, 1982]

Investigator:

D. Valle, M.D.  
Peds. Genetics Clinic  
The Johns Hopkins Hosp.  
Balt., MD 21205

Study Design. Open study of a single patient to assess the safety and efficacy of L-carnitine in systemic CDS.

Results.

Global evaluation. Drug was "life-saving" to patient.

Clinical impression. Patient was in extreme borderline CV status despite chronic Rx with digoxin and digrelin. Within one month, there was obvious improvement. After 3 months of therapy, the patient's clinical status was "normal."

Objective data. Cardiomegaly as measured on CXR improved markedly. Blood levels normalized, from 4.3 mg/dl pre treatment, to 21.0-32.0 after treatment (n' 20.8-44.5).

Adverse Reactions. None reported.

Sponsor's Conclusions. Markedly successful treatment in this patient.

Reviewer's Conclusions. Concur with above.

n. Study #17.

Investigator:

E. Honkus, M.D.  
Dept. of Peds.  
Div. of Neonatology  
Univ. of Miami  
School of Medicine  
Miami, FL 33101

Study Design. Open study with two patients, one with Leigh's encephalopathy (patient #1), the other with kwashiorkor (patient #2), of the safety and efficacy of L-carnitine.

Patient #1 received a total oral daily dose of 990 mg of L-carnitine. Patient #2 was maintained on 2540 mg/day.

Results.

Global evaluation. Patient #1 died approximately two months after beginning therapy; demise unrelated to drug administration, according to investigator. Patient #2 improved in response to treatment with L-carnitine.

Clinical impression. In patient #2, markedly improved nutrition during period of therapy was felt to be major factor in clinical improvement; unknown how much drug contributed.

Adverse Reactions. Death of one patient, felt unrelated to drug.

Sponsor's conclusions. Drug was beneficial to patient with Kwashiorkor.

Reviewer's conclusions. Unevaluable.

I. Study #18.

Investigator:

L.D. Prockop, M.D.  
Division of Neurology  
Univ. of So. Fla.  
Tampa, FL

Study Design. Open study of a single patient with myopathic CNS, severe respiratory failure and muscle weakness of the safety and efficacy of L-carnitine. Patient was treated with 10,000 mg qd D,L-carnitine initially, tapered to 4000 mg D,L-carnitine, now maintained on 1980 mg/d L-carnitine.

Results.

Global impression. According to investigator, drug was life-saving to patient.

Clinical impression. Patient's long-standing muscle weakness, flaccid paralysis and hypoxic brain damage were reversed substantially by drug therapy.

Objective. Patient was able to be weaned from ventilator, regained 67% of predicted respiratory function and approximately 90% of normal muscle strength after drug therapy.

Adverse reaction. Question of mild distal numbness of toes, transient, felt remotely likely to be related to drug.

J. Study #19.

Investigator:

C. Sansaricq, M.D.  
Dept. of Peds.  
NYU Med. Ctr.  
New York, NY 10016

Study Design. Open study of a single patient with systemic CBS of the safety and efficacy of L-carnitine.

The one year old male patient received 165 - 530 mg of drug po qd.

Results. Patient died of respiratory failure ten days after initiation of therapy, felt by investigator unrelated to drug therapy. Also felt duration of therapy too short to evaluate effect.

Sponsor's conclusions. Unevaluable.

Reviewer's conclusions. Unevaluable.

K. Study #20.

Investigator:

B.G. Stands, M.D.  
Medical Park Peds. & Adolescence, P.A.  
Richland Medical Park  
Columbia, S.C.

Study Design. Open study of a single patient with systemic CBS, with dx based on subnormal levels of L-carnitine in muscle, liver, and blood. Patient receives dosage 990 mg/d.

Results.

Global evaluation. Patient dramatically improved.

Clinical impression. Drug ended recurrent comatose episodes.

Adverse reactions. None reported.

Sponsor's conclusions. Drug beneficial in this patient.

Reviewer's conclusions. Concur.

L. Study #21.

Investigator:

K. Chandar, M.D.  
Division of Neurology  
Mt. Sinai Hospital  
Cleveland, O

Study Design. Open study to assess the efficacy and safety of L-carnitine in a single patient (71 yo female) with myopathic CDS. Dosage ranged from 1980-11,880 mg po qd.

Results.

Global evaluation. Drug did not alter patient's condition.

Clinical impression. No apparent increase in muscle strength during study.

Sponsor's conclusions. No response in this patient.

Reviewer's conclusions. Concur.

A. Study #22.

Investigators:

R. Cruse, D.O.  
R.S.K. Young, M.D.  
Dept. of Peds. Neurology  
Hershey Med. Sch.  
Hershey, PA 17033

Study Design. Open study of two patients (3.5 and 6 yr) with systemic CDS of the safety and efficacy of L-carnitine. Patients initially received oral dose of 1380 mg, later reduced to 1320 mg.

Results.

Global evaluation. Both patients improved on drug.

Clinical impression. Episodic attacks of coma have ended in one patient, and the second remains without symptoms.

Adverse reactions. Transient loose stools in both patients; fishy odor noted, eliminated with decreased dose.

Sponsor's conclusions. Both patients with systemic CDS benefited.

Reviewer's conclusions. Concur.

N. Study #23.

Investigator:

D.L. Ehrenreich, M.D.  
Buffalo Med. Grp.  
Buffalo, NY 14203

Study Design. Open study of a single patient, 41 yo female, with lipid storage myopathy, of the safety and efficacy of L-carnitine. Drug was begun at po dose 4 g qd and gradually tapered to zero over 14 months.

Results.

Global evaluation. No effect of drug.

Clinical impression. "Shortly after instituting treatment with Theragram (while still on carnitine), there was a dramatic improvement. Carnitine levels were not found to be low in muscle or serum. Lipid storage myopathy was "cured" when repeat muscle bx performed. Improvement was maintained after treatment with L-carnitine stopped."

Adverse reactions. Moderate burning and pain in necks and left hip; felt only remote possibility of relation to drug.

Sponsor's conclusions. No effect in this patient.

Reviewer's conclusions. Concur.

O. Study #24.

Investigators:

R.C. Griggs, M.D.  
Dept. of Neurology  
University of Rochester School of Med. and Dentistry  
Rochester, NY 14642

Study Design. Open study to assess the safety and efficacy of L-carnitine in two male patients (ages 3 and 16), one with mitochondrial myopathy (#1, age 3), the other with myopathic CDS (#2, age 16). Patients dosages ranged from 1320-1980 mg po qd.

Results.

Global evaluation. No effect of drug in either patient.

Adverse reactions. Both patients experienced mild GI sx, possibly related to drug. Patient #1 died of respiratory failure, approx. 2.5 mo after beginning drug (no mention of relationship to drug).

Sponsor's conclusions. No effect in two patients, one with mitochondrial myopathy and one with myopathic CDS.

Reviewer's conclusions. Concur.

P. Study #25.

Investigators:

P. Hartlage, M.D.  
Depts. of Neuro and Meds.  
Medical College of GA  
Augusta, GA 30912

Study Design. Open study in two patients, 3 yo F with probable systemic CDS (#1) and 7 yo M with lipid myopathy. #1 received oral dosage 1980 mg qd; #2, 990 mg qd.

Results.

Global evaluation. No effect of drug on either patient.

Adverse reactions. Transient "intestinal flu" in patient #2.

Sponsor's conclusions. No effect of drug.

Reviewer's conclusions. Concur.

Q. Study #26. [Johns Hopkins Med. Jour. 151, 196, 1982]

Investigator:

H.W. Moser, M.D.  
J.F. Kennedy Inst. for Handicapped Children  
Balt., MD 21205

Study Design. Open study in two patients with adrenoleukodystrophy; #1, 18 yo M; #2, 5 yo M. Patients received dose of 1980 mg po qd (plus clofibrate).

Results.

Global evaluation. No effect of drug.

Clinical impression. No effect of drug.

Sponsor's conclusions. Drug ineffective for two patients with adrenoleukodystrophy.

Reviewer's conclusions. Concur.

R. Study #27.

Investigator:

C.A. Stanley, M.D.  
Division of Endocrinology/Diabetes  
Children's Hosp. of Phila.  
Phila., PA 19104

Study Design. Open study to assess the safety and efficacy of L-carnitine in the treatment of either long-chain acyl-CoA dehydrogenase or medium-chain acyl-CoA dehydrogenase deficiency. Patients received approximately 100 mg/kg drug po qd.

Results.

Global evaluation. No effect of drug in the 3 patients with medium chain acyl-CoA dehydrogenase deficiency. Improved the condition of the patient with long-chain acyl-CoA dehydrogenase.

Clinical impression. The patient with long-chain acyl-CoA dehydrogenase deficiency was treated with L-carnitine and special diet. There was gradual improvement over 8-12 weeks. Heart size was reduced to upper normal level and muscle strength was much improved. ? How much is relative contribution of diet vs. drug.

Adverse reactions. None reported.

Sponsor's conclusions. Drug and diet treatment resulted in improvement in one patient with long-chain acyl-CoA dehydrogenase deficiency. Three patients with medium-chain acyl-CoA dehydrogenase were unresponsive to treatment.

Reviewer's conclusions. Concur, though as noted, it is difficult to know the relative contribution of drug vs. diet in the responsive patient.

S. Study #28.

Investigator:

J. Patrick, M.D.  
Children's Hospital of Eastern Ontario  
Ottawa, Canada

Study Design. Open study of L-carnitine in two patients, one (#1) with "hepatic malfunction" (1.5 yo F), the other (#2) with Hirschsprung's disease of long segment/snort bowel syndrome/jaundice (1 yo M). Patients received 350-660 mg po qd of drug.

Results.

Global impression. In patient #1, drug was life-saving; in patient #2, treatment improved patient's condition.

Clinical impression. Patient #1: in response to treatment, patient lost fetor hepaticus, became more alert, and began taking solid foods. A's, she tolerated high protein, high carbohydrates, low fat intake, and had decreased hypotonia and improvement in sitting, balance and equilibrium reaction. Increase in muscle bulk and strength was observed and ketogenesis was increased. Patient #2: was in pre-morbid state before treatment; patient expired approx. 3 weeks after initiation of drug.

Objective. In #1, treatment increased both total and free serum L-carnitine. After 1 yr of treatment, total serum level was increased from 20.0 to 56.3 nmol/L while the free level was increased from 12.2 to 51.1.

Adverse reactions. Patient #1 experienced vomiting and increased seizures (? related to drug). #2 experienced respiratory distress, hepatic failure and expired after approx. 3 weeks on drug.

Sponsor's conclusions. Treatment with drug was life-saving to one patient with hepatic malfunction/carnitine deficiency.

Reviewer's conclusions. Concur.

T. Study # 29.

Investigator:

J. DiLiberti, M.D.  
Emanuel Hosp.  
Portland, OR 97227

J.R. Schimschock, M.D.  
2525 N.W. Lovejoy  
Portland, OR 97210

Study Design. Open study of L-carnitine in two patients with systemic ODS; #1 an 11 mo old F, #2 a 7 yo F. #1 received doses of 990 mg po qd, #2 1980 mg po qd.

Results.

Global evaluation. Treatment dramatically improved both patients.

Clinical impression. #1 seems less prone to decompensations, infectious and metabolic, since beginning drug. #2 is neurologically and socially improved.

Sponsor's conclusions. Treatment was beneficial to both patients with systemic ODS.

Reviewer's conclusions. Concur.

U. Study #30. [Lancet 6/19/82, pp. 1411-2]

Investigator:

C. Roe, M.D.  
Divison of Peds. Metab.  
Duke Univ. Med. Ctr.  
Durham, NC 27710

Study Design. Open study of one patient, a 6.5 mo old M, with propionicacidemia (propionyl CoA carboxylase deficiency). Patient was treated with po drug, 100mg/kg for 8 montns; dose was then reduced to 50 mg/kg.

Results.

Global evaluation. Drug dramatically improved patient's condition.

Clinical impression. Markedly improved performance on Denver Development Test and increased muscle strength.

Adverse reactions. Transient diarrhea, possibly related to drug; intermittent hyperammonemia, unlikely to be related to drug; apneic episodes, unlikely to be related to drug.

Sponsor's conclusions. Drug was beneficial to this patient with propionicacidemia.

Reviewer's conclusions. Concur.

V. Study #31. [Feds. Res. 15, 633, 1981]

Investigator:

C.L. Hoppe<sup>1</sup>, M.D.  
Division of Clin. Pharm.  
V.A. Med. Ctr.  
Cleveland, O 44106

Study Design. Open study of L-carnitine in two-patients with systemic CDS; #1 was a 14 yo F, #2 a 9 mo. M. #1 received 30-125 mg po qd; #2 received 100 mg/kg/d.

Results.

Global evaluation. Drug was life saving to patient #1 and improved the condition of #2.

Clinical impression. Treatment reportedly greatly improved skeletal muscle and hepatic and cardiac function in #1. In #2, no recurrence of pre-treatment acute problems, including hypotonia, weakness, hepatomegaly, and hyperglycemia.

Objective. In #1, plasma levels of L-carnitine were increased from 2 to 3<sup>1</sup> after treatment.

Adverse reactions. In patient #2, transient moderate diarrhea and pneumonia were reported; felt only remotely likely to be related to drug by investigator.

Sponsor's conclusions. L-carnitine was life-saving in one patient, beneficial to the other.

Reviewer's conclusions. Concur. Also, I have a higher index of suspicion than the investigator that the diarrhea in #2 was drug-related.

X. Study # 32.

Investigator:

C. Imbus, M.D.  
Rancho Los Amigos Hosp.  
Downey, CA 90242

Study Design. Open study in a single 5.5 yo M patient with myopathic CDS. Patient received total po dose of 1650-1900 mg of drug.

## Results.

Global evaluation. Drug improved patient's condition.

Clinical impression. Investigator reports that patient has fewer adventitial movements and experienced a remarkable weight gain.

Adverse reactions. Treatment with drug was begun in 12/81. Severe diarrhea was reported the same month. The diarrhea subsided after the dose of L-Carnitine was reduced from 1980 mg to 1650 mg qd. Dose was later increased to 1980 mg with recurrence of diarrhea. In 3/82, fever, vomiting, coughing and draining of ears was reported. Vomiting was reported in 1/82. The investigator does not indicate his index of suspicion about drug-relatedness.

Sponsor's conclusions. Drug was beneficial to this single patient with myopathic CDS.

Reviewer's conclusions. Concur. It appears that the diarrhea reported is drug-related.

- Y. Safety Considerations. Of the 66 patients presented in the clinical protocols of the NDA, for 57 there was reported one or more adverse reaction. The overwhelming majority of those were in two categories: (1) GI symptoms, usually mild-moderate diarrhea and; (2) "fishy" body odor. In no case reported did either of these constitute or cause major morbidity or mortality, and symptoms were often diminished or eliminated by a reduction in dose.

Seven deaths occurred in patients in the various protocols, with cause of death listed variously as cardiorespiratory distress/progressive hepatic failure (1), pneumonia (1), hypotension and hypoventilation (1), respiratory arrest/failure (4). In all cases, the relationship of the patient's demise to drug administration was felt by the respective investigators to be remote; in most cases, the patients were extremely ill when therapy was begun. The age range in these patients was 14 mo to 71 years; likewise, there appeared nothing systematic in their diagnoses or dosage of drug.

In summary, safety of this natural product in the patient population for whom it is intended and at the dosages administered (up to 1g/d) appears to be demonstrated. Adverse reactions were common, consisting overwhelmingly of GI symptoms (approximately 30-40% of patients) or fishy body odor (approximately 10%), but these tended to be transient and mild.

- Z. Efficacy Considerations.

The patients included in the various studies include numerous diagnoses, many of them extremely rare, as shown on the next page.

INDICATION	NUMBER OF PATIENTS
Systemic carnitine deficiency	16
Myopathic carnitine deficiency	9
Duchenne Muscular Dystrophy	8
Lipid storage myopathy	7
Ragged Red Fiber Disease	5
Mitochondrial myopathy	2
Adrenal Leukodystrophy	2
Medium chain Acyl CoA dehydrogenase deficiency	1
Long chain Acyl CoA dehydrogenase deficiency	1
Propionyl CoA carboxylase deficiency	1
Motor Neuron Disease	1
Oculopharyngeal Muscular Dystrophy	1
Vacuolar myopathy	2
Carnitine responsive muscle fatigue and cramps	1
Phosphorylase deficiency	1
Myophosphorylase deficiency	1
Cardiac myopathy	1
Leigh's Encephalopathy	1
Kwashiorkor	1
Hepatic malfunction	1
Hirschsprung's Disease	1
<b>TOTAL</b>	<u>66</u>

Below is shown a tabulation of the investigators' global assessments of all patients, arranged by diagnosis; these are the pivotal data on which efficacy can be judged:

GLOBAL ASSESSMENT

INDICATION	LIFE- SAVING	DRAMATIC IMPROVEMENT	IMPROVEMENT	NO ALTERATION	WORSENING	NO RATING
systemic carnitine deficiency	4	5	3	2	0	2
myopathic carnitine deficiency	1	1	5	7	0	0
lipid storage myopathy	0	2	1	1	0	0
acid E-d Fiber Disease	0	0	1	2	0	2
mitochondrial myopathy	0	0	0	1	0	1
acid leukodystrophy	0	0	0	2	0	0
long chain Acyl CoA dehydrogenase deficiency	0	0	0	3	0	0
short chain Acyl CoA dehydrogenase deficiency	0	0	1	0	0	0
mitochondrial CoA carboxylase deficiency	0	1	0	0	0	0
or Neuron Disease	0	0	0	1	0	0
or limb Muscular Dystrophy	0	0	0	4	0	4
or laryngeal Muscular Dystrophy	0	0	0	0	0	1
or ocular myopathy	0	0	0	1	0	1
or citrate responsive muscle fatigue and cramps	0	0	1	0	0	0
or phosphorylase deficiency	0	0	0	0	0	1
or phosphorylase deficiency	0	0	0	1	0	0
or distal myopathy	0	0	0	0	0	1
or Leigh's Encephalopathy	0	0	0	0	0	1
or Shierker	0	0	1	0	0	0
or gastric malfunction	1	0	0	0	0	0
or Schönerberg Disease	0	0	1	0	0	0

000271

It is evident that L-carnitine appears to have been consistently effective only for patients with diagnoses of systemic carnitine deficiency (improvement, dramatic effect or lifesaving in 12/14, or 85% of evaluated patients) and myopathic carnitine deficiency (improvement, dramatic effect or lifesaving in 7/9, or 78%, of patients). For patients with other diagnoses, the numbers are too small to draw meaningful conclusions about efficacy, especially since in many cases the precise diagnosis itself is likely to be in doubt.

#### VI. CONCLUSIONS

- A. Scientific. The difficulties inherent in evaluating studies such as those submitted to this NDA for a determination of efficacy are obvious: there is a paucity of objective data; the total numbers of patients and number from any one institution are small; the diagnoses are somewhat vague both biochemically and clinically. There are, however, two considerations that are critical. First, there are the consistent judgements of experienced academic and other clinical investigators that the drug improves, often dramatically, sometimes in a lifesaving way, patients' clinical status. Second, many of the patients with the diagnoses for which efficacy appears to have been demonstrated (systemic and myopathic carnitine deficiency, respectively) are extremely ill, moribund or even in extremis, and no consistent, significant alternative therapy exists. And last, L-carnitine appears to be quite safe in this patient population in the range of dosages administered.

It should be noted that the evidence for efficacy in myopathic CDS is less compelling than that for systemic CDS. Five of the 7 patients reported in the NDA with myopathic CDS who improved on carnitine are from a single study, #13, and four of these were treated with glucocorticoids during at least part of the time of administration of carnitine. This makes interpretation of the effect of carnitine alone difficult; on the other hand, the principal investigator for this study is quite experienced and well-versed in the nuances of myopathic CDS (as is evidenced by the large number of patients he has accumulated).

In summary, data from the studies described herein are adequate to support the safety and effectiveness of L-carnitine in the treatment of systemic carnitine deficiency or myopathic carnitine deficiency.

- B. Regulatory. This NDA should be approved.

VII. PATIENT INFORMATION INSERT

The draft insert is approvable with the following corrections:

1. Under Indications and Usage on p. 1, the sentence should read, ". . . in the treatment of systemic deficiency or myopathic deficiency of L-carnitine."
2. Under Dosage and Administration on p. 1, it should be noted that decreasing the dosage often diminishes or eliminates drug-related patient body odor or gastrointestinal symptoms when present.
3. Under Dosage and Administration, Infants and children on p. 1, "oral" is misspelled.

H.I. Miller, M.D.

cc:

NDA Orig.  
HFN-180  
HFN-130  
HFN-130/HMiller/0133C

CHEM

REV

REVIEW OF CHEMISTRY AND MANUFACTURING CONTROLS

NDA #18-948

Division: MEDP, HFN 810

Chemist Review #4

Applicant: SIGMA-Tau, Inc.  
Address: Hialeah, New Jersey 07713

Reviewing Chemist: M.K. Bennett

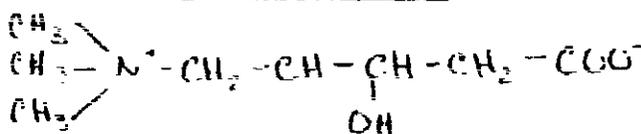
Date Completed: November, 1985

Product Name(s): L-CARNITINE Carnitor<sup>R</sup>

Dosage Form(s) and Route(s) of Administration: Oral

Pharmacological Category and/or Principal Indication: amino acid  
~~amino acid~~ L-beta-hydroxy-gamma-trimethylamino butyric acid inner salt

Structural Formula and Chemical Name:



Initial Submission: March 21, 1983 substantive Amendment June 14, 1983, See Chemist Review #2.

Amendments: See Chemist Review #3, 11/9/84, 12/27/84, 1-7-85, 2-5-84, 3-1-85, 6-14-85, 7-14-85, 7-25-85, 7-25-85, 7-31-85, 9-13-85, 10-17-85, 10-23-85.

Remarks: Amendments cleared most of the deficiencies in the chemistry and manufacturing controls. 1

Labeling and labels are approvable with chemical name revised as indicated above - need FPL.

Conclusions and Recommendations: From chemistry and manufacturing controls, this application may be approved. Applicant should be requested to submit FPL.

*H. E. ?* for M. Bennett  
Martin K. Bennett, P.D.

cc:

Orig. NDA

HFN-810

HFN-810/MKBennett/11/18/85/sw/12/6/85

R/D init. by: DJKertesz/11/18/85

Wang No. 0319S

18

PHARM

REV

Greg

NDA 18-948

April 22, 1985

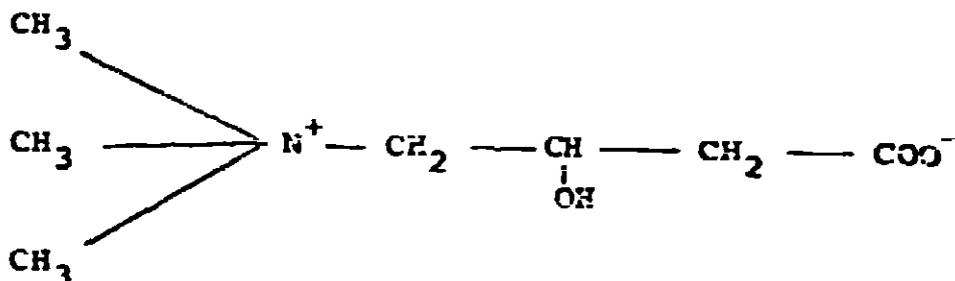
SIGMA-TAU, Inc.  
North Beer Street  
Holmdel, New Jersey 07733

Original Submission Date: February 8, 1983  
Amendment: (3.1) June 27, 1983  
Withdrawn October 23, 1983.  
Resubmitted: August 13, 1984

Pharmacologist Review and Evaluation of Pharmacology and Toxicology Data

Drug: L-Carnitine Tablets: The drug substance is the synthetic form of natural L-carnitine.

Chemical Formula: L-beta-hydroxy-trimethylammonium butyric acid inner salt



Source of Drug: Sigma-Tau Industrie Farmaceutiche Riunite Spa, Rome, Italy.

Proposed Clinical Use: The correction of systemic carnitine deficiencies.

Related IND: 17,819 L-Carnitine Tablets and Oral Solution.

Background Information: The original submission of the NDA contained a review by the sponsor of some 20 publications dealing with the pharmacological functions and the toxicological aspect of carnitine, and reports from toxicity tests performed by

These were:

- . acute tests in mice and rats,
- . subacute tests in rabbits, one with I.V. doses of d,l-carnitine for 24 days, and one with l-carnitine I.V. for 30 days
- . chronic tests: one in rats with l-carnitine administered I.V. for 180 days and one in dogs with l-carnitine administered intramuscularly for 180 days.
- . Reproduction/Teratogenicity tests in rats and rabbits with I.V. and I.M. administration.
- . Mutagenicity tests (Ames) in rats )

MAY 8 1985

While the review by the sponsor and the reports of the preclinical test furnished a considerable amount of information on the drug's properties they did not fulfill the requirements of the Agency to an extent to be considered acceptable for the support of the NDA, principally by the technical considerations of the submitted preclinical tests, such as the small numbers of animals used in them, the inappropriate routes of drug administration not applicable for the intended clinical usage, and the inadequate duration of drug administration applied in these tests.

Consequently the sponsor was informed that the then submitted preclinical test results could not be accepted, and he was notified on June 17, 1983 that chronic tests in dogs and rats of 12 months duration had to be performed according to FDA Guidelines, also reproduction/teratogenicity tests in rats and rabbits.

In a later telephone conversation between Dr. Klein of Sigma-Tau and Dr. Sobel, Dr. Klein was informed that the teratogenicity and carcinogenicity tests could be deferred and perhaps waived, and that long-range studies were not required for this drug, because of its nature and proposed clinical usage for correction of carnitine deficiencies.

Studies for the pharmacological characterization of l-carnitine to be conducted by or for the sponsor also were not requested by us because it was felt that the voluminous amount of publications covering clinical and animal pharmacological aspects obtained by intensive modern investigations in the last decade would provide sufficient information for the description of these functions of carnitine and would serve as support for the NDA. A collection of over 40 publications, 29 of them dealing with tests in animals published in 1982 was submitted in the amendment of August 4, 1983, and 4 more volumes with publications were submitted in the amendment of December 27, 1984.

#### Pharmacology of L-Carnitine:

The study of the voluminous material submitted in the NDA and its amendments was facilitated by the fact that modern text books on biochemistry do contain detailed descriptions on carnitine, its biosynthesis, functions in lipid metabolism. To mention a few:

"Biochemistry, The Molecular Basis of Cell Structure and Function", by Albert Lehninger, The John Hopkins University, Worth Publishers Inc. Second Edition, 1975.

"Principles of Biochemistry" by Abraham White, Distinguished Scientist, Syntex Research, et al., McGraw-Hill Book Company, Fifth edition 1973.

the function of carnitine in the mitochondria where they are

tion, from two essential functional contribution from precursor form of carnitine, metabolic actions into carnitine. groups from the cytoplasm into carnitine transferase located on the inner membrane catalyzes the conversion of the long-chain acyl-CoA into acylcarnitine. The mitochondrial acyl-CoA by the action of carnitine on the inner membrane. The acyl-CoA enters the matrix.

steps by their steps-by-step biochemistry," of A. Lehninger on

#### Mitochondria

fatty acids into mitochondria from

activation of the free fatty acid yield fatty acyl-CoA, a step activation of the fatty acid,

from the fatty acyl-CoA to the inner membrane followed by the transport of the acyl carnitine across the membrane and

from the fatty acyl carnitine to the matrix occurs on the inner surface of the

formation of acyl-CoA thioesters by acid chain length. These short-chain acyl-CoA synthetase activates long-chain acyl-CoA synthetase, and long-chain acyl-CoA synthetase activates long-chain acyl-CoA synthetase, and long-chain acyl-CoA synthetase activates long-chain acyl-CoA synthetase, and long-chain acyl-CoA synthetase activates long-chain acyl-CoA synthetase. The last step is the activation of saturated fatty acids as well as

### Transfer of Carnitine:

Long-chain saturated fatty acids have only a limited ability to cross the inner membrane as CoA thioesters but their entry is greatly stimulated by carnitine. This substance was long known to be present in animal tissue but its importance went unrecognized until it was found to be an essential growth factor for the mealworm *Tenebrio Molitor*.

I. H. Fritz and others showed that the stimulation of fatty acid oxidation by carnitine is due to the action of the enzyme carnitine acetyltransferase which catalyzes the transfer of the fatty acyl group from its thioester linkage with CoA to an oxygen-ester linkage with the hydroxyl group of carnitine. The acyl carnitine ester so formed then passes through the inner membrane into the matrix, presumably via a specific transport system.

### Transfer to Intramitochondrial CoA:

In the last stage of the entry process the acyl group is transferred from carnitine to mitochondrial CoA by the action of a secondary type of carnitine acyltransferase located on the inner surface of the inner membrane:



This complex entry mechanism, often called the fatty acid shuttle has the effect of keeping the extramitochondrial and intramitochondrial pools of CoA and of fatty acids separated. The intramitochondrial fatty acid - CoA now becomes the substrate of the fatty acid oxidation system which is situated in the inner matrix compartment.

In a second pathway, the acetyl group of acetyl-CoA is enzymatically transferred to carnitine which acts as the carrier of fatty acids into mitochondria preparatory to their oxidation. Acetylcarnitine passes from the mitochondrial matrix through the mitochondrial membrane into cytosol and acetyl-CoA is then regenerated to transfer of the acetyl group from acetylcarnitine to cytosol CoA."

While the majority of the submitted publications deals with actions of the long-chain carnitine acyltransferases which according to several investigations are localized to mitochondria in liver, heart, kidney and skeletal muscle, some interest exists now also for the short - and intermediate chain CAT and carnitine acetyltransferase activities that have been demonstrated to be present not only in mitochondria but also in peroxisomes and microsomes in the liver (Ch. Hoppel, "Carnitine and carnitine palmitoyltransferase in fatty acid oxidation and ketosis", in Federation Proceedings vol 41, no. 12, 1982, quoting from Markwell et al: "The subcellular distribution of carnitine acyltransferases in mammalian liver and kidney. A new peroxisomal enzyme". J. Biol. Chem. 248:3426, 1973).

The issue of the occurrence of short - and medium chain CATs in peroxisomes was treated by Bieber and co-workers in the article "Possible functions of short-chain and medium-chain carnitine acyltransferases", in Federation Proceedings 41:2858, 1982. These investigators stated that they had demonstrated both short - and medium-chain CAT activity in microsomes, peroxisomes and mitochondria in pig and rat liver. They made reference to a report by Lazarow and DeDuve "A fatty acyl-CoA oxidizing system in rat liver peroxisomes: enhancement by clofibrate, a hypolipidemic drug", in Proc. Natl. Acad. Sci. USA 73:2043, 1976. They postulated that the enzymes in peroxisomes are active in the shuttling of beta-oxidation chain-shortened products out of the peroxisomes into the liver, and for possible other functions.

The action of clofibrate on the functions of peroxisomes in rat livers was also described by Lund and Bremer in their publication "Carnitine Acyl Transferase - Effect of malonyl-CoA, Fasting and Clofibrate feeding in Mitochondria from Different Tissues" in Biochemica et Biophysica Acta 750:164, 1983. According to this study, clofibrate increased the carnitine acyltransferase activity of intact peroxisomes in a manner different from that resulting from fasting, and their conclusion was that clofibrate feeding showed a preferential increase in activity on the medium - and long-chain substrates. The authors also noted the significant increase in the size of the livers caused by clofibrate but unfortunately they did not attempt to correlate this phenomenon to the function of clofibrate in any direction. The authors were biochemists, not pathologists.

The effect of clofibrate was investigated also by Small et al., reported in "Localization of Carnitine Acyltransferases and Acyl - beta Oxidation enzymes in Small Intestinal Microperoxisome of Normal and Clofibrate Treated Mice," in Biochemistry International vol. 7 no.2, 1983. This study was conducted at the University of Manchester, Department of Biochemical Sciences. The authors stated that in the rat, mouse and human liver, fatty acid is oxidized in two subcellular sites, the mitochondria and the peroxisomes. The first enzyme of the peroxisomal fatty acid oxidation system is an H<sub>2</sub>O<sub>2</sub> producing acyl-CoA oxidase whose activity is greatly increased in rats and mice by hypolipidemic drugs (clofibrate). The possibility was raised by the authors that the intestinal peroxisomes might modify the chain length of luminal fatty acids that subsequently are esterified and released into the blood stream as chylomicra. The close distribution of the peroxisomes to endoplasmic reticulum was brought up at the very end but not further elucidated with respect to the functions of the endoplasmic reticulum.

Another article dealing with the functions of the peroxisomes and their regulation is that by Debeer and Mannerts of the University of Leuven, Belgium ("The Mitochondrial and Peroxisomal Pathways of Fatty Acid Oxidation in Rat Livers", in *Diabete and Metabolisme* (Paris) vol. 9:134-140, 1983) that need mentioning because it described and debated the action of malonyl-CoA as modulator of the carnitine-acetyltransferase system, and of the processes in peroxisomal fatty acid oxidation. It is pointed out that until recently, long-chain fatty acid oxidation in liver was considered to occur exclusively in mitochondria and "its regulation was generally considered to be inversely related to the regulation of triglycerol synthesis," but that it is now recognized that peroxisomes are also capable of oxidizing long-chain fatty acids, while inactive towards fatty acids shorter than octanoic acid. This article is cited here in this review mainly because it too addresses the action of clofibrate and of other chemicals in increasing peroxisomal beta-oxidation that also share the property of causing peroxisomal proliferation and lowering serum triglyceride levels in circulation. The authors concluded that the contributions from peroxisomes to fatty acid oxidation is minor compared to that from mitochondria but it is not clear from their discussions whether the action of clofibrate and related substances enhances the contribution rate and by what mechanisms it causes the histological proliferation of hepatocytes. The investigations by Farrel and Bieber in the same area, of the properties and effects of hypolipidemic drugs on mouse liver peroxisomes ("Carnitine Octanoyl Transferase of Mouse Liver Peroxisomes" in *Arch. Biochem. & Biophys.* 222:123-132, 1983) arrived at the conclusion, among others in this very complex report, that peroxisomes from drug-treated mice were broken at a higher rate than those of controls (60% vs 20%) indicating that hypolipidemic drugs enhance peroxisomal membrane fragility. The drugs used in these trials were clofibrate, nafenopin and WY-14,643.

The matter of the peroxisomes and their responses to hypolipidemic drugs is of interest beyond that of their contributions to lipid metabolism because the now emerging experience in the cited publications appears to give a new picture and concepts for the actions of the hypolipidemic drugs even of different chemical nature, as peroxisome proliferators and their tumorigenic properties that may need a complete revision for their significance.

A very interesting publication for a specific action of carnitine is that by Casillas and co-workers, at the Department of Chemistry, New Mexico State University entitled "Carnitine content of rabbit epididymal spermatozoa in organ culture", in *Jour. Reprod. Fert.* 65:247, 1982. After reporting previous investigations by other workers, starting with that of Marquis and Fritz in 1965 in rats, they report their own with bull, ram and rabbit spermatozoa describing a very high concentration of carnitine in the epididymis and

demonstrating the role of carnitine in the maturation process of spermatozoa during their passage through the epididymis. The presence of carnitine in the epididymis is established, and that its production is under the regulation by testosterone. In the present study, the presence and action of carnitine in different sections of the epididymis was investigated by an in-vitro set-up using single epididymal tubules. This action of testosterone was abolished by addition of cyproterone to the culture medium. Carnitine was taken up by spermatozoa in tubules taken from the capit but not from the caudal section (probably because the caudal spermatozoa have completed the maturation process and are not needing the stimulus from carnitine). (It has been known for a long time that sperm undergo a maturation process during their passage through the epididymis that resulted in initiation of their capacity to swim and to become capable of fertilization (capacitation). This action was correctly ascribed to mitochondria located at the base of spermatozoa.) The publication by Huckle and Tamplin confirms the findings of the cited investigators and expands the knowledge of carnitine functions on sperm ("Purification and Properties of carnitine acetyltransferases from bovine spermatozoa and heart" in Archives of Biochemistry and Biophysics vol. 226, no. 1:94, 1983).

For a corresponding situation in the female it was found by Costa and Stevenson that concentrations of carnitine in the ovary of rats and of acetylcarnitine increased 3-fold after gonadotropin stimulation, and in normally ovulating ovaries during the periods of rapid steroidogenesis in the luteal phase. ("Changes in Coenzyme A and carnitine concentration in superovulated rats". BBA 792:130, 1984). The carnitine appears to be produced in peroxisomes located in ovarian tissue.

#### Other Investigations in Animal Models:

Other publications among the submitted material describing a wide variety of investigations in animals are not reviewed in this review because they concerned mostly specific phases of carnitine functions with limited significance for the fundamental analysis of the pharmacological spectrum of carnitine, and those with clinical aspects, were greatly overshadowed by the large volume of similar investigations already performed in human. Their omission is justified, and also necessitated by existing circumstances, also for the sake of conserving space in the review document, and time of the reviewer. For eventual later utilization and review, the following publications are cited here, all by P. P. Bell, from Diabetes-Atherosclerosis Research, The Upjohn Company and his co-authors from the staffs of several Medical Universities. All these publications were submitted in the material furnished by Sigma-Tau:

Carnitine metabolism in *Macaca arctoides*: the effects of dietary change and fasting on serum triglycerides, unesterified carnitine, esterified (acyl) carnitine and beta-hydroxybutyrate, in Am. J. Clin. Nutr. 36:115-121, 1982.

The Effect of Diet on Plasma Carnitine, Triglycerides, Cholesterol and Arterial Carnitine Levels in Cynomolgus Monkeys. *Comp. Biochem. Physiol.* 75 B: 211-215, 1983.

Plasma and Liver Carnitine (Free and Esterified) Levels and their Interrelationships in Moderately Hypercholesterolemic Monkeys (*Macaca arctoides*). *Can. J. Biochem. Cell Biol.* 61:328-332, 1983.

Carnitine Esters: Novel Inhibitors of Plasma Lecithin: Cholesterol Acyltransferase in Experimental Animals But Not in Man (*Homo Sapiens*). *Int. J. Biochem.* 15:133-136, 1983.

The last cited article of Bell et al. states among other facets that carnitine esters possess surface active properties at certain concentrations and cites for this issue the publication by Cho and Proulx on "Studies on mechanisms of hemolysis by acyl carnitines, lecithins and acyl-cholines", *Biochem. Biophys. Acta* 225:214, 1971.

#### CONCLUSION FOR THE PHARMACOLOGY PART

The material for the description of the pharmacology properties of carnitine differs from that which usually is submitted for a new drug substance when original investigations conducted with that drug are presented. This approach was not requested for carnitine in view of the long history of its existence both, as a nutrient, and for clinical utilization for correction of deficiency induced myopathies by other pathological conditions. It is more important that the submitted literature is of recent vintage and deals in depth with the complex mechanism of the function of the drug as a carrier, in collaboration from the now known enzyme systems, of fatty acids, mostly of the long-chain type, into the site of their oxidation, the mitochondria. This phenomenon is now firmly and unequivocally established, even though admittedly there probably will be new discoveries forthcoming. Among these one can expect additional knowledge on the roles of the peroxisomes, and very likely also of the endoplasmic reticulum.

But for the consideration of the safety of carnitine in its planned clinical use, and for the fulfilment of the requirements for adequate pharmacological data, it appears reasonable and justified to consider the furnished and reviewed information as satisfactory, from the standpoint of Pharmacology, to serve this purpose.

#### TOXICOLOGY:

The supplement to the NDA of November 9, 1984, vol. 6.1 contains the reports entitled:

"L-Carnitine 52 Weeks Oral Toxicity Study in Sprague-Dawley Rats" and

"L-Carnitine Oral Toxicity Study in Beagle Dogs - Repeated Daily Dosage for 52 Weeks."

These are the two toxicity studies that were requested by us for the support of the NDA.

52-Weeks Oral Toxicity Study in Sprague-Dawley Rats:

The report for this study is in the amendment Vol. 6.1 in the submission of November 9, 1984 of NDA 18-948.

This investigation was conducted by

Manager

institute is authorized by the

toxicological studies on pharmaceutical specialties and that the reported study was conducted on behalf of Sigma Tau, Pomezia, Rome, Italy.

In the foreword to the report, the General makes the statement that the

to conduct

A declaration by the management is submitted attesting that the study was conducted in compliance with the GLP Regulations, together with a Quality Assurance Statement with a schedule of performed inspections, and a list of Scientists involved in this study.

Study Plan and Methodology:

Test Animals: Charles River CD (SD) BR rats

30 males and 30 females per dose group weighing at start of treatment, males 230-233 gms., females 163-165 gms.

Dosage: administered in the diet daily for 52 consecutive weeks.

Group 1 control

Group 2 150 mg/kg/day

Group 3 450 mg/kg/day

Group 4 1350 mg/kg/day

The concentrations of the drug in the diet were established each week from the weekly body weights, and the drug intake was calculated from the daily intake of the diet established by the consumed portion of the daily offered feed dose.

An interim sacrifice of 5 animals/sex/group was performed at completion of week 13 of treatment, with a complete work-up.

At the end of week 52, 5 animals/sex/group were selected to continue without treatment for 4 weeks to study after-effects, or recovery from induced effects respectively, with sacrifice of these animals at week 57. The remaining 20 animals/sex/group were sacrificed after completion of 52 weeks of treatment.

Results:

Clinical Observations:

No mortality was reported.

Fecal changes: No changes in fecal consistency were reported (dissimilar to the liquid feces noted in the study with dogs)

Behavior: No variations in treated animals from that of controls. The only anomaly noted was the occurrence of an abnormal, excessive growth of incisor teeth more frequent in males than in females, and in males its occurrence appeared to be dose related: 2 in controls, 3 in group 2, 2 in group 3, 4 in group 4. In females the incidence was one in controls and group 2, 2 in group 3 but none in group 4.

Body Weight Gains:

The enclosed graphs and tabulations for this parameter yield an interesting picture for the action of the drug. The tables are a condensation made by me of the data obtained weekly by presenting, for brevity sake, only the weights obtained at critical points of the study, namely before initiation of treatment, then at week 13 (at the 3-months interim sacrifice) followed by week 26, and 52, at the terminal sacrifice, and weeks 54 and 57 for the 5 males and 5 females from each dose group on the recovery phase.

It is evident that the low dose had a greater growth stimulating effect in both sexes compared to controls, most distinct in females and less effective in males where it became stimulating over controls only in the last 8 weeks of treatment while in females it had induced greater gains than that of controls almost from the start, and continuing to the end of treatment. The mid-dose had in males the greatest stimulatory action, but in females it was less than that of the low dose.

In contrast to this action by the low and mid-dose was the response to the high dose in both sexes where it reduced gains of body weights affecting to a greater extent the females than the males.

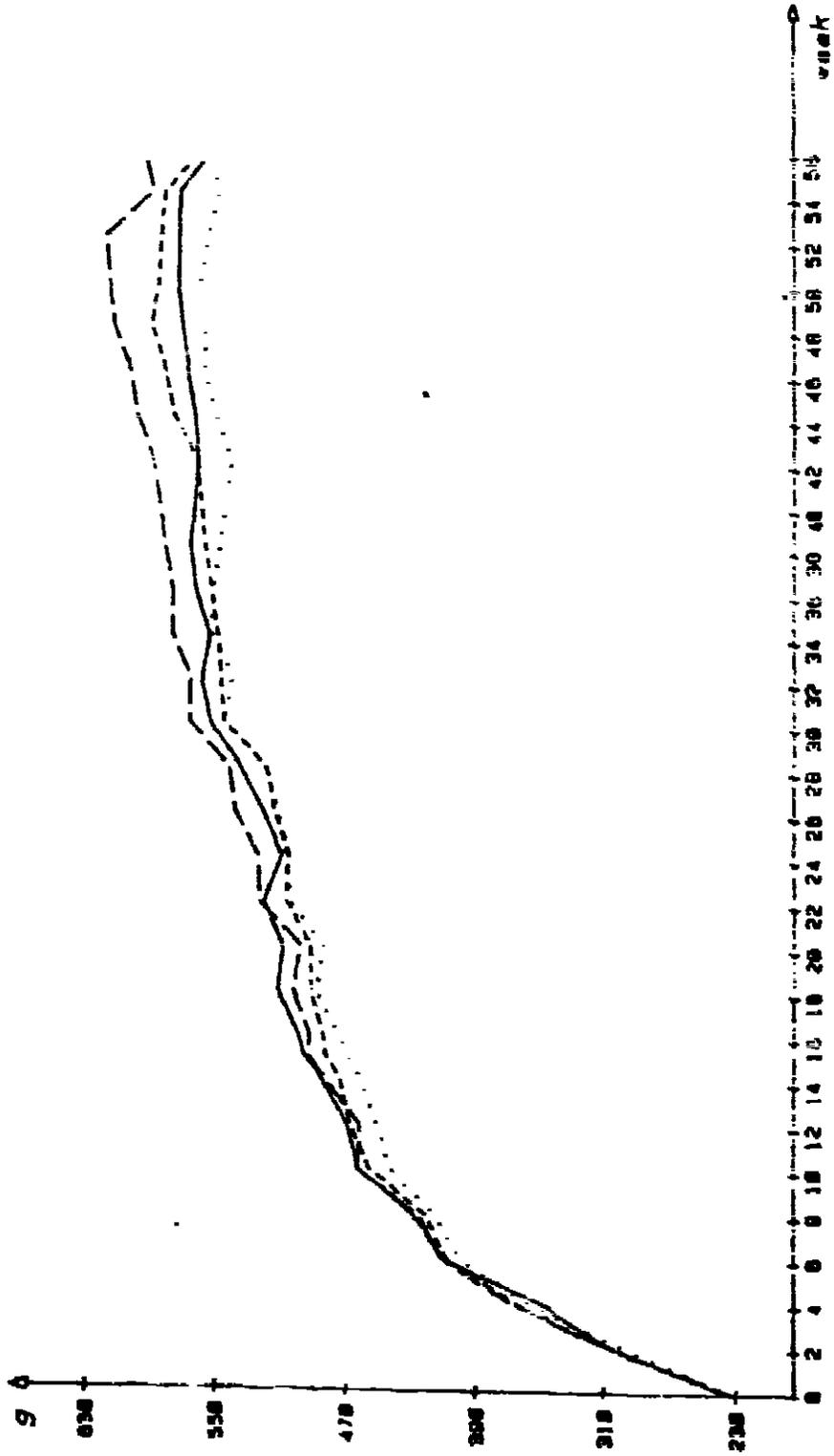
The beneficial action of the drug on weight gains was clearly demonstrated by the response to the withdrawal of the drug after week 52 when the by-then achieved body weights of the low and mid-dose groups of both sexes were not maintained but were actually reduced.

In the high-dose males a tendency for body weight loss was noticeable after week 50 and this tendency continued in the no-treatment period with a slight recovery and reversal setting in later in the no-treatment period (week 54). In the high-dose females a definite resumption of body weight gains set in immediately after removal of the drug from the diet.

# Body Weights of Male Rats

Fig. No. 1  
 Exp. 001032  
 Body weight

Group	Dose (Rate)
Grp 1	C
Grp 2	150 mg/kg/d
Grp 3	400 mg/kg/d
Grp 4	1300 mg/kg/d



000039

# Body weights of Male Rats

Table no. 1(p1)  
Exp. no. 081631  
Body weights (g) : Mean  
25 E.  
(M)

Mean (gms)

Week day	GR 1	GR 2	GR 3	GR 4
-2 -10	159.03 1.22 (30)	161.04 1.29 (30)	168.17 1.34 (30)	168.00 1.44 (30)
0	233.87 1.04 (30)	233.53 2.23 (30)	236.04 2.54 (30)	231.33 2.56 (30)
13 91	475.97 1.10 (30)	471.07 7.15 (30)	471.43 5.94 (30)	455.80 6.18 (30)
26 112	517.84 6.88 (25)	511.92 8.30 (25)	535.64 8.01 (25)	589.48 6.30 (25)
52 314	566.41 7.24 (25)	578.84 8.56 (25)	612.20 11.31 (25)	549.48 5.13 (25)
54 170	506.50 16.20 (5)	575.20 7.10 (5)	501.80 16.74 (5)	542.00 18.40 (5)
57 173	552.40 17.91 (5)	559.60 0.65 (5)	517.00 19.00 (5)	546.80 7.51 (5)

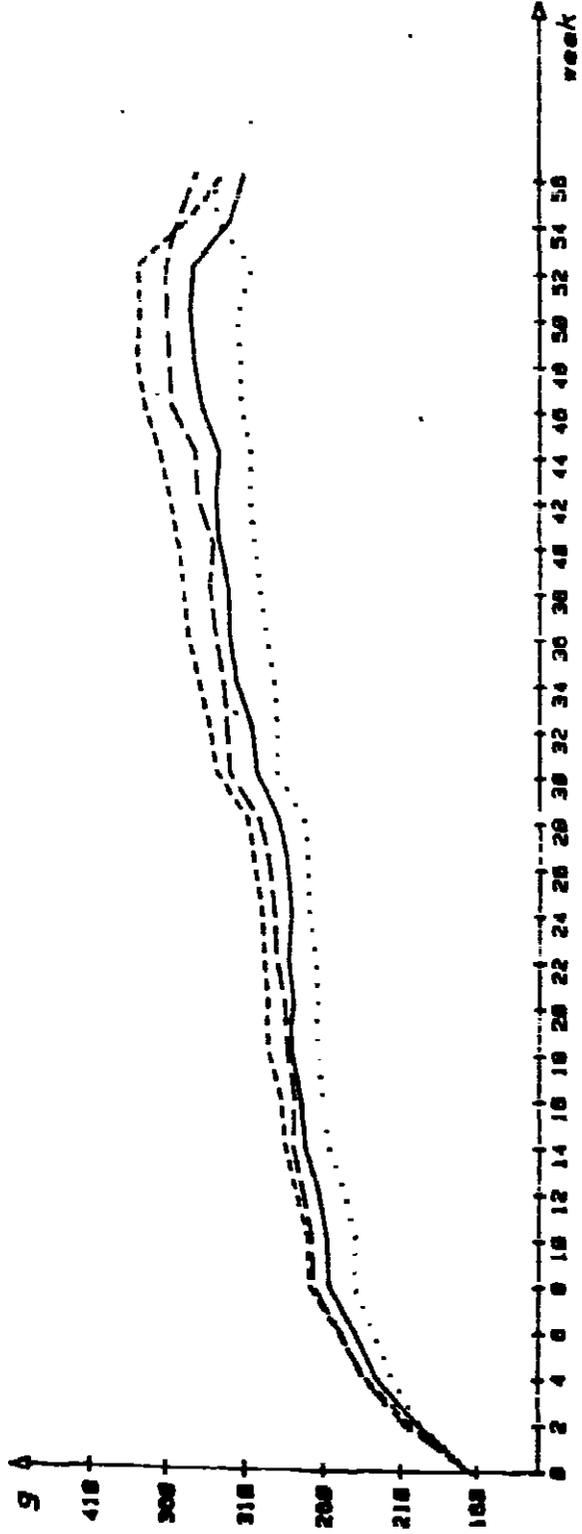
Continued

# Body Weights of Female Rats

Fig. n. 2  
Exp. 81832

Body weight	Females (Rate)
Grp 1	c. 1/3 1/4 1/2
Grp 2	1/50
Grp 3	1/50
Grp 4	1/3 1/4 1/2

(1111) 211



# Body Weights of Female Rats.

Table no. 2 (p.1)

Exp. no. B1632

Body weight (g) | Mean  
S.E.  
(N)

Female (Rats)

Week	day	GR 1	GR 2	GR 3	GR 4
-2	-9	133.43 1.25 (30)	132.00 1.25 (30)	132.73 1.25 (30)	132.87 1.17 (30)
0	0	163.10 2.61 (30)	165.63 2.01 (30)	163.97 2.13 (30)	163.23 1.59 (30)
13	91	258.97 6.73 (30)	272.90 5.91 (30)	268.17 5.34 (30)	243.88 4.46 (30)
26	182	303.32 6.09 (25)	303.96 6.77 (25)	295.16 5.86 (25)	249.92 5.24 (25)
52	364	343.00 9.52 (25)	370.12 10.12 (25)	360.04 8.21 (25)	305.64 7.20 (25)
54	370	319.00 23.03 (5)	345.20 25.46 (5)	351.60 11.34 (5)	327.60 10.20 (5)
56	392	310.20 25.40 (5)	323.40 24.40 (5)	340.80 11.30 (5)	320.60 19.31 (5)

This apparently favorable picture for the drug's action on body weight gain is somewhat obscured by the observation that the control animals, especially the females, also showed a weight loss in the recovery period.

This problem can be eliminated by comparing the body weights of the treated animals achieved by the end of the treatment period (week 52) to the weights after the 4 weeks "recovery" period:

Males

Week	Controls	Low	Mid	High
52	566	578	612	549
57	552	559	587	546

Females

52	343	378	360	305
56	310	323	349	328

A decline in feed intake was noted in controls, low and mid dose animals during the 4 weeks recovery period, but in the high dose animals the feed intake was slightly elevated.

Hematology

The values obtained for this parameter correspond rather well to the picture obtained from the body weight gain performance. The hematology values in males and females in the low and mid dose were unaffected throughout the entire treatment period and no differences resulted in the period without drug. Only in the high dose males appeared slight variations from mean normal values for erythrocytes, hemoglobin and hematocrit values, and by week 57 all these values were again at a normal range.

Blood Chemistry Tests:

Conducted for 18 parameters, their results can be interpreted to depict effects from the pharmacological properties of the drug and not indicative for any direct toxic action.

In female rats, the only deviations of significance from normal and control values were the changes in the mean triglyceride values (shown as mg/100 ml)

Week	Control	Low	Mid	High
26	77.25	87.25	94.5	73.5
52	103.0	119.1	117.1	87.12
57	69.4	66.4	87.2	102.6

The values for total cholesterol were considered to be not statistically significant to indicate a drug action, and are shown here only in an attempt by the reviewer to line them up with the action of the drug on triglycerides.

26	122.8	136.7	130.9	138.4
52	119.68	135.8	125.7	130.3
57	134.6	129.2	125.6	132.4

While the data for the triglycerides show a stimulatory action by the low and mid dose and the loss of this effect with withdrawal of the drug, the effect of the high dose by week 52 would indicate a suppressive action (perhaps by a negative feed back mechanism) that was removed in the no-treatment period from week 52 to week 57, but the data for total cholesterol would indicate that the drug had neither a stimulatory nor an inhibitory action on cholesterol metabolism, and the noted changes in the triglyceride levels might be results of the action of carnitine on the metabolism and transportation of fatty acids. It has to be remembered that the high triglyceride values occurred at the time when the body weight gains of the low-and-dose groups were high but that of high dose group low.

For a comparison, the mean triglyceride levels in males are shown in the next tabulation followed by a tabulation of their cholesterol values.

Triglyceride values in males:

<u>Week</u>	<u>Control</u>	<u>Low</u>	<u>Mid</u>	<u>High Doses</u>
26	94.5	101.8	110.24	72.7
52	130.0	164.4	180.4	121.2
57	103.6	118.6	143.4	88.7

The Cholesterol values in males:

<u>Week</u>	<u>Control</u>	<u>Low</u>	<u>Mid</u>
26	82.3	80.8	96.0 +
52	90.8	95.2	110.1
57	92.6	105.2	100.7

For the triglyceride values, the picture is essentially the same as in females; elevated in the low-and-mid dose group, reduced in the high dose group by week 52, with a drop in these values after drug withdrawal, with the difference that the high dose group showed a drop during the recovery period.

The data for the cholesterol levels show a difference from that seen in females in that they show an elevation during treatment from all doses, with, no significant change in the recovery period. With respect to the bodyweight gains in relation to these two parameters, the mid-dose had caused the highest gains during treatment, lesser gains in the low-dose group, and reduction by the high-dose, followed by a drop-off in gains in the low and mid dose after drug withdrawal, but slight improvement in the high-dose group.

With respect to other parameters of blood chemistry, the males had a higher incidence order than females in several that however by their appearance during the treatment and disappearance in the recovery period did not give any indication for a direct toxic potential of the drug.

Among these, the increase of total bilirubin levels in all treatment groups was interpreted by the investigators to be caused by sporadic elevations in single animals but remaining within the normal (physiological) range. The SGOT levels decreased in a dose related order from 73.12 units in the low dose, to 70.4 in the mid-, and 59.4 units in the high-dose by week 52, hardly indicative for any toxic involvements, and SGPT values remained unaltered.

With these low orders of occurrence of deviations from normal and control levels, the events in the recovery period by necessity were unremarkable.

Urinalysis: Unremarkable.

Ophthalmology: Unremarkable

Organ Weights and their Histopathology at weeks 13 and 52:

Organs recovered at the interim sacrifice at week 13 of 5 animals/sex/group had weights in the normal range and were similar to that of controls, by absolute and relative values. Their histological structures did not reveal any derangements indicative for drug actions on them.

Similarly, the organ weights from the animals sacrificed at the end of treatment (week 52) did not reflect a distinct drug action because of the irregular occurrence of the noted differences in weights that also were of minor extent:

In Males:

The livers of animals from the low- and mid-dose groups were slightly heavier by their mean absolute weight than those of controls, and also those from the high dose animals, but their relative values were of the same (or similar) order for all 4 groups.

The kidneys of only the high dose group had a slight but statistically significant elevation of the mean relative weight value compared with the control values but not by their mean absolute weights.

The spleen weights were elevated by absolute mean values in the low and mid dose group, of not statistical significance and not shown by their relative values.

In Females:

A similar picture for organ weight changes (or their absence) prevails also for the females.

For the liver, a statistically significant elevation of its absolute weight was reported for the mid dose confirmed by the mean relative value. For the low dose, elevation of the absolute weight was similar to that of the mid-dose group but it was not considered statistically significant by the statistician. It is interesting that the absolute mean weight of the high dose group was actually lower than that of controls.

The kidneys showed a statistically significant elevated mean weight in the low dose group only; that of the high dose group was lower than that of the low and mid dose group by the absolute values and, statistically, significantly higher by its relative weigh value.

The spleen was slightly (but statistically significant) elevated in the mid dose by absolute and relative weight values.

Results of the recovery period to week 57:

The presentation by the sponsor of the organ weights of the animals that were continued in the recovery period did not show values with statistical significance when compared with the control values (by the Dunnett's test) but this mode of presentation does not show whether there was a change in organ weights resulting from drug withdrawal that would reveal a drug action effect. Therefore the following tabulation was prepared by the reviewer to provide a clearer picture for this action, (or its absence.)

In this tabulation, the body weights were included in order to bring out again the fact that the increased body weight gains of the low and mid-dose groups of both sexes appeared to indicate a favorable drug action, and to put this phenomenon into a perspective relationship to organ weights as an indicator for a drug action or its absence. Since the control groups had shown the somewhat enigmatic drop in body weights during the recovery period, the organ weights from the control groups can be disregarded, and make the comparison only between the values of week 52 and 57.

It can be seen that for the low and mid dose groups there was an opposite trend for the body weights and the liver weights, in that the elevated liver weights were time-wise related to the higher body weight gains of these groups while the reduced liver weights in the recovery period were associated with the reduced body weight gains (in the absence of the drug). Inversely, the low weight gain performance of the high-dose males occurred when their liver weights were lower than those of the low and mid-dose groups. In the females, an improvement of the body weight gains appeared in the high-dose during the recovery period, but the liver weights were unaltered.

From these events it would seem that the liver weights were not directly and adversely affected by the drug, and that whatever changes there were, could be connected with the pharmacological functions of the drug that for instance caused the elevation of plasma triglycerides.

The data for the spleen and kidney values do not indicate any drug effect on these organs judging by the absence of persistent differences between the week 52 and week 57 levels. The rare incidences of the slight differences resulting from statistical significance analyses are considered to be incidental, as for instance the elevated mean value of the spleen of the mid-dose females that was attributed by the investigators to one single case of a very enlarged organ described as having extended areas of sclerosis of incidental origine found in female 152 which also had a mammary gland adenocarcinoma.

Organ Weights at Week 52 and 57 (gms) of Male and Female Rats

I. Males

<u>Week</u>	<u>Organ</u>	<u>Control</u>	<u>Low</u>	<u>Mid</u>	<u>High</u>
52	<u>Bodyweight</u>	541	555	584	518
57		534	535	549	516
52	<u>Liver abs.</u>	11.08	13.23	13.24	11.85
	rel.%	2.20	2.38	2.27	2.29
57	<u>Liver abs.</u>	11.49	11.39	12.39	11.73
	rel.%	2.15	2.13	2.25	2.28
52	<u>Kidney abs.</u>	3.20	3.38	3.38	3.32
	rel.%	.59	.61	.58	.64 <sup>XX</sup>
57	<u>Kidney abs.</u>	3.05	3.26	3.29	3.32
	rel.%	.57	.61	.60	.64
52	<u>Spleen abs.</u>	.74	.84	.86	.78
	rel.%	.14	.15	.15	.15
57	<u>Spleen abs.</u>	.70	.78	.77	.16
	rel.%	.13	.15	.14	.23

II. Females

<u>Week</u>	<u>Organ</u>	<u>Control</u>	<u>Low</u>	<u>Mid</u>	<u>High</u>
52	Bodyweight	323	367	343	291
57	Bodyweight	298	308	324	314
52	Liver abs.	8.31	9.15	9.71 <sup>x</sup>	8.14
	rel. %	2.38	2.50	2.85 <sup>x</sup>	2.79
57	Liver abs.	8.13	6.98 <sup>x</sup>	7.60	8.35
	rel. %	2.75	2.29	2.34	2.66
52	Kidney abs.	2.12	2.40 <sup>x</sup>	2.28	2.24
	rel. %	.66	.66	.67	.77 <sup>xx</sup>
57	Kidney abs.	2.07	1.92	2.12	2.13
	rel. %	.78	.64	.65	.68
52	Spleen abs.	.46	.48	.69 <sup>x</sup>	.49
	rel. %	.14	.13	.21	.17
57	Spleen abs.	.45	.49	.48	.55
	rel. %	.15	.16	.15	.17



Study Set-Up and Methodology:

Thirty-two purebred beagle dogs, (16 males, 16 females) supplied by  
were acclimated for 6 weeks, vaccinated,  
and treated with anthelmintic piperazine applied at 3 months intervals also  
during the test.

Source of Drug: Sigma-Tau, Rome.

Dosage: 4 males and 4 females in each dose group of control, 300, 600 and  
1200 mg/.g/day administered in gelatine capsules.

Tests conducted: Blood and urine samples taken during weeks 6, 12, 26 and 52,  
24 hours after drug administration.

Body Weights: weekly

Food Consumption: Recorded daily.

Ophthalmoscopy: Performed by means of a Keeler indirect ophthalmoscope before  
treatment and during week 6, 12, 25 and 51.

The test procedures applied in this study were described in detail. Interim  
reports covering clinical observations, results of hematology, blood chemistry  
and urinalysis were submitted for the test periods at 13 and 26 weeks, with  
the final report covering the final data for these parameters and the results  
of the terminal autopsies.

Results:

Mortality:

No cases of drug induced mortality.

One control female died during week 3 of dosing from acute diarrhea and severe  
dehydration. The complete autopsy work-up indicated histological changes  
from dehydration resulting from diarrhea and the congestion of the mucosal  
surface of the gastro-intestinal tract, but the primary cause for these  
lesions was not established. This animal was replaced by a new entry.

A high-dose male was found in week 15 to have a severe strangulated,  
inoperable hernia, and he was euthanatized. The autopsy disclosed a 14 cm  
long strangulation of the small intestine. The Microscopy report of the  
jejunum said: "Marked congestion of the mucosa and lamina propria", but the  
exact site of this finding was not identified, probably it was in the  
strangulated section. Other findings in this dog were a focus of hepatocyte  
necrosis, and in the kidney a focus of basophilic tubules. The noted changes  
in the right testis are probably associated with the strangulating hernia.

Clinical Signs:

Incidence of Liquid Feces: This symptom, first reported at the 4-weeks interim, continued to be present throughout the test, and increased in incidence with continuation of treatment, shown in the tabulation from the 26 weeks and 52-weeks reports. It is expressed as percentages of the number of instances maximally possible:

Dosage	Percentage Incidence	
	26 weeks	52 weeks
Control	0.22	1.1
300	12.7	15.6
600	35.8	44.1
1200	68.5	57.4

The comment for this parameter by the investigators was that the occurrence of liquid feces had no effects on the health conditions of the animals during the entire dosing period. This comment is important for the evaluation of a possible adverse drug action that would compromise the utility of the drug. It has to be added that the first Pharmacology Review for this drug on the occasions of the 4-weeks and 13-weeks interim reports did stress the high occurrence of liquid feces in all treated groups and expressed the view that this response to the drug may indicate an irritating action on the gastrointestinal lining and its consequences. The reviewing pharmacologist at that stage did not have the benefit of results from interim sacrifices at that period and therefore the presumptive conclusion was justified. However, as will be shown later, the results of the terminal sacrifice demonstrate that the suspected irritation of the gastrointestinal mucosa was not confirmed by the histopathological findings. It will be discussed later in this review that the liquid state of the feces may have resulted from the action of the drug on the lipid metabolism and might be specific for this species, because no such effect was present in rats treated with similar dosages of the drug.

Body Weight Gains:

The enclosed Table 9 taken from the report, with the data of individual weights and group means for dose groups, and weight gains, and the graphs made from these data show that in both sexes there was a flattening of the weight gain curves after the stage of rapid growth (up to approximately week 20.) Since this decrease in weight gain rates from then on is also shared by the control groups, it obviously reflects a natural event, as the onset of declining growth is not dose dependent: in the high dose males it starts already by week 16, and in females of the mid-and high dose group at about the same time, and the general pattern is similar for all groups and both sexes.

(Because the weights of the males are higher than those of females, the arrangement in the table presenting a group mean for each dose group by combining the weights of males and females is, in my opinion, a faulty method, unusable for effect evaluation purposes.)

It is evident that the weight gains of the control groups (both sexes) are higher than those of the treated groups. The males of the mid dose group come closest to the control group, while in females it is the low dose group; the mid dose females made lower gains than the high dose group while the high dose males made the smallest gains.

This variation for the sexes could be ascribed to the wide variation in gains made by individual dogs, that is distinctly shown in the graphs for individual dogs:

Dog 663, Female, low dose, was the only animal with a weight loss at term (shown in the table). It was explained by the investigators that there were no indications for sickness or excessive liquid feces, but that this dog was the oldest of the entire population and was also the heaviest of all. A drug action did not seem to be the cause for this variation. The others in this group showed a rather similar growth performance.

A similar situation existed in the high dose group of males where dog 677 showed a distinctly reduced growth performance already after week 11 with continued decline of weight, followed by a slight recovery, but with a total gain much below that of the other group members. At the terminal autopsy, this dog was found to be the only animal with a severe local erosion in the stomach wall, but lesions in the intestinal tract were not found. The record for incidence of liquid feces did not indicate any aggravation in this respect.

The only other animal not making normal weight gains is dog 672, female, mid dose, that after an initial normal performance up to week 12 gradually lost weight through most of the treatment period at a steady rate, but eventually started a recovery after week 44.

The biochemical and hematological data of the poor performers did not show deviations from normal levels. It seems therefore reasonable to conclude that the individual variations in body weight gains did not result from any adverse drug actions but were resulting from individual genetic make-up for growth.

#### Hematology:

The overall picture for this parameter is that there is no indication for a drug effect. An apparent elevation in the values for hemoglobin and RBC of males and females in all dose groups and a drop in the values of total WBC with progressing treatment when compared with the values obtained before treatment, at first would appear to be a favorable drug action, but a similar "response" appears also in the control groups at each test interval.

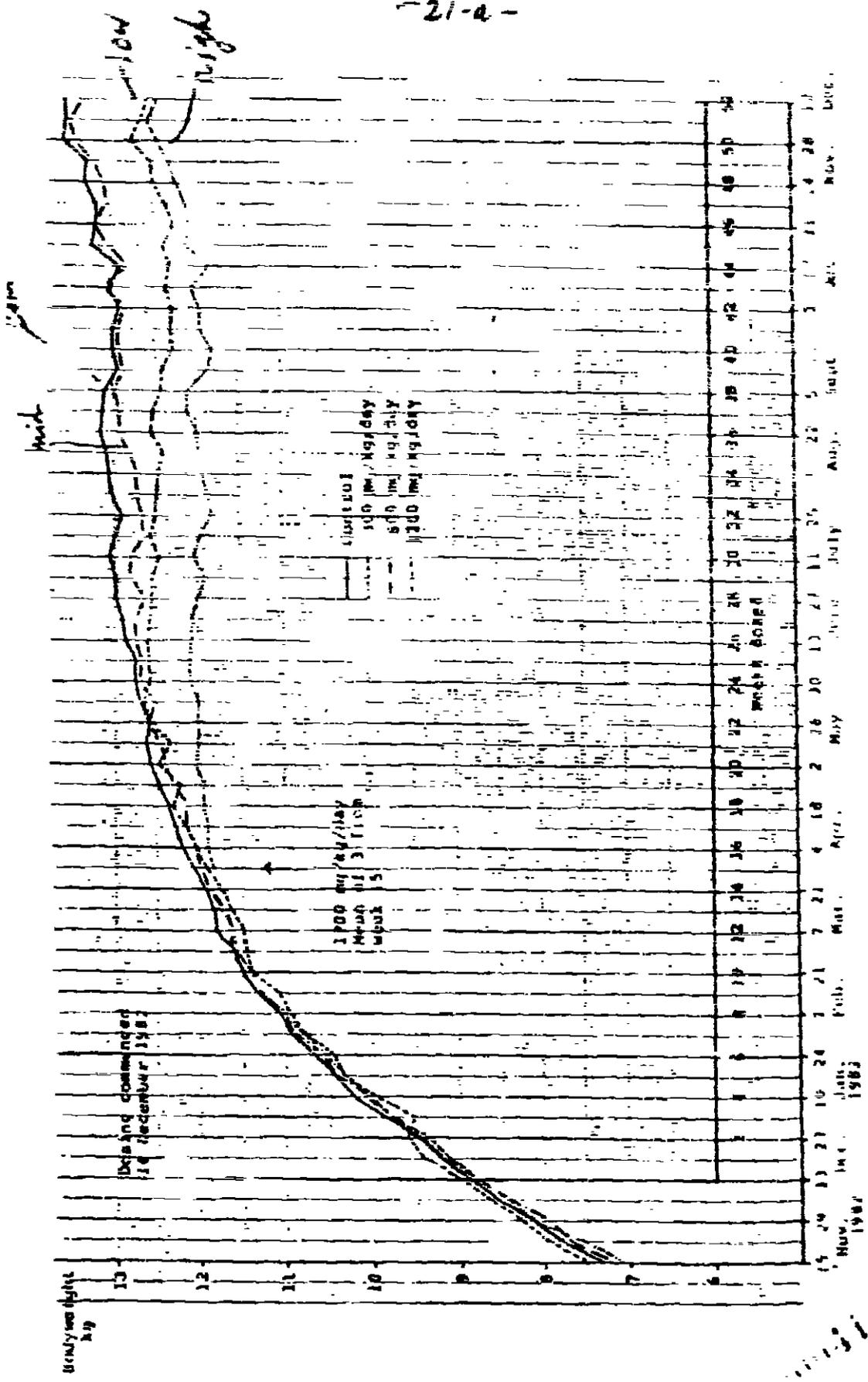


FIGURE 24

Individual bodyweights - Control - males

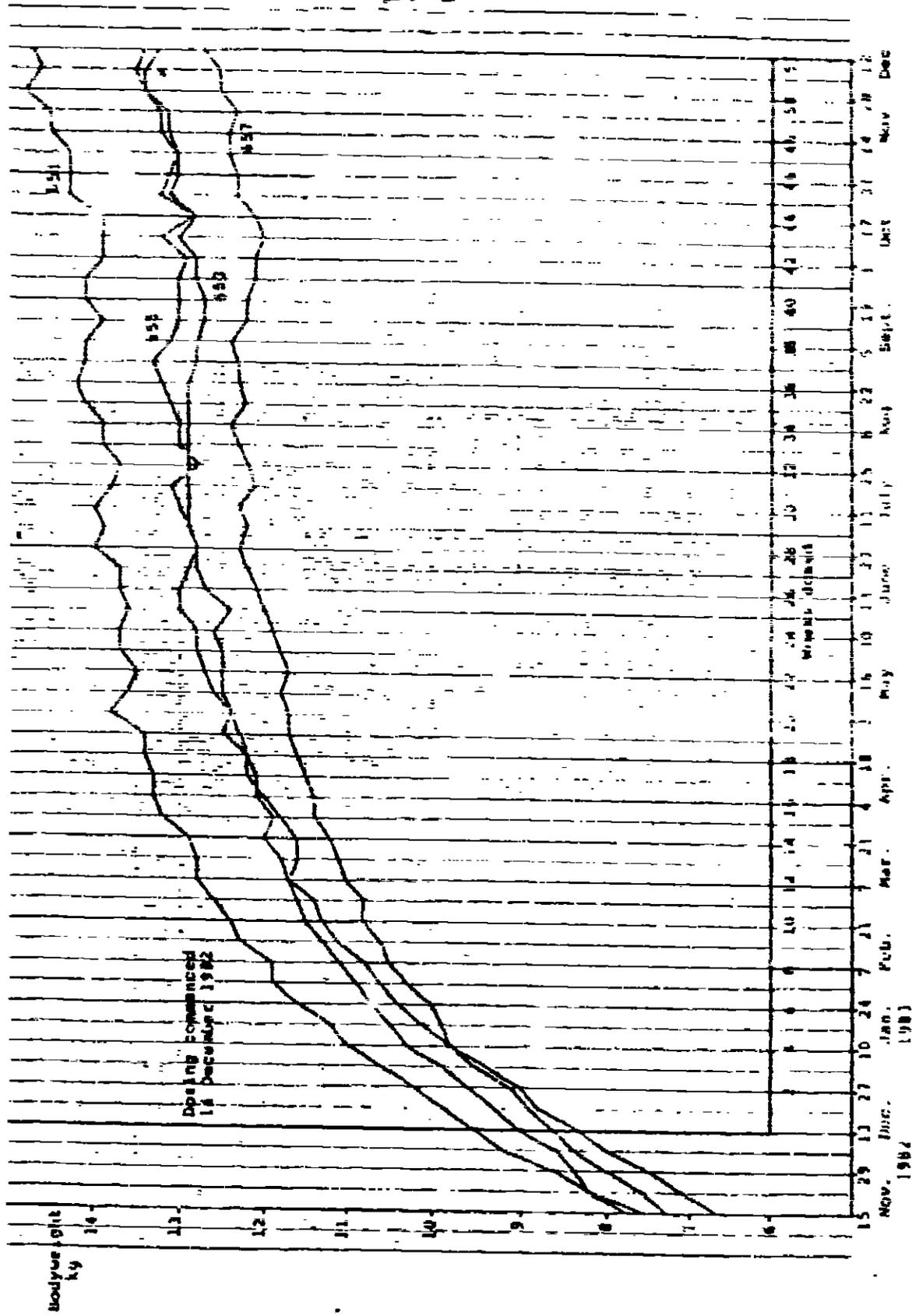


FIGURE 34  
Individual bodyweights - 100 mg/kg/day - males

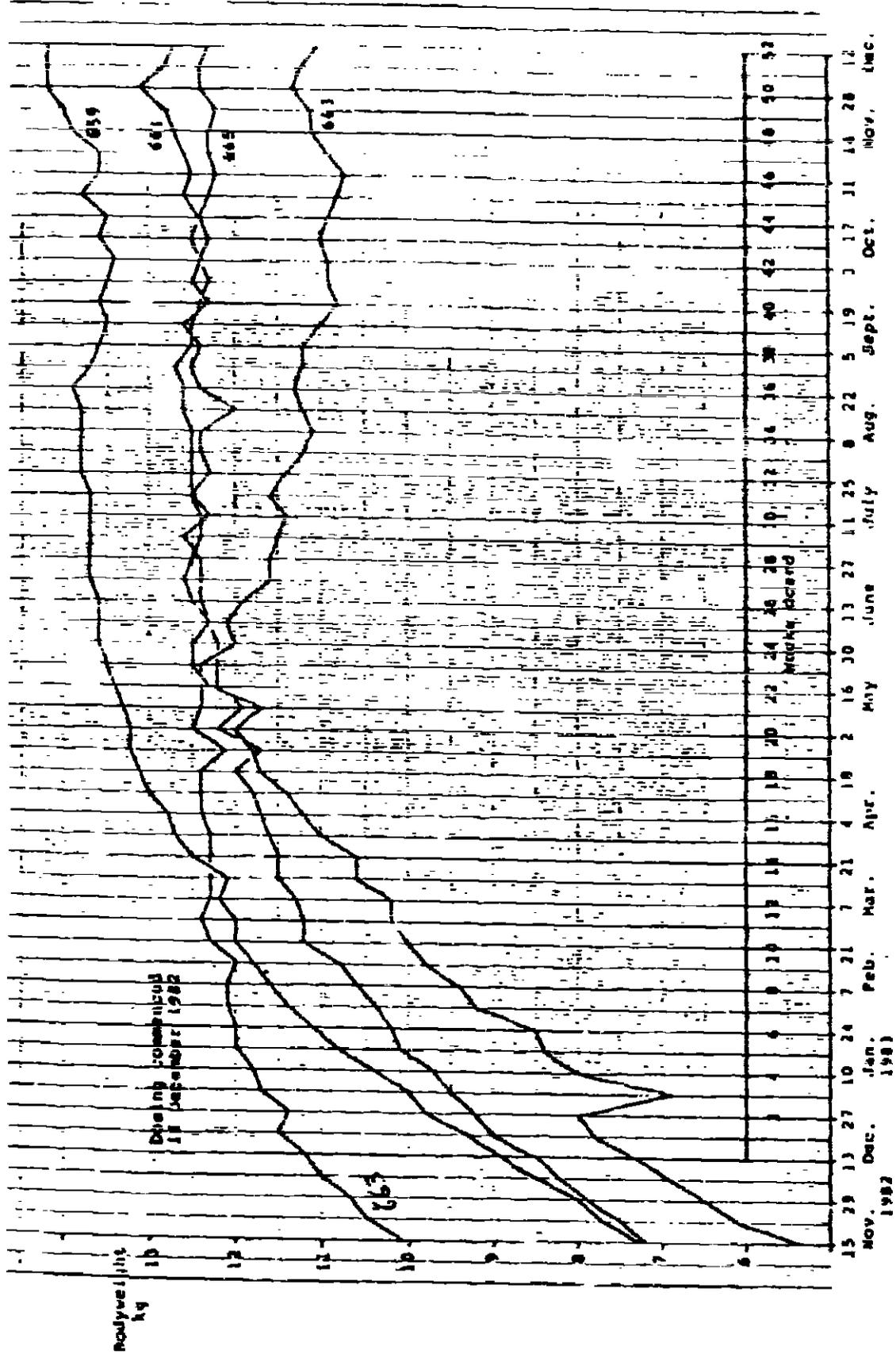


FIGURE 40  
Individual bodyweights - 600 mg/kg/day - male

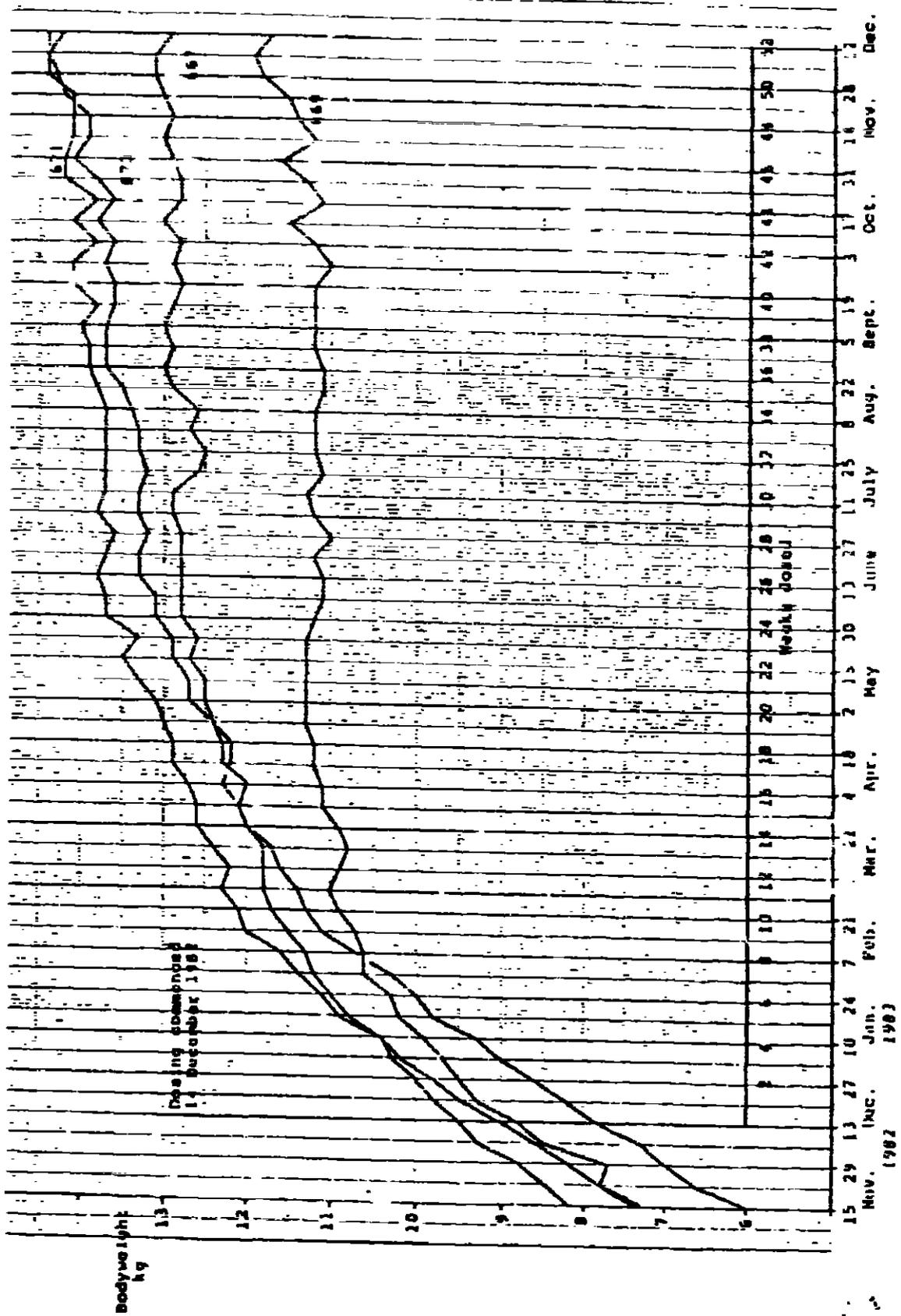
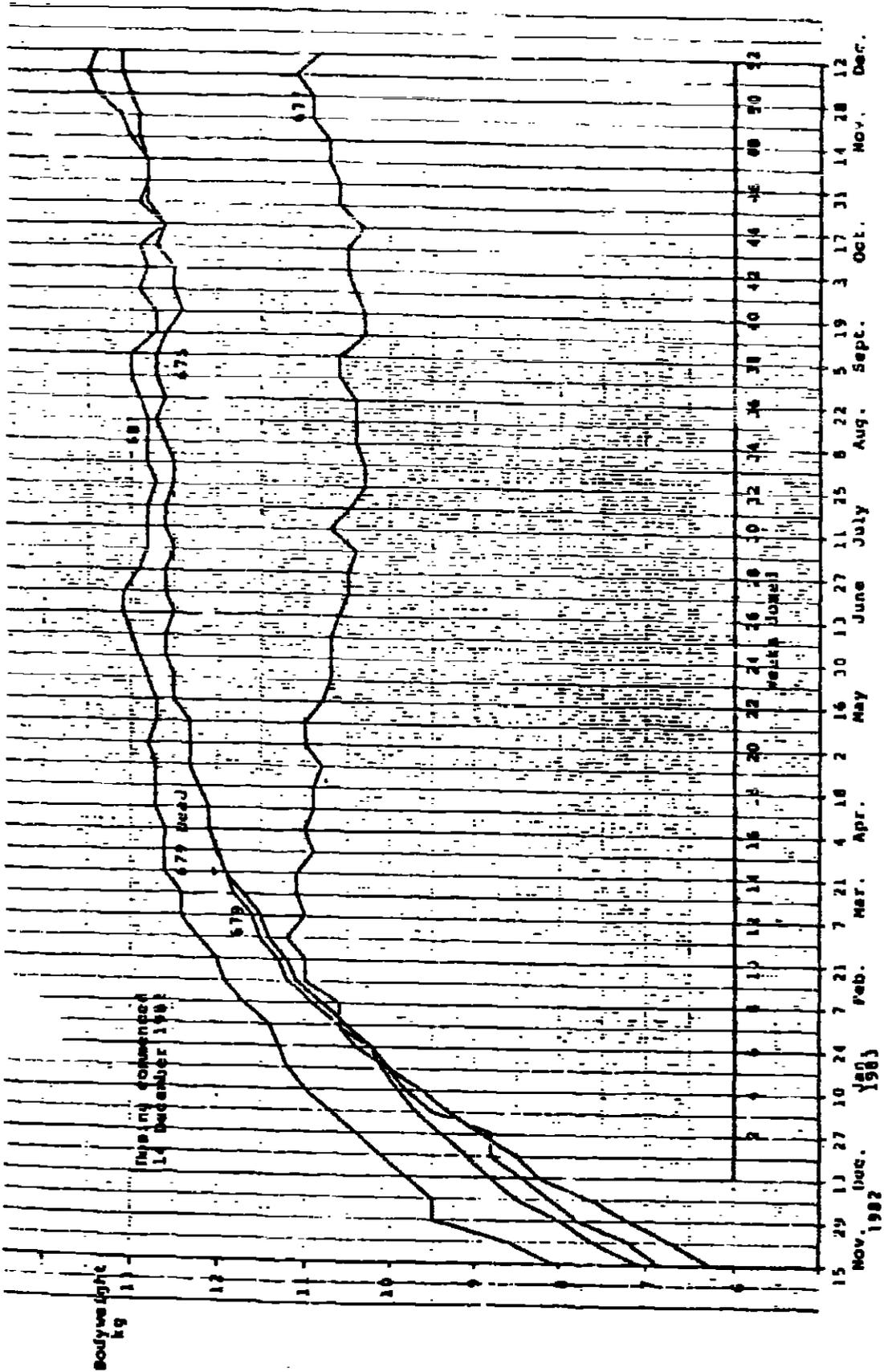


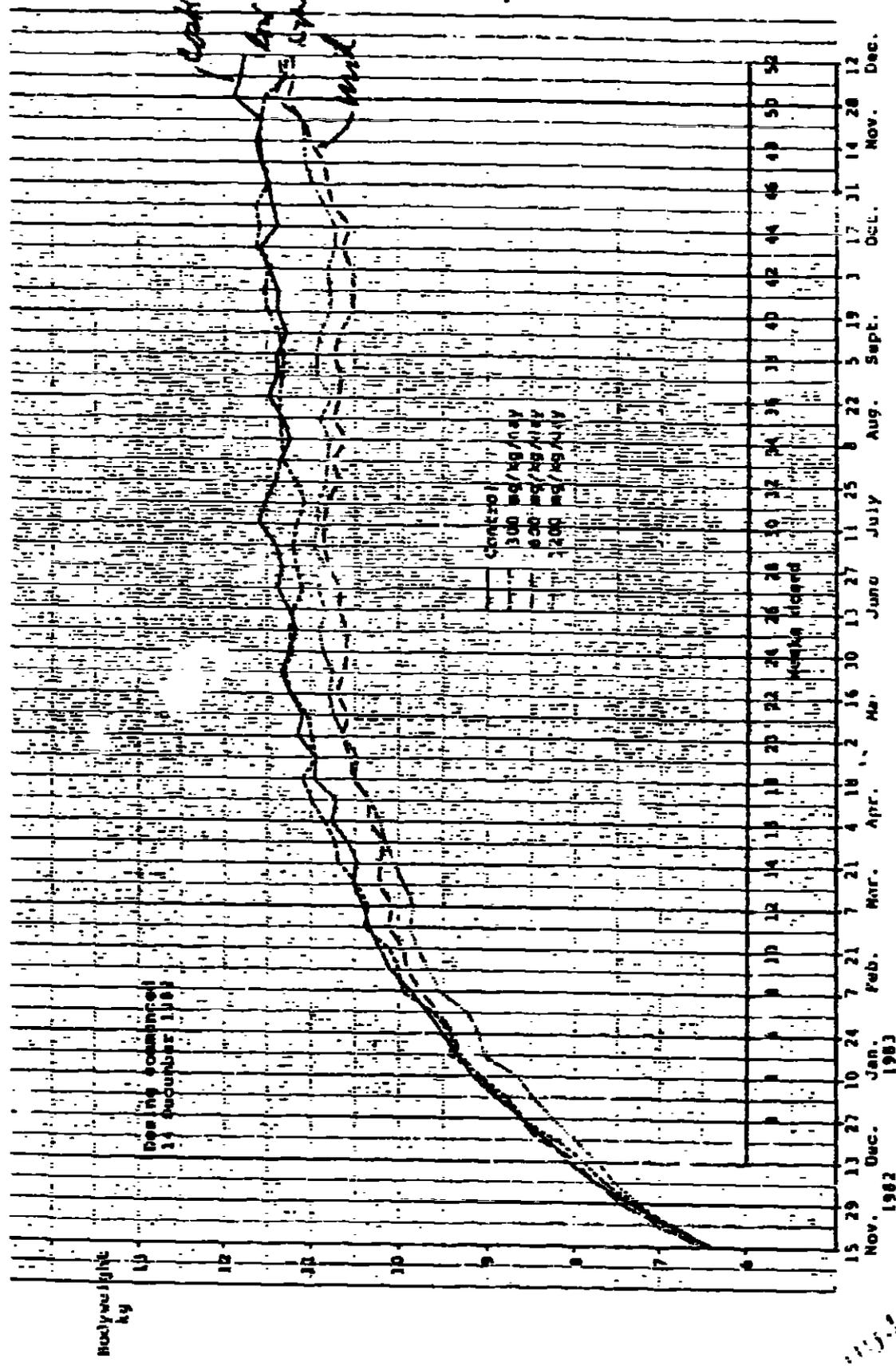
FIGURE 5a  
Individual bodyweights - 1200 mg/kg/day - males



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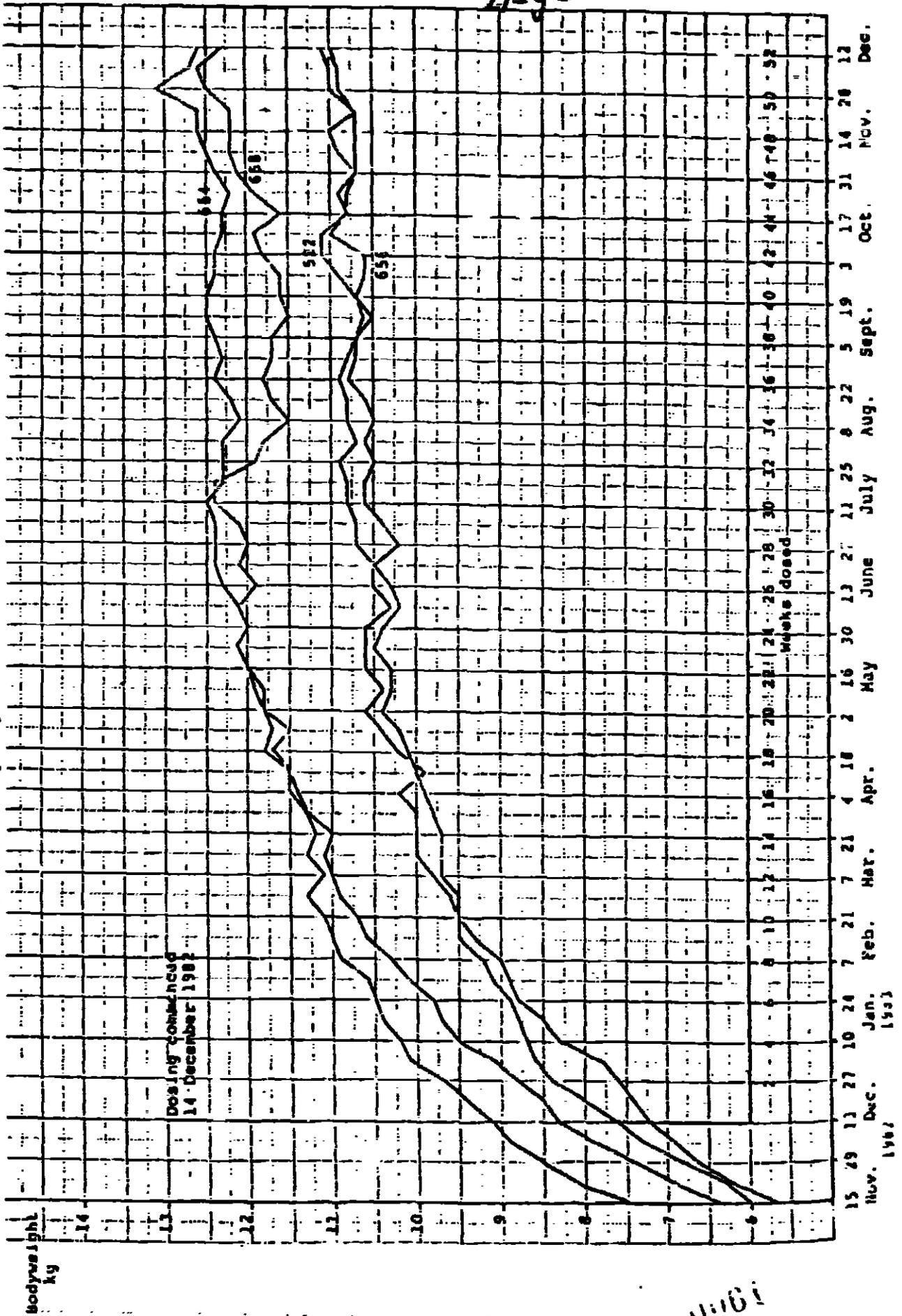
21-f

FIGURE 1b  
Group mean bodyweights - females



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FIGURE 2b  
Individual bodyweights - Control - females



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FIGURE 3b

Individual bodyweights - 300 mg/kg/day - females

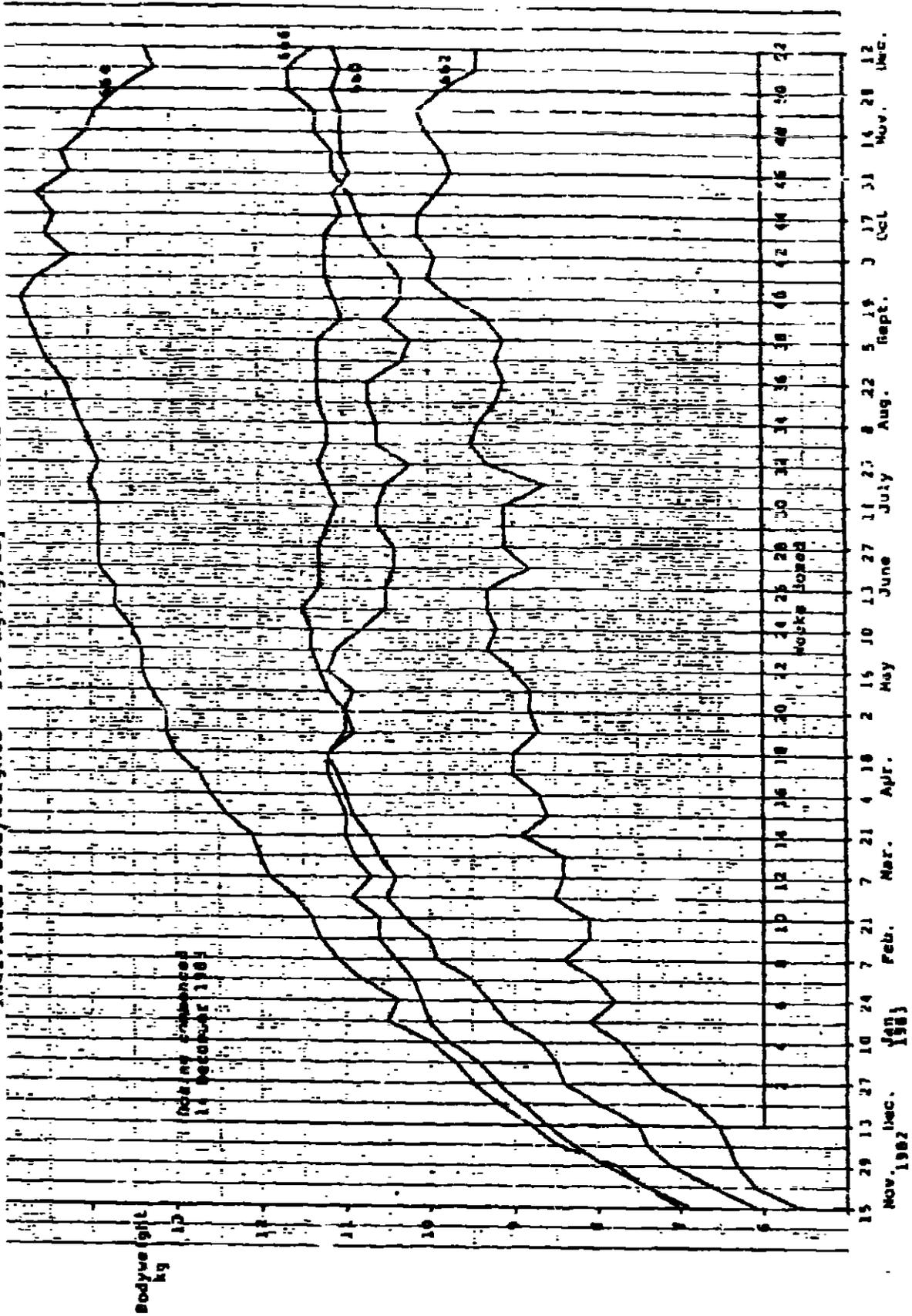


FIGURE 4b  
Individual bodyweights - 600 mg/kg/day - females

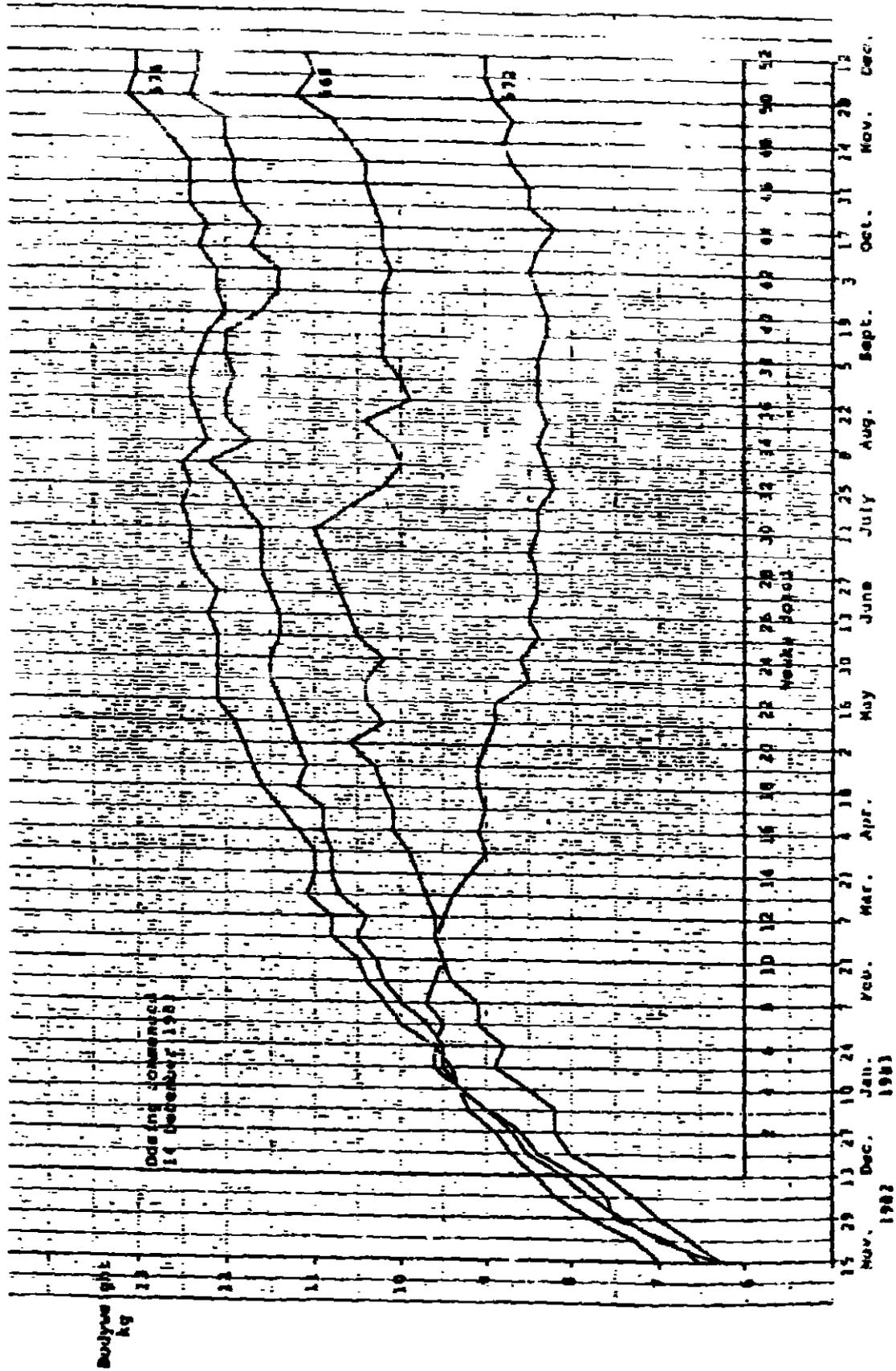
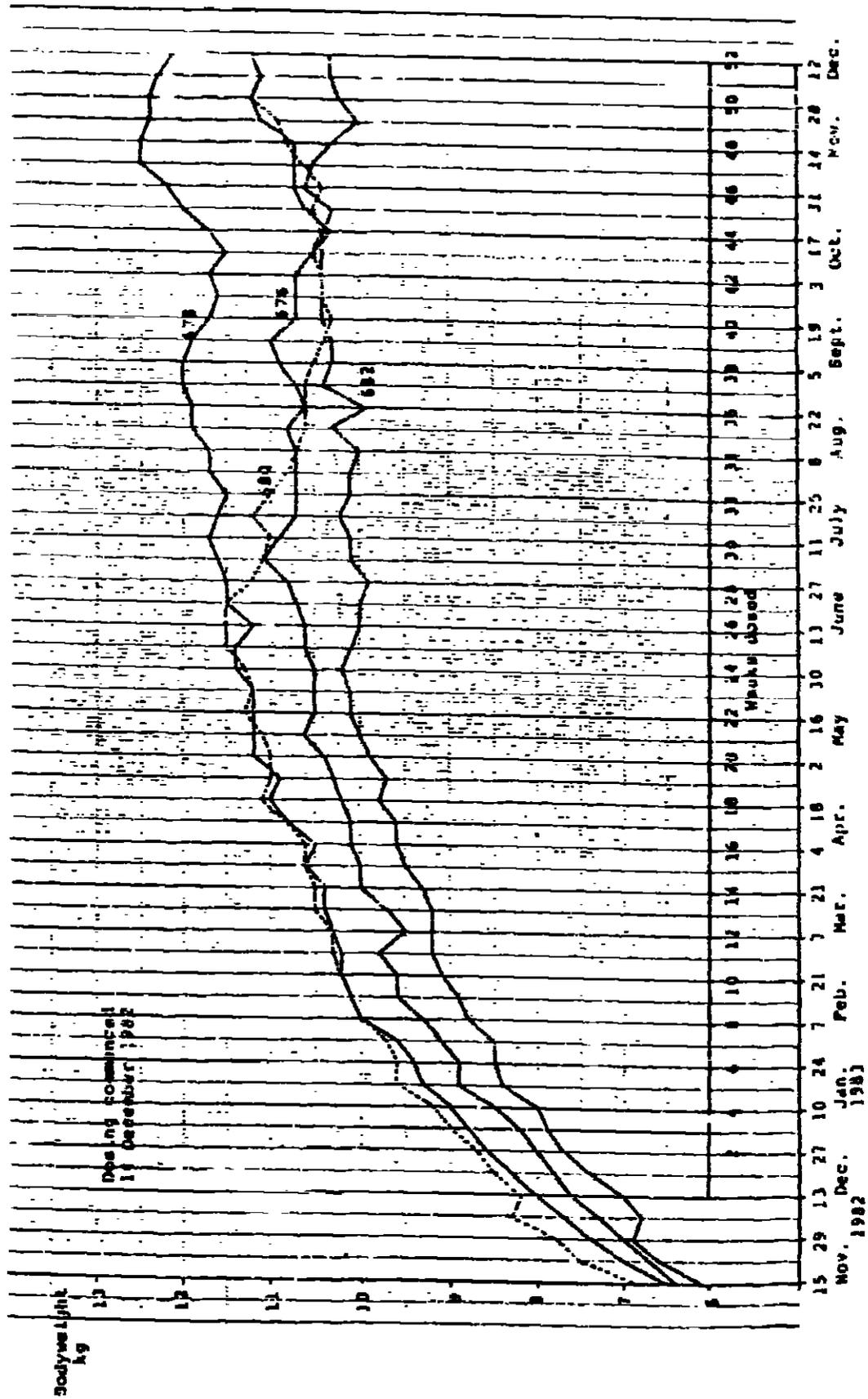


FIGURE 5b  
Individual bodyweights - 1200 mg/kg/day - females



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Initial weight (g) and weight change after 52 weeks of dosing

Dosage mg/kg/day	Dog No./ sex	Initial weight	Weight after 52 weeks	Weight change
Control	651g	9500	14700	5200
	653g	9000	13200	4200
	655g	8300	13400	5100
	657g	8600	12700	4100
	522g*	7600	11100	3500
	654g	9100	17500	3500
	656g	7200	11800	3800
	658g	8300	12300	4000
	Male mean	8850	13500	4650
	Female mean	8050	11750	3700
Group Mean	8450	12625	4175	
300	659g	8900	14200	5300
	661g	7300	12700	5400
	663g	11700	11000	-200
	665g	8500	12300	3800
	660g	8600	11100	2500
	662g	6500	9400	2900
	664g	8800	13300	4500
	666g	7500	11300	3800
	Male mean	8975	12550	3575
	Female mean	7850	11275	3425
Group mean	8413	11913	3500	
600	667g	9500	12900	3400
	669g	8800	11700	2900
	671g	9000	14200	5200
	673g	7800	14400	6600
	668g	7600	11100	3500
	670g	8100	12300	4200
	672g	8400	9000	600
	674g	7900	13000	5100
	Male mean	8775	13300	4525
	Female mean	8000	11350	3350
Group mean	8388	12325	3938	
1200	675g	8200	13400	5200
	677g	8200	10800	2000
	681g	9800	13100	3300
	676g	7600	11200	3600
	678g	8000	12100	4100
	680g	8200	11200	3000
	682g	7000	10300	3300
	Male mean	8933	12433	3500
	Female mean	7700	11200	3500
	Group mean	8229	11729	3500

\* Replacement animal - treatment commenced on Day 19 of study

Initial weight (g) and weight change after 52 weeks of dosing

Dosage mg/kg/day	Dog No./sex	Initial weight	Weight after 52 weeks	Weight change
Control	651♂	9500	14700	5200
	653♂	9000	13200	4200
	655♂	8500	13400	5100
	657♂	8600	12700	4100
	522♀*	7600	11100	3500
	654♀	9100	12600	3500
	656♀	7200	11000	3800
	658♀	6300	12300	4000
	Male mean	8850	13500	4650
	Female mean	8050	11750	3700
Group Mean	8450	12625	4175	
300	659♂	8900	14200	5300
	661♂	7300	12700	5400
	663♂	11200	11000	-200
	665♂	8500	12300	3800
	660♀	8600	11100	2500
	662♀	6500	9400	2900
	664♀	8800	13300	4500
	666♀	7500	11300	3800
	Male mean	8975	12550	3575
	Female mean	7850	11275	3425
Group mean	8413	11913	2500	
600	667♂	9500	12900	3400
	669♂	8800	11700	2900
	671♂	9000	14200	5200
	673♂	7800	14400	6600
	668♀	7600	11100	3500
	670♀	8100	12300	4200
	672♀	8400	9000	600
	674♀	7900	13000	5100
	Male mean	8775	13300	4525
	Female mean	8000	11350	3350
Group mean	8388	12325	3938	
1200	675♂	8200	10400	5200
	677♂	8800	10800	2000
	681♂	9800	13100	3300
	676♀	7600	11200	3600
	678♀	8000	12100	4100
	680♀	8700	11200	3000
	682♀	7000	10300	3300
	Male mean	8933	12433	3500
	Female mean	7700	11200	3500
	Group mean	8229	11729	3500

\* Replacement animal - treatment commenced on Day 19 of study

### Biochemistry

Also for this area, a drug effect is not evident. It has to be mentioned that Table 13a, Biochemistry - group mean values for males and Table 13b for females shows a sudden considerable change in the values for AP (alkaline phosphatase) in week 25 from the values given for pre-treatment and weeks 6 and 12. During these intervals, the AP values were fairly uniform for all dose and control groups ranging from 29 to 45 mU/ml but the data given for week 25 and week 51 as group mean values are all in the range above 100 mU/ml, namely for week 25, control group 138 mU, and 140, 146 and 184 for the low, mid and high dose respectively, and for week 51 as 113, 111, 123 and 178 mU/ml respectively. This change was probably caused by a change in the equipment used for this particular test that is stated in a footnote to the table.

The next table is part of the table in the report giving the values for biochemistry parameters intended to show the absence of an action of carnitine on the metabolism of lipids. The only possibility of an action is that on triglycerides but it is confused by the high pretreatment values.

All other biochemistry values including those for SGPT and SGOT are in the normal ranges. Glucose levels as mg/dl are lower at week 51 than they were before treatment but here again the control dogs too had a drop with progression of treatment.

### Urinalysis:

performed at weeks, 6, 12, 25 and 51: Unremarkable.

### Ophthalmoscopy:

Examinations of the eyes of all animals were performed before start of treatment and during weeks 6, 12, 25 and 51, by means of a Keeler indirect ophthalmoscope. Results were presented for the cited interims and final stage of the test. The findings were diagnosed by the investigator as being incidental and that no trends indicative for a drug effect were seen.

### Terminal Findings:

All animals were killed by exsanguination under pentobarbitone anesthesia.

Bone Marrow: Smears prepared from specimen obtained by sternal puncture from all animals were diagnosed to be normal.

### Organ Weights:

From all animals: brain, heart, lung, liver, spleen, pancreas, thymus, uterus/prostates, kidneys, thyroids, adrenals, gonads.

Female Dogs

Week number	Dose (mg/kg/day)	Cholest- erol mg/dl	HDL mg/dl	$\beta$ -Lipo- protein UD x1000	Tri- glyceride mg/dl	NEFA mg/dl
Pre-dose	Control	142	136	103	44	0.55
	300	144	131	137	53*	0.50
	600	143	131	133	55*	0.54
	1200	143	126	125	55*	0.58
Week 6	Control	132	132	87	41	0.61
	300	133	128	122	51	0.56
	600	144	134	109	52	0.62
	1200	148	115	109	51	0.49
Week 12	Control	117	122	73	51	0.62
	300	139	136	108	54	0.66
	600	148	143	144(*)	56	0.70
	1200	136	114	0	63*	0.57
Week 25	Control	127	128	81	30	0.63
	300	151	149	113	41	0.82
	600	149	144	101	38	0.76
	1200	179	172	142	36	0.55
Week 51	Control	108	108	104	38	0.55
	300	192(**)	177(**)	147	44	0.36
	600	153	132	159	5	0.61
	1200	138	125	118	37	0.50

Male Dogs

Week number	Dose (mg/kg/day)	Cholest- erol mg/dl	HDL mg/dl	$\beta$ -Lipo- protein UD x1000	Tri- glyceride mg/dl	NEFA mg/dl
Pre-dose	Control	179	164	132	42	0.39
	300	141	133	160	54*	0.51
	600	146	135	149	50**	0.44
	1200	170	150	152	50**	0.44
Week 6	Control	168	152	109	47	0.51
	300	150	136	144	55	0.63
	600	143	138	138	54	0.60
	1200	154	139	125	51	0.45
Week 12	Control	151	145	87	50	0.50
	300	132	135	142(*)	55	0.41
	600	142	133	109	52	0.46
	1200	153	142	127	60**	0.63
Week 25	Control	149	149	106	30	0.41
	300	135	143	139	34	0.60
	600	146	139	98	31	0.62
	1200	164	150	125	40	0.71
Week 51	Control	141	132	106	40	0.42
	300	145	133	157	47	0.45
	600	136	125	145	64(*)	0.45
	1200	128	125	157	53	0.40

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Organ weights - group mean values for selected parameters and results of statistical analysis

Organ	Dosage level mg/kg/day	Absolute weight (g)	Weight adjusted for final bodyweight (g)	Results of statistical analysis
Kidneys	Control	57.19	55.45	
	300	52.64	53.47	NS
	600	56.84	56.42	NS
	1200	56.80	58.31	NS
Liver	Control	383.90	371.99	
	300	338.39	344.09	NS
	600	375.33	373.08	NS
	1200	354.93	365.27	NS
Heart	Control	107.01	105.59	
	300	88.20	89.31	(*)
	600	101.36	100.81	NS
	1200	90.91	92.93	NS
Testes	Control	32.14	-	
	300	25.59	-	(*)
	600	31.89	-	NS
	1200	30.06	-	NS

NS No significance

(\*) 5% level of probability ('t' test, not confirmed by Williams' test)

No significant changes related to drug dosage were noted.

Histopathology Findings:

Tissues for Histopathological Study:

Samples (or the whole) of the following tissues from each animal were preserved together with any tissue showing macroscopic abnormality:

Adrenals	ileum	sternum
aorta (arch and abdominal)	jejunum	spleen
brain (cerebral cortex, thalamic nuclei, mid-brain, medulla and cerebellum)	kidneys	stomach (body and antrum)
colon (upper and lower)	liver	testes or ovaries
duodenum	lungs	thymus
eyes and optic nerves*	lymph nodes (cervical and mesenteric)	thyroids
gall bladder	mammary gland	tongue
heart	oesophagus	trachea
	pancreas	urinary bladder
	pituitary	uterus or prostate
	salivary gland	femur and articular surface
	sciatic nerve	rectum
	skeletal muscle	vagina
	skin	spinal cord

The findings were described: for each dog as macroscopic and microscopic changes from normal and a summary tabulation of these was prepared by the investigators. All reports were signed by

The Incidence of Liquid Feces:

The occurrence of liquid feces was the outstanding unfavorable result from the treatment of dogs with carnitine. In view of this effect and its weight on acceptability of the drug, also because of the suspected irritating action on the gastrointestinal mucosa, this problem is addressed first of all, in an attempt to reach its resolution and clarification.

The only case of an established lesion in the stomach was that of dog 677, a male on high dose, with the microscopic finding of a deep erosion of approx. 15x5 mm, and two erosions up to 12x5 mm. The microscopic findings read: "Focal erosion of the luminal mucosa with associated inflammatory cell infiltration." The comment for macroscopy of the jejunum said: "minimal congestion of mucosal surface", and the microscopy protocol said "Within normal limits". For the stomach lesion, the comment in the tabulation says: "The gastric erosion involved the mucosa and lamina propria but did not extend through the muscularis mucosae in the sections examined. The toxicological significance of this finding is equivocal."

One other case of a microscopic finding in the stomach was in dog 663 on the low dose as "foci of mineralization in the mucosa probably of no significance since it also was seen in one control dog, and a change in the jejunum found in dog 675, high dose, described as "marked cytoplasmic vacuolation of epithelium of tips of villi." The significance of this finding was not addressed by the pathologist,

It appears to be of important to compare these described minor intestinal changes in the 3 dogs with the severe changes found in the female control dog that had to be sacrificed because of severe time diarrhea of unknown etiology in which the gastrointestinal tract was found to show marked congestion, with free blood on mucosal surfaces in the entire tract, and large areas of mucosal erosion in the ileum/jejunum seen on microscopy, with marked necrosis of the laminal mucosae and inflammatory cell infiltration into the mucosa, but without lesions in the stomach.

Since liquid feces had occurred also in dogs that did not have any histologically established intestinal lesions, it seems reasonable to theorize that the cause for liquid feces might result indirectly from an action of the drug on the lipid metabolism leading to steatorrhea from the excessive high doses of the drug applied in these studies. It might also be due to the excretion of the excessive doses of carnitine in the feces, but these possibilities were not investigated.

#### Macroscopic Post Mortem Findings:

##### Kidney:

The Summary prepared by the investigators for the entire dog study contains under this subheading (p.000010) the following comment:

"Macroscopic abnormalities detected that may be related to administration of L-carnitine were restricted to a pallor of the inner cortex of the kidneys in 2 of 8 animals receiving 300 mg/kg/day and 4 of 7 animals receiving 1200 mg/kg/day".

Apparently no search was made for any relation of these macroscopic findings to findings in these cases by their histopathological examination, and therefore this had to be done by the reviewer, with the following results:

The animals in which the above macroscopy findings were made were dog 662 and 666, in the 300 mg/kg/day group, and dogs 675, 676, 678, and 682 in the 1200 mg/kg/day group. The only histological findings that could apply as being possibly related to the macroscopic findings were: for the 2 mid-dose dogs: Moderate extensive cytoplasmic vacuolation of the

corticomedullary tubules," and for the 4 high dose dogs: "Marked extensive cytoplasmic vacuolation of the corticomedullary tubules." However, the same description of this change in the kidneys was made also for 3 dogs in the low-dose group that did not have the macroscopically seen pallor varying in degree from slight to marked extensive cytoplasmic vacuolation, and the same incidence was found in 1 mid-dose animals, and in 2 high-dose dogs, that all were without the pallor finding. Therefore there is no relationship between the macroscopic and the histopathological findings.

As to the significance of the microscopic findings, the most severe kidney lesion was found in one control male (655) with the macroscopic finding for the kidney saying: "Pitted appearance to capsular surface with strands extending into cortex from surface pitting", and the microscopic finding described as "Wedge-shaped areas of cortical scarring in both kidneys characterized by areas of fibrosis, loss of tubules, atrophic glomeruli, and focal inflammatory cell infiltration. An association between these can be recognized.

One other control dog (522) also had the microscopy finding "Slight vacuolation of cytoplasm of some corticomedullary tubules," therefore it is clear that this change is common among control and treated dogs but perhaps it is slightly aggravated by the drug in a somewhat dose related extent.

A distinct kidney lesion was found also in one mid-dose dog (673), without any macroscopic abnormalities, that was microscopically detected and described as "loss of the papillary tip with re-epithelialisation over the necrotic tip and degenerating medullary tubules, with marked subepithelial mononuclear cell infiltration in the pelvis." This dog had a better than the mean growth performance for this dose group, therefore the lesion in the kidney is of little clinical significance, and not a drug effect judging from its singular appearance. Therefore it can be concluded that the minor gross change pointed out by the investigator as possibly treatment related was an error of judgement.

An other microscopical change within the corticomedullary tubules that was dealt with by the pathologist was the incidence of fat droplets in the epithelium of these tubules.

The comment by the pathologist said: "Fat droplets in the epithelium of the corticomedullary tubules, also seen as vacuolations in the H&E sections, were present in control and treated dogs. There were moderate/marked amounts in some treated dogs compared to slight/minimal amounts in control dogs. The toxicological significance of this increased number of fat droplets is uncertain as moderate/marked amounts of fat droplets are not uncommon in untreated dogs."

Incidence and degree of fat droplets in the corticomedullary tubules.

	Control		300 mg/kg/day		600 mg/kg/day		1200 mg/kg/day	
	1	2	1	2	1	2	1	2
Slight/minimal amounts of fat droplets	3	4	4	1	2	3	2	1
Moderate/marked amounts of fat droplets				3	1	1	1	3
Total dogs examined	4	4	4	4	4	4	3	4

A comparison of the incidence of fat droplets with that of the vacuolation in the histopathology reports does not confirm by their similar incidence in individual dogs that they are the same histological structures. It is equivocal whether they represent an adverse drug action, or if they are related to the physiological action of the drug on fat metabolism.

Other observations on histological structure changes of kidneys were described as follows:

- Control 655: Wedge-shaped areas of cortical scarring in both kidneys characterized by areas of fibrosis, loss of tubules, atrophic glomeruli, focal inflammatory cell infiltration
- Low Dose 654: marked subepithelial mononuclear cell infiltration in pelvis
- 659: Occasional dilated tubules contain amphophilic material.
- 661: Area of marked interstitial mononuclear and eosinophilic infiltration.
- 666: Areas of necrosis with marked inflammatory cell infiltration
- Mid Dose 667: minimal subepithelial mononuclear cell infiltration in the pelvis of one kidney.
- 671: occasional dilated tubules containing amphophilic material.
- 673: left k.: loss of papillary tip with re-epithelialization over necrotic tip and degenerating medullary tubules. Marked subepithelial mononuclear cell infiltration in the pelvis.  
right k.: areas of interstitial mononuclear cell infiltration, some with associated basophilic cortical tubules.
- 674: Area of interstitial mononuclear cell infiltration
- High Dose: 675: occasional dilated cortical tubules containing amphophilic material.

It can be concluded that these are single, sporadically occurring minor "changes from normal" not characteristic for a drug action.

Liver:

The histology of liver tissues did not show any change from normal. Foci of hepatic necrosis with associated mononuclear cell infiltration were found only in two control dogs.

Portal mononuclear/eosinophilic infiltration noted in 2 dogs of the low dose group only.

Minimal dilatation of centrilobular sinusoids found in one high dose dog (677) that was the only dog with the gastric erosions, and it also had parasitic granuloma foci in the lungs, and erythrophagocytosis in lymph nodes. All these changes were of singular incidence, not indicative for a drug action.

Other Organs:

None of the other investigated critical organs had histopathological changes that would indicate a toxic potential of the drug, or profound alterations in their structure from the pharmacophysiological actions of the massive doses of carnitine.

Pharmacokinetics:

Blood specimen taken at weeks, 6, 12, 26 and 52, and specimen from liver, kidney, heart, skeletal muscle and urine and feces samples taken at ultimate post-mortem, from all animals were forwarded to the sponsor. A report for the analyses of these specimen was not in the submitted NDA documents.

Teratogenicity Tests:

Teratogenicity tests in rats and rabbits were reported in the original NDA. They were conducted in the Laboratory of

They followed in general the FDA Guidelines for Teratogenicity/Reproduction Studies. In the rat study, 10 rats per group were treated with 25 or 50 mg/kg/day by the intramuscular route from day 3 to day 14, and sacrificed on day 19. In the rabbit test, 10 animals per group were treated also with 25 and 50 mg/kg/day I.M., from day 6 to day 29, with sacrifice on day 29.

No adverse effects on gestation, fetal development and postnatal viability were noted.

There was at first some concern about the adequacy of these tests for their validity to support safety of the drug use by pregnant women, but it was later agreed that they are acceptable and that no new tests would be needed.

#### Mutagenicity Tests:

Mutagenicity tests with *Salmonella typhimurium*, *Saccharomyces cerevisiae*, and *Schizosaccharomyces pombe* in-vitro and in vivo, were conducted by the same institution that conducted the described toxicity tests in rats with l-carnitine. These had been reported in the original NDA.

An additional test, a Micronucleus test in mice, conducted at the same institution, was reported in the amendment of November 2, 1983 to the NDA.

From the detailed description of the tests procedures etc. in the original NDA (with authorized translation into English,) it appears that this institution is competent to conduct this class of research, but documentation for compliance with GLP requirements is not provided. (This defect should not hold up further processing of the NDA.) All mutagenicity tests were negative for a mutagenic potential of l-carnitine.

#### SUMMARY AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

For the aspects of the pharmacological properties of carnitine, with regard of its biological functions as carrier of long-chain fatty acids, the information obtained from the literature material furnished by the sponsor amply demonstrates the essential role of carnitine for lipid metabolism in normal and deficiency conditions and thereby projected its efficacy for correcting a state of carnitine deficiency of various origins, such as defective synthesis from its precursors, or genetic defects in the availability of the enzyme systems needed to form the complex of carnitine with the fatty acids that is essential for their translocation into mitochondria, the site for their oxidation into energy. Some of the submitted literature actually goes beyond this phase and intended use of carnitine by dealing with the functions of carnitine in complex with mid-chain length fatty acids, and the involvement of peroxisomes in this process, beside mitochondria, and furthermore, for the now emerging evidence for the pathways of actions of hypolipidemic drugs such as clofibrate, as stimulators of peroxisome activity beside that of H<sub>2</sub>O<sub>2</sub> release.

For the aspects of toxicity and safety, the one-year toxicity tests in rats and mice, performed at our request, have to be viewed from the angle that they do not imitate the conditions of the proposed clinical use of the drug, correction of carnitine deficient, and the dosages were multiples of the proposed clinical dosage. Even with this milieu, unfavorable to the drug, the outcome of both studies is remarkable for the absence of overt adverse results from the drug that would predict serious health hazards from doses in excess of the clinically effective dose.

The most pronounced result is in the dogs in the form of the continuous presence of liquid feces even from the low dose and seen very early in the early phase of treatment. However, it is felt that it is not certain that these were symptoms for a drug induced irritation and even a damage to the gastrointestinal mucosa was alleviated when the health of the dogs remained unaffected, with only a slight depression of the body weight gains by the mid and high dose, and resolved by the histological findings at the time necropsy that did not reveal any distortions of the histological architecture of the mucosal components.

The cause for the liquid feces that is limited to the dogs and was not evident in rats with the same doses, remains only speculative, mostly because of the unavailability at present of the analyses of the pharmacokinetic samples. The existence of disturbances from the stool caused by a disturbance of lipid metabolism by the excessive carnitine doses is a theoretical possibility resulting in extreme stool-softening and eventually a liquid state.

As to the clinical consideration, it also is possible that the effects in dogs might be related to the actions of carnitine in human where the causal pathways apparently are not established either.

In view of the fact that the actions of carnitine on the gastrointestinal system are accepted and are cited in the labeling for carnitine, the effects in the dogs should not constitute a reason for any further actions at the present stage (but it would seem advantageous to find some remedy against the undesirable side effects after finding the causes for them).

There were several other differences in responses to the drug between the two species. In the rats, the drug at the low- and mid-dose had a stimulating action on body weight gain by elevating them above that of the control animals, but the high dose had the opposite effect by depressing the body weight gains of the high dose group animals to a range below that of controls. The beneficial function of the low- and mid dose levels of the drug was further supported by the observation that these two groups of animals developed a reduced rate of body weight gain when drug administration was withdrawn, and inversely, when the high-dose rats showed improved weight gains when the drug was withdrawn. The causal origine for both effects, stimulation by the low and mid-dose, suppression by the high dose, was not investigated, except that there was no change in the feed intake at the change of treatment. While the stimulating action could be explained by a more effective metabolism from the drug, the suppressive effect is difficult to explain, a negative feed back perhaps. It should be noted that the animals in the high dose group did not show clinical signs for any adverse actions.

The only other parameter with a noticeable indication for a drug action was the elevation of triglycerides in the low and mid dose groups coincident with the elevated body weight gains in these groups, and lower levels in the high dose group, accompanied with a drop in triglycerides levels with withdrawal of the drug in the low and mid-dose group, and an elevation in the high-dose group. An association between these two phenomena is difficult to construct. It may indicate the stimulatory action of the triglycerides as the active stimulus secondary to a action of carnitine on triglyceride activation. I was unable to obtain advice, or a source of information from the literature for this action of carnitine, and whether it is an unfavorable action. It might occur only with the excessive doses of carnitine, and therefore would not occur with the clinical use. A modulating action on various lipid levels was not seen in the rat study.

Also in the dogs, levels for cholesterol, HDL, betalipoprotein and PFA values were not affected by carnitine at the doses used.

No adverse effects were indicated by organ weights and histopathology in either species to an extent that would predict a hazardous actions in the human.

#### Teratogenicity Tests:

Two tests, one in rats and one in rabbits were, as could be expected from the nature of the drug, uneventful with regard to actions on pregnancy and fetal development.

#### Mutagenicity Tests:

These tests by 4 different methodologies did not indicate a mutagenic property of carnitine.

#### Publications by Sigma-Tau:

The submitted literature material contains several articles, published and unpublished, by staff members of the Research Laboratories of Sigma-Tau S.p.A., Pomezia, Italy that by themselves would not "carry" the NDA but are reviewed here for the sake of documenting that this firm had comprehensive experience in the field of carnitine and its functions.

N. Siliprandi and Maria T. Ramacci, Institute of Biological Chemistry, University of Padova, and Sigma-Tau Laboratories, "Carnitine as a Drug Affecting Lipid Metabolism." (apparently an In-House manuscript.)

This was primarily a review of the functions of carnitine from publications that was informative for describing the complexities of the actions involved in the mechanism of the drug, and its derangements by pathological and deficiency conditions leading to myopathies and myocardial anomalies. Of interest is their reference in the Conclusion, to clofibrate by saying: "With regard to the alterations of lipid metabolism, it is appropriate to point out that some drugs, such as clofibrate which typically affect lipid metabolism, strongly modify the biosynthesis and distribution of carnitine as well as the carnitine transport system, a fact highly relevant to their mode of action. However, pharmacological and clinical studies using carnitine have the advantage over those using other drugs because of the fairly well-known background of carnitine biochemistry" (Ref.: Fante and Parvin, *Biochim. Biophys. Acta*, 100,209-214, 1980)

F. Maccari, Pessotto, P., Ramacci, M.T. Angalucci L., of Sigma-Tau and Istituto di Farmacologia, University of Rome: "The Effects of Exogenous L-Carnitine on Fat-Diet Induced Hyperlipidemia in the Rat" (no literature reference). The purpose of this study was to investigate the reducing effect of carnitine on diet-induced hyperlipidemia, and by what mechanism, on the premise that in the absence of carnitine only short- and medium-chain fatty acids can enter the mitochondrial matrix, and that long-chain acyl-CoA preferentially participates in extramitochondrial reactions such as triglyceride synthesis that occurs in lipid storage myopathies. A TG rich diet with 25% olive oil was the test diet, treatment was 500 mg/kg carnitine by gavage. Tissue distribution search was for triglycerides, total cholesterol, phospholipids, carnitine, and acetyl-carnitine, in heart, muscle, liver, kidney.

The TG-rich diet induced modifications in lipoprotein pattern by increased serum chylomicron content and of pre-beta lipoproteins, triglycerides, FFA, and phospholipids in controls, and their reduction by the extraneous carnitine. The results in the various organs were similar. An excess of carnitine caused its loss in urine.

Zago, E., Maccari F and Ramacci M.T. ("Carnitine-Insulin Treatment in Diabetic Rats." The capacity of exogenous L-carnitine to restore impaired glucose and lipid metabolism in diabetes was investigated in rats. This property had been found to exist by other investigators. In this short notice it is reported that streptozotocin-diabetic rats were treated with carnitine 250 mg/kg p.o., alone or with insulin 20 U/kg s.c., for 4 days. Carnitine by itself moderately reduced hyperglycemia in addition to lowering FFA, and markedly, triglycerides in serum. Insulin alone also reduced significantly glucose, FFA and triglycerides and the combination of both reduced FFA and triglycerides, and was more markedly effective in normalizing hyperglycemia than by their separate actions.

Zago, E., Maccari, P., Sestini, G., and Sestini, G. "Altered Persistence to Developing Ketosis during Fasting in Obese Zucker Rats Treated with L-Carnitine." The fasting state is characterized by an enhanced release of free fatty acid from adipose tissue which are then oxidized in the liver into ketone bodies. A process controlled by the hepatic acetyltransferase activities modulated by the glucagon levels. According to some investigators, studies in man and animals are said to have shown that in the obese normal state reduction in increased mobilization is induced without readily developing a state of ketosis. The investigators theorized that the hyperinsulinemia (hyperinsulinemia) "prevented" ketogenic process is caused by a defective control mechanism in the liver. In their test obese and lean Zucker rats were treated with 500 mg/kg carnitine orally. In untreated obese rats, free and acetyl carnitine levels were significantly higher than in lean rats without a definite difference in serum FFA, but acetoacetate and hydroxybutyrate levels were reduced in the obese rats with triglycerides being elevated. Treatment with carnitine significantly increased serum FFA, and acetyl carnitine in both groups, but acetoacetate and hydroxybutyrate were enhanced in the obese, but significantly reduced in the lean rats. The conclusion by the investigators is not quite clear. They say that the decreased production of ketone bodies can be ascribed to the persistence of hyperinsulinemia but that carnitine stimulates oxidation of the fatty acids when carried into liver mitochondria.

The next article entitled "Ketone Body Modifications after L-Carnitine Treatment in Obese and Diabetic Rats" by the same authors is a continuation of the theme in it. The effects of L-carnitine was again studied in streptozotocin diabetic Wistar rats treated with 500 mg/kg p.o. daily in the diabetic, and 500 mg/kg oral in the obese and lean Zucker rats.

Diabetic rats exhibited a considerable increase of blood ketone bodies which was significantly reduced by the exogenous carnitine. Liver carnitine acetyltransferase was unaffected. Diabetes provoked a decrease in the serum of free carnitine but an increase in serum acetylcarnitine.

The results in the obese and lean Zucker rats showed that blood levels of ketone bodies in obese rats are lower than in lean rats whereas both free carnitine and acetylcarnitine values were increased in the obese rats. Carnitine administration had opposite effects in the obese and lean rats: it increased serum ketone levels in obese, but decreased it in lean rats.

The investigators discussed these results by the following considerations: In diabetic rats the level of blood ketone bodies is high while in obese rats it is low, but in both conditions FFA levels are elevated above normal. This phenomenon is explained by the condition that the excess of fatty acids in the diabetic rat arriving at the liver are rapidly oxidized and only partially transformed into ketone bodies, while in obese rats the excess fatty acids are

not oxidised in the liver but are esterified into triglycerides. The difference in the metabolism is ascribed to the high glucagon/insulin ratio typical of diabetes that stimulates the activity of the translocatory carnitine system and transfer to the inner mitochondrial membrane and oxidation, whereas the low glucagon/insulin ratio typical for (genetic) obesity inhibits the activity of this system. Therefore, the differences in the metabolic alterations in diabetic and obese conditions in the rat can be explained as consequences of an altered control on carnitine translocating system (and the ensuing alteration in carnitine function?). The decreased ketone bodies content in blood after administration of carnitine to diabetic rats was explained by an increased utilization of the ketone bodies for oxidation by extrahepatic tissue mitochondria (probably the heart and kidney) with carnitine - stimulated mitochondrial CoA.

Zago E and M. T. Ramacci. "Effects of L-Carnitine on Glucose Uptake and Utilization in Rats and Rabbits." The authors stated that it is known from work by other investigators that carnitine is involved in the stimulation of pyruvate dehydrogenase activity as a consequence of an induced decrease of the acetyl-CoA: CoA ratio, and this study was to investigate whether carnitine also could affect carbohydrate metabolism in healthy and in diabetic animals. The test was run in-vitro on rat diaphragms in Krebs-Henseleit buffer, for glucose uptake and its disappearance from the medium within 6 minutes of incubation. In the in-vivo study run on rats and rabbits, fasted rats were treated with oral carnitine 250 mg/kg, 60 minutes before an oral glucose load; for rabbits a glucose infusion of 10 mg/kg/min for 90 minutes was followed one week later by a combination load of carnitine plus glucose. The results of the in-vitro test showed that glucose uptake in the diaphragm was increased by carnitine but to a lesser degree than by insulin. With a combination of both, the uptake was slightly above that by insulin alone.

In the in-vivo rat study, pretreatment before the glucose load with carnitine resulted in a significant decrease of the hyperglycemic peak. The investigators claim that these results correspond to the results in human where a reduction of glycosuria in hyperlipidemic diabetic patients was obtained with carnitine. They believe that the carnitine action reflects a stimulation of pyruvate-dehydrogenase activity owing to a decreased acetyl-CoA: CoA ratio. It was speculated that two separate mechanisms exist: insulin induced glucose uptake in the cell, and carnitine improved the oxidative utilization of the cellular glucose.

Fanelli, Ottorino: "Carnitine and Acetyl-Carnitine, Natural Substances Endowed with Interesting Pharmacological Properties" in Life Sciences Vol. 23, pp.2563-2570, 1978. This article deals with the inotropic and anti-fatigue effects of d,l-carnitine, l-carnitine and d,l-acetyl-carnitine in rat after a fatigue test, and in isolated heart of rabbits. The introduction gave a very good summary of important findings reported in the current literature.

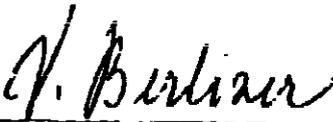
For the isolated heart test, freshly obtained hearts were perfused with solutions of the different drug substances, and contractile force, coronary flow and heart rate were calculated. All substances were found to have similar effects that were dose related in effectiveness.

In the fatigue test performed on a rotarod apparatus, in a dose range from 20.0 to 800 mg/kg, d,l-acetyl carnitine was most effective but at 800 mg/kg it induced a motor activity excitation effect and adversely affected the animals.

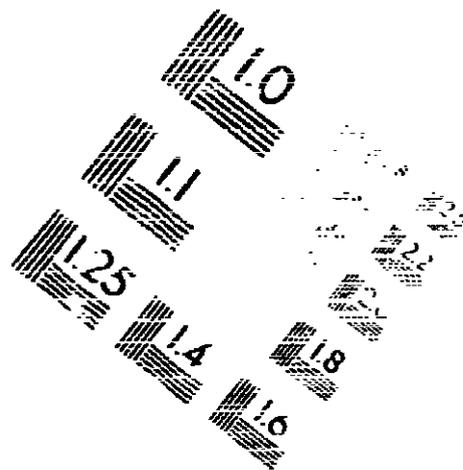
CONCLUSION and RECOMMENDATION:

The essential role of l-carnitine in fat metabolism is unequivocally established by the numerous investigations reported in the world-literature, and its safety is supported by the toxicology studies and teratogenicity tests conducted for the NDA by the sponsor.

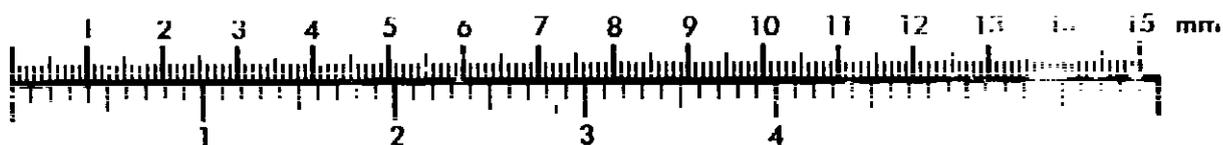
From the standpoint of Pharmacology, approval of the NDA can be recommended.

  
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V. Berliner, Ph.D.

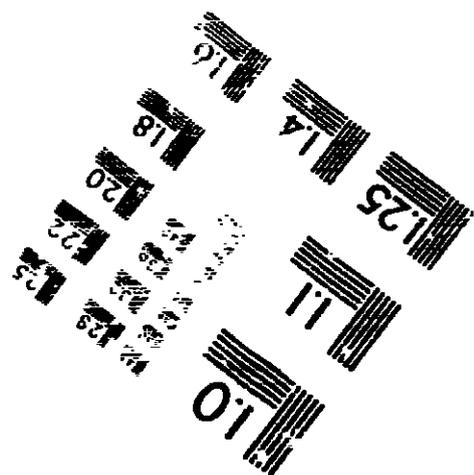
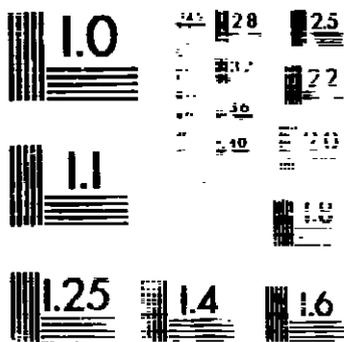
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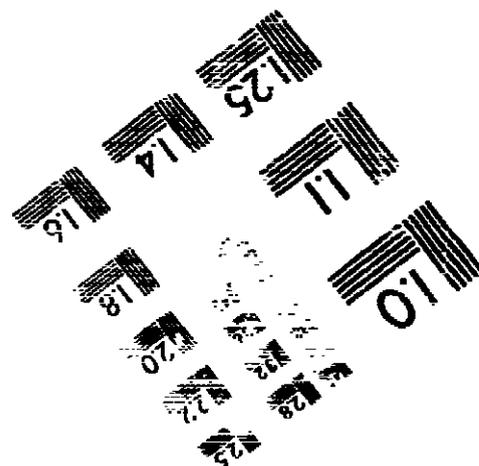
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NDA 18-948

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Summary Basis of Approval

NDA 18-948

Drug Generic Name:  
L-Carnitine Tablets

Applicant:  
Sigma-Tau, Inc.  
Holmdel, New Jersey 07733

Drug Trade Name:  
Carnitor

I. Indication for Use:

L-carnitine is indicated in the treatment of primary systemic carnitine deficiency.

II. Dosage form, route of administration and recommended dosage:

Tablets; oral.

Adults: 990 mg two or three times a day depending on clinical response.

Infants and Children: 50 to 100 mg/kg/day in divided doses with a maximum of 3 g/day.

III. Manufacturing and Controls:

A. Manufacturing and Controls

1. The synthesis of the new drug substance involves the reaction of known materials under well-defined and acceptable conditions and purified by use of standard methods.
2. Specifications and testing by appropriate methodology insure its compliance with the required standards of identity, strength, quality and purity.

B. Stability

Studies are ongoing and based on the data submitted at this time an expiration dating of 5 years at room temperature is justified.

C. Methods Validation

The methods validation is now being processed.

D. Labeling

The technical aspects of the labeling and labels are satisfactory.

E. Establishment Inspection

The facilities, equipment, manufacturing controls are in compliance with current good manufacturing practices and with conditions, commitments and requirements contained in the new drug application as indicated by appropriate reports of inspections from the Manufacturing Review Branch in the Division of Compliance.

F. Environmental Impact Analysis Report (EIAR)

EIAR indicates that the product does not have adverse environmental effects.

G. Bioavailability Requirements

Deferred by Division of Biopharmaceutics until after approval.

IV. Pharmacology:

L-Carnitine is the synthetic l-isomer of d,l-carnitine discovered 80 years ago as a constituent of muscle tissue that at first was used as a nutrient agent. It was sixty years later that its essential action in the metabolism of fatty acids was recognized. D-L-Carnitine is synthesized in liver, kidney and testis, from two essential amino acids, lysine and methionine. The l-isomer is the biologically active form.

Its biological function is to activate the transport of long-chain fatty acids such as palmitic acid across the inner mitochondrial membrane into the mitochondrial matrix where they undergo beta-oxidation resulting in the production of energy. For example, palmitic acid released from adipose tissue or derived from diet is activated by outer membrane ATP-dependent palmitoyl-CoA synthetase to form palmitoyl-CoA. Such long-chain fatty acyl-CoA esters have only a limited ability to cross the mitochondrial membrane barrier and their entry is facilitated by outer carnitine palmitoyltransferase which catalyzes a transesterification reaction in which the palmitoyl moiety from CoA is transferred to carnitine forming palmitoylcarnitine. This ester then crosses the inner mitochondrial membrane through action of a translocase. A second transesterification reaction now takes place wherein "inner" palmitoyltransferase, located in the inner mitochondrial membrane, regenerates palmitoyl-CoA for subsequent beta-oxidation and releases carnitine for a repetition of its catalytic role in overall fatty acid transport (After: Broquist, H.P. and P.R. Borum: "Carnitine Synthesis Nutritional Implications." Adv. Nutrit. Research 4:181-204, 1982).

The biophysiological functions of l-carnitine have been studied by numerous investigators in several animal species during the last decade. A recent discovery is the essential role played by carnitine (and regulated by androgen) in epididymal mitochondria, in the maturation of spermatozoa, and in the ovary in the maturation and ovulation of follicles in rats, rabbits and monkeys. Evidence is also accumulating for a role of carnitine in gluconeogenesis.

Publications submitted in the NDA describing pharmacological parameters of l-carnitine in the animal model were used for the evaluation of the efficacy of l-carnitine. Some of these were performed by the staff of Sigma-Tau in their laboratories in Italy.

Toxicology Tests were performed in rats and dogs, both of 52 weeks duration, with doses of 150, 450 and 1350 mg/kg/day in the diet in the rat study and 300, 600 and 1200 mg/kg/day in capsules in the dog study. (The clinical dose is 990 mg one to 3 times per day.)

Adverse actions from l-carnitine were limited to a slight depression of body weight gains of rats on the high dose, in contrast to a growth promoting action of the low- and mid-dose. There was also a slight reduction of body weight gains in dogs by the mid- and high-dose. In both species the reduction of body weight gains was not accompanied by any clinical signs of affected health conditions. Liquid feces were observed in dogs. There was no associated structural damage to the gastrointestinal mucosa, and the mechanism of action was not established.

Serum triglycerides were elevated in rats given the low- and mid-doses of l-carnitine. There were no related changes in the livers and kidneys.

Teratogenicity tests performed in rats and rabbits did not reveal any effects on the dams and their offspring.

Four sets of mutagenicity tests were negative.

V. Medical:

A. Introduction:

Utilization of lipid for energy requires the presence of l-carnitine for optimum transfer of long-chain fatty acids into the mitochondrial matrix, where beta-oxidation takes place. Generally, the requirements for carnitine are met by endogenous synthesis and/or dietary intake. In normal adults diet and endogenous synthesis are adequate to meet the requirements for carnitine. Systemic (blood and tissue) carnitine deficiency is manifested clinically as muscle weakness, cardiac failure, hypoglycemia and/or liver insufficiency. The clinical manifestations may resemble cardiac fibroelastosis, Reyes Syndrome, or muscular dystrophy. The enzymopathy has not been defined, and the variety of manifestations suggest that there may be several enzymopathies that fall into the category of primary systemic carnitine deficiency. The reported cases have in common low serum and/or tissue levels of carnitine. Some of the reported cases responded very dramatically to administration of exogenous l-carnitine.

Secondary carnitine deficiency occurs in organic acidurias, chronic hemodialysis, severe malnutrition, Fanconi's syndrome and valproic acid therapy. These conditions might benefit from carnitine therapy, but data have not been submitted. Diagnosis of primary systemic carnitine deficiency requires accurate quantitation of both free and acylcarnitine in serum, urine and tissue. Patients with high urine ratios of acylcarnitine to free carnitine may have carnitine deficiency secondary to defective organic acid metabolism. Because of incomplete biochemical characterization, it is not clear whether carnitine deficiency is primary or secondary in some of the submitted cases.

B. Studies to provide evidence of safety and effectiveness.

There are 16 cases identified as systemic carnitine deficiency which were treated with carnitine, 11 of which provide evidence of effectiveness. An additional 50 cases not identified as systemic carnitine deficiency were treated with carnitine and provide evidence of safety only.

- a. Cases identified as systemic carnitine deficiency are listed below, whether or not they were evaluated as showing effectiveness. Five of the ten investigators had more than one case.

S. Cederbaum, M.D., NEJMed 1980, 303:1389.

1. A 3.5 year old male patient with low carnitine levels in liver, muscle and blood was treated first with d,l-carnitine 1500-4000 mg/d p.o. and then with l-carnitine 1980 mg/d. He improved in affect, frequency of infections and in listlessness, and had diminished cardiomegaly. Liver and serum carnitine levels increased, but muscle carnitine remained low. Carnitine therapy evaluation: life-saving. He had transient diarrhea which resolved with decreased dosage. This patient has been continued on l-carnitine, 75 mg/kg/d for four years with continued benefit. Total plasma carnitine is low normal (30 micromolar). S. Cederbaum, et al., NEJMed 1984, 310, 1395.

R.R. Chun, M.D., et al., NEJMed 1981, 305:385. Two patients with systemic carnitine deficiency were treated with l-carnitine 990 to 1650 mg/d.

2. A 14 month old male patient died of respiratory arrest after about 2 weeks of treatment. L-carnitine evaluation: did not alter this patient's condition.
3. A 9 year old female had a deteriorating cardiomyopathy, familial in nature with mitral insufficiency and syncope. She was considered a candidate for a cardiac transplant, but after treatment with l-carnitine, cardiac function normalized, digitalis was discontinued and heart size returned to normal. Carnitine treatment was evaluated: produced dramatic improvement.

A. Slonim, J. Peds. 1981, 99:551. Three patients with systemic carnitine deficiency were treated with 330 to 1320 mg/d l-carnitine (100 mg/kg/d).

4. A 2 year old male had nonketotic hypoglycemia before treatment with carnitine. After treatment he had no hypoglycemic episodes, his height, weight and appetite increased, and he appeared to be more normal. Plasma carnitine increased to normal, and on fasting he produced ketones. Carnitine treatment evaluation: produced dramatic improvement.
5. A 3 year old female was walking and speaking better after treatment with carnitine, and had no episodes of hypoglycemia. Carnitine treatment evaluation: life-saving. She had a viral infection during treatment.
6. A 4 1/2 year old male was treated too short a time to evaluate response.

- D. Valle, M.D., J. Peds. 1982, 101:700.
7. A 5 1/2 year old male with low blood and muscle levels of carnitine was treated with 3960 mg one day and then 2980 mg/d. He had moderate cardiomegaly and minimal heart failure despite treatment with digoxin and diuretics. Within a week of starting carnitine, heart size decreased and failure disappeared. After 5 months of treatment, he was normal. Carnitine therapy evaluation: life-saving. The patient had moderate diarrhea which was dose related, and developed body odor.
- C. Sansaricq, NYU Med. Center.
8. A 1 year old male with systemic carnitine deficiency was treated with 165 to 330 mg l-carnitine orally. He died of respiratory failure after 10 days of therapy. Carnitine therapy evaluation: too short a time to evaluate.
- B.O. Stands, M.D., Richland Medical Park, Columbia, SC.
9. A 2 1/2 year old female with systemic carnitine deficiency was treated with 990 mg l-carnitine/d. She had several episodes of severe coma and was seriously ill before treatment but has had no further problems and is developing normally. Carnitine therapy evaluation: produced dramatic improvement. She had cough, runny nose and diarrhea of moderate severity.
- R. Cruse, D.O. and S.K. Young, M.D., Hershey Med. School. Two patients with systemic carnitine deficiency were treated with 1320 to 1980 mg l-carnitine.
10. A 3 1/2 year old female had two attacks of coma before and none in the year after initiating treatment. She is stronger. Carnitine therapy evaluation: produced improvement. She had loose stools which ceased after one week. Fishy odor was eliminated by decreasing the dose of l-carnitine. She also had urinary tract infection and virus.
  11. A 6 year old female remained "asymptomatic," and carnitine therapy evaluation: produced improvement. She also had loose stools and fishy odor.
- P. Hartlage, M.D., Medical College of Georgia.
12. A 3 year old female with probable systemic carnitine deficiency was treated with 1980 mg l-carnitine/d orally. She did not benefit from carnitine therapy.

J. DiLiberti, M.D. and J.R. Schimschock, M.D. of Portland, Ore. Two patients with systemic carnitine deficiency received l-carnitine 990 or 1980 mg/d.

13. An 11 month old female had two episodes of hypoglycemia with hepatic, cerebral and muscle dysfunction. No subsequent attacks despite infections. Carnitine therapy evaluation: produced improvement. She had flu with vomiting, rash on trunk and back, fever and emesis, lethargy.
14. A 7 year old female after carnitine treatment had increased attention span, began following sequential commands and was more compliant and affectionate. She had better leg control and became toilet trained. Carnitine therapy evaluation: produced dramatic improvement.

C.L. Hoppel, Peds. Res. 1981, 15:633. Two patients with systemic carnitine deficiency were treated with l-carnitine.

15. A 14 year old female showed greatly improved skeletal muscle function, hepatic function, and cardiac function. Growth and development are normal. Carnitine therapy evaluation: life-saving.
16. A 9 month old male had no recurrence of acute hypotonia, weakness, hepatomegaly and hyperglycemia (sic), but duration of therapy was not sufficient to determine long-term effect on muscle and cardiac function. He had moderate diarrhea, which resolved in 2-3 days, and severe pneumonia.

Carnitine therapy was rated life-saving for 4 patients, dramatic for 4, and producing improvement for 3. The other 5 did not respond or were not treated long enough to evaluate. Six had diarrhea, and four had body odor. The dose of carnitine was decreased because of the adverse effects in some patients, but it was not discontinued for adverse effects in any patients.

B. Cases providing supportive evidence of safety of carnitine administration.

The additional 50 patients were frequently very ill, and several deaths occurred in this group, but were attributable to the underlying disease (Leigh's encephalopathy, mitochondrial myopathy, Hirschprung's Disease with short bowel syndrome and liver failure). There were reports of 7 cases of fishy body odor, 3 cases of tremulousness, sweating, faintness, hyperventilation and dizziness, one case each of mild gastrointestinal symptoms, "intestinal flu", vomiting and increased seizures, respiratory distress and hepatic failure,

transient diarrhea, hyperammonemia and apneic spells, severe diarrhea which subsided with lower dose, fever and vomiting with draining ears, stiffness and headaches, numbness of toes, and moderate burning and pain in heels and left hip. There is little reason to think any of the symptoms except gastrointestinal symptoms and body odor are related to carnitine therapy.

VI. Approved Package Insert:

A copy of the package insert is attached.

PI

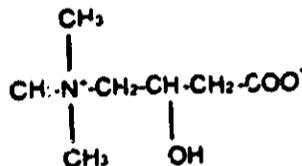
# CARNITOR\*

(L-carnitine)

Tablets

## DESCRIPTION

L-carnitine is L-beta-hydroxy-gamma-trimethylamino butyric acid (inner salt). It is a white powder with a melting point of 196-197°C and is readily soluble in water. The L-isomer of carnitine is the biologically active form.



## CLINICAL PHARMACOLOGY

L-carnitine is essential for the transport of long chain fatty acids across the inner mitochondrial membrane into the mitochondrial matrix where they undergo  $\beta$ -oxidation.

## INDICATIONS AND USAGE

L-carnitine is indicated in the treatment of primary systemic carnitine deficiency.

## CONTRAINDICATIONS

None known.

## WARNINGS

None.

## PRECAUTIONS

Mutagenicity tests have been performed in *Salmonella typhimurium*, *Saccharomyces cerevisiae*, and *Schizosaccharomyces pombe* that do not indicate that L-carnitine is mutagenic.

Long-term animal studies have not been conducted to evaluate the carcinogenicity of the compound.

Pregnancy Category B. Reproductive studies have been performed in rats and rabbits using parenteral administration at doses equivalent on a mg/kg

basis to the suggested oral adult dosage and have revealed no harm to the fetus due to L-carnitine. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

#### **ADVERSE REACTIONS**

The most frequent type of adverse reaction occurring during treatment with L-carnitine is gastrointestinal.

In clinical studies, 41% of patients reported one or more gastrointestinal complaints which tended to be transient.

Typical complaints included nausea, diarrhea, and abdominal distress. Patient odor was the next most frequent adverse effect which had an incidence of 11%. Decreasing the dosage often diminishes or eliminates drug-related patient body odor or gastrointestinal symptoms when present.

#### **DOSAGE AND ADMINISTRATION**

**Adults:** The recommended oral dosage for adults is 990 mg two or three times a day, depending on clinical response.

**Infants and children:** The recommended oral dosage for infants and children is between 50 and 100 mg/kg/day in divided doses, with a maximum of 3 grams/day. The exact dosage will depend on clinical response.

#### **BIOAVAILABILITY**

The bioavailability/pharmacokinetics of L-carnitine tablets have not been determined in well controlled studies.

#### **HOW SUPPLIED**

L-carnitine is supplied as 330 mg, individually foil wrapped tablets in boxes of 90. Store at room temperature.

#### **CAUTION**

Federal (USA) law prohibits dispensing without a prescription.

\* TM application pending

 **sigma-tau**, Inc. 723 North Beers Street  
Holmdel, New Jersey 07733

MED. REVIEW

NDA 18,948  
Carnitine  
Sigma-Tau

Submitted: 2/8/83  
Rec'd by MO: 3/3/83

MOR of NDA

I. GENERAL INFORMATION

- A. Name of drug: L-carnitine
- B. Pharm. category: naturally-occurring cofactor necessary for fatty acid oxidation in mitochondria
- C. Proposed indications: carnitine deficiency syndromes
- D. Dosage form: tablets, each containing 330 mg L-carnitine
- E. Source and method of preparation: see chem review

II. MANUFACTURING CONTROLS

See chemistry review

III. PHARMACOLOGY

See pharmacology review

IV. CLINICAL BACKGROUND

CDS, carnitine-deficiency syndrome, has been recognized for about ten years; its etiology(ies) are still not well established. CDS is considered by many to be a specific disorder of fat metabolism. Patients have been divided into two groups: (1) those with normal serum concentrations and low carnitine content in muscle, possibly resulting from a deficit in carnitine uptake [myopathic carnitine deficiency syndrome], and (2) those with systemic deficiency with decreased content of carnitine in blood and one or more tissues - a generalized metabolic disorder that may be due to a defect in carnitine synthesis [systemic carnitine deficiency syndrome]. It is apparent that any deficiencies of carnitine metabolism may result in similar signs and symptoms as impaired lipid metabolism. A diet low in fat and high in carbohydrate may thus be of use in CDS of any kind. Similarly the treatment of acute metabolic crisis in CDS requires correction of acidosis and administration of substantial amounts of IV glucose. Also, the frequent cardiac involvement in CDS may be understandable on the basis of impaired fatty acid influx into heart muscle mitochondria.

JUN 29 1983

~~JUN 29 1983~~

While the syndrome is rare, there are a number of patients documented in the literature with various types of CDS who have shown dramatic improvement - both objective and subjective - after carnitine therapy.

Carnitine is present in meat and fish and, except for gastrointestinal symptoms, primarily diarrhea, and fish-like body odor, adverse reactions have been virtually unknown.

## V. CLINICAL STUDIES

The studies reported are prototypic for an orphan drug product. The numbers of patients in the studies are small and the data available for each study are limited and largely subjective.

In general, what is known for each patient is: (1) diagnosis; (2) dosage and length of time of drug administration; (3) "Physician's Global Evaluation" of whether (a) carnitine was life-saving to the patient, (b) carnitine dramatically improved the patient's condition, (c) carnitine improved the patient's condition, (d) carnitine did not alter the patient's condition, or (e) carnitine worsened the patient's condition; (4) subjective response with regard to muscle strength, general activity, and neurological response; (5) cardiac size; and (6) tissue levels of L-carnitine. The studies will be described and evaluated in as much detail as possible on the basis of the data presented.

All patients were monitored for any adverse reaction, and all possible such reactions were recorded on the case report form. For each adverse reaction the investigator was asked to rate the severity of the reaction as mild, moderate, or severe, and to estimate the causal relationship of the effect and the drug as remote, possible, probable or definite.

### A. Study #10.

Investigator: S. Cederbaum, M.D.  
Div. of Medical Genetics  
Neuropsychiatric Inst.  
Center of Health Sciences  
760 Westwood Plaza  
L.A., CA

Study Design. Open study to assess the efficacy and safety of carnitine in the treatment of systemic CDS of a single patient, 3.5 yo M. Dx was based on determination of the level of L-carnitine in liver, muscle, and blood: all levels were low. Patient began on D,L-carnitine, at dosages ranging from 1500-4000 mg po qd; currently on 1980 mg qd L-carnitine. [Reported in NEJM 303, 1389, 1980]

Results.

Global evaluation - according to Dr. Cederbaum, carnitine was life-saving to the patient and dramatically improved the patient's condition.

Subjective response - marked improvement in affect, frequency of infections and in listlessness.

Objective response - diminished cardiomegaly; markedly increased liver and serum carnitine levels (to or above nl), muscle carnitine levels low and virtually unchanged with therapy (vol. 1, p. 102).

Adverse reactions - diarrhea, transient, resolved with decreased dosage.

Sponsor's conclusions. "Carnitine provided a successful treatment to the one patient with systemic CDS who participated in the study. The quality of this patient's life has been greatly improved by the administration of the drug. . . ."

Reviewer's conclusions. I agree that there appears to be a marked subjective and objective improvement in this patient. Moreover, there is the laboratory correlation of increased liver and serum carnitine levels. Thus, the drug appears safe and effective in this patient.

B. Study #11. [Neurology 32, 1106, 1982]

Investigator: T. Snyder, M.D.  
Dept. of Neurology  
Med. Ctr. of Vt.  
Burlington, VT 05401

Study Design. Open study to assess the efficacy and safety of L-carnitine in the treatment of lipid storage myopathy.

Two patients with lipid storage myopathy entered the study. The dx of lipid storage myopathy was based on histochemistry of muscle biopsies. The concentration of L-carnitine in the muscle of one patient was 16.8 nmoles/mg protein, 7.8 nmoles/mg protein in the other (nl 11-21). For the first week, the first patient received an oral, total daily dosage of 14.85 gm of drug in 3 divided doses. The dose was then reduced to 4.95 gm/d divided into 3 doses.

For the first four days the second patient received an oral, total daily dosage of 14.85 gm of L-carnitine divided into 3 equal doses. The dose was then reduced to 4.95 g/day.

Results.

Global evaluation - both patients showed dramatic improvement in symptomatology.

Subjective - first patient had marked improvement in his functional abilities with markedly increased muscle bulk and strength; second patient had marked improvement in muscle strength but not in bulk, with improved sense of well-being and "functional abilities."

Adverse reactions - none.

Sponsor's conclusions - "L-carnitine provided a successful treatment to two patients with lipid storage myopathy."

Reviewer's conclusions. Once again, the safety and efficacy of L-carnitine appears to have been demonstrated in 2/2 patients. Note, however, that in only one of these was true carnitine deficiency demonstrated.

C. Study #12. [NEJM 305, 385, 1981]

Investigators:

R.R. Chun, M.D.  
H.A. Peters, M.D.  
M.L. Katcher, M.D.  
Univ. of Wisconsin Hosp. and Clinics  
Madison, WI 53792  
M.E. Tripp, M.D.  
Univ. of Chicago  
Chicago, IL

Study Design. Open study in 8 patients with different syndromes to examine the safety and efficacy of L-carnitine.

Patients had following diagnoses:

Patient #1	systemic CDS
#2	systemic CDS
#3	motor neuron disease
#4	Duchenne Muscular Dystrophy
#5	myopathic CDS; Duchenne muscular dystrophy; palmitoyl transferase deficiency
#6	Duchenne Muscular Dystrophy
#7	Duchenne Muscular Dystrophy
#8	Duchenne Muscular Dystrophy

Patients received total daily doses of 990 to 1650 mg.

Results.

Global evaluation. Patient 1 - treatment with drug did not alter the patient's condition but too early to adequately evaluate. Patient 2 - treatment dramatically improved the patient's condition. Patients 3,5,6,7 - treatment did not alter their condition. Patient 4 - too early to evaluate. Patient 8 - not evaluated.

Subjective evaluation. Patient #2 had deteriorating familial cardiomyopathy, with MI and syncope, and was reportedly considered a candidate for a cardiac transplant. After treatment with drug, cardiac function has normalized, digitalis has been discontinued, and heart size has returned to normal.

Adverse reactions. Patient #1 died of respiratory arrest after being treated with drug for approximately 2 weeks; felt only remote possibility of relationship to drug. Patient #2 had mild GI sx felt possibly related to treatment with drug; in addition, this patient had odd body odor, probably related to drug. Patient #4 noted stiffness and headaches.

Sponsor's conclusions. Treatment with L-carnitine dramatically improved the condition of one patient with systemic CDS.

Reviewer's conclusions. Agree with sponsor's conclusions, as far as they go. In addition, 0/3 evaluable patients with Duchenne Muscular Dystrophy and 0/1 with "motor neuron disease" responded.

D. Study #13.

Investigator:

W.K. Engel, M.D.  
Hospital of the Good Samaritan  
L.A. CA 90017

Study Design. Open study of 22 patients with following dx:

Patient #1	myopathic CDS
#2	carnitine responsive muscle fatigue and cramps
#3	probable lipid myopathy and neuropathy
#4	myopathic carnitine deficiency
#5	myopathic carnitine deficiency
#6	myopathic carnitine deficiency
#7	increased lipid in muscle at birth
#8	lipid myopathy
#9	mitochondrial myopathy
#10	Duchenne M.D.
#11	Duchenne M.D.
#12	Duchenne M.D.
#13	Oculopharyngeal M.D.
#14	Ragged Red Fibers-increased lipid on bx
#15	myopathic carnitine deficiency
#16	Ragged red fiber disease
#17	vacuolar myopathy
#18	phosphorylase deficiency
#19	myophosphorylase deficiency
#20	Ragged red fiber disease
#21	Ragged red fiber myopathy with increased lipid
#22	vacuolar myopathy with lipid droplets

Patients received total daily po doses of up to 15 g of drug.

### Results.

Global evaluation. In the five patients with myopathic CDS (#s 1,4,5,6, and 15), one patient experienced dramatic improvement and four patients experienced improvement. In the other patients, there was apparently no effect of treatment.

Adverse reactions. Some 31 adverse reactions were reported in 10 patients. All but three in three patients were mild-moderate GI effects or fishy body odor. The remaining three were tremulousness/sweating/faintness/hyperventilation and dizziness (2).

Sponsor's conclusions. All five patients with myopathic carnitine deficiency benefited by treatment with drug; in one patient the response was dramatic.

Reviewer's conclusions. On the basis of such meager data, I cannot dispute the sponsor's conclusions. It would have been of interest to know whether laboratory values correlated with the reported clinical response.

It should also be noted that in this pivotal study (5 of the 7 patients overall in the NDA with myopathic CDS showing improvement are from this study) that 4 of the 5 patients were on glucocorticoids during at least part of the time of administration of carnitine. This makes difficult interpretation of the effect of carnitine.

E. Study #14. [J. Peds. 99, 551, 1981]

Investigator:

A. Slonim, M.D.  
Dept. of Peds. Endocrinology  
Vanderbilt Univ. Hosp.  
Nashville, TN 37232

Study Design. Open study to assess efficacy and safety of L-carnitine in CDS in 3 patients with systemic CDS and one with myopathic CDS.

Patients received between 330 and 1320 mg of po drug qd or 100 mg/kg/d.

Adverse reactions. None reported.

Results.

Global impression and other data. Two patients with systemic CDS responded to treatment with drug (walking, speaking improved; without further episodes of hypoglycemia). The one patient with myopathic CDS did not respond to treatment. It was felt to be too early to evaluate the other patient with systemic CDS.

Adverse reactions. Single episode of a "viral illness."

Sponsor's conclusions. Treatment with drug was beneficial to two patients with systemic CDS. One patient with myopathic carnitine deficiency did not respond to treatment.

Reviewer's conclusions. Marked efficacy appears to have been demonstrated in 2/2 patients with systemic CDS. Ineffective in the one patient with myopathic CDS. Again, no safety problems.

F. Study #15 - only patient in study unevaluable.

G. Study #16. [J. Peds. 101, 700, 1982]

Investigator:

D. Valle, M.D.  
Peds. Genetics Clinic  
The Johns Hopkins Hosp.  
Balt., MD 21205

Study Design. Open study of a single patient to assess the safety and efficacy of L-carnitine in systemic CDS.

Results.

Global evaluation. Drug was "life-saving" to patient.

Clinical impression. Patient was in extreme borderline CV status despite chronic Rx with digoxin and diuretics. Within one month, there was obvious improvement. After 5 months of therapy, the patient's clinical status was "normal."

Objective data. Cardiomegaly as measured on CXR improved markedly. Blood levels normalized, from 4.3 nmol/ml pre-treatment, to 21.6-32.0 after treatment (nl 20.8-44.5).

Adverse Reactions. None reported.

Sponsor's Conclusions. Markedly successful treatment in this patient.

Reviewer's Conclusions. Concur with above.

H. Study #17.

Investigator:

E. Monkus, M.D.  
Dept. of Peds.  
Div. of Neonatology  
Univ. of Miami  
School of Medicine  
Miami, FL 33101

Study Design. Open study with two patients, one with Leigh's encephalopathy (patient #1), the other with kwashiorkor (patient #2), of the safety and efficacy of L-carnitine.

Patient #1 received a total oral daily dose of 990 mg of L-carnitine. Patient #2 was maintained on 2640 mg/day.

Results.

Global evaluation. Patient #1 died approximately two months after beginning therapy; demise unrelated to drug administration, according to investigator. Patient #2 improved in response to treatment with L-carnitine.

Clinical impression. In patient #2, markedly improved nutrition during period of therapy was felt to be major factor in clinical improvement; unknown how much drug contributed.

Adverse Reactions. Death of one patient, felt unrelated to drug.

Sponsor's conclusions. Drug was beneficial to patient with Kwashiorkor.

Reviewer's conclusions. Unevaluable.

I. Study #18.

Investigator:

L.D. Prockop, M.D.  
Division of Neurology  
Univ. of So. Fla.  
Tampa, FL

Study Design. Open study of a single patient with myopathic CDS, severe respiratory failure and muscle weakness of the safety and efficacy of L-carnitine. Patient was treated with 10,000 mg qd D,L-carnitine initially, tapered to 4000 mg D,L-carnitine, now maintained on 1980 mg/d L-carnitine.

Results.

Global impression. According to investigator, drug was life-saving to patient.

Clinical impression. Patient's long-standing muscle weakness, flaccid paralysis and hypoxic brain damage were reversed substantially by drug therapy.

Objective. Patient was able to be weaned from ventilator, regained 67% of predicted respiratory function and approximately 90% of normal muscle strength after drug therapy.

Adverse reaction. Question of mild distal numbness of toes, transient, felt remotely likely to be related to drug.

J. Study #19.

Investigator:

C. Sansaricq, M.D.  
Dept. of Peds.  
NYU Med. Ctr.  
New York, NY 10016

Study Design. Open study of a single patient with systemic CDS of the safety and efficacy of L-carnitine.

The one year old male patient received 165 - 330 mg of drug po qd.

Results. Patient died of respiratory failure ten days after initiation of therapy, felt by investigator unrelated to drug therapy. Also felt duration of therapy too short to evaluate effect.

Sponsor's conclusions. Unevaluab!e.

Reviewer's conclusions. Unevaluab!e.

K. Study #20.

Investigator:

B.O. Stands, M.D.  
Medical Park Peds. & Adolescence, P.A.  
Richland Medical Park  
Columbia, S.C.

Study Design. Open study of a single patient with systemic CDS, with dx based on subnormal levels of L-carnitine in muscle, liver, and blood. Patient receives dosage 990 mg/d.

Results.

Global evaluation. Patient dramatically improved.

Clinical impression. Drug ended recurrent comatose episodes.

Adverse reactions. None reported.

Sponsor's conclusions. Drug beneficial in this patient.

Reviewer's conclusions. Concur.

L. Study #21.

Investigator:

K. Chandar, M.D.  
Division of Neurology  
Mt. Sinai Hospital  
Cleveland, O

Study Design. Open study to assess the efficacy and safety of L-carnitine in a single patient (71 yo female) with myopathic CDS. Dosage ranged from 1980-11,880 mg po qd.

Results.

Global evaluation. Drug did not alter patient's condition.

Clinical impression. No apparent increase in muscle strength during study.

Sponsor's conclusions. No response in this patient.

Reviewer's conclusions. Concur.

H. Study #22.

Investigators:

R. Cruse, D.O.  
R.S.K. Young, M.D.  
Dept. of Peds. Neurology  
Hershey Med. Sch.  
Hershey, PA 17033

Study Design. Open study of two patients (3.5 and 6 yr) with systemic CDS of the safety and efficacy of L-carnitine. Patients initially received oral dose of 1980 mg, later reduced to 1320 mg.

Results.

Global evaluation. Both patients improved on drug.

Clinical impression. Episodic attacks of coma have ended in one patient, and the second remains without symptoms.

Adverse reactions. Transient loose stools in both patients; fishy odor noted, eliminated with decreased dose.

Sponsor's conclusions. Both patients with systemic CDS benefited.

Reviewer's conclusions. Concur.

N. Study #23.

Investigator:

D.L. Ehrenreich, M.D.  
Buffalo Med. Grp.  
Buffalo, NY 14203

Study Design. Open study of a single patient, 41 yo female, with Lipid storage myopathy, of the safety and efficacy of L-carnitine. Drug was begun at po dose 4 g qd and gradually tapered to zero over 14 months.

Results.

Global evaluation. No effect of drug.

Clinical impression. "Shortly after instituting treatment with Theragram (while still on carnitine), there was a dramatic improvement. Carnitine levels were not found to be low in muscle or serum. Lipid storage myopathy was "cured" when repeat muscle bx performed. Improvement was maintained after treatment with L-carnitine stopped."

Adverse reactions. Moderate burning and pain in heels and left hip; felt only remote possibility of relation to drug.

Sponsor's conclusions. No effect in this patient.

Reviewer's conclusions. Concur.

O. Study #24.

Investigators:

R.C. Griggs, M.D.  
Dept. of Neurology  
University of Rochester School of Med. and Dentistry  
Rochester, NY 14642

Study Design. Open study to assess the safety and efficacy of L-carnitine in two male patients (ages 3 and 15), one with mitochondrial myopathy (#1, age 3), the other with myopathic CDS (#2, age 16). Patients dosages ranged from 1320-1980 mg po qd.

Results.

Global evaluation. No effect of drug in either patient.

Adverse reactions. Both patients experienced mild GI sx, possibly related to drug. Patient #1 died of respiratory failure, approx. 2.5 mo after beginning drug (no mention of relationship to drug).

Sponsor's conclusions. No effect in two patients, one with mitochondrial myopathy and one with myopathic CDS.

Reviewer's conclusions. Concur.

P. Study #25.

Investigators:

P. Hartlage, M.D.  
Depts. of Neuro and Peds.  
Medical College of GA  
Augusta, GA 30912

Study Design. Open study in two patients, 3 yo F with probable systemic CDS (#1) and 7 yo M with lipid myopathy. #1 received oral dosage 1980 mg qd; #2, 990 mg qd.

Results.

Global evaluation. No effect of drug on either patient.

Adverse reactions. Transient "intestinal flu" in patient #2.

Sponsor's conclusions. No effect of drug.

Reviewer's conclusions. Concur.

Q. Study #26. [Johns Hopkins Med. Jour. 151, 196, 1982]

Investigator:

H.W. Moser, M.D.  
J.F. Kennedy Inst. for Handicapped Children  
Balt., MD 21205

Study Design. Open study in two patients with adrenoleukodystrophy; #1, 18 yo M; #2, 5 yo M. Patients received dose of 1980 mg po qd (plus clofibrate).

Results.

Global evaluation. No effect of drug.

Clinical impression. No effect of drug.

Sponsor's conclusions. Drug ineffective for two patients with adrenoleukodystrophy.

Reviewer's conclusions. Concur.

R. Study #27.

Investigator:

C.A. Stanley, M.D.  
Division of Endocrinology/Diabetes  
Children's Hosp. of Phila.  
Phila., PA 19104

Study Design. Open study to assess the safety and efficacy of L-carnitine in the treatment of either long-chain acyl-CoA dehydrogenase or medium-chain acyl-CoA dehydrogenase deficiency. Patients received approximately 100 mg/kg drug po qd.

Results.

Global evaluation. No effect of drug in the 3 patients with medium chain acyl-CoA dehydrogenase deficiency. Improved the condition of the patient with long-chain acyl-CoA dehydrogenase.

Clinical impression. The patient with long-chain acyl-CoA dehydrogenase deficiency was treated with L-carnitine and special diet. There was gradual improvement over 8-12 weeks. Heart size was reduced to upper normal level and muscle strength was much improved. ? How much is relative contribution of diet vs. drug.

Adverse reactions. None reported.

Sponsor's conclusions. Drug and diet treatment resulted in improvement in one patient with long-chain acyl-CoA dehydrogenase deficiency. Three patients with medium-chain acyl-CoA dehydrogenase were unresponsive to treatment.

Reviewer's conclusions. Concur, though as noted, it is difficult to know the relative contribution of drug vs. diet in the responsive patient.

S. Study #28.

Investigator:

J. Patrick, M.D.  
Children's Hospital of Eastern Ontario  
Ottawa, Canada

Study Design. Open study of L-carnitine in two patients, one (#1) with "hepatic malfunction" (1.5 yo F), the other (#2) with Hirschsprung's disease of long segment/short bowel syndrome/jaundice (1 yo M). Patients received 350-660 mg po qd of drug.

Results.

Global impression. In patient #1, drug was life-saving; in patient #2, treatment improved patient's condition.

Clinical impression. Patient #1: in response to treatment, patient lost fetor hepaticus, became more alert, and began taking solid foods. Also, she tolerated high protein, high carbohydrates, low fat intake, and had decreased hypotonia and improvement in sitting, balance and equilibrium reaction. Increase in muscle bulk and strength was observed and ketogenesis was increased. Patient #2: was in pre-morbid state before treatment; patient expired approx. 3 weeks after initiation of drug.

Objective. In #1, treatment increased both total and free serum L-carnitine. After 1 yr of treatment, total serum level was increased from 20.0 to 56.3 nmol/L while the free level was increased from 12.2 to 51.1.

Adverse reactions. Patient #1 experienced vomiting and increased seizures (? related to drug). #2 experienced respiratory distress, hepatic failure and expired after approx. 3 weeks on drug.

Sponsor's conclusions. Treatment with drug was life-saving to one patient with hepatic malfunction/carnitine deficiency.

Reviewer's conclusions. Concur.

T. Study # 29.

Investigator:

J. DiLiberti, M.D.  
Emanuel Hosp.  
Portland, OR 97227

J.R. Schimschock, M.D.  
2525 N.W. Lovejoy  
Portland, OR 97210

Study Design. Open study of L-carnitine in two patients with systemic CDS; #1 an 11 mo old F, #2 a 7 yo F. #1 received doses of 990 mg po qd, #2 1980 mg po qd.

Results.

Global evaluation. Treatment dramatically improved both patients.

Clinical impression. #1 seems less prone to decompensations, infectious and metabolic, since beginning drug. #2 is neurologically and socially improved.

Sponsor's conclusions. Treatment was beneficial to both patients with systemic CDS.

Reviewer's conclusions. Concur.

U. Study #30. [Lancet 6/19/82, pp. 1411-2]

Investigator:

C. Roe, M.D.  
Divison of Peds. Metab.  
Duke Univ. Med. Ctr.  
Durham, NC 27710

Study Design. Open study of one patient, a 6.5 mo old M, with propionicacidemia (propionyl CoA carboxylase deficiency). Patient was treated with po drug, 100mg/kg for 8 months; dose was then reduced to 50 mg/kg.

Results.

Global evaluation. Drug dramatically improved patient's condition.

Clinical impression. Markedly improved performance on Denver Development Test and increased muscle strength.

Adverse reactions. Transient diarrhea, possibly related to drug; intermittent hyperammonemia, unlikely to be related to drug; apneic episodes, unlikely to be related to drug.

Sponsor's conclusions. Drug was beneficial to this patient with propionicacidemia.

Reviewer's conclusions. Concur.

V. Study #31. [Peds. Res. 15, 633, 1981]

Investigator:

C.L. Hoppe<sup>1</sup>, M.D.  
Division of Clin. Pharm.  
V.A. Med. Ctr.  
Cleveland, O 44106

Study Design. Open study of L-carnitine in two patients with systemic CDS; #1 was a 14 yo F, #2 a 9 mo. M. #1 received 30-125 mg po qd; #2 received 100 mg/kg/d.

Results.

Global evaluation. Drug was life saving to patient #1 and improved the condition of #2.

Clinical impression. Treatment reportedly greatly improved skeletal muscle and hepatic and cardiac function in #1. In #2, no recurrence of pre-treatment acute problems, including hypotonia, weakness, hepatomegaly, and hyperglycemia.

Objective. In #1, plasma levels of L-carnitine were increased from 2 to 3<sup>1</sup> after treatment.

Adverse reactions. In patient #2, transient moderate diarrhea and pneumonia were reported; felt only remotely likely to be related to drug by investigator.

Sponsor's conclusions. L-carnitine was life-saving in one patient, beneficial to the other.

Reviewer's conclusions. Concur: Also, I have a higher index of suspicion than the investigator that the diarrhea in #2 was drug-related.

X. Study # 32.

Investigator:

C. Imbus, M.D.  
Rancho Los Amigos Hosp.  
Downey, CA 90242

Study Design. Open study in a single 5.5 yo M patient with myopathic CDS. Patient received total po dose of 1650-1900 mg of drug.

Results.

Global evaluation. Drug improved patient's condition.

Clinical impression. Investigator reports that patient has fewer adventitial movements and experienced remarkable weight gain.

Adverse reactions. Treatment with drug was begun in 12/81. Severe diarrhea was reported the same month. The diarrhea subsided after the dose of L-carnitine was reduced from 1980 mg to 1650 mg qd. Dose was later increased to 1980 mg with recurrence of diarrhea. In 3/82, fever vomiting, coughing and draining of ears was reported. Vomiting was reported in 1/82. The investigator does not indicate his index of suspicion about drug-relatedness.

Sponsor's conclusions. Drug was beneficial to this single patient with myopathic CDS.

Reviewer's conclusions. Concur. It appears that the diarrhea reported is drug-related.

- Y. Safety Considerations. Of the 66 patients presented in the clinical protocols of the NDA, for 37 there was reported one or more adverse reaction. The overwhelming majority of those were in two categories: (1) GI symptoms, usually mild-moderate diarrhea and; (2) "fishy" body odor. In no case reported did either of these constitute or cause major morbidity or mortality, and symptoms were often diminished or eliminated by a reduction in dose.

Seven deaths occurred in patients in the various protocols, with cause of death listed variously as cardiorespiratory distress/progressive hepatic failure (1), pneumonia (1), hypotension and hypoventilation (1), respiratory arrest/failure (4). In all cases, the relationship of the patient's demise to drug administration was felt by the respective investigators to be remote; in most cases, the patients were extremely ill when therapy was begun. The age range in these patients was 14 mo to 71 years; likewise, there appeared nothing systematic in their diagnoses or dosage of drug.

In summary, safety of this natural product in the patient population for whom it is intended and at the dosages administered (up to 15 g/d) appears to be demonstrated. Adverse reactions were common, consisting overwhelmingly of GI symptoms (approximately 30-40% of patients) or fishy body odor (approximately 10%), but these tended to be transient and mild.

- Z. Efficacy Considerations.

The patients included in the various studies include numerous diagnoses, many of them extremely rare, as shown on the next page:

Results.

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Clinical impression. Investigator reports that patient has fewer adventitial movements and experienced remarkable weight gain.

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Sponsor's conclusions. Drug was beneficial to this single patient with myopathic CDS.

Reviewer's conclusions. Concur. It appears that the diarrhea reported is drug-related.

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In summary, safety of this natural product in the patient population for whom it is intended and at the dosages administered (up to 1g/d) appears to be demonstrated. Adverse reactions were common, consisting overwhelmingly of GI symptoms (approximately 30-40% of patients) or fishy body odor (approximately 10%), but these tended to be transient and mild.

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INDICATION	NUMBER OF PATIENTS
Systemic carnitine deficiency	16
Myopathic carnitine deficiency	9
Duchenne Muscular Dystrophy	8
Lipid storage myopathy	7
Ragged Red Fiber Disease	5
Mitochondrial myopathy	2
Adrenal Leukodystrophy	2
Medium chain Acyl CoA dehydrogenase deficiency	3
Long chain Acyl CoA dehydrogenase deficiency	1
Propionyl CoA carboxylase deficiency	1
Motor Neuron Disease	1
Oculopharyngeal Muscular Dystrophy	1
Vacuolar myopathy	2
Carnitine responsive muscle fatigue and cramps	1
Phosphorylase deficiency	1
Myophosphorylase deficiency	1
Cardiac myopathy	1
Leigh's Encephalopathy	1
Kwashiorkor	1
Hepatic malfunction	1
Hirschsprung's Disease	1
TOTAL	<u>66</u>

Below is shown a tabulation of the investigators' global assessments of all patients, arranged by diagnosis; these are the pivotal data on which efficacy can be judged:

GLOBAL ASSESSMENT

INDICATION	LIFE-SAVING	DRAMATIC IMPROVEMENT	IMPROVEMENT	NO ALTERATION	WORSENING	NO RATING
systemic carnitine deficiency	4	5	3	2	0	2
hepatic carnitine deficiency	1	1	5	2	0	0
lipid storage myopathy	0	2	1	4	0	0
oxid Red Fiber Disease	0	0	1	2	0	2
mitochondrial myopathy	0	0	0	1	0	1
galactose leukodystrophy	0	0	0	2	0	0
long chain Acyl CoA dehydrogenase deficiency	0	0	0	3	0	0
medium chain Acyl CoA dehydrogenase deficiency	0	0	1	0	0	0
propionyl CoA carboxylase deficiency	0	1	0	0	0	0
Infantile Neuronal Disease	0	0	0	1	0	0
Bonne Muscular Dystrophy	0	0	0	4	0	4
laryngeal Muscular Dystrophy	0	0	0	0	0	1
ocular myopathy	0	0	0	1	0	1
histamine responsive muscle fatigue and cramps	0	0	1	0	0	0
phosphorylase deficiency	0	0	0	0	0	1
phosphorylase deficiency	0	0	0	1	0	0
disiac myopathy	0	0	0	0	0	1
juvenile Encephalopathy	0	0	0	0	0	1
shierker	0	0	1	0	0	0
mitochondrial malfunction	1	0	0	0	0	0
Scheppung Disease	0	0	1	0	0	0

00270

It is evident that L-carnitine appears to have been consistently effective only for patients with diagnoses of systemic carnitine deficiency (improvement, dramatic effect or lifesaving in 12/14, or 85% of evaluated patients) and myopathic carnitine deficiency (improvement, dramatic effect or lifesaving in 7/9, or 78%, of patients. For patients with other diagnoses, the numbers are too small to draw meaningful conclusions about efficacy, especially since in many cases the precise diagnosis itself is likely to be in doubt.

## VI. CONCLUSIONS

- A. Scientific. The difficulties inherent in evaluating studies such as those submitted to this NDA for a determination of efficacy are obvious: there is a paucity of objective data; the total numbers of patients and number from any one institution are small; the diagnoses are somewhat vague both biochemically and clinically. There are, however, two considerations that are critical. First, there are the consistent judgements of experienced academic and other clinical investigators that the drug improves, often dramatically, sometimes in a lifesaving way, patients' clinical status. Second, many of the patients with the diagnoses for which efficacy appears to have been demonstrated (systemic and myopathic carnitine deficiency, respectively) are extremely ill, moribund or even in extremis, and no consistent, significant alternative therapy exists. And last, L-carnitine appears to be quite safe in this patient population in the range of dosages administered.

It should be noted that the evidence for efficacy in myopathic CDS is less compelling than that for systemic CDS. Five of the 7 patients reported in the NDA with myopathic CDS who improved on carnitine are from a single study, #13, and four of these were treated with glucocorticoids during at least part of the time of administration of carnitine. This makes interpretation of the effect of carnitine alone difficult; on the other hand, the principal investigator for this study is quite experienced and well-versed in the nuances of myopathic CDS (as is evidenced by the large number of patients he has accumulated).

In summary, data from the studies described herein are adequate to support the safety and effectiveness of L-carnitine in the treatment of systemic carnitine deficiency or myopathic carnitine deficiency.

- B. Regulatory. This NDA should be approved.

VII. PATIENT INFORMATION INSERT

The draft insert is approvable with the following corrections:

1. Under Indications and Usage on p.1, the sentence should read, ". . . in the treatment of systemic deficiency or myopathic deficiency of L-carnitine."
2. Under Dosage and Administration on p. 1, it should be noted that decreasing the dosage often diminishes or eliminates drug-related patient body odor or gastrointestinal symptoms when present.
3. Under Dosage and Administration, Infants and children on p.1, "oral" is misspelled.

H.I. Miller, M.D.

cc:

NDA Orig.  
HFN-180  
HFN-130  
HFN-130/HMiller/0133C

PHARM. REVIEW

NDA 18-948

April 22, 1985

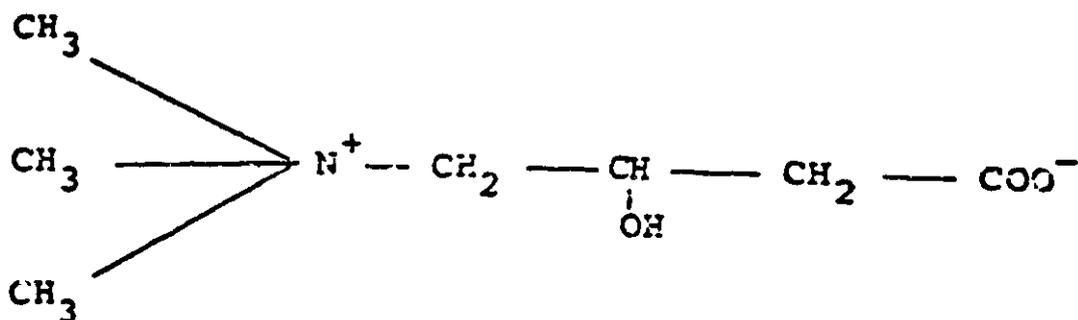
SIGMA-TAU, Inc.  
North Beer Street  
Holmdel, New Jersey 07733

Original Submission Date: February 8, 1983  
Amendment: (3.1) June 27, 1983  
Withdrawn October 23, 1983.  
Resubmitted: August 13, 1984

Pharmacologist Review and Evaluation of Pharmacology and Toxicology Data

Drug: L-Carnitine Tablets: The drug substance is the synthetic form of natural L-carnitine.

Chemical Formula: L-beta-hydroxy-trimethylammonium butyric acid inner salt



Source of Drug: Sigma-Tau Industrie Farmaceutiche Riunite Spa, Rome, Italy.

Proposed Clinical Use: The correction of systemic carnitine deficiencies.

Background Information: The original submission of the NDA contained a review by the sponsor of some 20 publications dealing with the pharmacological functions and the toxicological aspect of carnitine, and reports from toxicity tests performed by

These were:

- . acute tests in mice and rats,
- . subacute tests in rabbits, one with I.V. doses of d,l-carnitine for 24 days, and one with l-carnitine I.V. for 30 days
- . chronic tests: one in rats with l-carnitine administered I.V. for 180 days and one in dogs with l-carnitine administered intramuscularly for 180 days.
- . Reproduction/Teratogenicity tests in rats and rabbits with I.V. and I.M. administration.
- . Mutagenicity tests (Ames) in rats

MAY 8 1985

While the review by the sponsor and the reports of the preclinical test furnished a considerable amount of information on the drug's properties they did not fulfill the requirements of the Agency to an extent to be considered acceptable for the support of the NDA, principally by the technical considerations of the submitted preclinical tests, such as the small numbers of animals used in them, the inappropriate routes of drug administration not applicable for the intended clinical usage, and the inadequate duration of drug administration applied in these tests.

Consequently the sponsor was informed that the then submitted preclinical test results could not be accepted, and he was notified on June 17, 1983 that chronic tests in dogs and rats of 12 months duration had to be performed according to FDA Guidelines, also reproduction/teratogenicity tests in rats and rabbits.

In a later telephone conversation between Dr. Klein of Sigma-Tau and Dr. Sobel, Dr. Klein was informed that the teratogenicity and carcinogenicity tests could be deferred and perhaps waived, and that long-range studies were not required for this drug, because of its nature and proposed clinical usage for correction of carnitine deficiencies.

Studies for the pharmacological characterization of l-carnitine to be conducted by or for the sponsor also were not requested by us because it was felt that the voluminous amount of publications covering clinical and animal pharmacological aspects obtained by intensive modern investigations in the last decade would provide sufficient information for the description of these functions of carnitine and would serve as support for the NDA. A collection of over 40 publications, 29 of them dealing with tests in animals published in 1982 was submitted in the amendment of August 4, 1983, and 4 more volumes with publications were submitted in the amendment of December 27, 1984.

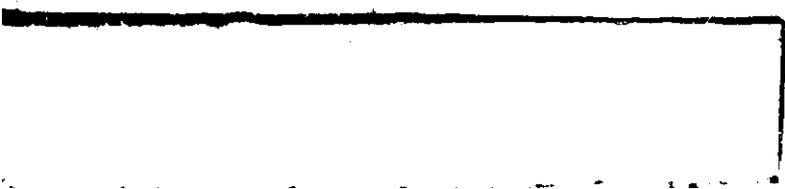
#### Pharmacology of L-Carnitine:

The study of the voluminous material submitted in the NDA and its amendments was facilitated by the fact that modern text books on biochemistry do contain detailed descriptions on carnitine, its biosynthesis, functions in lipid metabolism. To mention a few:

"Biochemistry, The Molecular Basis of Cell Structure and Function", by Albert Lehninger, The John Hopkins University, Worth Publishers Inc. Second Edition, 1975.

"Principles of Biochemistry" by Abraham White, Distinguished Scientist, Syntex Research, et al., McGraw-Hill Book Company, Fifth edition 1973.

.....



The majority of the publications are based on the function of carnitine in the transfer of long-chain fatty acids into the mitochondria where they are oxidized to serve as a source of energy.

Carnitine is synthesized, under normal condition, from two essential aminoacids, lysine and methionine, with additional functional contribution from ascorbate, niacin and B<sub>6</sub>. The intermediate precursor form of carnitine, gamma-butyrobetaine, is hydroxylated by enzymatic actions into carnitine. Carnitine acts as the carrier for fatty acyl groups from the cytoplasm into mitochondria where the enzyme acetyl carnitine transferase located on the external surface of the inner membrane catalyses the conversion of the cytoplasmic long-chain acyl CoA and carnitine into acylcarnitine. The acylcarnitine is reconverted to intramitochondrial acyl-CoA by the action of carnitine palmitoyl transferase located in the inner membrane. The acyl-CoA is now available for beta-oxidation in the matrix.

A more detailed description of these processes by their steps-by-step progression cited is given in the textbook "Biochemistry," of A. Lehninger on p. 546-547, quoted here "in toto":

#### "Activation of Entry of Fatty Acids into Mitochondria

There are three stage in the entry of fatty acids into mitochondria from extramitochondrial cytoplasm:

- (1) the enzymatic ATP-driven esterification of the free fatty acid with extramitochondrial CoA to yield fatty acyl-CoA, a step often referred to as the activation of the fatty acid,
- (2) the transfer of the acyl group from the fatty acyl-CoA to the carrier molecule carnitine followed by the transport of the acyl carnitine across the inner membrane and
- (3) the transfer of the acyl group from the fatty acyl carnitine to intramitochondrial CoA which occurs on the inner surface of the inner membrane.

#### Activation of Fatty Acids:

At least three different enzymes catalyse formation of acyl-CoA thioesters each being specific for a given range of fatty acid chain length. These enzymes are called acyl-CoA synthetases. Acetyl-CoA synthetase activates acetic, propionic and acrylic acids; medium-chain acyl-CoA synthetase activates fatty acids with 4 to 12 carbon atoms, and long-chain acyl-CoA synthetase activates fatty acids with 12 to 22 or more carbon atoms. The last two enzymes activate both saturated and unsaturated fatty acids as well as 2- and 3-hydroxy acids.

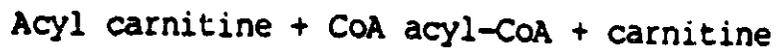
Transfer of Carnitine:

Long-chain saturated fatty acids have only a limited ability to cross the inner membrane as CoA thioesters but their entry is greatly stimulated by carnitine. This substance was long known to be present in animal tissue but its importance went unrecognized until it was found to be an essential growth factor for the mealworm *Tenebrio Molitor*.

I. B. Fritz and others showed that the stimulation of fatty acid oxidation by carnitine is due to the action of the enzyme carnitine acetyltransferase which catalyzes the transfer of the fatty acyl group from its thioester linkage with CoA to an oxygen-ester linkage with the hydroxyl group of carnitine. The acyl carnitine ester so formed then passes through the inner membrane into the matrix, presumably via a specific transport system.

Transfer to Intramitochondrial CoA:

In the last stage of the entry process the acyl group is transferred from carnitine to mitochondrial CoA by the action of a secondary type of carnitine acyltransferase located on the inner surface of the inner membrane:



This complex entry mechanism, often called the fatty acid shuttle has the effect of keeping the extramitochondrial and intramitochondrial pools of CoA and of fatty acids separated. The intramitochondrial fatty acid - CoA now becomes the substrate of the fatty acid oxidation system which is situated in the inner matrix compartment.

In a second pathway, the acetyl group of acetyl-CoA is enzymatically transferred to carnitine which acts as the carrier of fatty acids into mitochondria preparatory to their oxidation. Acetylcarnitine passes from the mitochondrial matrix through the mitochondrial membrane into cytosol and acetyl-CoA is then regenerated to transfer of the acetyl group from acetylcarnitine to cytosol CoA."

While the majority of the submitted publications deals with actions of the long-chain carnitine acyltransferases which according to several investigations are localized to mitochondria in liver, heart, kidney and skeletal muscle, some interest exists now also for the short - and intermediate chain CAT and carnitine acetyltransferase activities that have been demonstrated to be present not only in mitochondria but also in peroxisomes and microsomes in the liver (Ch. Hoppel, "Carnitine and carnitine palmitoyltransferase in fatty acid oxidation and ketosis", in Federation Proceedings vol 41, no. 12, 1982, quoting from Markwell et al: "The subcellular distribution of carnitine acyltransferases in mammalian liver and kidney. A new peroxisomal enzyme". J. Biol. Chem. 248:3426, 1973).

The issue of the occurrence of short - and medium chain CATs in peroxisomes was treated by Bieber and co-workers in the article "Possible functions of short-chain and medium-chain carnitine acyltransferases", in Federation Proceedings 41:2858, 1982. These investigators stated that they had demonstrated both short - and medium-chain CAT activity in microsomes, peroxisomes and mitochondria in pig and rat liver. They made reference to a report by Lazarow and DeDuve "A fatty acyl-CoA oxidizing system in rat liver peroxisomes: enhancement by clofibrate, a hypolipidemic drug", in Proc. Natl. Acad. Sci. USA 73:2043, 1976. They postulated that the enzymes in peroxisomes are active in the shuttling of beta-oxidation chain-shortened products out of the peroxisomes into the liver, and for possible other functions.

The action of clofibrate on the functions of peroxisomes in rat livers was also described by Lund and Bremer in their publication "Carnitine Acyl Transferase -- Effect of malonyl-CoA, Fasting and Clofibrate feeding in Mitochondria from Different Tissues" in Biochemica et Biophysica Acta 750:164, 1983. According to this study, clofibrate increased the carnitine acyltransferase activity of intact peroxisomes in a manner different from that resulting from fasting, and their conclusion was that clofibrate feeding showed a preferential increase in activity on the medium - and long-chain substrates. The authors also noted the significant increase in the size of the livers caused by clofibrate but unfortunately they did not attempt to correlate this phenomenon to the function of clofibrate in any direction. The authors were biochemists, not pathologists.

The effect of clofibrate was investigated also by Small et al., reported in "Localization of Carnitine Acyltransferases and Acyl - beta Oxidation enzymes in Small Intestinal Microperoxisome of Normal and Clofibrate Treated Mice," in Biochemistry International vol. 7 no.2, 1983. This study was conducted at the University of Manchester, Department of Biochemical Sciences. The authors stated that in the rat, mouse and human liver, fatty acid is oxidized in two subcellular sites, the mitochondria and the peroxisomes. The first enzyme of the peroxisomal fatty acid oxidation system is an  $H_2O_2$  producing acyl-CoA oxidase whose activity is greatly increased in rats and mice by hypolipidemic drugs (clofibrate). The possibility was raised by the authors that the intestinal peroxisomes might modify the chain length of luminal fatty acids that subsequently are esterified and released into the blood stream as chylomicra. The close distribution of the peroxisomes to endoplasmic reticulum was brought up at the very end but not further elucidated with respect to the functions of the endoplasmic reticulum.

Another article dealing with the functions of the peroxisomes and their regulation is that by Debeer and Mannerts of the University of Leuven, Belgium ("The Mitochondrial and Peroxisomal Pathways of Fatty Acid Oxidation in Rat Livers", in *Diabete and Metabolisme* (Paris) vol. 9:134-140, 1983) that need mentioning because it described and debated the action of malonyl-CoA as modulator of the carnitine-acetyltransferase system, and of the processes in peroxisomal fatty acid oxidation. It is pointed out that until recently, long-chain fatty acid oxidation in liver was considered to occur exclusively in mitochondria and "its regulation was generally considered to be inversely related to the regulation of triglycerol synthesis," but that it is now recognized that peroxisomes are also capable of oxidizing long-chain fatty acids, while inactive towards fatty acids shorter than octanoic acid. This article is cited here in this review mainly because it too addresses the action of clofibrate and of other chemicals in increasing peroxisomal beta-oxidation that also share the property of causing peroxisomal proliferation and lowering serum triglyceride levels in circulation. The authors concluded that the contributions from peroxisomes to fatty acid oxidation is minor compared to that from mitochondria but it is not clear from their discussions whether the action of clofibrate and related substances enhances the contribution rate and by what mechanisms it causes the histological proliferation of hepatocytes. The investigations by Farrel and Bieber in the same area, of the properties and effects of hypolipidemic drugs on mouse liver peroxisomes ("Carnitine Octanoyl Transferase of Mouse Liver Peroxisomes" in *Arch. Biochem. & Biophys.* 222:123-132, 1983) arrived at the conclusion, among others in this very complex report, that peroxisomes from drug-treated mice were broken at a higher rate than those of controls (60% vs 20%) indicating that hypolipidemic drugs enhance peroxisomal membrane fragility. The drugs used in these trials were clofibrate, nafenopin and WY-14,643.

The matter of the peroxisomes and their responses to hypolipidemic drugs is of interest beyond that of their contributions to lipid metabolism because the now emerging experience in the cited publications appears to give a new picture and concepts for the actions of the hypolipidemic drugs even of different chemical nature, as peroxisome proliferators and their tumorigenic properties that may need a complete revision for their significance.

A very interesting publication for a specific action of carnitine is that by Casillas and co-workers, at the Department of Chemistry, New Mexico State University entitled "Carnitine content of rabbit epididymal spermatozoa in organ culture", in *Jour. Reprod. Fert.* 65:247, 1982. After reporting previous investigations by other workers, starting with that of Marquis and Fritz in 1965 in rats, they report their own with bull, ram and rabbit spermatozoa describing a very high concentration of carnitine in the epididymis and

demonstrating the role of carnitine in the maturation process of spermatozoa during their passage through the epididymis. The presence of carnitine in the epididymis is established, and that its production is under the regulation by testosterone. In the present study, the presence and action of carnitine in different sections of the epididymis was investigated by an in-vitro set-up using single epididymal tubules. This action of testosterone was abolished by addition of cyproterone to the culture medium. Carnitine was taken up by spermatozoa in tubules taken from the caput but not from the caudal section (probably because the caudal spermatozoa have completed the maturation process and are not needing the stimulus from carnitine). (It has been known for a long time that sperm undergo a maturation process during their passage through the epididymis that resulted in initiation of their capacity to swim and to become capable of fertilization (capacitation). This action was correctly ascribed to mitochondria located at the base of spermatozoa.) The publication by Huckle and Tamblin confirms the findings of the cited investigators and expands the knowledge of carnitine functions on sperm ("Purification and Properties of carnitine acetyltransferases from bovine spermatozoa and heart" in Archives of Biochemistry and Biophysics vol. 226, no. 1:94,1983).

For a corresponding situation in the female it was found by Costa and Stevenson that concentrations of carnitine in the ovary of rats and of acetylcarnitine increased 3-fold after gonadotropin stimulation, and in normally ovulating ovaries during the periods of rapid steroidogenesis in the luteal phase. ("Changes in Coenzyme A and carnitine concentration in superovulated rats". BBA 792:130,1984). The carnitine appears to be produced in peroxisomes located in ovarian tissue.

#### Other Investigations in Animal Models:

Other publications among the submitted material describing a wide variety of investigations in animals are not reviewed in this review because they concerned mostly specific phases of carnitine functions with limited significance for the fundamental analysis of the pharmacological spectrum of carnitine, and those with clinical aspects, were greatly overshadowed by the large volume of similar investigations already performed in human. Their omission is justified, and also necessitated by existing circumstances, also for the sake of conserving space in the review document, and time of the reviewer. For eventual later utilization and review, the following publications are cited here, all by F. P. Bell, from Diabetes-Atherosclerosis Research, The Upjohn Company and his co-authors from the staffs of several Medical Universities. All these publications were submitted in the material furnished by Sigma-Tau:

Carnitine metabolism in *Macaca arctoides*: the effects of dietary change and fasting on serum triglycerides, unesterified carnitine, esterified (acyl) carnitine and beta-hydroxybutyrate, in Am. J. Clin. Nutr. 36:115-121,1982.

The Effect of Diet on Plasma Carnitine, Triglycerides, Cholesterol and Arterial Carnitine Levels in Cynomolgus Monkeys. *Comp. Biochem. Physiol.* 75 B: 211-215, 1983.

Plasma and Liver Carnitine (Free and Esterified) Levels and their Interrelationships in Moderately Hypercholesterolemic Monkeys (*Macaca arctoides*). *Can. J. Biochem. Cell Biol.* 61:328-332, 1983.

Carnitine Esters: Novel Inhibitors of Plasma Lecithin: Cholesterol Acyltransferase in Experimental Animals But Not in Man (*Homo Sapiens*). *Int. J. biochem.* 15:133-136, 1983.

The last cited article of Bell et al. states among other facets that carnitine esters possess surface-active properties at certain concentrations and cites for this issue the publication by Cho and Proulx on "Studies on mechanisms of hemolysis by acyl carnitines, lecithins and acyl-cholines", *Biochem. Biophys. Acta* 225:214, 1971.

#### CONCLUSION FOR THE PHARMACOLOGY PART

The material for the description of the pharmacology properties of carnitine differs from that which usually is submitted for a new drug substance when original investigations conducted with that drug are presented. This approach was not requested for carnitine in view of the long history of its existence both, as a nutrient, and for clinical utilization for correction of deficiency induced myopathies by other pathological conditions. It is more important that the submitted literature is of recent vintage and deals in depth with the complex mechanism of the function of the drug as a carrier, in collaboration from the now known enzyme systems, of fatty acids, mostly of the long-chain type, into the site of their oxidation, the mitochondria. This phenomenon is now firmly and unequivocally established, even though admittedly there probably will be new discoveries forthcoming. Among these one can expect additional knowledge on the roles of the peroxisomes, and very likely also of the endoplasmic reticulum.

But for the consideration of the safety of carnitine in its planned clinical use, and for the fulfilment of the requirements for adequate pharmacological data, it appears reasonable and justified to consider the furnished and reviewed information as satisfactory, from the standpoint of Pharmacology, to serve this purpose.

#### TOXICOLOGY:

The supplement to the NDA of November 9, 1984, vol. 6.1 contains the reports entitled:

"L-Carnitine 52 Weeks Oral Toxicity Study in Sprague-Dawley Rats" and

"L-Carnitine Oral Toxicity Study in Beagle Dogs - Repeated Daily Dosage for 52 Weeks."

These are the two toxicity studies that were requested by us for the support of the NDA.

52-Weeks Oral Toxicity Study in Sprague-Dawley Rats:

The report for this study is in the amendment Vol. 6.1 in the submission of November 9, 1984 of NDA 18-948.

This investigation was conducted by

Manager In the foreword to the report, the General  
institute is authorized by the makes the statement that the  
toxicological studies on pharmaceutical specialties and that the reported  
study was conducted on behalf of Sigma Tau, Pomezia, Rome, Italy.

A declaration by the management is submitted attesting that the study was conducted in compliance with the GLP Regulations, together with a Quality Assurance Statement with a schedule of performed inspections, and a list of Scientists involved in this study.

Study Plan and Methodology:

Test Animals: Charles River CD (SD) BR rats  
30 males and 30 females per dose group weighing at start of treatment, males 230-233 gms., females 163-165 gms.

Dosage: administered in the diet daily for 52 consecutive weeks.

Group 1 control  
Group 2 150 mg/kg/day  
Group 3 450 mg/kg/day  
Group 4 1350 mg/kg/day

The concentrations of the drug in the diet were established each week from the weekly body weights, and the drug intake was calculated from the daily intake of the diet established by the consumed portion of the daily offered feed dose.

An interim sacrifice of 5 animals/sex/group was performed at completion of week 13 of treatment, with a complete work-up.

At the end of week 52, 5 animals/sex/group were selected to continue without treatment for 4 weeks to study after-effects, or recovery from induced effects respectively, with sacrifice of these animals at week 57. The remaining 20 animals/sex/group were sacrificed after completion of 52 weeks of treatment.

Results:

Clinical Observations:

No mortality was reported.

Fecal changes: No changes in fecal consistency were reported (dissimilar to the liquid feces noted in the study with dogs)

Behavior: No variations in treated animals from that of controls. The only anomaly noted was the occurrence of an abnormal, excessive growth of incisor teeth more frequent in males than in females, and in males its occurrence appeared to be dose related: 2 in controls, 3 in group 2, 2 in group 3, 4 in group 4. In females the incidence was one in controls and group 2, 2 in group 3 but none in group 4.

Body Weight Gains:

The enclosed graphs and tabulations for this parameter yield an interesting picture for the action of the drug. The tables are a condensation made by me of the data obtained weekly by presenting, for brevity sake, only the weights obtained at critical points of the study, namely before initiation of treatment, then at week 13 (at the 3-months interim sacrifice) followed by week 26, and 52, at the terminal sacrifice, and weeks 54 and 57 for the 5 males and 5 females from each dose group on the recovery phase.

It is evident that the low dose had a greater growth stimulating effect in both sexes compared to controls, most distinct in females and less effective in males where it became stimulating over controls only in the last 8 weeks of treatment while in females it had induced greater gains than that of controls almost from the start, and continuing to the end of treatment. The mid-dose had in males the greatest stimulatory action, but in females it was less than that of the low dose.

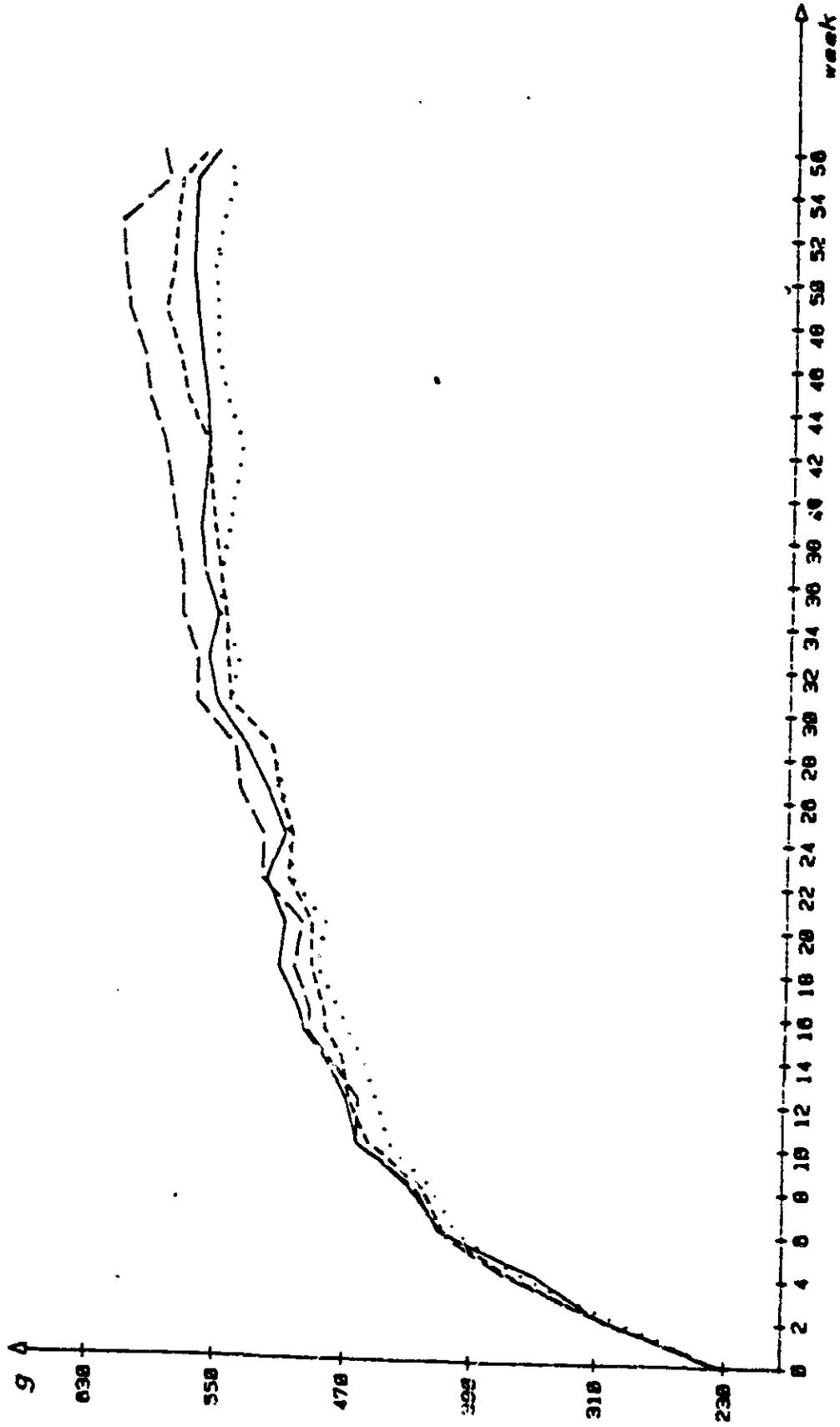
In contrast to this action by the low and mid-dose was the response to the high dose in both sexes where it reduced gains of body weights affecting to a greater extent the females than the males.

The beneficial action of the drug on weight gains was clearly demonstrated by the response to the withdrawal of the drug after week 52 when the by-then achieved body weights of the low and mid-dose groups of both sexes were not maintained but were actually reduced.

In the high-dose males a tendency for body weight loss was noticeable after week 50 and this tendency continued in the no-treatment period with a slight recovery and reversal setting in later in the no-treatment period (week 54). In the high-dose females a definite resumption of body weight gains set in immediately after removal of the drug from the diet.

# Body Weights of Male Rats

Fig. n. 1  
 Exp. 001032  
 Body weight      Malice (Rate)  
 Gr. 1                      C  
 Gr. 2                      150 mg/kg/d  
 Gr. 3                      450 mg/kg/d  
 Gr. 4                      1350 mg/kg/d



000039

# Body weights of MALE RATS

Table no. 1 (p1)

Exp. no. 001632

Body weight (g) : Mean  
±S.E.  
(N)

Males (Rats)

Week day	GR 1	GR 2	GR 3	GR 4
-2 -10	159.03 1.27 (30)	161.03 1.29 (30)	160.17 1.34 (30)	160.00 1.39 (30)
0	233.07 1.04 (30)	233.53 2.23 (30)	230.03 2.56 (30)	231.33 2.56 (30)
13 91	475.97 5.10 (30)	471.07 7.15 (30)	471.43 5.96 (30)	455.00 6.16 (30)
26 102	517.04 6.00 (25)	511.92 8.30 (25)	535.64 8.01 (25)	507.48 6.30 (25)
52 304	566.20 7.24 (25)	578.04 8.56 (25)	612.20 11.31 (25)	599.40 5.13 (25)
54 310	566.00 16.09 (5)	575.20 7.12 (5)	503.20 16.74 (5)	542.00 10.42 (5)
57 313	552.60 17.93 (5)	559.60 8.65 (5)	507.00 19.09 (5)	546.00 7.51 (5)

11-2-

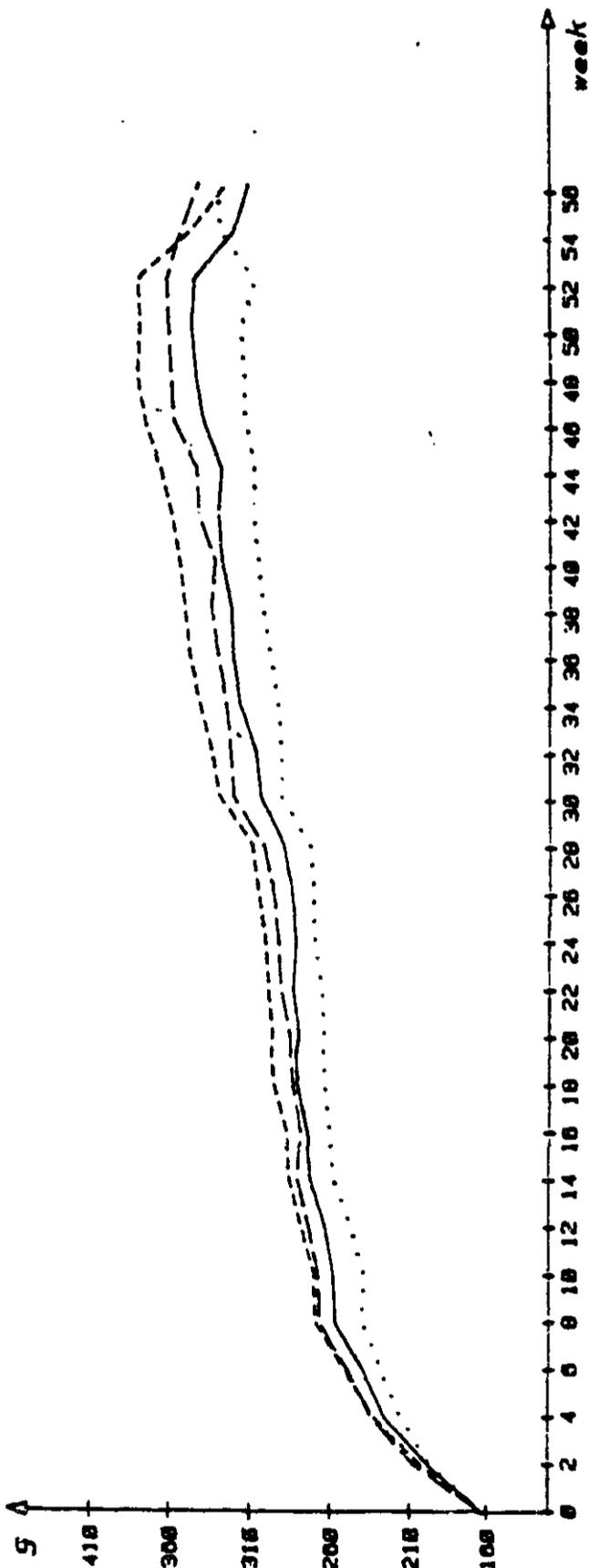
001632

# Body Weights of Female Rats

Fig. n. 2  
Exp. 01632

Body weight	Females (Rate)
Grp 1	2
Grp 2	1.50
Grp 3	4.50
Grp 4	13.50

(0000)10



# Body Weights of Female Rats.

-11-d-

Table no. 2(p.1)

Exp. no. 01632

Body weight (g) : Mean  
S.E.  
(N)

Females (Kois)

Week day	GR0 1	GR0 2	GR0 3	GR0 4
-2 -9	133.43 1.22 (30)	132.00 1.25 (30)	132.73 1.25 (30)	132.07 1.17 (30)
0	163.10 2.61 (30)	165.63 2.01 (30)	163.97 2.13 (30)	163.23 1.59 (30)
13 91	250.97 6.73 (30)	272.90 5.91 (30)	260.17 5.34 (30)	243.80 4.66 (30)
26 182	203.32 6.09 (25)	303.96 6.77 (25)	295.16 5.86 (25)	269.92 5.24 (25)
52 364	343.00 9.52 (25)	370.12 10.12 (25)	360.04 6.21 (25)	305.64 7.20 (25)
54 370	319.00 23.03 (5)	345.20 25.46 (5)	351.60 11.34 (5)	327.60 18.20 (5)
56 392	310.20 25.40 (5)	323.40 24.40 (5)	340.80 11.30 (5)	328.60 19.31 (5)

This apparently favorable picture for the drug's action on body weight gain is somewhat obscured by the observation that the control animals, especially the females, also showed a weight loss in the recovery period.

This problem can be eliminated by comparing the body weights of the treated animals achieved by the end of the treatment period (week 52) to the weights after the 4 weeks "recovery" period:

Males

Week	Controls	Low	Mid	High
52	566	578	612	549
57	552	559	587	546

Females

52	343	378	360	305
56	310	323	349	328

A decline in feed intake was noted in controls, low and mid dose animals during the 4 weeks recovery period, but in the high dose animals the feed intake was slightly elevated.

Hematology:

The values obtained for this parameter correspond rather well to the picture obtained from the body weight gain performance. The hematology values in males and females in the low and mid dose were unaffected throughout the entire treatment period and no differences resulted in the period without drug. Only in the high dose males appeared slight variations from mean normal values for erythrocytes, hemoglobin and hematocrit values, and by week 57 all these values were again at a normal range.

Blood Chemistry Tests:

Conducted for 18 parameters, their results can be interpreted to depict effects from the pharmacological properties of the drug and not indicative for any direct toxic action.

In female rats, the only deviations of significance from normal and control values were the changes in the mean triglyceride values (shown as mg/100 ml)

Week	Control	Low	Mid	High
26	77.25	87.25	94.5	73.5
52	103.0	119.1	117.1	87.12
57	69.4	66.4	87.2	102.6

The values for total cholesterol were considered to be not statistically significant to indicate a drug action, and are shown here only in an attempt by the reviewer to line them up with the action of the drug on triglycerides.

26	122.8	136.7	130.9	138.4
52	119.68	135.8	125.7	130.3
57	134.6	129.2	125.6	132.4

While the data for the triglycerides show a stimulatory action by the low and mid dose and the loss of this effect with withdrawal of the drug, the effect of the high dose by week 52 would indicate a suppressive action (perhaps by a negative feed back mechanism) that was removed in the no-treatment period from week 52 to week 57, but the data for total cholesterol would indicate that the drug had neither a stimulatory nor an inhibitory action on cholesterol metabolism, and the noted changes in the triglyceride levels might be results of the action of carnitine on the metabolism and transportation of fatty acids. It has to be remembered that the high triglyceride values occurred at the time when the body weight gains of the low-and-dose groups were high but that of high dose group low.

For a comparison, the mean triglyceride levels in males are shown in the next tabulation followed by a tabulation of their cholesterol values.

Triglyceride values in males:

<u>Week</u>	<u>Control</u>	<u>Low</u>	<u>Mid</u>	<u>High Doses</u>
26	94.5	101.8	110.24	72.7
52	130.0	164.4	180.4	121.2
57	103.6	118.6	143.4	88.7

The Cholesterol values in males:

<u>Week</u>	<u>Control</u>	<u>Low</u>	<u>Mid</u>
26	82.3	80.8	6.0 +
52	90.8	95.2	110.1
57	92.6	105.2	100.7

For the triglyceride values, the picture is essentially the same as in females; elevated in the low-and-mid dose group, reduced in the high dose group by week 52, with a drop in these values after drug withdrawal, with the difference that the high dose group showed a drop during the recovery period.

The data for the cholesterol levels show a difference from that seen in females in that they show an elevation during treatment from all doses, with no significant change in the recovery period. With respect to the bodyweight gains in relation to these two parameters, the mid-dose had caused the highest gains during treatment, lesser gains in the low-dose group, and reduction by the high-dose, followed by a drop-off in gains in the low and mid dose after drug withdrawal, but slight improvement in the high-dose group.

With respect to other parameters of blood chemistry, the males had a higher incidence order than females in several that however by their appearance during the treatment and disappearance in the recovery period did not give any indication for a direct toxic potential of the drug.

Among these, the increase of total bilirubin levels in all treatment groups was interpreted by the investigators to be caused by sporadic elevations in single animals but remaining within the normal (physiological) range. The SGOT levels decreased in a dose related order from 73.12 units in the low dose, to 70.4 in the mid-, and 59.4 units in the high-dose by week 52, hardly indicative for any toxic involvements, and SGPT values remained unaltered.

With these low orders of occurrence of deviations from normal and control levels, the events in the recovery period by necessity were unremarkable.

Urinalysis: Unremarkable.

Ophthalmology: Unremarkable

Organ Weights and their Histopathology at weeks 13 and 52:

Organs recovered at the interim sacrifice at week 13 of 5 animals/sex/group had weights in the normal range and were similar to that of controls, by absolute and relative values. Their histological structures did not reveal any derangements indicative for drug actions on them.

Similarly, the organ weights from the animals sacrificed at the end of treatment (week 52) did not reflect a distinct drug action because of the irregular occurrence of the noted differences in weights that also were of minor extent:

In Males:

The livers of animals from the low-and mid-dose groups were slightly heavier by their mean absolute weight than those of controls, and also those from the high dose animals, but their relative values were of the same (or similar) order for all 4 groups.

The kidneys of only the high dose group had a slight but statistically significant elevation of the mean relative weight value compared with the control values but not by their mean absolute weights.

The spleen weights were elevated by absolute mean values in the low and mid dose group, of not statistical significance and not shown by their relative values.

In Females:

A similar picture for organ weight changes (or their absence) prevails also for the females.

For the liver, a statistically significant elevation of its absolute weight was reported for the mid dose confirmed by the mean relative value. For the low dose, elevation of the absolute weight was similar to that of the mid-dose group but it was not considered statistically significant by the statistician. It is interesting that the absolute mean weight of the high dose group was actually lower than that of controls.

The kidneys showed a statistically significant elevated mean weight in the low dose group only; that of the high dose group was lower than that of the low and mid dose group by the absolute values and, statistically, significantly higher by its relative weigh value.

The spleen was slightly (but statistically significant) elevated in the mid dose by absolute and relative weight values.

Results of the recovery period to week 57:

The presentation by the sponsor of the organ weights of the animals that were continued in the recovery period did not show values with statistical significance when compared with the control values (by the Dunnett's test) but this mode of presentation does not show whether there was a change in organ weights resulting from drug withdrawal that would reveal a drug action effect. Therefore the following tabulation was prepared by the reviewer to provide a clearer picture for this action, (or its absence.)

In this tabulation, the body weights were included in order to bring out again the fact that the increased body weight gains of the low and mid-dose groups of both sexes appeared to indicate a favorable drug action, and to put this phenomenon into a perspective relationship to organ weights as an indicator for a drug action or its absence. Since the control groups had shown the somewhat enigmatic drop in body weights during the recovery period, the organ weights from the control groups can be disregarded, and make the comparison only between the values of week 52 and 57.

It can be seen that for the low and mid dose groups there was an opposite trend for the body weights and the liver weights, in that the elevated liver weights were time-wise related to the higher body weight gains of these groups while the reduced liver weights in the recovery period were associated with the reduced body weight gains (in the absence of the drug). Inversely, the low weight gain performance of the high-dose males occurred when their liver weights were lower than those of the low and mid-dose groups. In the females, an improvement of the body weight gains appeared in the high-dose during the recovery period, but the liver weights were unaltered.

From these events it would seem that the liver weights were not directly and adversely affected by the drug, and that whatever changes there were, could be connected with the pharmacological functions of the drug that for instance caused the elevation of plasma triglycerides.

The data for the spleen and kidney values do not indicate any drug effect on these organs judging by the absence of persistent differences between the week 52 and week 57 levels. The rare incidences of the slight differences resulting from statistical significance analyses are considered to be incidental, as for instance the elevated mean value of the spleen of the mid-dose females that was attributed by the investigators to one single case of a very enlarged organ described as having extended areas of sclerosis of incidental origine found in female 152 which also had a mammary gland adenocarcinoma.

Organ Weights at Week 52 and 57 (gms) of Male and Female Rats

I. Males

Week	Organ	Control	Low	Mid	High
52	<u>Bodyweight</u>	541	555	584	518
57		534	535	549	516
52	<u>Liver abs.</u>	11.08	13.23	13.24	11.85
		rel.%	2.20	2.38	2.27
57	<u>Liver abs.</u>	11.49	11.39	12.39	11.73
		rel.%	2.15	2.13	2.25
52	<u>Kidney abs.</u>	3.20	3.38	3.38	3.32
		rel.%	.59	.61	.58
57	<u>Kidney abs.</u>	3.05	3.26	3.29	3.32
		rel.%	.57	.61	.60
52	<u>Spleen abs.</u>	.74	.84	.86	.78
		rel.%	.14	.15	.15
57	<u>Spleen abs.</u>	.70	.78	.77	.16
		rel.%	.13	.15	.14

II. Females

<u>Week</u>	<u>Organ</u>	<u>Control</u>	<u>Low</u>	<u>Mid</u>	<u>High</u>
52	Bodyweight	323	367	343	291
57	Bodyweight	298	308	324	314
52	Liver abs.	8.31	9.15	9.71 <sup>x</sup>	8.14
	rel. %	2.38	2.50	2.85 <sup>x</sup>	2.79
57	Liver abs.	8.13	6.98 <sup>x</sup>	7.60	8.35
	rel. %	2.75	2.29	2.34	2.66
52	Kidney abs.	2.12	2.40 <sup>x</sup>	2.28	2.24
	rel. %	.66	.66	.67	.77 <sup>xx</sup>
57	Kidney abs.	2.07	1.92	2.12	2.13
	rel. %	.78	.64	.65	.68
52	Spleen abs.	.46	.48	.69 <sup>x</sup>	.49
	rel. %	.14	.13	.21	.17
57	Spleen abs.	.45	.49	.48	.55
	rel. %	.15	.16	.15	.17

In his discussion of the observations for organ weight changes, the investigator cites as the only other organ found to have variations from normal values enlarged pituitaries but with weights that "fell within the normal range of physiological variability", found at term as one case in the control group, 3 in the low-dose group, 2 in mid-dose, and none in the high dose. All these were found by histopathology to be cases of pituitary adenomas, and additionally 2 low-dose and one high-dose female rat found after the recovery period. Their occurrence also in untreated animals, and their histology is not an indication for a carcinogenic potential of the drug.

Histopathology:

For this parameter the investigator stated that "the histopathologic examination did not reveal any changes which could be treatment related in any of the animals killed after 13 and 52 weeks of treatment and after the recovery period. All alterations found on an individual basis had features of common phenomena of spontaneous pathology and they were generally sporadic in nature or were of an intensity or frequency in rats treated at the higher dosage which is comparable to that of the control group rats."

A thorough inspection of the histopathology reports confirms this conclusive statement. Among the noted histopathological alterations common among control and treated rats were hemosiderosis of the spleen, mild perilobular fatty degeneration of livers, steatosis of single hepatocytes.

Neoplastic changes beside the already cited pituitary adenomas were one case of adrenal-cortical adenoma, a low-dose female, and one adrenal pheochromocytoma in a high-dose female.

The animals from the recovery period had essentially the same incidence and type of histological findings.

THE DOG STUDY WITH L-CARNITINE:

This report was prepared by th

where this study was conducted for Sigma Tau. The report is dated 21 August, 1984 and signed by the responsible personnel of Compliance with the GLP Regulations was assured by the study director, the Quality Assurance Audit Statement, with a log for the QAU Study Inspection dates signed by is submitted.

The report was addressed to Dr. M. T. Ramacci, Sigma-Tau Industrie Pharmaceutiche, Riunite S.P.A., 47 Viale, Shakespeare, Rome, Italy.

N 18948 - 2

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Study Set-Up and Methodology:

Thirty-two purebred beagle dogs, (16 males, 16 females) supplied by \_\_\_\_\_ were acclimated for 6 weeks, vaccinated, and treated with anthelmintic piperazine applied at 3 months intervals also during the test.

Source of Drug: Sigma-Tau, Rome.

Dosage: 4 males and 4 females in each dose group of control, 300, 600 and 1200 mg/kg/day administered in gelatine capsules.

Tests conducted: Blood and urine samples taken during weeks 6, 12, 26 and 52, 24 hours after drug administration.

Body Weights: weekly

Food Consumption: Recorded daily.

Ophthalmoscopy: Performed by means of a Keeler indirect ophthalmoscope before treatment and during week 6, 12, 25 and 51.

The test procedures applied in this study were described in detail. Interim reports covering clinical observations, results of hematology, blood chemistry and urinalysis were submitted for the test periods at 13 and 26 weeks, with the final report covering the final data for these parameters and the results of the terminal autopsies.

Results:

Mortality:

No cases of drug induced mortality.

One control female died during week 3 of dosing from acute diarrhea and severe dehydration. The complete autopsy work-up indicated histological changes from dehydration resulting from diarrhea and the congestion of the mucosal surface of the gastro-intestinal tract, but the primary cause for these lesions was not established. This animal was replaced by a new entry.

A high-dose male was found in week 15 to have a severe strangulated, inoperable hernia, and he was euthanatized. The autopsy disclosed a 14 cm long strangulation of the small intestine. The Microscopy report of the jejunum said: "Marked congestion of the mucosa and lamina propria", but the exact site of this finding was not identified, probably it was in the strangulated section. Other findings in this dog were a focus of hepatocyte necrosis, and in the kidney a focus of basophilic tubules. The noted changes in the right testis are probably associated with the strangulating hernia.

Clinical Signs:

Incidence of Liquid Feces: This symptom, first reported at the 4-weeks interim, continued to be present throughout the test, and increased in incidence with continuation of treatment, shown in the tabulation from the 26-weeks and 52-weeks reports. It is expressed as percentages of the number of instances maximally possible:

Dosage	Percentage Incidence	
	26 weeks	52 weeks
Control	0.02	1.1
300	12.7	15.6
600	35.8	44.1
1200	68.5	57.4

The comment for this parameter by the investigators was that the occurrence of liquid feces had no effects on the health conditions of the animals during the entire dosing period. This comment is important for the evaluation of a possible adverse drug action that would compromise the utility of the drug. It has to be added that the first Pharmacology Review for this drug on the occasions of the 4-weeks and 13-weeks interim reports did stress the high occurrence of liquid feces in all treated groups and expressed the view that this response to the drug may indicate an irritating action on the gastrointestinal lining and its consequences. The reviewing pharmacologist at that stage did not have the benefit of results from interim sacrifices at that period and therefore the presumptive conclusion was justified. However, as will be shown later, the results of the terminal sacrifice demonstrate that the suspected irritation of the gastrointestinal mucosa was not confirmed by the histopathological findings. It will be discussed later in this review that the liquid state of the feces may have resulted from the action of the drug on the lipid metabolism and might be specific for this species, because no such effect was present in rats treated with similar dosages of the drug.

Body Weight Gains:

The enclosed Table 9 taken from the report, with the data of individual weights and group means for dose groups, and weight gains, and the graphs made from these data show that in both sexes there was a flattening of the weight gain curves after the stage of rapid growth (up to approximately week 20.) Since this decrease in weight gain rates from then on is also shared by the control groups, it obviously reflects a natural event, as the onset of declining growth is not dose dependent: in the high dose males it starts already by week 16, and in females of the mid- and high dose group at about the same time, and the general pattern is similar for all groups and both sexes.

(Because the weights of the males are higher than those of females, the arrangement in the table presenting a group mean for each dose group by combining the weights of males and females is, in my opinion, a faulty method, unusable for effect evaluation purposes.)

It is evident that the weight gains of the control groups (both sexes) are higher than those of the treated groups. The males of the mid dose group come closest to the control group, while in females it is the low dose group; the mid dose females made lower gains than the high dose group while the high dose males made the smallest gains.

This variation for the sexes could be ascribed to the wide variation in gains made by individual dogs, that is distinctly shown in the graphs for individual dogs:

Dog 663, Female, low dose, was the only animal with a weight loss at term (shown in the table). It was explained by the investigators that there were no indications for sickness or excessive liquid feces, but that this dog was the oldest of the entire population and was also the heaviest of all. A drug action did not seem to be the cause for this variation. The others in this group showed a rather similar growth performance.

A similar situation existed in the high dose group of males where dog 677 showed a distinctly reduced growth performance already after week 11 with continued decline of weight, followed by a slight recovery, but with a total gain much below that of the other group members. At the terminal autopsy, this dog was found to be the only animal with a severe local erosion in the stomach wall, but lesions in the intestinal tract were not found. The record for incidence of liquid feces did not indicate any aggravation in this respect.

The only other animal not making normal weight gains is dog 672 female, mid dose, that after an initial normal performance up to week 12 gradually lost weight through most of the treatment period at a steady rate, but eventually started a recovery after week 44.

The biochemical and hematological data of the poor performers did not show deviations from normal levels. It seems therefore reasonable to conclude that the individual variations in body weight gains did not result from any adverse drug actions but were resulting from individual genetic make-up for growth.

#### Hematology:

The overall picture for this parameter is that there is no indication for a drug effect. An apparent elevation in the values for hemoglobin and RBC of males and females in all dose groups and a drop in the values of total WBC with progressing treatment when compared with the values obtained before treatment, at first would appear to be a favorable drug action, but a similar "response" appears also in the control groups at each test interval.

Group mean bodyweights - males

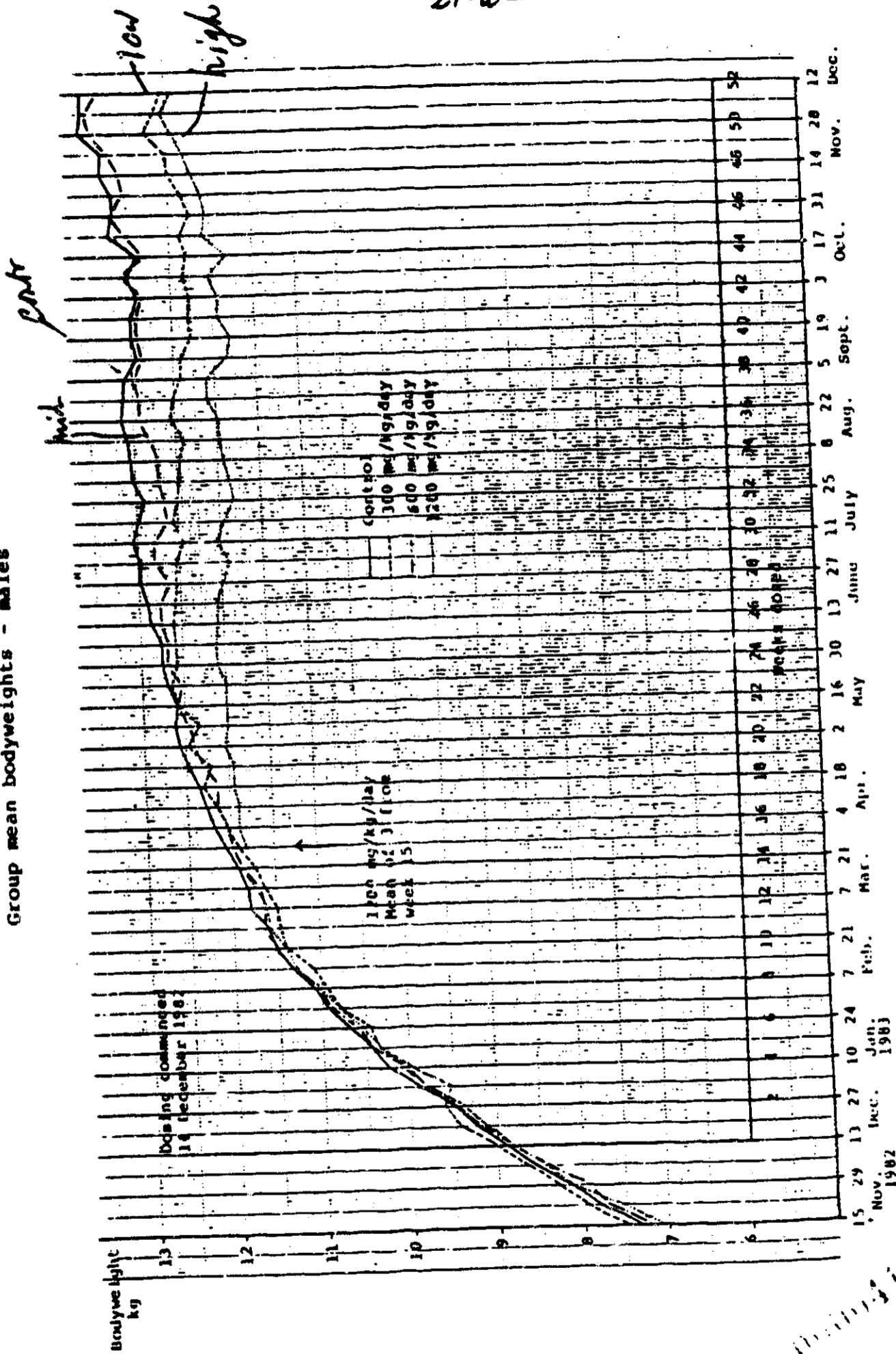
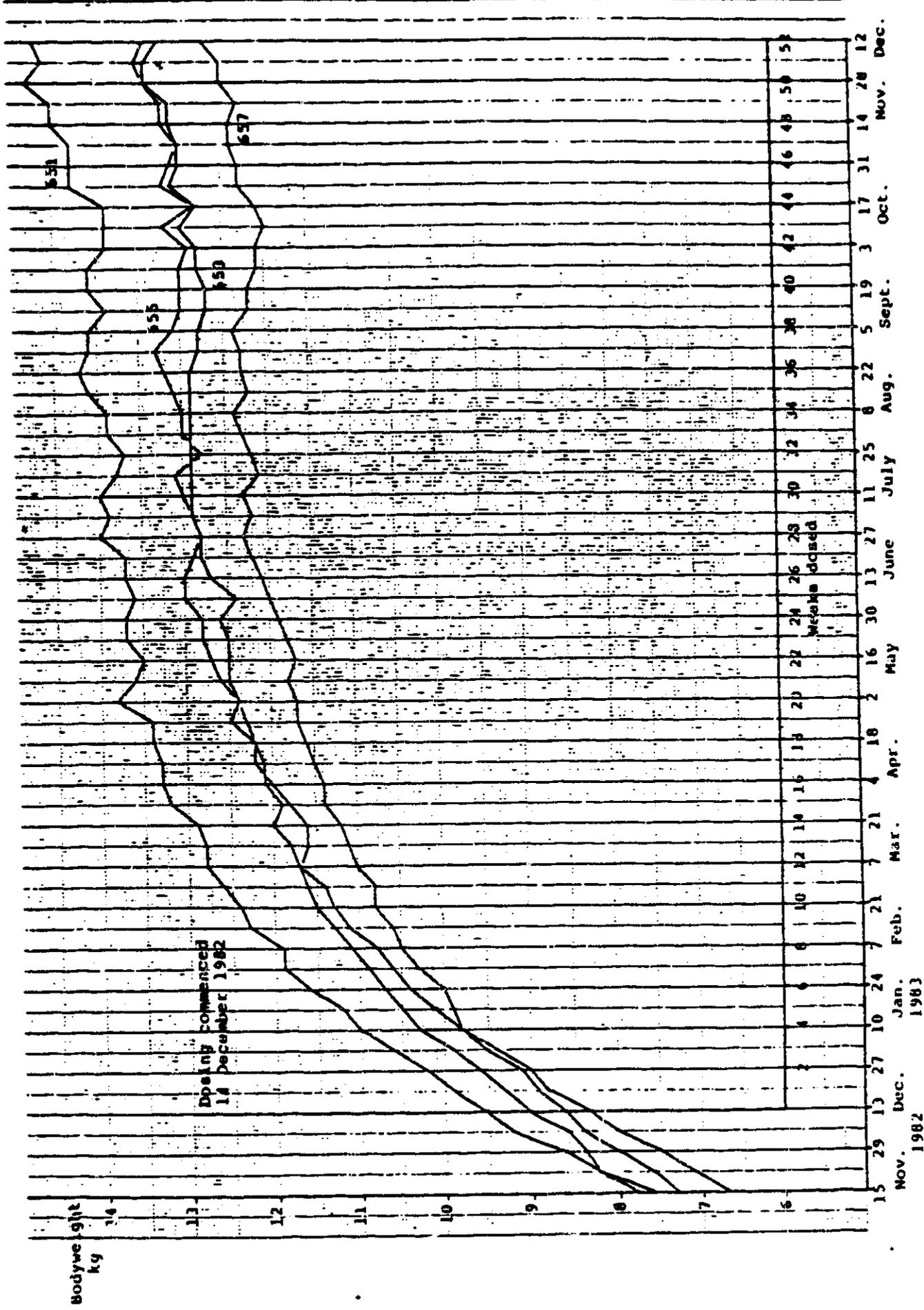


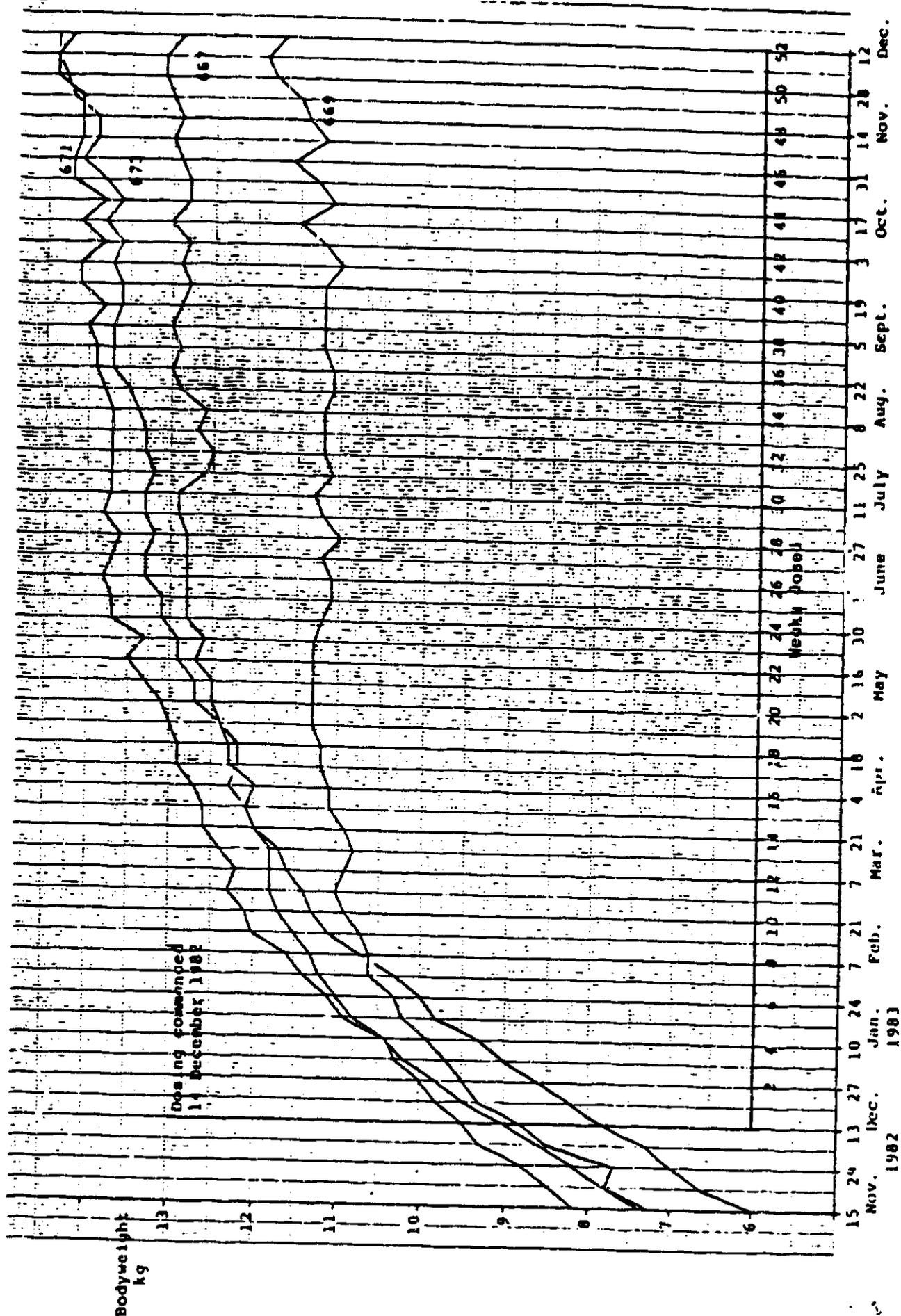
FIGURE 2a  
Individual bodyweights - Control - males



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FIGURE 4a  
Individual bodyweights - 600 mg/kg/day - males





21-f

FIGURE 1b  
Group mean bodyweights - females

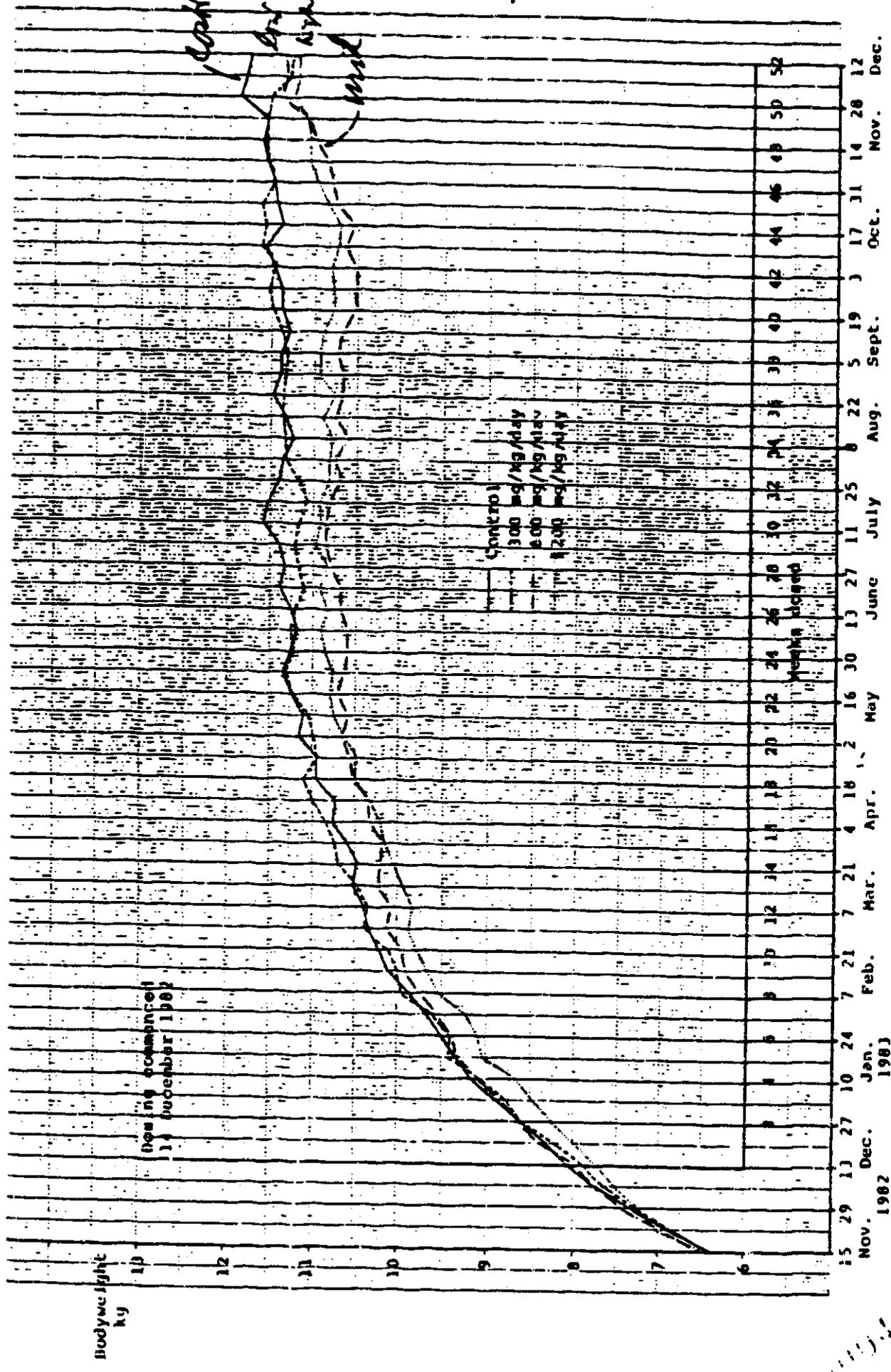




FIGURE 3b

Individual bodyweights - 300 mg/kg/day - females

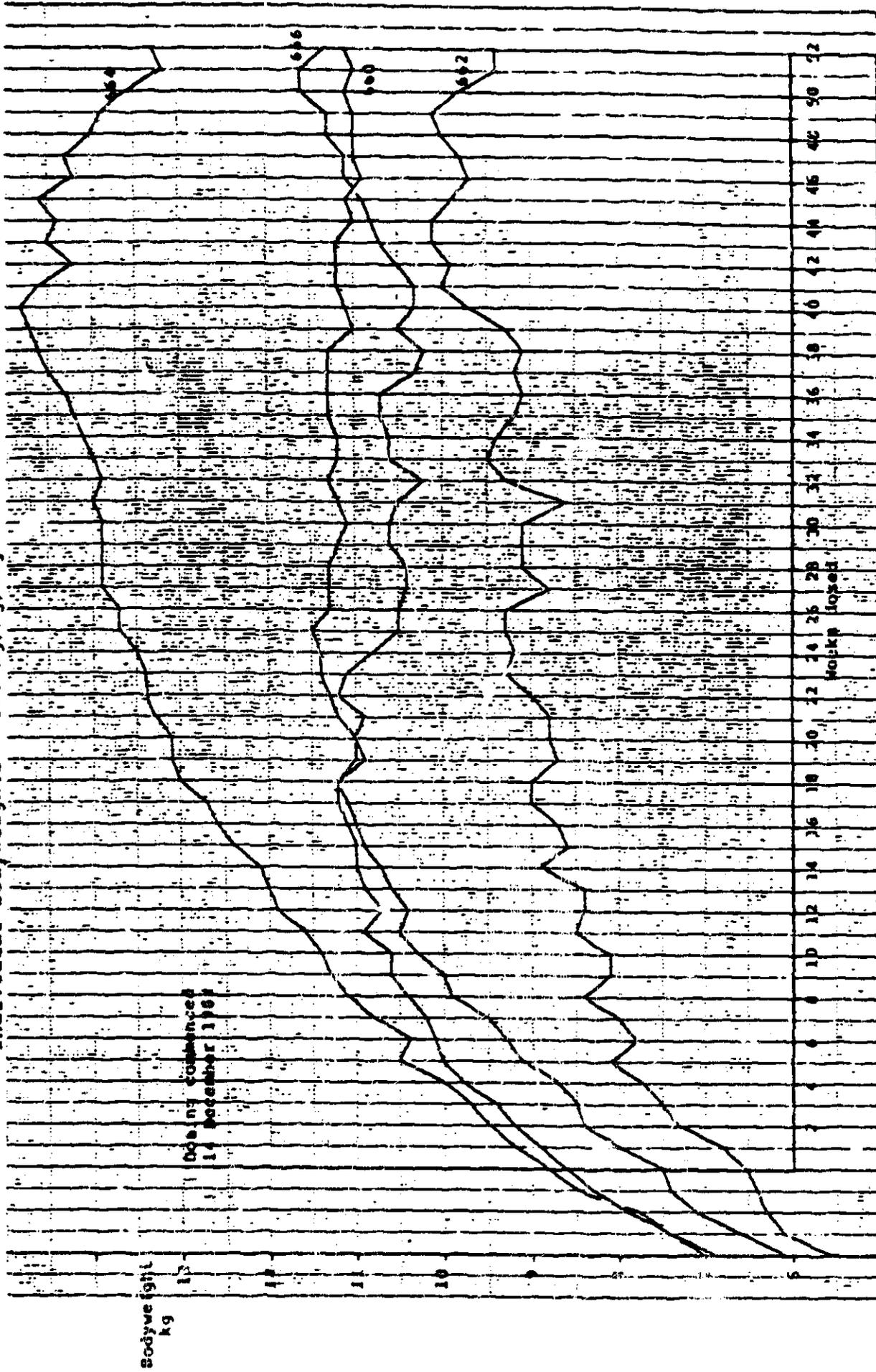
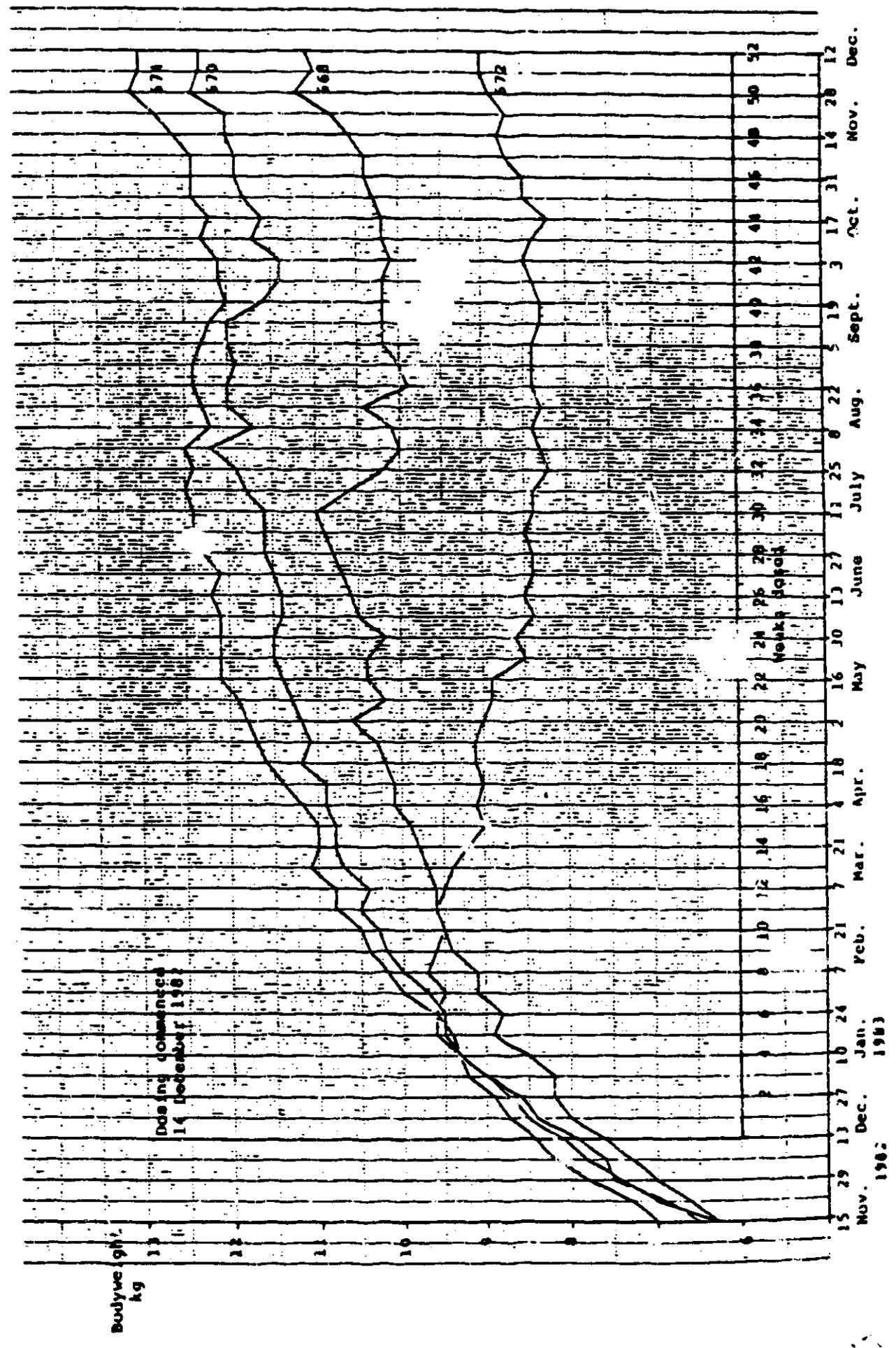
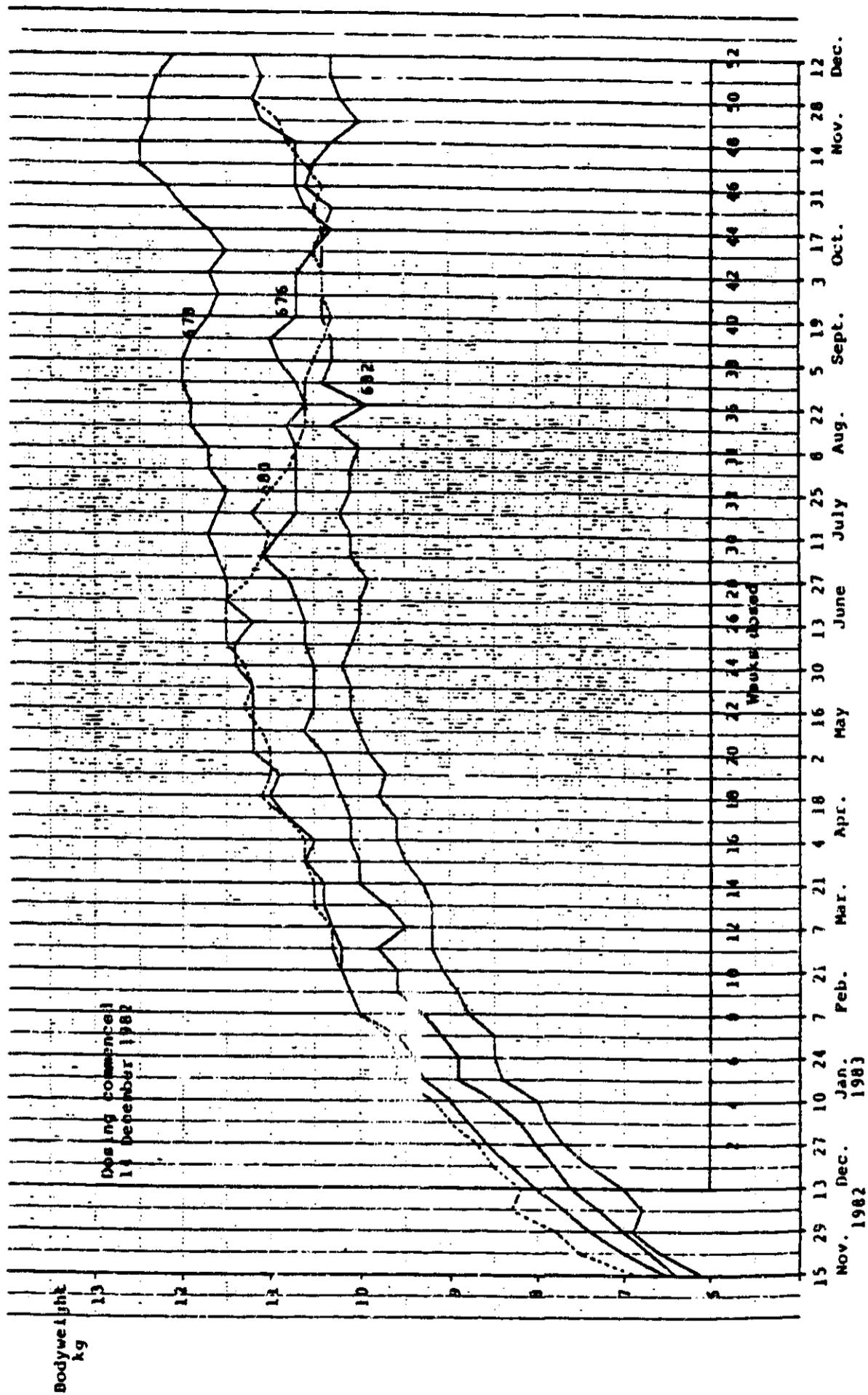


FIGURE 4b  
Individual bodyweights - 600 mg/kg/day - females



21-j

FIGURE 5b  
Individual bodyweights - 1200 mg/kg/day - females



-21-k-

Initial weight (g) and weight change after 52 weeks of dosing

Dosage mg/kg/day	Dog No./sex	Initial weight	Weight after 52 weeks	Weight change
Control	651♂	9500	14700	5200
	653♂	9000	13200	4200
	655♂	8300	13400	5100
	657♂	8600	12700	4100
	522♀*	7600	11100	3500
	654♀	9100	12600	3500
	656♀	7200	11000	3800
	658♀	8300	12300	4000
	Male mean	8850	13500	4650
	Female mean	8050	11750	3700
Group Mean	8450	12625	4175	
300	659♂	8900	14200	5300
	661♂	7300	12700	5400
	663♂	11200	11000	-200
	665♂	8500	12300	3800
	660♀	8600	11100	2500
	662♀	6500	9400	2900
	664♀	8800	13300	4500
	666♀	7500	11300	3800
	Male mean	8975	12550	3575
	Female mean	7850	11275	3425
Group mean	8413	11913	3500	
600	667♂	9500	12900	3400
	669♂	8800	11700	2900
	671♂	9000	14200	5200
	673♂	7800	14400	6600
	668♀	7600	11100	3500
	670♀	8100	12300	4200
	672♀	8400	9000	600
	674♀	7900	13000	5100
	Male mean	8775	13300	4525
	Female mean	8000	11350	3350
Group mean	8388	12325	3938	
1200	675♂	8200	13400	5200
	677♂	8800	10800	2000
	681♂	9800	13100	3300
	676♀	7600	11200	3600
	678♀	8000	12100	4100
	680♀	8200	11200	3000
	682♀	7000	10300	3300
	Male mean	8933	12433	3500
	Female mean	7700	11200	3500
	Group mean	8229	11729	3500

\* Replacement animal - treatment commenced on Day 19 of study

TABLE 9 -21-k-

Initial weight (g) and weight change after 52 weeks of dosing

Dosage mg/kg/day	Dog No./sex	Initial weight	Weight after 52 weeks	Weight change
Control	651♂	9500	14700	5200
	653♂	9000	13200	4200
	655♂	8300	13400	5100
	657♂	8600	12700	4100
	522♀*	7600	11100	3500
	654♀	9100	12600	3500
	656♀	7200	11000	3800
	658♀	8300	12300	4000
	Male mean	8850	13500	4650
	Female mean	8050	11750	3700
Group Mean	8450	12625	4175	
300	659♂	8900	14200	5300
	661♂	7300	12700	5400
	663♂	11200	11000	-200
	665♂	8500	12300	3800
	660♀	8600	11100	2500
	662♀	6500	9400	2900
	664♀	8800	13300	4500
	666♀	7500	11300	3800
	Male mean	8975	12550	3575
	Female mean	7850	11275	3425
Group mean	8413	11913	3500	
600	667♂	9500	12900	3400
	669♂	8800	11700	2900
	671♂	9000	14200	5200
	673♂	7800	14400	6600
	668♀	7600	11100	3500
	670♀	8100	12300	4200
	672♀	8400	9000	600
	674♀	7900	13000	5100
	Male mean	8775	13300	4525
	Female mean	8000	11350	3350
Group mean	8388	12325	3938	
1200	675♂	8200	13400	5200
	677♂	8800	10800	2000
	681♂	9800	13100	3300
	676♀	7600	11200	3600
	678♀	8000	12100	4100
	680♀	8200	11200	3000
	682♀	7000	10300	3300
	Male mean	8933	12433	3500
	Female mean	7700	11200	3500
	Group mean	8229	11729	3500

\* Replacement animal - treatment commenced on Day 19 of study

Biochemistry:

Also for this area, a drug effect is not evident. It has to be mentioned that Table 13a, Biochemistry - group mean values for males, and Table 13b for females shows a sudden considerable change in the values for AP (alkaline phosphatase) in week 25 from the values given for predosing and weeks 6 and 12. During these intervals, the AP values were fairly uniform for all dose and control groups ranging from 29 to 35 mU/ml but the data given for week 25 and week 51 as group mean values are all in the range above 100 mU/ml, namely for week 25, control group 132 mU, and 140, 146 and 184 for the low, mid and high dose respectively, and for week 51 as 113, 111, 123 and 178 mU/ml respectively. This change was probably caused by a change in the equipment used for this particular test that is stated in a footnote to the table.

The next table is part of the table in the report giving the values for biochemistry parameters intended to show the absence of an action of carnitine on the metabolism of lipids. The only possibility of an action is that on triglycerides but it is confused by the high pretreatment values.

All other biochemistry values including those for SGPT and SGOT are in the normal ranges. Glucose levels as mg/dl are lower at week 51 than they were before treatment but here again the control dogs too had a drop with progression of treatment.

Urinalysis:

performed at weeks, 6, 12, 25 and 51: Unremarkable.

Ophthalmoscopy:

Examinations of the eyes of all animals were performed before start of treatment and during weeks 6, 12, 25 and 51, by means of a Keeler indirect ophthalmoscope. Results were presented for the cited interims and final stage of the test. The findings were diagnosed by the investigator as being incidental and that no trends indicative for a drug effect were seen.

Terminal Findings:

All animals were killed by exsanguination under pentobarbitone anesthesia.

Bone Marrow: Smears prepared from specimen obtained by sternal puncture from all animals were diagnosed to be normal.

Organ Weights:

From all animals: brain, heart, lung, liver, spleen, pancreas, thymus, uterus/prostates, kidneys, thyroids, adrenals, gonads.

Female Dogs

Week number	Dosage mg/kg/day	Cholest-erol mg/dl	HDL mg/dl	β-Lipo-protein OD x1000	Tri-glyceride mg/dl	MSFA mEq/l
Pre-dose	Control	142	136	103	44	0.55
	300	144	131	137	53*	0.50
	600	143	131	133	55*	0.54
	1200	143	126	125	55*	0.58
Week 6	Control	132	132	87	41	0.61
	300	133	128	122	51	0.56
	600	144	134	109	52	0.62
	1200	148	135	109	51	0.49
Week 12	Control	117	122	73	51	0.82
	300	139	136	108	54	0.66
	600	148	142	114(*)	56	0.70
	1200	136	134	100	63*	0.57
Week 25	Control	127	128	81	30	0.63
	300	151	149	113	41	0.62
	600	149	144	101	38	0.76
	1200	179	172	142	36	0.55
Week 51	Control	108	108	104	38	0.55
	300	192(**)	177(**)	147	44	0.36
	600	153	132	159	50	0.41
	1200	138	125	118	37	0.50

Male Dogs

Week number	Dosage mg/kg/day	Cholest-erol mg/dl	HDL mg/dl	β-Lipo-protein OD x1000	Tri-glyceride mg/dl	MSFA mEq/l
Pre-dose	Control	179	164	132	42	0.39
	300	141	133	160	54*	0.51
	600	146	135	149	50**	0.44
	1200	170	150	152	50**	0.44
Week 6	Control	168	152	109	47	0.51
	300	150	136	144	55	0.63
	600	143	138	130	54	0.60
	1200	154	139	125	51	0.45
Week 12	Control	151	145	87	50	0.58
	300	132	135	142(*)	55	0.41
	600	142	133	109	52	0.46
	1200	153	147	127	66**	0.63
Week 25	Control	149	149	106	30	0.41
	300	135	143	139	39	0.60
	600	146	139	98	31	0.62
	1200	164	150	125	40	0.71
Week 51	Control	141	132	106	40	0.42
	300	145	133	157	47	0.45
	600	136	125	145	64(*)	0.45
	1200	128	125	157	53	0.48

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TABLE 15 - 12-6

Organ weights - group mean values for selected parameters and results of statistical analysis

Organ	Dosage level mg/kg/day	Absolute weight (g)	Weight adjusted for final bodyweight (g)	Results of statistical analysis
Kidneys	Control	57.19	55.45	NS NS NS NS
	300	52.64	53.47	
	600	56.84	56.42	
	1200	56.80	58.31	
Liver	Control	383.90	371.99	NS NS NS NS
	300	338.39	344.09	
	600	375.93	373.08	
	1200	354.93	365.27	
Heart	Control	107.91	105.59	(*) NS NS NS
	300	88.20	89.31	
	600	101.36	100.81	
	1200	90.91	92.93	
Testes	Control	32.14	-	(*) NS NS NS
	300	25.59	-	
	600	31.89	-	
	1200	30.06	-	

NS No significance

(\*) 5% level of probability ('t' test, not confirmed by Williams' test)

No significant changes related to drug dosage were noted.

Histopathology Findings:

Tissues for Histopathological Study:

Samples (or the whole) of the following tissues from each animal were preserved together with any tissue showing macroscopic abnormality:

Adrenals	ileum	sternum
aorta (arch and abdominal)	jejunum	spleen
brain (cerebral cortex, thalamic nuclei, mid-brain, medulla and cerebellum)	kidneys	stomach (body and antrum)
colon (upper and lower)	liver	testes or ovaries
duodenum	lungs	thymus
eyes and optic nerves*	lymph nodes (cervical and mesenteric)	thyroids
gall bladder	mammary gland	tongue
heart	oesophagus	trachea
	pancreas	urinary bladder
	pituitary	uterus or prostate
	salivary gland	femur and articular surface
	sciatic nerve	rectum
	skeletal muscle	vagina
	skin	spinal cord

The findings were described: for each dog as macroscopic and microscopic changes from normal and a summary tabulation of these was prepared by the investigators. All reports were signed by

The Incidence of Liquid Feces:

The occurrence of liquid feces was the outstanding unfavorable result from the treatment of dogs with carnitine. In view of this effect and its weight on acceptability of the drug, also because of the suspected irritating action on the gastrointestinal mucosa, this problem is addressed first of all, in an attempt to reach its resolution and clarification.

The only case of an established lesion in the stomach was that of dog 677, a male on high dose, with the microscopic finding of a deep erosion of approx. 15x5 mm, and two erosions up to 12x5 mm. The microscopic findings read: "Focal erosion of the luminal mucosa with associated inflammatory cell infiltration." The comment for macroscopy of the jejunum said: "minimal congestion of mucosal surface", and the microscopy protocol said "Within normal limits". For the stomach lesion, the comment in the tabulation says: "The gastric erosion involved the mucosa and lamina propria but did not extend through the muscularis mucosae in the sections examined. The toxicological significance of this finding is equivocal."

One other case of a microscopic finding in the stomach was in dog 663 on the low dose as "foci of mineralization in the mucosa probably of no significance since it also was seen in one control dog, and a change in the jejunum found in dog 675, high dose, described as "marked cytoplasmic vacuolation of epithelium of tips of villi." The significance of this finding was not addressed by the pathologist,

It appears to be of important to compare these described minor intestinal changes in the 3 dogs with the severe changes found in the female control dog that had to be sacrificed because of severe time diarrhea of unknown etiology in which the gastrointestinal tract was found to show marked congestion, with free blood on mucosal surfaces in the entire tract, and large areas of mucosal erosion in the ileum/jejunum seen on microscopy, with marked necrosis of the laminal mucosae and inflammatory cell infiltration into the mucosa, but without lesions in the stomach.

Since liquid feces had occurred also in dogs that did not have any histologically established intestinal lesions, it seems reasonable to theorize that the cause for liquid feces might result indirectly from an action of the drug on the lipid metabolism leading to steatorrhea from the excessive high doses of the drug applied in these studies. It might also be due to the excretion of the excessive doses of carnitine in the feces, but these possibilities were not investigated.

#### Macroscopic Post Mortem Findings:

##### Kidney:

The Summary prepared by the investigators for the entire dog study contains under this subheading (p.000010) the following comment:

"Macroscopic abnormalities detected that may be related to administration of L-carnitine were restricted to a pallor of the inner cortex of the kidneys in 2 of 8 animals receiving 300 mg/kg/day and 4 of 7 animals receiving 1200 mg/kg/day".

Apparently no search was made for any relation of these macroscopic findings to findings in these cases by their histopathological examination, and therefore this had to be done by the reviewer, with the following results:

The animals in which the above macroscopy findings were made were dog 662 and 666, in the 300 mg/kg/day group, and dogs 675, 676, 678, and 682 in the 1200 mg/kg/day group. The only histological findings that could apply as being possibly related to the macroscopic findings were: for the 2 mid-dose dogs: Moderate extensive cytoplasmic vacuolation of the

corticomedullary tubules," and for the 4 high dose dogs: "Marked extensive cytoplasmic vacuolation of the corticomedullary tubules." However, the same description of this change in the kidneys was made also for 3 dogs in the low-dose group that did not have the macroscopically seen pallor varying in degree from slight to marked extensive cytoplasmic vacuolation, and the same incidence was found in 3 mid-dose animals, and in 2 high-dose dogs, that all were without the pallor finding. Therefore there is no relationship between the macroscopic and the histopathological findings.

As to the significance of the microscopic findings, the most severe kidney lesion was found in one control male (655) with the macroscopic finding for the kidney saying: "Pitted appearance to capsular surface with strands extending into cortex from surface pitting", and the microscopic finding described as "Wedge-shaped areas of cortical scarring in both kidneys characterized by areas of fibrosis, loss of tubules, atrophic glomeruli, and focal inflammatory cell infiltration. An association between these can be recognized.

One other control dog (522) also had the microscopy finding "Slight vacuolation of cytoplasm of some corticomedullary tubules," therefore it is clear that this change is common among control and treated dogs but perhaps it is slightly aggravated by the drug in a somewhat dose related extent.

A distinct kidney lesion was found also in one mid-dose dog (673), without any macroscopic abnormalities, that was microscopically detected and described as "loss of the papillary tip with re-epithelialisation over the necrotic tip and degenerating medullary tubules, with marked subepithelial mononuclear cell infiltration in the pelvis." This dog had a better than the mean growth performance for this dose group, therefore the lesion in the kidney is of little clinical significance, and not a drug effect judging from its singular appearance. Therefore it can be concluded that the minor gross change pointed out by the investigator as possibly treatment related was an error of judgement.

An other microscopical change within the corticomedullary tubules that was dealt with by the pathologist was the incidence of fat droplets in the epithelium of these tubules.

The comment by the pathologist said: "Fat droplets in the epithelium of the corticomedullary tubules, also seen as vacuolations in the H&E sections, were present in control and treated dogs. There were moderate/marked amounts in some treated dogs compared to slight/minimal amounts in control dogs. The toxicological significance of this increased number of fat droplets is uncertain as moderate/marked amounts of fat droplets are not uncommon in untreated dogs."

Incidence and degree of fat droplets in the corticomedullary tubules.

	Control		300 mg/kg/day		600 mg/kg/day		1200 mg/kg/day	
	♂	♀	♂	♀	♂	♀	♂	♀
Slight/minimal amounts of fat droplets	3	4	4	1	2	3	2	1
Moderate/marked amounts of fat droplets				3	1	1	1	3
Total dogs examined	4	4	4	4	4	4	3	4

A comparison of the incidence of fat droplets with that of the vacuolation in the histopathology reports does not confirm by their similar incidence in individual dogs that they are the same histological structures. It is equivocal whether they represent an adverse drug action, or if they are related to the physiological action of the drug on fat metabolism.

Other observations on histological structure changes of kidneys were described as follows:

- Control 655: Wedge-shaped areas of cortical scarring in both kidneys characterized by areas of fibrosis, loss of tubules, atrophic glomeruli, focal inflammatory cell infiltration
- Low Dose 654: marked subepithelial mononuclear cell infiltration in pelvis
- 659: Occasional dilated tubules contain amphophilic material.
- 661: Area of marked interstitial mononuclear and eosinophilic infiltration.
- 666: Areas of necrosis with marked inflammatory cell infiltration
- Mid Dose 667: minimal subepithelial mononuclear cell infiltration in the pelvis of one kidney.
- 671: occasional dilated tubules containing amphophilic material.
- 673: left k.: loss of papillary tip with re-epithelialization over necrotic tip and degenerating medullary tubules. Marked subepithelial mononuclear cell infiltration in the pelvis.  
right k.: areas of interstitial mononuclear cell infiltration, some with associated basophilic cortical tubules.
- 674: Area of interstitial mononuclear cell infiltration
- High Dose: 675: occasional dilated cortical tubules containing amphophilic material.

It can be concluded that these are single, sporadically occurring minor "changes from normal" not characteristic for a drug action.

Liver:

The histology of liver tissues did not show any change from normal. Foci of hepatic necrosis with associated mononuclear cell infiltration were found only in two control dogs.

Portal mononuclear/eosinophilic infiltration noted in 2 dogs of the low dose group only.

Minimal dilatation of centrilobular sinusoids found in one high dose dog (677) that was the only dog with the gastric erosions, and it also had parasitic granuloma foci in the lungs, and erythrophagocytosis in lymph nodes. All these changes were of singular incidence, not indicative for a drug action.

Other Organs:

None of the other investigated critical organs had histopathological changes that would indicate a toxic potential of the drug, or profound alterations in their structure from the pharmaco-physiological actions of the massive doses of carnitine.

Pharmacokinetics:

Blood specimen taken at weeks, 6, 12, 26 and 52, and specimen from liver, kidney, heart, skeletal muscle and urine and feces samples taken at the ultimate post-mortem, from all animals were forwarded to the sponsor. A report for the analyses of these specimen was not in the submitted NDA documents.

Teratogenicity Tests:

Teratogenicity tests in rats and rabbits were reported in the original NDA. They were conducted in the Laboratory of

They followed in general the FDA Guidelines for Teratogenicity/Reproduction Studies. In the rat study, 10 rats per group were treated with 25 or 50 mg/kg/day by the intramuscular route from day 3 to day 14, and sacrificed on day 19. In the rabbit test, 10 animals per group were treated also with 25 and 50 mg/kg/day I.M., from day 6 to day 29, with sacrifice on day 29.

No adverse effects on gestation, fetal development and postnatal viability were noted.

There was at first some concern about the adequacy of these tests for their validity to support safety of the drug use by pregnant women, but it was later agreed that they are acceptable and that no new tests would be needed.

Mutagenicity Tests:

Mutagenicity tests with *Salmonella typhimurium*, *Saccharomyces cerevisiae*, and *Schizosaccharomyces pombe* in-vitro and in vivo, were conducted by the same institution that conducted the described toxicity tests in rats with l-carnitine. These had been reported in the original NDA.

An additional test, a Micronucleus test in mice, conducted at the same institution was reported in the amendment of November 2, 1983 to the NDA.

From the detailed description of the tests procedures etc. in the original (with authorized translation into english,) it appears that this institution is competent to conduct this class of research, but documentation of compliance with GLP requirements is not provided. (This defect should not hold up further processing of the NDA.) All mutagenicity tests were negative for a mutagenic potential of l-carnitine.

SUMMARY AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

For the aspects of the pharmacological properties of carnitine, with regard of its biological functions as carrier of long-chain fatty acids, the information obtained from the literature material furnished by the sponsor amply demonstrates the essential role of carnitine for lipid metabolism in normal and deficiency conditions and thereby projected its efficacy for correcting a state of carnitine deficiency of various origins, such as defective synthesis from its precursors, or genetic defects in the availability of the enzyme systems needed to form the complex of carnitine with the fatty acids that is essential for their translocation into mitochondria, the site for their oxidation into energy. Some of the submitted literature actually goes beyond this phase and intended use of carnitine by dealing with the functions of carnitine in complex with mid-chain length fatty acids, and the involvement of peroxisomes in this process, beside mitochondria, and furthermore, for the now emerging evidence for the pathways of actions of hypolipidemic drugs such as clofibrate, as stimulators of peroxisome activity beside that of H<sub>2</sub>O<sub>2</sub> release.

For the aspects of toxicity and safety, the one-year toxicity tests in rats and dogs, performed at our request, have to be viewed from the angle that they do not imitate the conditions of the proposed clinical use of the drug, correction of carnitine deficient, and the dosages were multiples of the proposed clinical dosage. Even with this milieu, unfavorable to the drug, the outcome of both studies is remarkable for the absence of overt adverse results from the drug that would predict serious health hazards from doses in excess of the clinically effective dose.

The most pronounced result is in the dogs in the form of the continuous presence of liquid feces even from the low dose and setting-in already at the early phase of treatment. However, the at-first expressed concern that this were symptoms for a drug induced irritation and even a damage to the gastrointestinal mucosa was alleviated when the health of the dogs remained unaffected, with only a slight depression of the body weight gains by the mid and high dose, and resolved by the histological findings at the final necropsy that did not reveal any distortions of the histological architecture of the mucosal components.

The cause for the liquid feces that was limited to the dogs and was not evident in rats with roughly similar doses, remains only speculative, mostly because of the unavailability at present of the analyses of the pharmacokinetic samples. The existence of steatorrhea from FFA in the stool caused by a disturbance of lipid metabolism by the excessive carnitine doses is a theoretical possibility resulting in extreme stool-softening and eventually a liquid state.

As to the clinical consideration, it also is possible that the effects in dogs might be related to the actions of carnitine in human where the causal pathways apparently are not established either.

In view of the fact that the actions of carnitine on the gastrointestinal system are accepted and are cited in the labeling for carnitine, the effects in the dogs should not constitute a reason for any further actions at the present stage (but it would seem advantageous to find some remedy against the undesirable side effects after finding the causes for them).

There were several other differences in responses to the drug between the two species. In the rats, the drug at the low- and mid-dose had a stimulating action on body weight gain by elevating them above that of the control animals, but the high dose had the opposite effect by depressing the body weight gains of the high dose group animals to a range below that of controls. The beneficial function of the low - and mid dose levels of the drug was further supported by the observation that these two groups of animals developed a reduced rate of body weight gain when drug administration was withdrawn, and inversely, when the high-dose rats showed improved weight gains when the drug was withdrawn. The causal origine for both effects, stimulation by the low and mid-dose, suppression by the high dose, was not investigated, except that there was no change in the feed intake at the change of treatment. While the stimulating action could be explained by a more effective metabolism from the drug, the suppressive effect is difficult to explain, a negative feed back perhaps. It should be noted that the animals in the high dose group did not show clinical signs for any adverse actions.

The only other parameter with a noticeable indication for a drug action was the elevation of triglycerides in the low- and mid dose groups coincident with the elevated body weight gains in these groups, and lower levels in the high dose group, accompanied with a drop in triglycerides levels with withdrawal of the drug in the low- and mid-dose group, and an elevation in the high-dose group. An association between these two phenomena is difficult to construct. It may indicate the stimulatory action of the triglycerides as the active stimulus secondary to an action of carnitine on triglyceride activation. I was unable to obtain advice, or a source of information from the literature for this action of carnitine, and whether it is an unfavorable action. It might occur only with the excessive doses of carnitine, and therefore would not occur with the clinical use. A modulating action on various lipid levels was not seen in the rat study.

Also in the dogs, levels for cholesterol, HDL, betalipoprotein and FFA values were not affected by carnitine at the doses used.

No adverse effects were indicated by organ weights and histopathology in either species to an extent that would predict a hazardous actions in the human.

Teratogenicity Tests:

Two tests, one in rats and one in rabbits were, as could be expected from the nature of the drug, uneventful with regard to actions on pregnancy and fetal development.

Mutagenicity Tests:

These tests by 4 different methodologies did not indicate a mutagenic property of carnitine.

Publications by Sigma-Tau:

The submitted literature material contains several articles, published and unpublished, by staff members of the Research Laboratories of Sigma-Tau S.p.A., Pomezia, Italy that by themselves would not "carry" the NDA but are reviewed here for the sake of documenting that this firm had comprehensive experience in the field of carnitine and its functions.

N. Siliprandi and Maria T. Ramacci, Institute of Biological Chemistry, University of Padova, and Sigma-Tau Laboratories, "Carnitine as a Drug Affecting Lipid Metabolism," (apparently an In-House manuscript.)

This was primarily a review of the functions of carnitine from publications that was informative for describing the complexities of the actions involved in the mechanism of the drug, and its derangements by pathological and deficiency conditions leading to myopathies and myocardial anomalies. Of interest is their reference in the Conclusion, to clofibrate by saying: "With regard to the alterations of lipid metabolism, it is appropriate to point out that some drugs, such as clofibrate which typically affect lipid metabolism, strongly modify the biosynthesis and distribution of carnitine as well as the carnitine transport system, a fact highly relevant to their mode of action. However, pharmacological and clinical studies using carnitine have the advantage over those using other drugs because of the fairly well-known background of carnitine biochemistry" (Ref.: Pande and Parvin, *Biochim, Biophys. Acta*, 100,209-214, 1980)

F. Maccari, Pessotto, P., Ramacci, M.T. Angalucci L., of Sigma-Tau and Istituto di Farmacologia, University of Rome: "The Effects of Exogenous L-Carnitine on Fat-Diet Induced Hyperlipidemia in the Rat" (no literature reference). The purpose of this study was to investigate the reducing effect of carnitine on diet-induced hyperlipidemia, and by what mechanism, on the premise that in the absence of carnitine only short- and medium-chain fatty acids can enter the mitochondrial matrix, and that long-chain acyl-CoA' preferentially participates in extramitochondrial reactions such as triglyceride synthesis that occurs in lipid storage myopathies. A TG rich diet with 25% olive oil was the test diet, treatment was 500 mg/kg carnitine by gavage. Tissue distribution search was for triglycerides, total cholesterol, phospholipids, carnitine, and acetyl-carnitine, in heart, muscle, liver, kidney.

The TG-rich diet induced modifications in lipoprotein pattern by increased serum chylomicron content and of pre-beta lipoproteins, triglycerides, FFA, and phospholipids in controls, and their reduction by the extraneous carnitine. The results in the various organs were similar. An excess of carnitine caused its loss in urine.

Zago, E., Maccari F and Ramacci M.T. ("Carnitine-Insulin Treatment in Diabetic Rats." The capacity of exogenous l-carnitine to restore impaired glucose and lipid metabolism in diabetes was investigated in rats. This property had been found to exist by other investigators. In this short notice it is reported that streptozotocin-diabetic rats were treated with carnitine 250 mg/kg p.o., alone or with insulin 20 U/kg s.c., for 4 days. Carnitine by itself moderately reduced hyperglycemia in addition to lowering FFA, and markedly, triglycerides in serum. Insulin alone also reduced significantly glucose, FFA and triglycerides and the combination of both reduced FFA and triglycerides, and was more markedly effective in normalizing hyperglycemia than by their separate actions.

Zago, E, Maccari, Pessotto and Ramacci,: "Reduced Resistance to Developing Ketosis during fasting in Obese Zucker Rats Treated with L-Carnitine." The fasting state is characterized by an enhanced release of free fatty acids from adipose tissue which are then oxidized in the liver into ketone bodies, a process controlled by the carnitine acyltransferase activities modulated by the glucagon: insulin ratio, according to some investigators. Studies in man and animals are said to have shown that in the obese normal state reduced or increased FFA mobilization is induced without readily developing a state of ketosis. The investigators theorized that the impaired (rather more accurately "prevented) ketogenic process is caused by a defective carnitine release mechanism in the liver. In this test obese and lean Zucker rats were treated with 500 mg/kg carnitine orally. In untreated obese rats, free and acetyl carnitine levels were significantly higher than in lean rats without a definite difference in serum FFA, but acetoacetate and hydroxybutyrate levels were reduced in the obese rats with triglycerides being elevated. Treatment with carnitine significantly increased serum FFA and acetyl carnitine in both groups, but acetoacetate and hydroxybutyrate were enhanced in the obese, but significantly reduced in the lean rats. The conclusion by the investigators is not quite clear. They say that the deficient(?) production of ketone bodies can be ascribed to the persistence of hyperinsulinemia but that carnitine stimulates oxidation of the fatty acids when carried into liver mitochondria.

The next article entitled "Ketone Body Modifications after L-Carnitine Treatment in Obese and Diabetic Rats" by the same authors is a continuation of the theme in it. The effects of L-carnitine was again studied in streptozotocin diabetic Wistar rats treated with 250 mg/kg p.o. daily in the diabetic, and 500 mg/kg oral in the obese and lean Zucker rats.

Diabetic rats exhibited a considerable increase of blood ketone bodies which was significantly reduced by the exogenous carnitine. Liver carnitine acetyltransferase was unaffected;... diabetes provoked a decrease in the serum of free carnitine but an increase in serum acetylcarnitine.

The results in the obese and lean Zucker rats showed that blood levels of ketone bodies in obese rats are lower than in lean rats whereas both free carnitine and acetylcarnitine values were increased in the obese rats. Carnitine administration had opposite effects in the obese and lean rats: it increased serum ketone levels in obese, but decreased it in lean rats.

The investigators discussed these results by the following considerations: In diabetic rats the level of blood ketone bodies is high while in obese rats it is low, but in both conditions FFA levels are elevated above normal. This phenomenon is explained by the conclusion that the excess of fatty acids in the diabetic rat arriving at the liver are rapidly oxidized and only partially transformed into ketone bodies, while in obese rats the excess fatty acids are

not oxidised in the liver but are esterified into triglycerides. The difference in the metabolism is ascribed to the high glucagon/insulin ratio typical of diabetes that stimulates the activity of the translocatory carnitine system and transfer to the inner mitochondrial membrane and oxidation, whereas the low glucagon/insulin ratio typical for (genetic) obesity inhibits the activity of this system. Therefore, the difference in the metabolic alterations in diabetic and obese conditions in the rat can be explained as consequences of an altered control on carnitine translocating system (and the ensuing alteration in carnitine function?). The decreased ketone bodies content in blood after administration of carnitine to diabetic rats was explained by an increased utilization of the ketone bodies for oxidation by extrahepatic tissue mitochondria (probably the heart and kidney) with carnitine - stimulated mitochondrial CoA.

Zago E and M. T. Ramacci: "Effects of L-Carnitine on Glucose Uptake and Utilization in Rats and Rabbits." The authors stated that it is known from work by other investigators that carnitine is involved in the stimulation of pyruvate dehydrogenase activity as a consequence of an induced decrease of the acetyl - CoA: CoA ratio, and this study was to investigate whether carnitine also could affect carbohydrate metabolism in healthy and in diabetic animals. The test was run in-vitro on rat diaphragms in Krebs-Hanseleit buffer, for glucose uptake and its disappearance from the medium within 6 minutes of incubation. In the in-vivo study run on rats and rabbits, fasted rats were treated with oral carnitine 250 mg/kg, 60 minutes before an oral glucose load; for rabbits a glucose infusion of 10 mg/kg/min for 90 minutes was followed one week later by a combination load of carnitine plus glucose. The results of the in-vitro test showed that glucose uptake in the diaphragm was increased by carnitine but to a lesser degree than by insulin. With a combination of both, the uptake was slightly above that by insulin alone.

In the in-vivo rat study, pretreatment before the glucose load with carnitine resulted in a significant decrease of the hyperglycemic peak. The investigators claim that these results correspond to the results in human where a reduction of glycosuria in hyperlipidemic diabetic patients was obtained with carnitine. They believe that the carnitine action reflects a stimulation of pyruvate-dehydrogenase activity owing to a decreased acetyl-CoA: CoA ratio. It was speculated that two separate mechanisms exist: insulin induced glucose uptake in the cell, and carnitine improved the oxidative utilization of the cellular glucose.

Fanelli, Ottorino: "Carnitine and Acetyl-Carnitine, Natural Substances Endowed with Interesting Pharmacological Properties" in Life Sciences Vol. 23, pp.2563-2570, 1978. This article deals with the inotropic and anti-fatigue effects of d,l-carnitine, l-carnitine and d,l-acetyl-carnitine in the rat after a fatigue test, and in isolated heart of rabbits. The introduction gave a very good summary of important findings reported in the current literature.

For the isolated heart test, freshly obtained hearts were perfused with solutions of the different drug substances, and contractile force, coronary flow and heart rate were calculated. All substances were found to have inotropic effects that were dose related in effectiveness.

In the fatigue test performed on a rotarod apparatus, in a dose range from 200 to 600 mg/kg, d,l-acetyl carnitine was most effective but at 800 mg/kg it induced a motor activity excitation effect and adversely affected the animals.

CONCLUSION and RECOMMENDATION:

The essential role of l-carnitine in fat metabolism is unequivocally established by the numerous investigations reported in the world-literature, and its safety is supported by the toxicology studies and teratogenicity tests conducted for the NDA by the sponsor.

From the standpoint of Pharmacology, approval of the NDA can be recommended.

  
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V. Berliner, Ph.D.

cc:  
Orig. NDA  
HFN-810  
HFN-340  
HFN-810/Pharmacology  
HFN-810/VBerliner/4/22/85/sw/4/23/85  
Wang No. 1436D  
HFN-340/W. Galbraith