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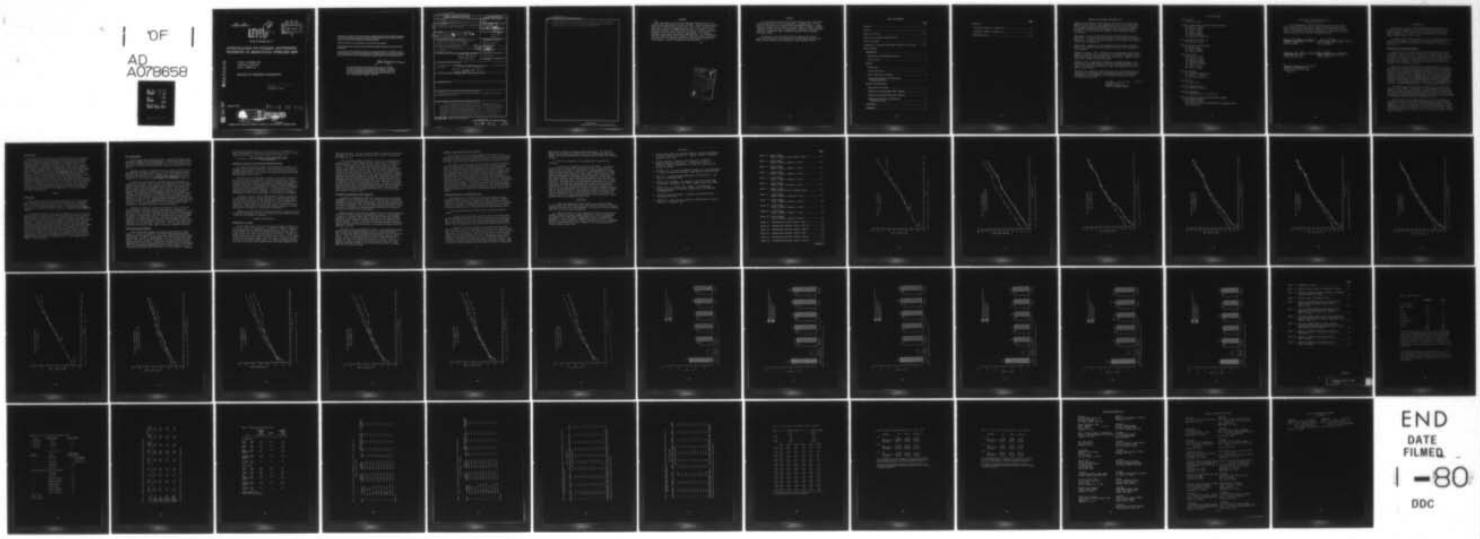

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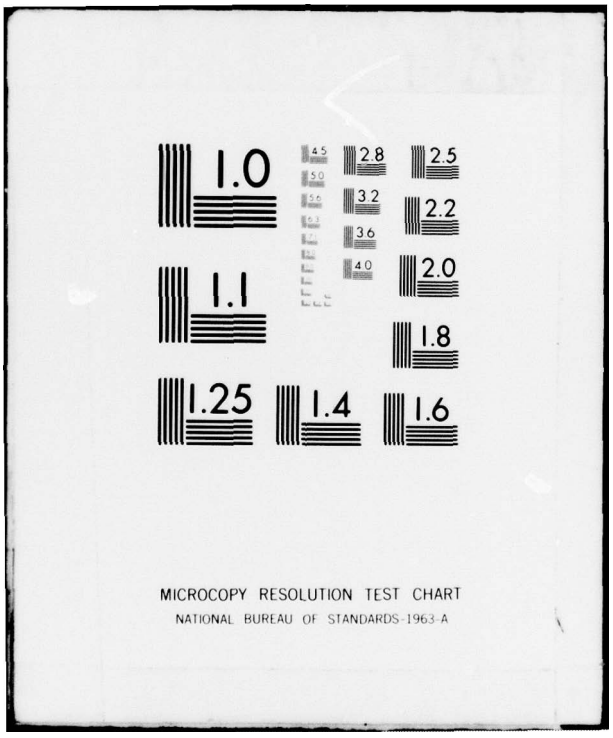
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INSTITUTE REPORT NO. 71

INVESTIGATION OF POSSIBLE ANTITHIAMIN PROPERTIES IN IRRADIATION STERILIZED BEEF

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DIVISION OF NUTRITION TECHNOLOGY

AUGUST 1979

LETTERMAN ARMY INSTITUTE OF RESEARCH PRESIDIO OF SAN FRANCISCO CALIFORNIA 94129

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Comittee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

John H. Marshall Jr. 30 Aug 79
(Date)

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Male and female rats (156 each) were made thiamin-deficient by feeding a semipurified diet devoid of thiamin. They were then repleted with various beef-containing test diets: frozen beef, thermally processed beef, electron or gamma-irradiated beef. All repletion diets contained carefully controlled levels of thiamin. Recovery rates were monitored by growth (weight gain) and measurements of a thiamin-dependent blood enzyme (erythrocyte transketolase). The responses of

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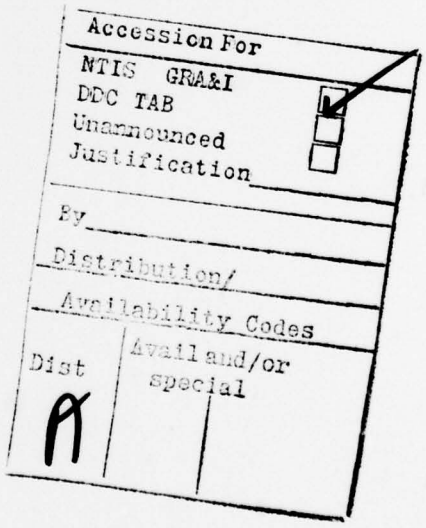
ABSTRACT

Male and female rats (156 each) were made thiamin-deficient by feeding a semipurified diet devoid of thiamin. They were then repleted with various beef-containing test diets: frozen beef, thermally processed beef, electron or gamma-irradiated beef. All repletion diets contained carefully controlled levels of thiamin. Recovery rates were monitored by growth (weight gain) and measurements of a thiamin-dependent blood enzyme (erythrocyte transketolase). The responses of rats fed irradiated beef were similar to those fed frozen or thermally processed beef. No evidence was found of antithiamin substances in either gamma or electron irradiation sterilized beef.



PREFACE

The experimental portions of the study covered in this report were conducted during the period of 1 May 1978 - 1 October 1978. All raw data are being stored at Letterman Army Institute of Research. Anyone wishing to examine the raw data or to obtain copies of tables containing individual values, may do so by contacting: Commander, Letterman Army Institute of Research, ATTN: SGRD-ULN, Presidio of San Francisco, California 94129.

In addition to the personnel listed on page vi, the authors gratefully acknowledge the assistance of Ms. Cindy White and Ms. Anne Regh, typists; and Ms. Lottie Applewhite, LAIR technical editor.

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REPORT OF THE QUALITY ASSURANCE UNIT

Summary and Conclusions: This study and the draft of the final report dated 25 January 1979 have been examined and the study has been found to have been conducted as described in the protocol, and its addendum. The procedures used were those described in the standard operating procedures. The conclusions and reported results accurately reflect the raw data.

Inspections: Written records were not made of inspections until 20 March 1979. On that date record was made to best of memory of previous inspections. Following that date written record was made of all inspections at time of inspection.

August 1978: Inspection of diet preparations and examined notebooks where B_1 and proximate analysis data were recorded. No recommendations were necessary.

September 13 and 27, 1978: Inspection of the hemoglobin and hematocrit assays. Information as to the control used and the manufacturers target assay value was not being entered on the work sheets. Laboratory personnel were advised to enter these data.

November, 1978: Inspection of erythrocyte transketolase assay. SOPs were present and being followed. Raw data were date stamped but not signed. The technician performing the assay was asked to sign the data sheets, which he did.

From March 21 to March 29, 1979, the data of the final report dated 25 January 1979 were examined and found consistent with the protocol and its addendum. Where examined, the conclusions reflect the results recorded in the raw data.

William R. Wise Jr. 31 Aug 79

WILLIAM R. WISE, JR.
QUALITY ASSURANCE MONITOR

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Signatures of Principal Scientists
Involved in the Study

We, the undersigned, believe the study described in this report to be scientifically sound and the results and interpretations to be valid. The study was conducted to comply, to the best of our ability, with the Good Laboratory Practice Regulations for Nonclinical Laboratory Studies outlined by the Food and Drug Administration.

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INTRODUCTION

The testing of control and irradiated meats for antimetabolite activity against vitamins B_1 and B_6 is a requirement of the protocol entitled "Animal Feeding Protocol for Irradiation Sterilized Test Foods" originated by the Office for the Wholesomeness of Irradiated Foods, USAMRDC, dated 21 October 1975.

The purpose of the study reported here was to determine whether or not irradiation (gamma or electron) or thermal processing of beef produces factors which are antagonistic to vitamin B_1 (thiamin) in the diet of rats.

Background and Experimental Design

The protocol for the antithiamin study specified that rats were to be made deficient in thiamin (according to a pre-set weight gain criterion). They were then to be repleted with various meat-containing or dry diets which had identical (high or low) thiamin contents and the recovery rates compared. A decreased recovery rate in animals fed irradiated meat relative to those fed control meat would indicate the presence of an antithiamin substance.

Resumption of growth was the most obvious indicator of recovery. The other (more sensitive and specific) parameter of thiamin status was specified to be erythrocyte transketolase (ETK) activity. This enzyme requires a thiamin derivative (thiamin pyrophosphate) to function. The activity of the enzyme drops rapidly in red cells of animals fed diets deficient in thiamin. Also, *in vitro* addition of thiamin pyrophosphate cofactor generally produces a much greater (%) increase in enzymatic activity in hemolysates from deficient animals than from nondeficient controls. This *in vitro* stimulation, "TPP effect," is presumably indicative of the percent apoenzyme which is not saturated with cofactor.

The protocol specified that the meat diets contain 35% test meat (on a dry weight basis). Furthermore, it specified that each meat be tested at 2 levels of vitamin intake, a marginal level and a high level (5.0 and 20.0 mg thiamin/kg diet on a dry weight basis). The high vitamin diets were included to determine whether or not antithiamin substances (if detected) could be overcome by additional vitamin.

The test meat for this study was specified to be beef which had been heated to inactivate enzymes and then preserved by four different methods: 1) frozen (control), 2) canned (thermally processed), 3) gamma irradiated, 4) electron irradiated. The last three treatments produce shelf-stable products and are known to decrease vitamin content. Finally, the protocol specified that groups fed dry, semi-purified diets without meat be included.

Pilot Studies

Two pilot studies (using only semi-purified diets) were conducted in this laboratory. The first was done to familiarize the personnel with the necessary technical procedures and the experimental design. The second study was conducted because of uncertainty that 5.0 mg thiamin/kg diet, as specified in the protocol, was, in fact, marginal for restoration of erythrocyte transketolase. The National Research Council (NRC) estimates that the minimum level of dietary thiamin necessary to promote optimal growth in a non-deficient rat is 1.25 mg/kg diet (1). Therefore, the second pilot study evaluated differences in recovery rates of thiamin-deficient rats which were repleted at 5.0 and 20.0 mg/kg. Only a slight difference was found (data not shown). Although deficient rats repleted on a diet containing the NRC requirement (1.25 mg/kg) resumed growing at nearly normal rates, there was almost no recovery of erythrocyte transketolase. On the basis of the above observations, we felt that the marginal thiamin level should be lowered to 3.75 mg/kg to enhance differences between the groups during repletion. Upon obtaining the approval of LTC Hilmas and Dr. Raica, this change was made in the protocol.

METHODS

Animal Care

Male (study 1) and female (study 2) weanling rats (156 per study) were purchased from Charles River Breeding Laboratories, Wilmington, Mass. Each animal was identified by ear tag and housed individually in a room with a 12-hour light/dark cycle. All were given ad libitum water and fed a semi-purified diet (Table 1), containing 20 mg thiamin/kg (Diet A).

The schedule and diet codes for the studies are outlined in Table 2. After one week of quarantine and adaptation (Phase 1), 24 rats were randomly selected to remain on Diet A. All other animals were placed on Diet B, which was identical to Diet A except that thiamin had been omitted (Phase 2). Growth was monitored throughout the study by thrice weekly weighings. Animals on Diet B were considered to be deficient when the average daily weight gain was less than 0.5 grams. The deficient animals were then randomly divided into 11 groups of 12 animals each. The 24 Diet A animals were also divided into 2 groups of 12 each. One group of 12 Diet A rats and one group of 12 Diet B rats were bled by cardiac puncture and removed from the study. The remaining 10 groups of deficient animals were placed on 10 different repletion diets (C-L) and the remaining Diet A group was continued on Diet A. The repletion period (Phase 3) lasted 4 weeks.

Diet Preparations

All test meats were supplied by the U.S. Army Natick Research and Development Command, Natick, Massachusetts. They were processed according to the procedure outlined in Appendix A of the Animal Feeding Study Protocol for Irradiation Sterilized Beef, described in paragraph one of page one.

Proximate analyses of the beef (crude fat, protein, moisture, and ash) were done by standard methods (2). Calcium and phosphorus determinations were also done (3,4). Thiamin assays were done by a microbiological method which utilizes Lactobacillus viridescens as the test organism (5). The results of these assays are summarized in Table 3.

As specified by the protocol, the meat diets were formulated to contain 35% (dry weight) beef. The fat and protein levels of the semi-purified diets (Table 1) were adjusted to be similar to the meat diets, based on calculations from proximate analysis data. For each of the meat treatment groups (E through L) a dry premix with fat and protein omitted and containing the proper amount of thiamin was prepared in advance. When mixed with the corresponding meat (35% dry weight basis) the complete diets contained the specified levels of thiamin and were similar to the semi-purified diets, except that ground beef replaced the casein, lard, and corn oil. Analyzed thiamin contents of repletion diets are given in Table 4. The calcium/phosphate ratio of the meat diets was calculated to be 1.325 compared to 1.25 in the semi-purified diets; therefore, no adjustment was made in these two minerals.

Each meat was heated in a forced air oven set at 175 ± 5°C until the temperature in the meat center, as recorded by a thermocouple, reached 50-60°C. The meat and juices were then ground through a 1/4 inch plate and thoroughly mixed. Each meat was mixed with its corresponding dry premix in the specified ratio and the diets were prepared no longer in advance of feeding than two days. Each feed jar was weighed when placed into and removed from the cages to allow estimates of food consumption. The diets remained in the cages no longer than 48 hours before being replaced by fresh diet in clean jars. Feed jars containing each diet were also placed in empty cages to serve as evaporation controls.

Blood Sampling and Analyses

Blood samples were obtained by cardiac puncture from all rats on days 7, 14, and 28 of repletion. The animals were anesthetized with penthrane gas and samples (1.5 ml each) were collected into EDTA-containing syringes. Hematocrit determinations were done in duplicate on each sample. Aliquots of each were prepared, the red cells were washed and stored frozen until assayed. Erythrocyte transketolase (ETK) activity was determined in the presence and absence of added thiamin pyrophosphate (TPP) by an autoanalyzer adaptation of the method of Smeets et al. (6). (In-house modifications were the efforts of Mr. Paul Waring.) Enzymatic

activity was expressed as ETK = I.U./ml packed red cells where I.U. = μmole glyceraldehyde-3-phosphate produced per minute at 37°C. The stimulation in activity in the presence of TPP was calculated as:

$$\text{TPP Effect} = \frac{[\text{ETK stimulated} - \text{ETK unstimulated}]\ (100)}{\text{ETK unstimulated}}$$

Statistical Analysis of Erythrocyte Transketolase Data

Erythrocyte transketolase (ETK) data were analyzed separately for studies 1 and 2 (males and females). Within each sex, the data were analyzed separately for each of the three sample collection days in the repletion phase (days 7, 14, and 28). The variables analyzed were ETK, ETK-stimulated, and TPP effect.

The data were to be analyzed to determine the possible effects of diet (food group effect) and level of thiamin (vitamin level effect). The data were first scrutinized by plotting histograms and calculating standard deviations, skewness, and kurtosis to establish reasonably normal distributions. This examination revealed no gross abnormalities and that the usual transformations, e.g. logarithm, would not significantly increase the apparent normality. It was concluded that an assumption of normality was reasonable, considering the robustness of the statistical tests used with respect to the normality requirement.

A packaged computer program, BMDP Biomedical Computer Program P2V (7) was used to perform a two-way analysis of variance (ANOVA) using food and vitamin level as the grouping factors. The non-depleted diet group (A) was not included in the analysis of variance because its difference from the other food groups was strongly obvious from visual inspection. ANOVA was done both with and without inclusion of the dry diet groups, for reasons to be discussed under Results.

Comparisons between individual groups were done by Dunnett's method of multiple comparisons (8) using the appropriate mean square (MS) error values from the analyses of variance.

RESULTS AND DISCUSSION

Observations on Growth

Growth (body weight) curves for the males are shown in Figures 1-6 and for the females in Figures 7-12. Cessation of growth in aminals on the deficient diet was remarkably abrupt (group B in Figures 1 and 7). In all studies the criterion for deficiency (weight gain less than 0.5 g/day) was met 14-16 days after the beginning of Phase 2. Figures 2-6 (males) and Figures 8-12 (females) show the growth curves for all groups on the various repletion diets. The growth curve of Group A has been included in each figure (dashed line). A striking observation was the fact that all meat-fed groups gained faster than those repleted on the

semi-purified diets. In fact, within two weeks all groups on beef diets had caught up to the non-deficient groups and in Study 2 had actually surpassed Group A.

A few accidental deaths occurred as a result of the anesthesia or cardiac puncture. These losses, as well as the stress of bleeding all of the animals resulted in shifts in some growth curves after days 7 and 14. To eliminate the potentially misleading effects of animal deaths on group means, the growth data were recalculated and expressed as mean weight gain per group per day. Tables 5 and 6 summarize the average daily weight gain for the males and females for weekly periods as well as the overall means for the four weeks of repletion. Although some high-thiamin groups appeared to gain more during the first week of repletion than their low-thiamin counterparts, these differences were generally reversed during the second week. The variation was such that no consistent effect of vitamin level was found. Moreover, no obvious differences were observed among meat-fed groups. As would be expected from the growth curves, the average gains for animals fed semi-purified diets were less than those for the meat-fed groups. The daily mean weight gains from which the numbers in Tables 5 and 6 were calculated are included in Tables 7 and 8.

Erythrocyte Transketolase (ETK) Analyses

Erythrocyte transketolase (ETK) data (unstimulated) are summarized graphically by day in Figures 13-18. In each figure, the far left bar represents group A (non-deficient animals). Group B means (representing the deficient animals at day 0, before repletion) have been dotted in to aid visual comparisons. The bars are paired together according to food group and in each pair the open and diagonally-hatched bars represent low and high vitamin level, respectively.

Before repletion, the deficient animals (Group B) had ETK activities 20-25% of the levels observed in nondeficient controls. From Figures 13 and 16 it is obvious that within 7 days ETK activity had increased strikingly in all experimental groups regardless of the repletion diet. Smaller increases relative to group A were observed on days 14 and 28 in both studies. However, at 28 days all experimental group means (both high and low vitamin levels) were still lower than the means of Group A (Figures 15 and 18).

TPP effect data are summarized in Table 9. The differences between groups A and B are apparent, despite the large standard deviations. Once the deficient animals had been placed on the repletion diets, there were no longer any obvious differences between group A and the other groups. Thus, the apoenzyme present in the red cells appeared to be readily saturable when thiamin was returned to the diet. However, as pointed out above, at least 28 days were necessary before the total amount of apoenzyme increased to levels found in red cells of animals which had never been deficient.

Comments Concerning Statistical Analyses

As described above, the primary purpose of this study was to compare the recovery rates of thiamin-deficient rats which were repleted with diets containing irradiation-sterilized beef or thermally processed beef with those fed diets containing beef which had been stored frozen.

The protocol also specified that two groups of animals be repleted with dry diets similar in protein and fat content to the meat-based diets. As described in the Methods section, the thiamin content of each diet had been carefully pre-adjusted to the specified level. After the studies had been completed and the growth and food consumption data were examined, the dry diet groups were found to differ markedly from meat-fed groups. Animals placed on meat-containing diets promptly increased their food intake and grew faster than those animals repleted with non-meat diets. (Pallatibility was perhaps a factor.) Consequently, the semi-purified diet groups consumed less thiamin than did the corresponding meat-fed animals. Since the experimental design depends upon identical thiamin intakes to allow comparison of diet treatment effects, this report will focus on statistical analyses of the meat groups only. However, ANOVA was also done on the combined meat groups plus dry diet groups.

Statistical Analysis of Erythrocyte Data

Two-way analysis of variance (food group and vitamin level as grouping factors) was done on the ETK data from the meat-fed animals (Groups E-L) and P values are presented in Tables 10 (Study 1) and 11 (Study 2). The values in parentheses were obtained when dry diet groups C and D were included. Enzymatic parameters analyzed were ETK, ETK-stimulated, and TPP effect. These will be discussed separately below.

1. *ETK and ETK-stimulated* (both related to amount of enzyme present).

Vitamin level had highly significant effects ($P<0.05$) on ETK and ETK-stimulated throughout the studies, except on day 14 of Study 2, when P values were only 0.11 and 0.13. The significant vitamin effect was not surprising, since the high vitamin group means were uniformly higher than the corresponding low vitamin group means (Figures 13-18).

Among the meat diets, food group was without effect on ETK. (When dry diets C and D were included, a food effect was significant on days 7 and 14 of Study 1.) Thus, the meat diets did not appear to differ with respect to ETK response. Since the low vitamin, irradiated meat groups I and K were considered the crucial test groups, these two were examined more closely with respect to the corresponding frozen beef group (E). From Figures 13-18 it may be seen that some of group I and K means were slightly lower (and some higher) than group E means. However none of these differences even approached statistical significance

when tested by Dunnett's multiple comparisons method. This was true whether the variances were assumed to be equal (and MS_{error} taken from ANOVA) or whether variances were calculated separately when they appeared unequal.

2. TPP effect (related to the % unsaturation of enzyme with cofactor).

No effect of food group was found on TPP effect. In contrast to ETK activity results, there was also no effect of vitamin level, except on day 14 of Study 1. On that day, a food-vitamin level interaction appeared significant (Table 10). Closer examination of the data suggest a significant difference between Groups I and E ($P<0.01$). However, the physiological importance of this statistic is questionable for several reasons. Group E mean was particularly low on the day--in fact, even lower than that of the animals which had never been deficient (Group A). Although Group I mean was slightly high (8.97), it was still well within the range of values observed in normal non-deficient rats. Furthermore, the fact that dietary thiamin level had no significant effect on TPP effect, whereas ETK activity was highly dependent, suggests that the latter parameter is a more reliable indicator of thiamin status. Since no detrimental effect of irradiated test foods was found on absolute ETK activity, the one isolated difference found in TPP effect is likely to have been a chance occurrence which did not necessarily reflect thiamin status of the animals in that group.

CONCLUSIONS

1. Under the conditions of these studies, actual ETK enzymatic activity rather than TPP effect was a more sensitive indicator of thiamin status; ETK activity was highly dependent on the amount of dietary thiamin, while TPP effect was largely unaffected.

2. Thiamin deficient rats were repleted with diets containing beef (frozen, thermally processed, gamma irradiated or electron irradiated). No difference was found among the meat-fed groups in the parameters measured: growth, erythrocyte transketolase activity, and TPP effect. Specifically, neither gamma nor electron irradiated beef had a detrimental effect on thiamin status in rats, compared to rats fed non-irradiated (stored frozen) control beef.

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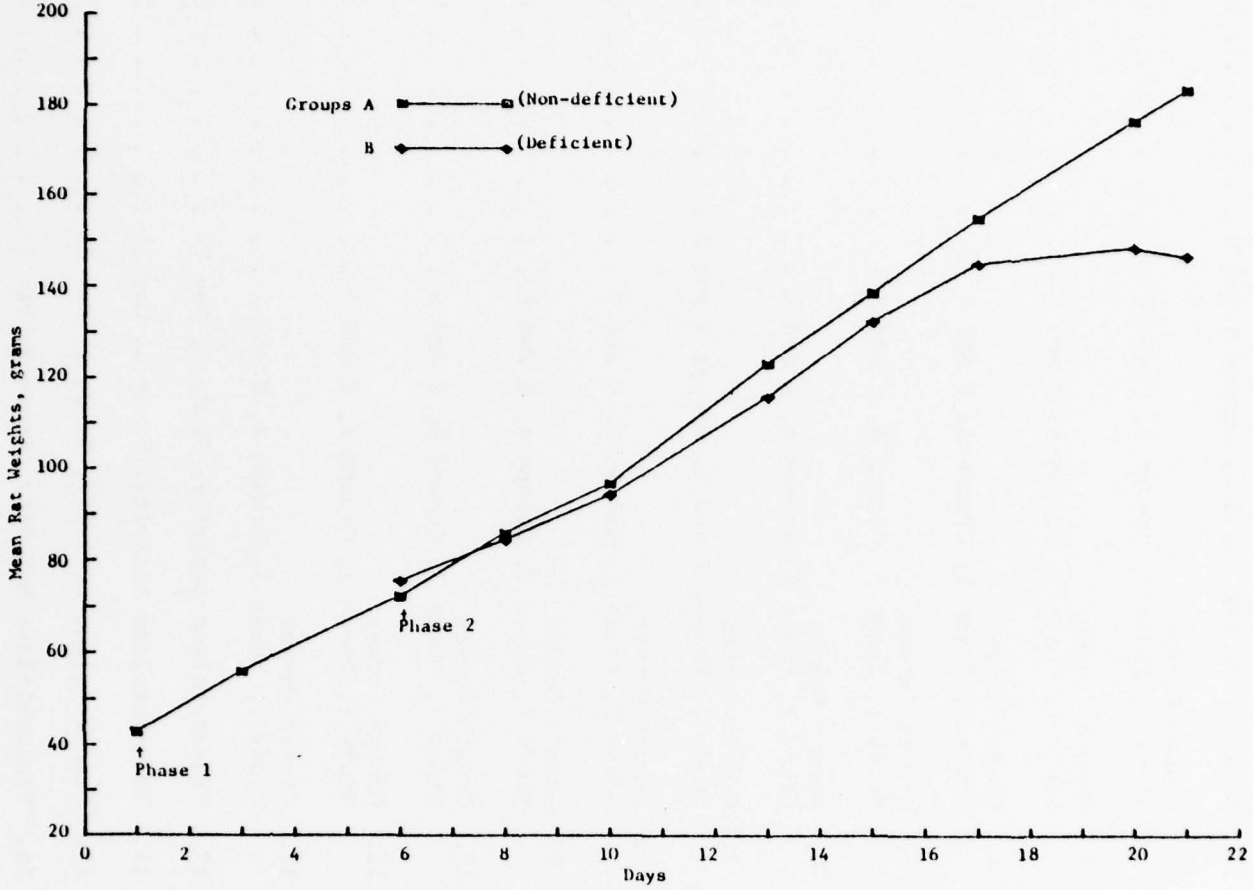

Figure 1. Growth Curves, Study 1 (Males), Phase 1 and 2, Groups A and B.

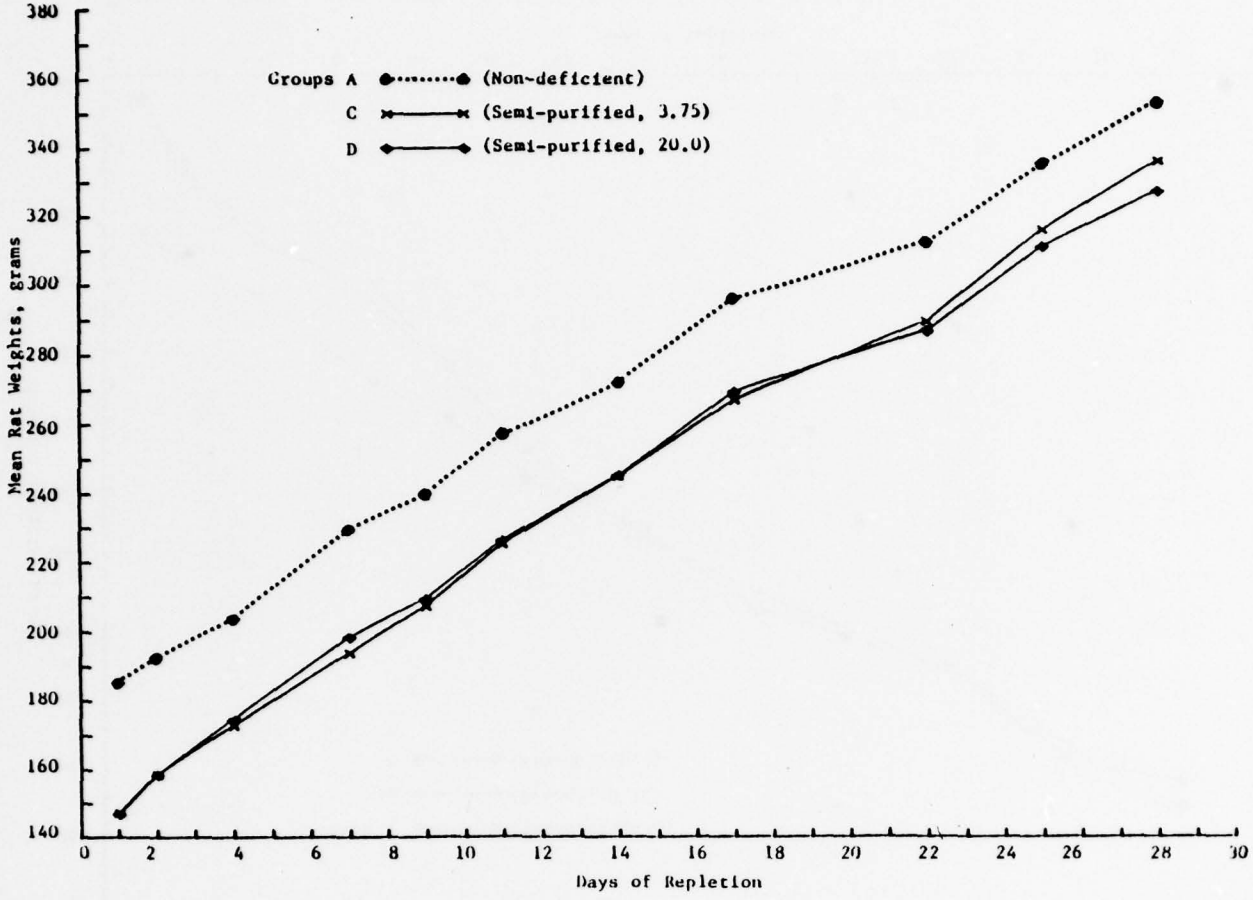

Figure 2. Growth Curves, Study 1 (Males), Phase 1 and 3, Groups A, C and D.

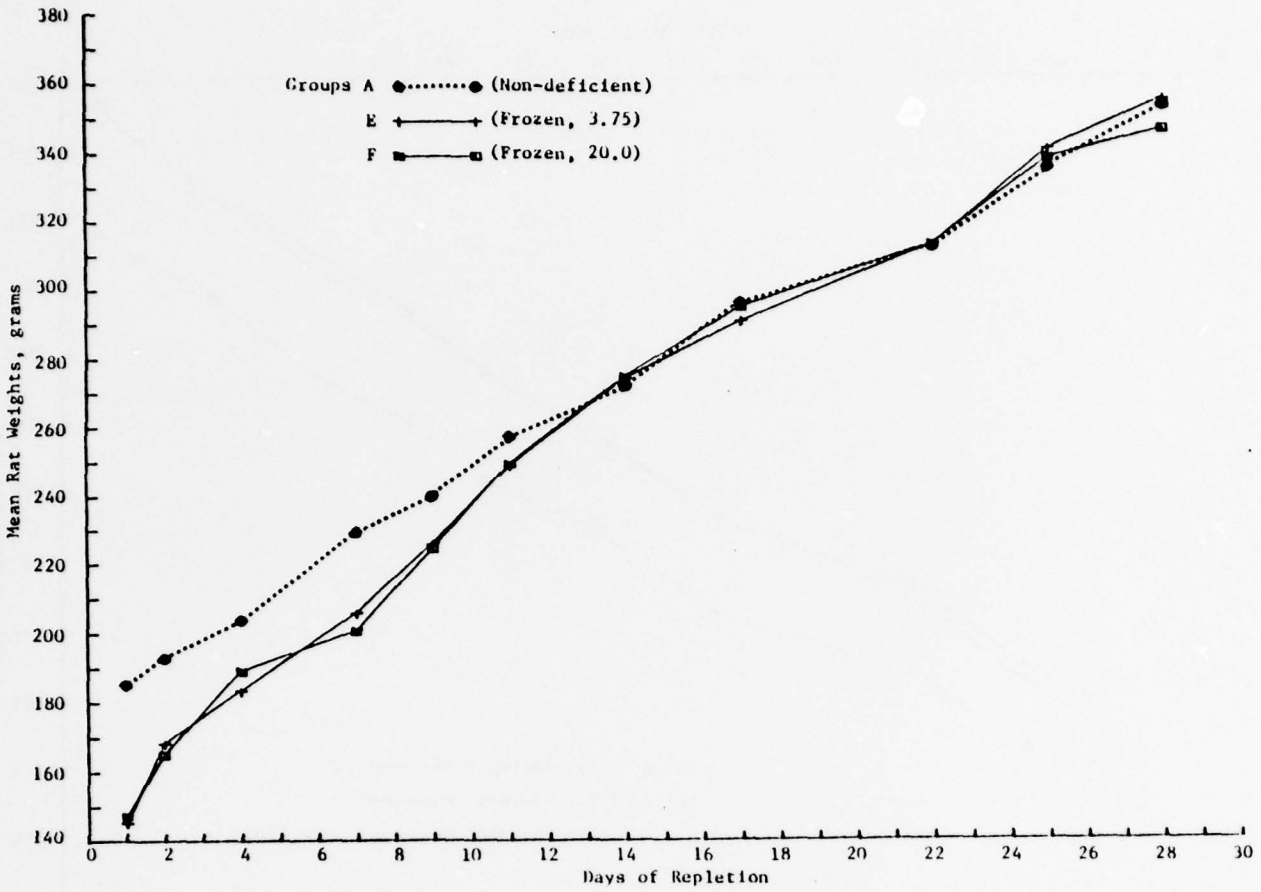

Figure 3. Growth Curves, Study 1 (Males), Phase 3, Groups A, E and F.

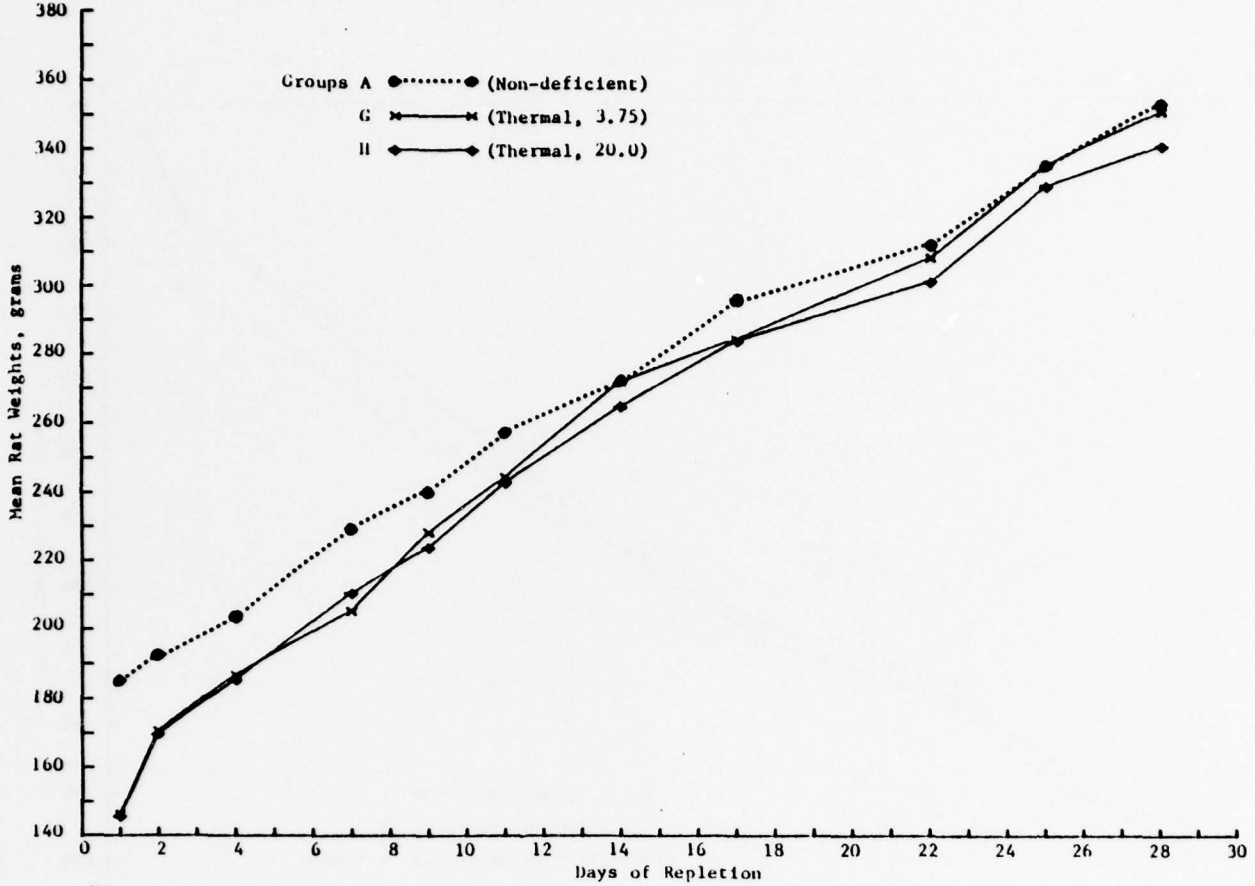

Figure 4. Growth Curves, Study I (Males), Phase 3, Groups A, G and H.

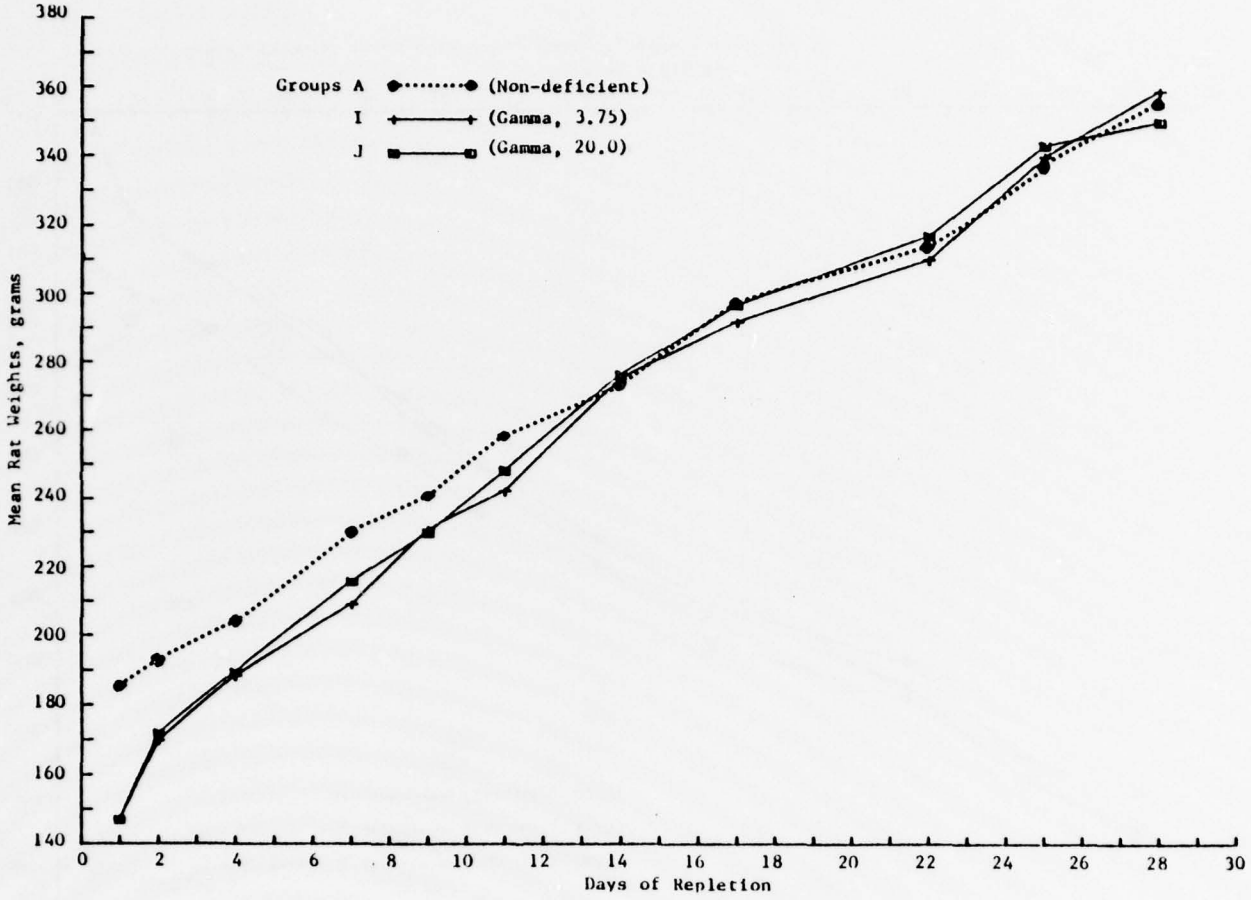

Figure 5. Growth Curves, Study I (Males), Phase 3, Groups A, I and J.

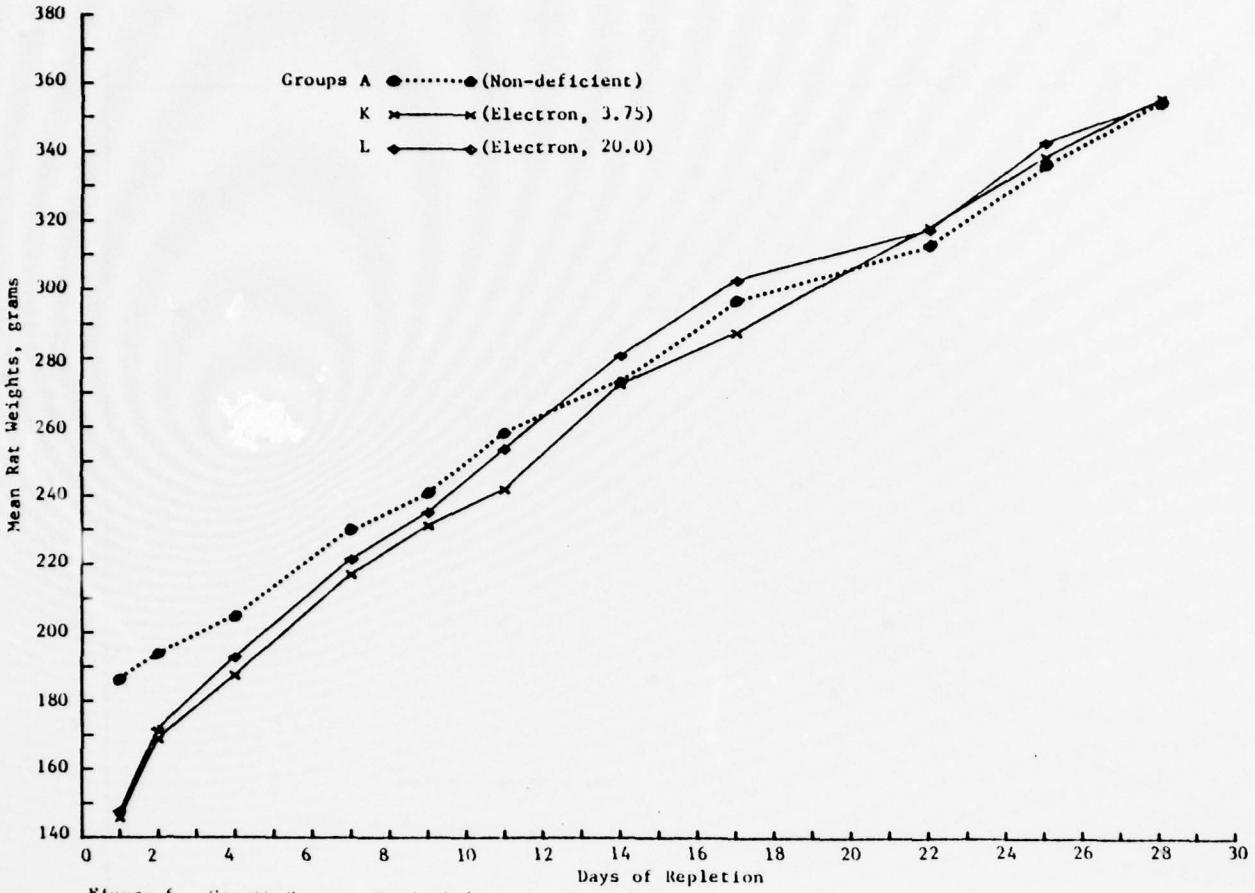

Figure 6. Growth Curves, Study 1 (Males), Phase 3, Groups A, K and L.

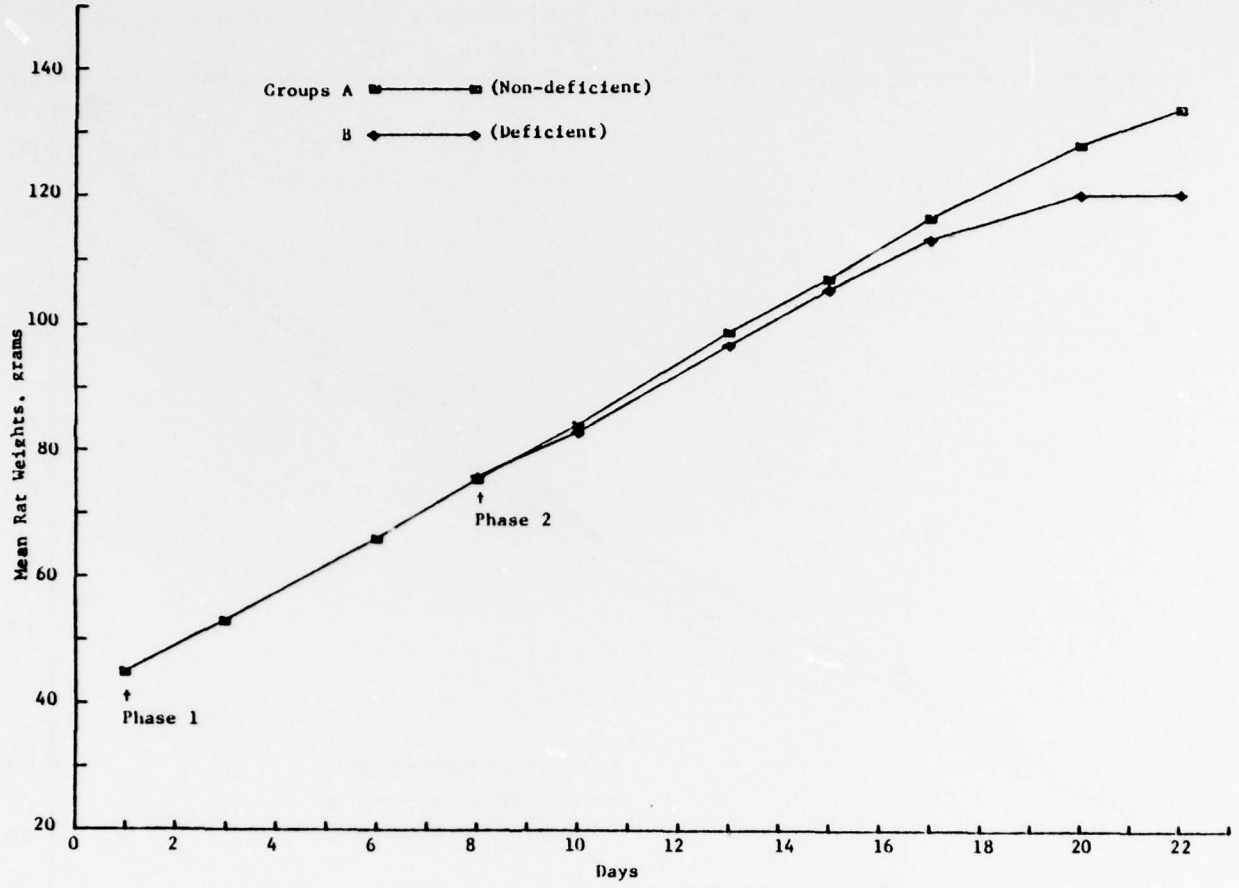

Figure 7. Growth Curves, Study 2 (Females), Phase 1 and 2, Groups A and B.

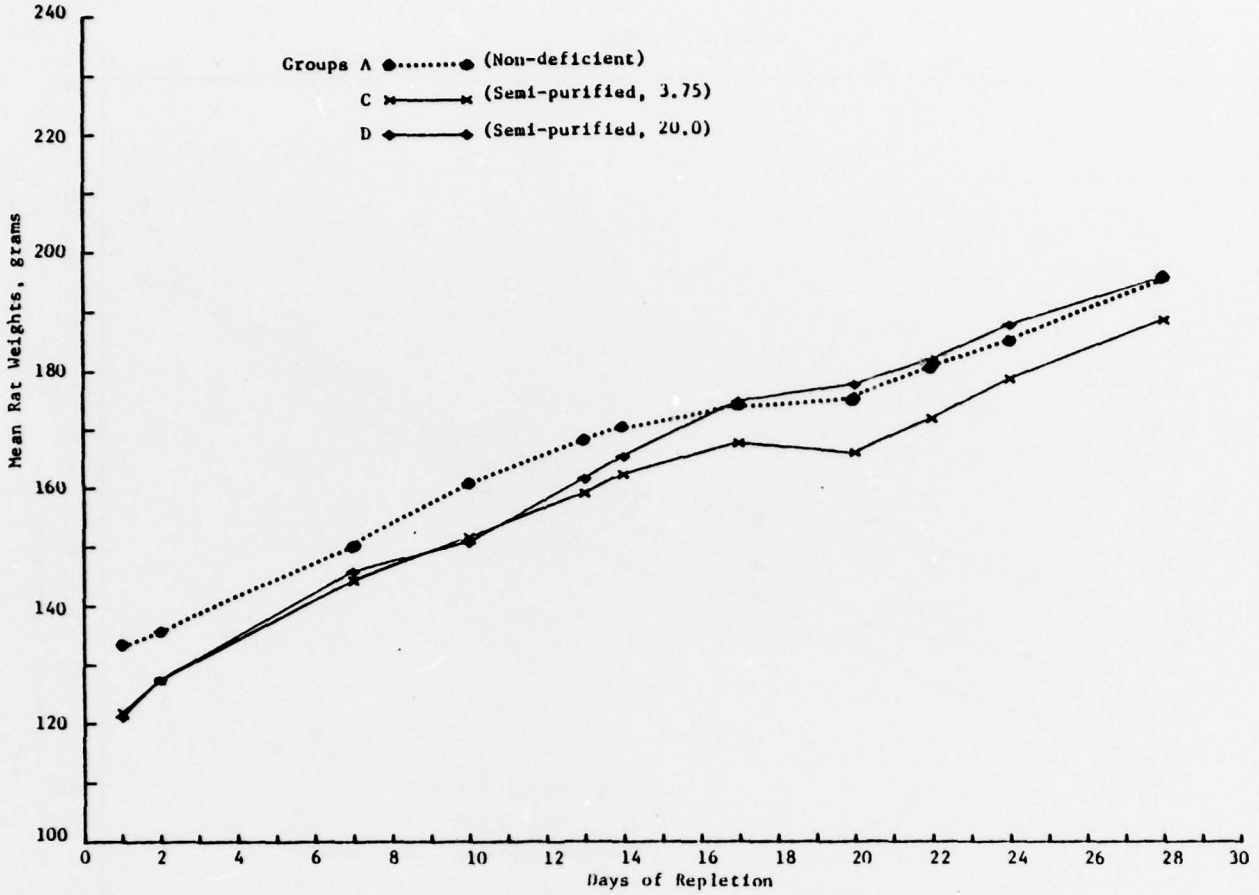

Figure 8. Growth Curves, Study 2 (Females), Phase 3, Groups A, C and D.

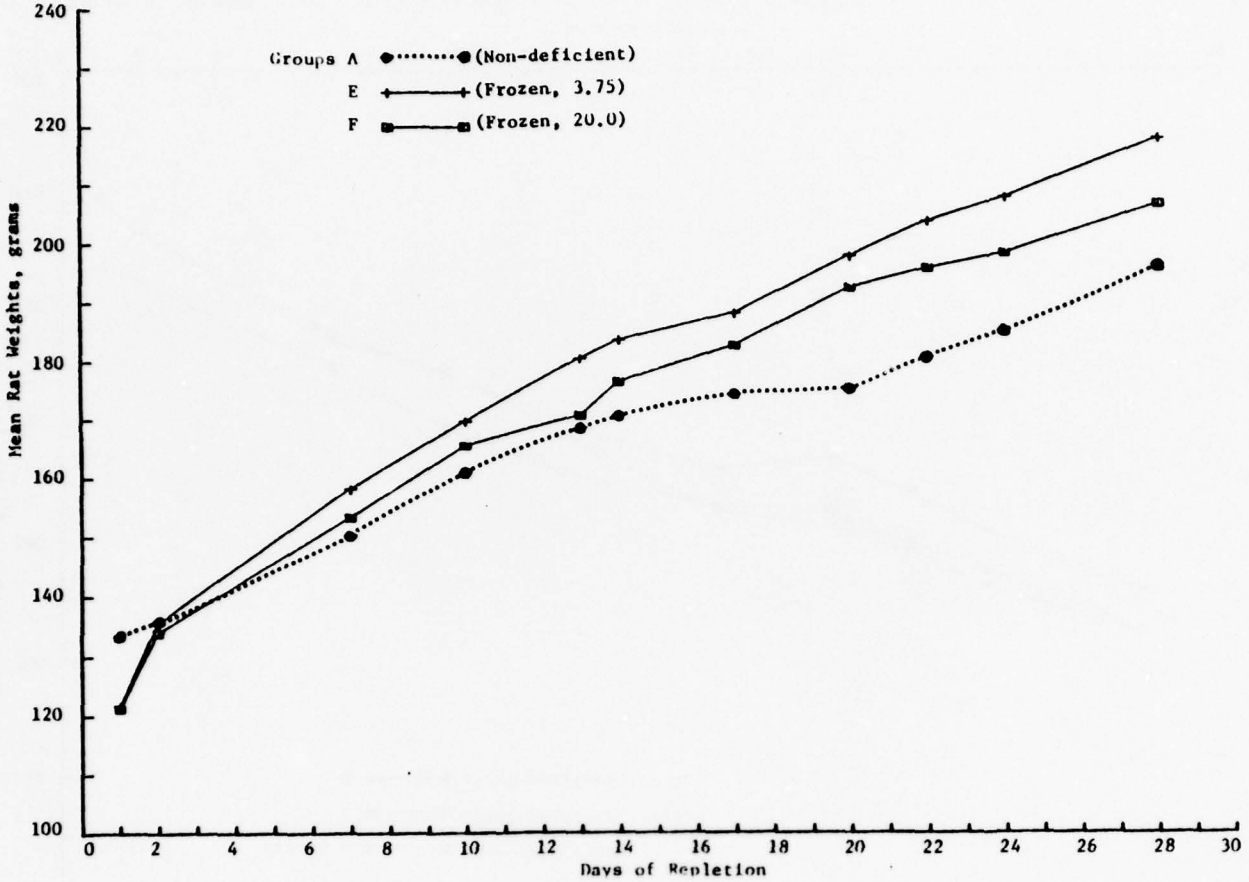

Figure 9. Growth Curves, Study 2 (Females), Phase 3, Groups A, E and F.

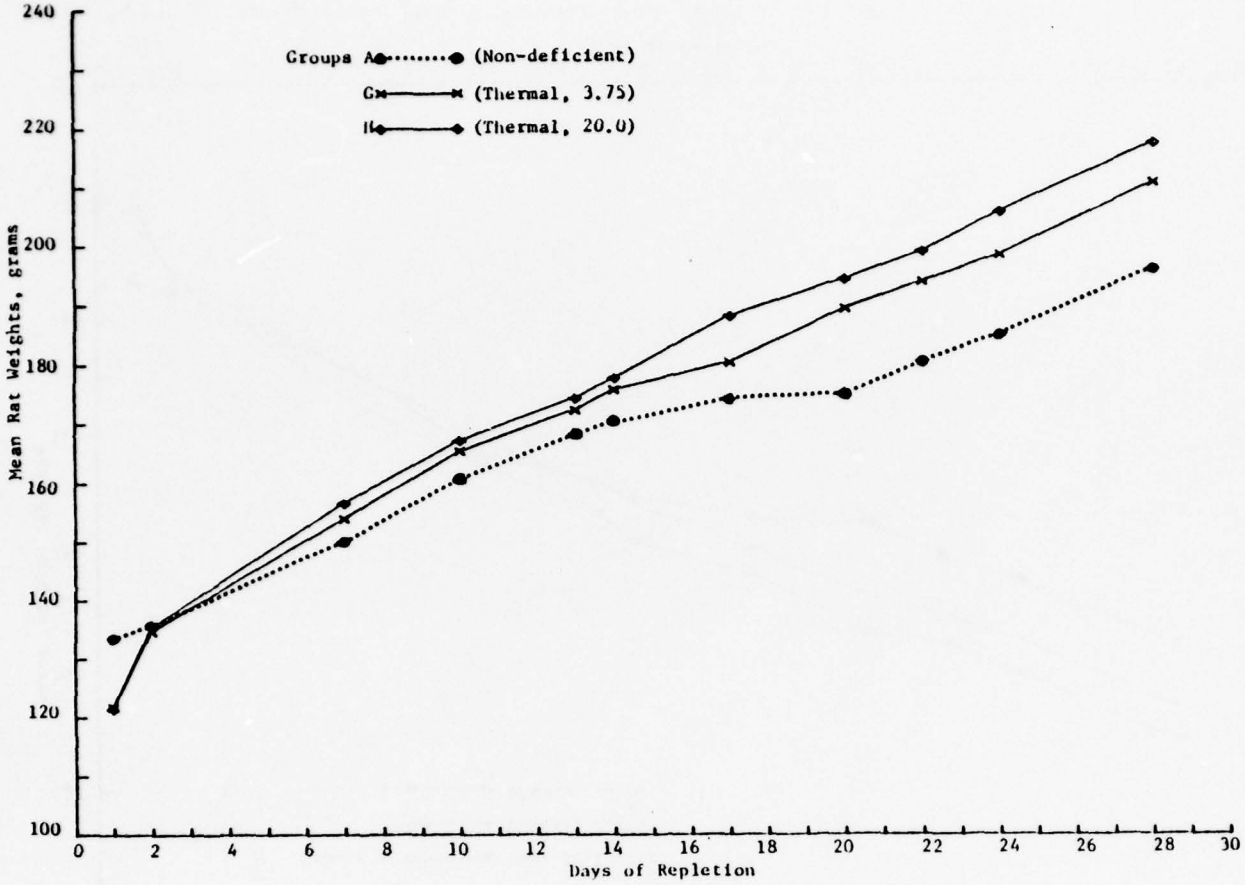

Figure 10. Growth Curves, Study 2 (Females), Phase 3, Groups A, G and H.

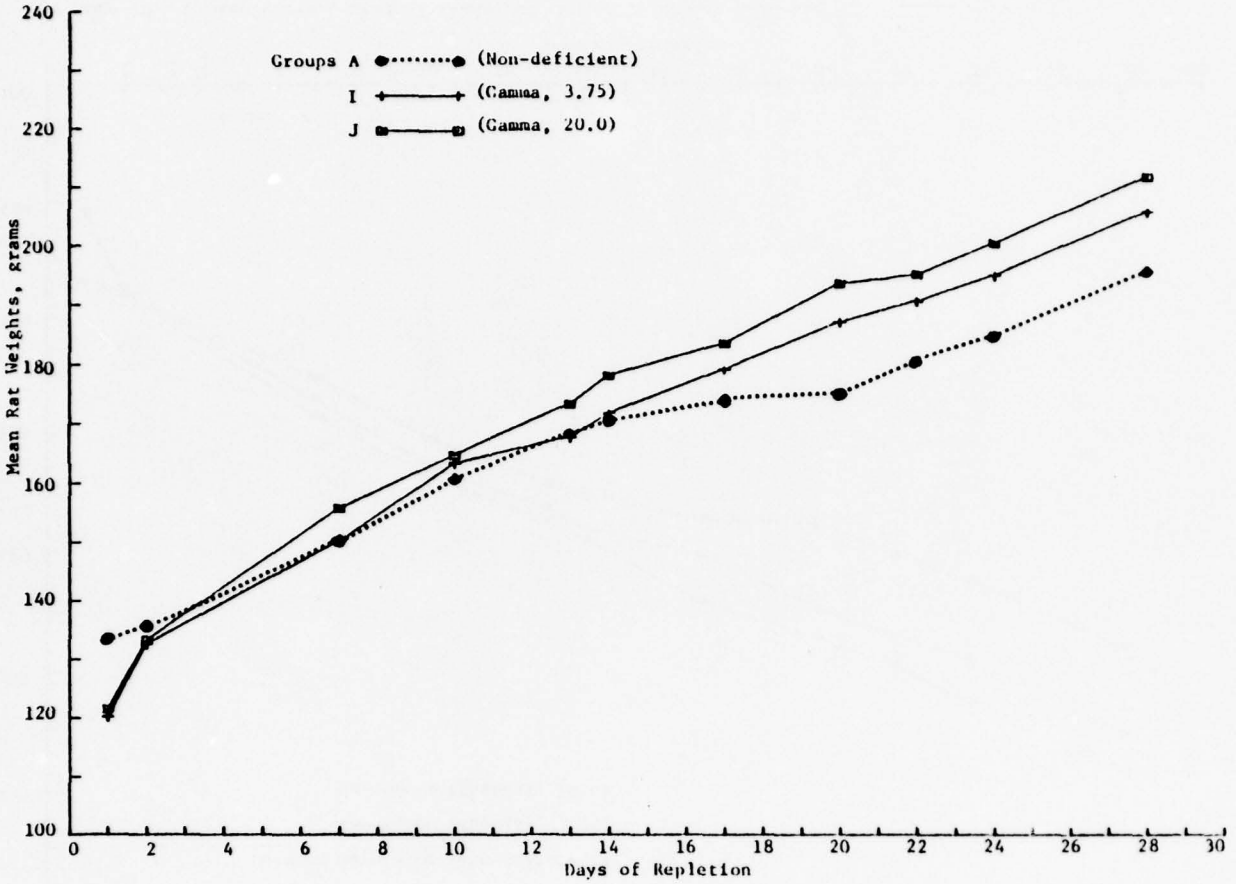

Figure 11. Growth Curves, Study 2 (Females), Phase 3, Groups A, I and J.

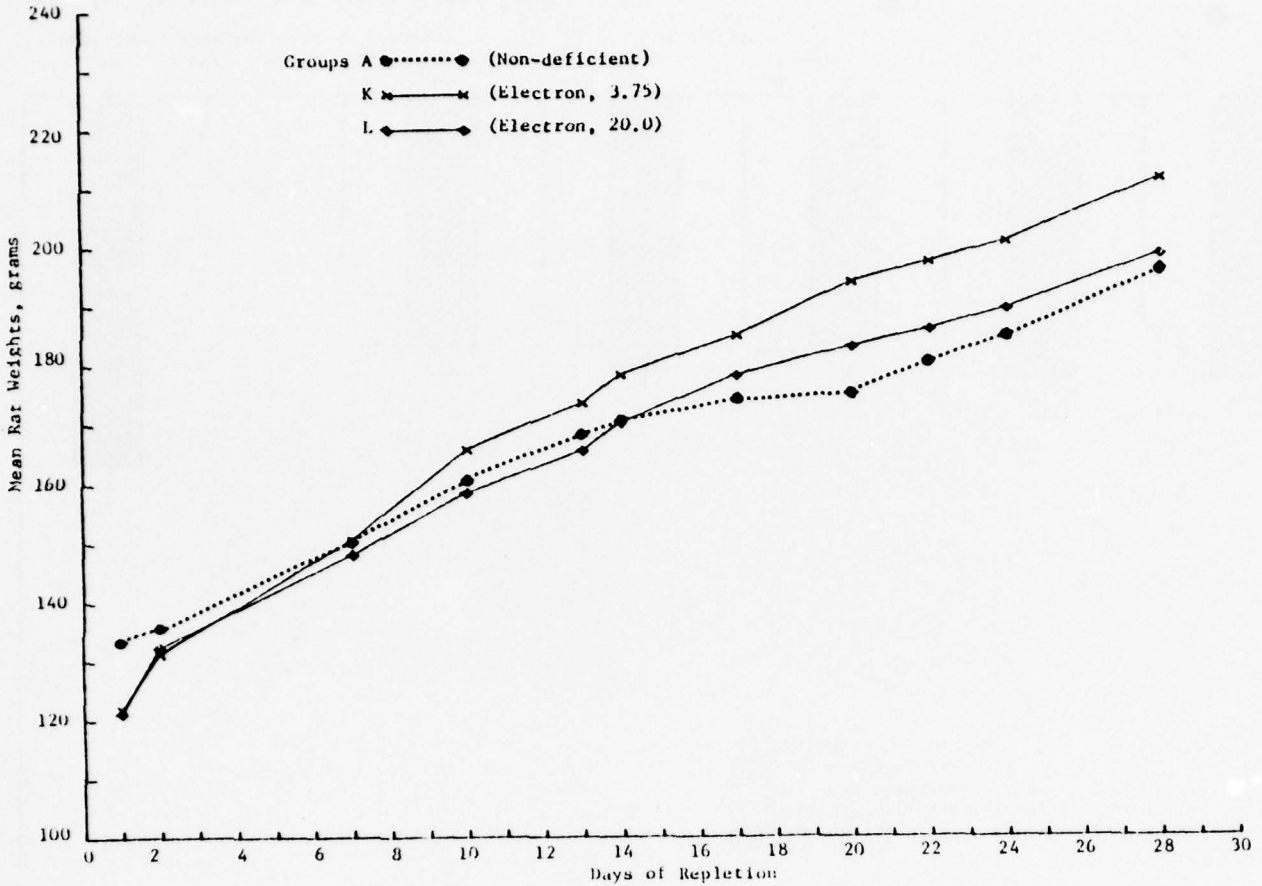

Figure 12. Growth Curves, Study 2 (Females), Phase 3, Groups A, K and L.

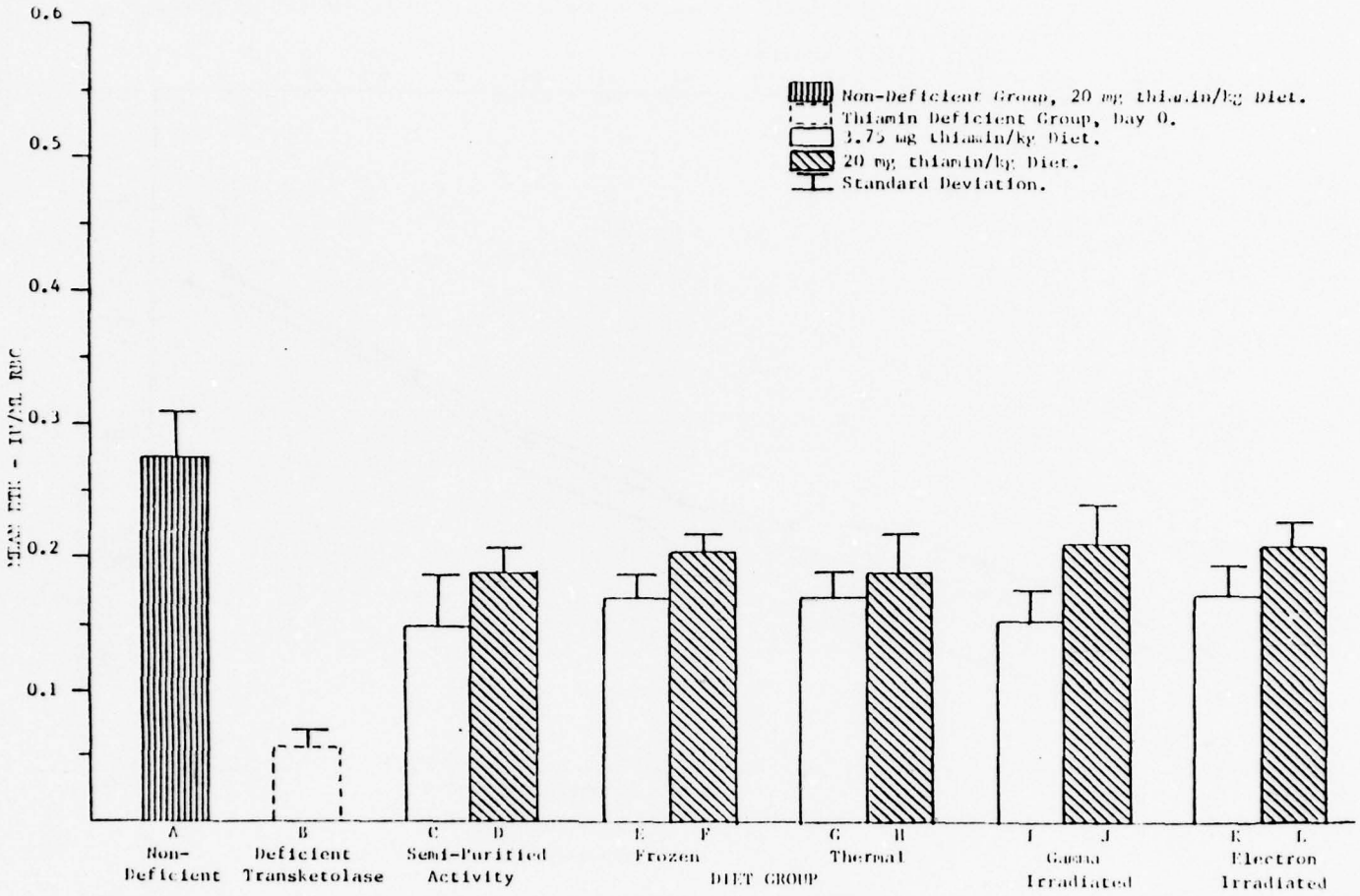

Figure 13. Transketolase Activity, Study 1 (Males), Day 7.

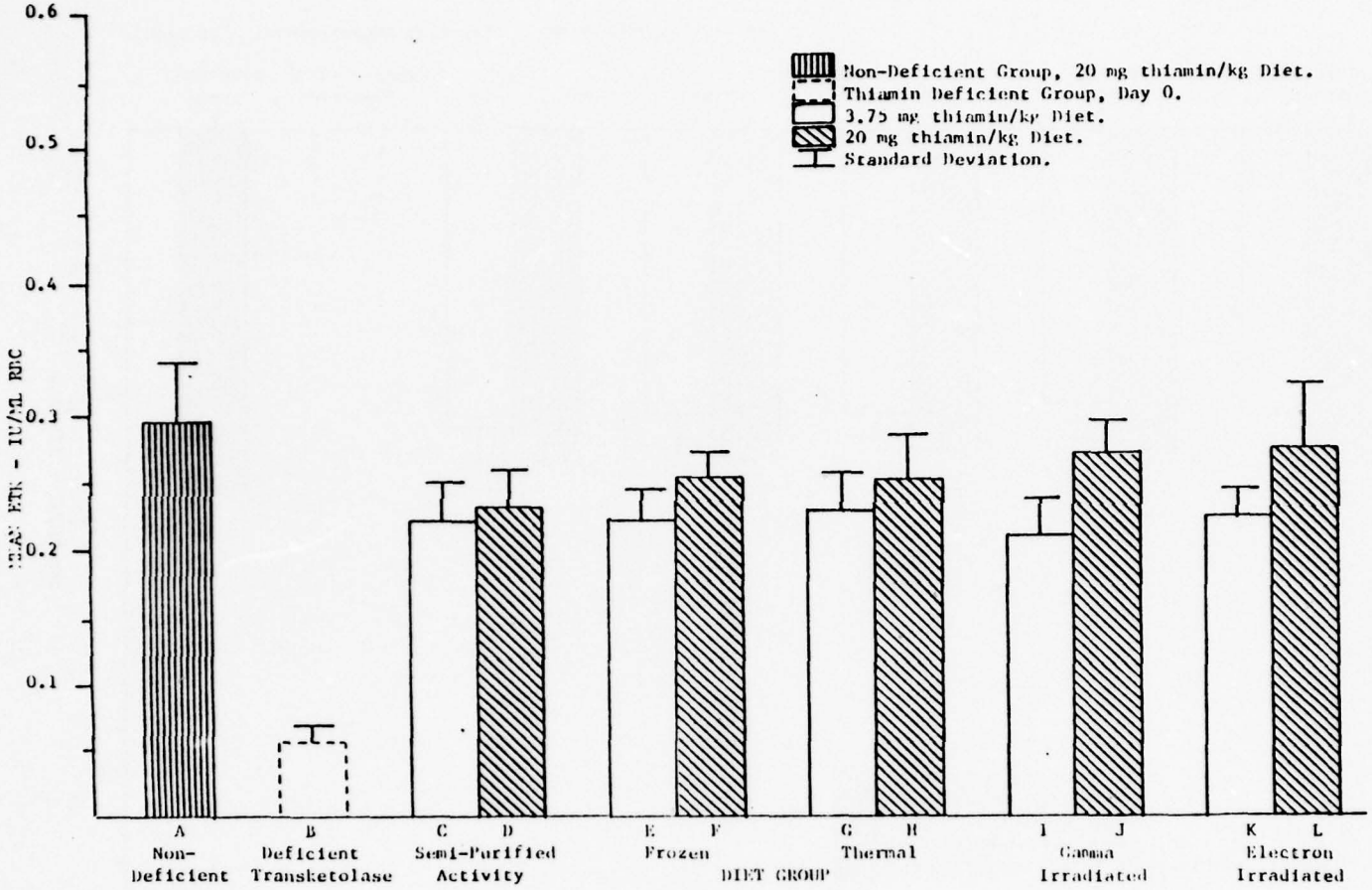

Figure 14. Transketolase Activity, Study 1 (Males), Day 14.

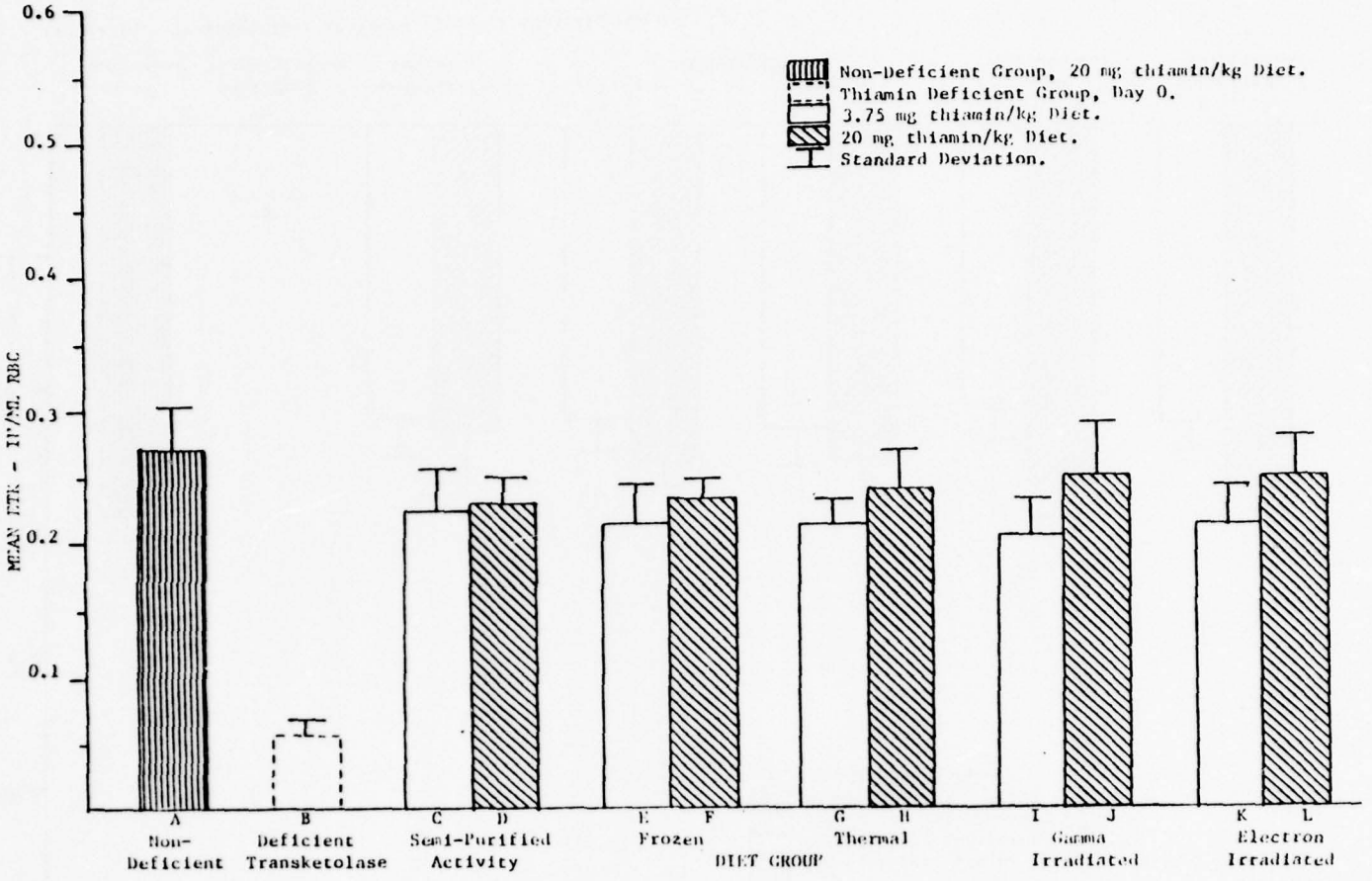

Figure 15. Transketolase Activity, Study 1 (Males), Day 28.

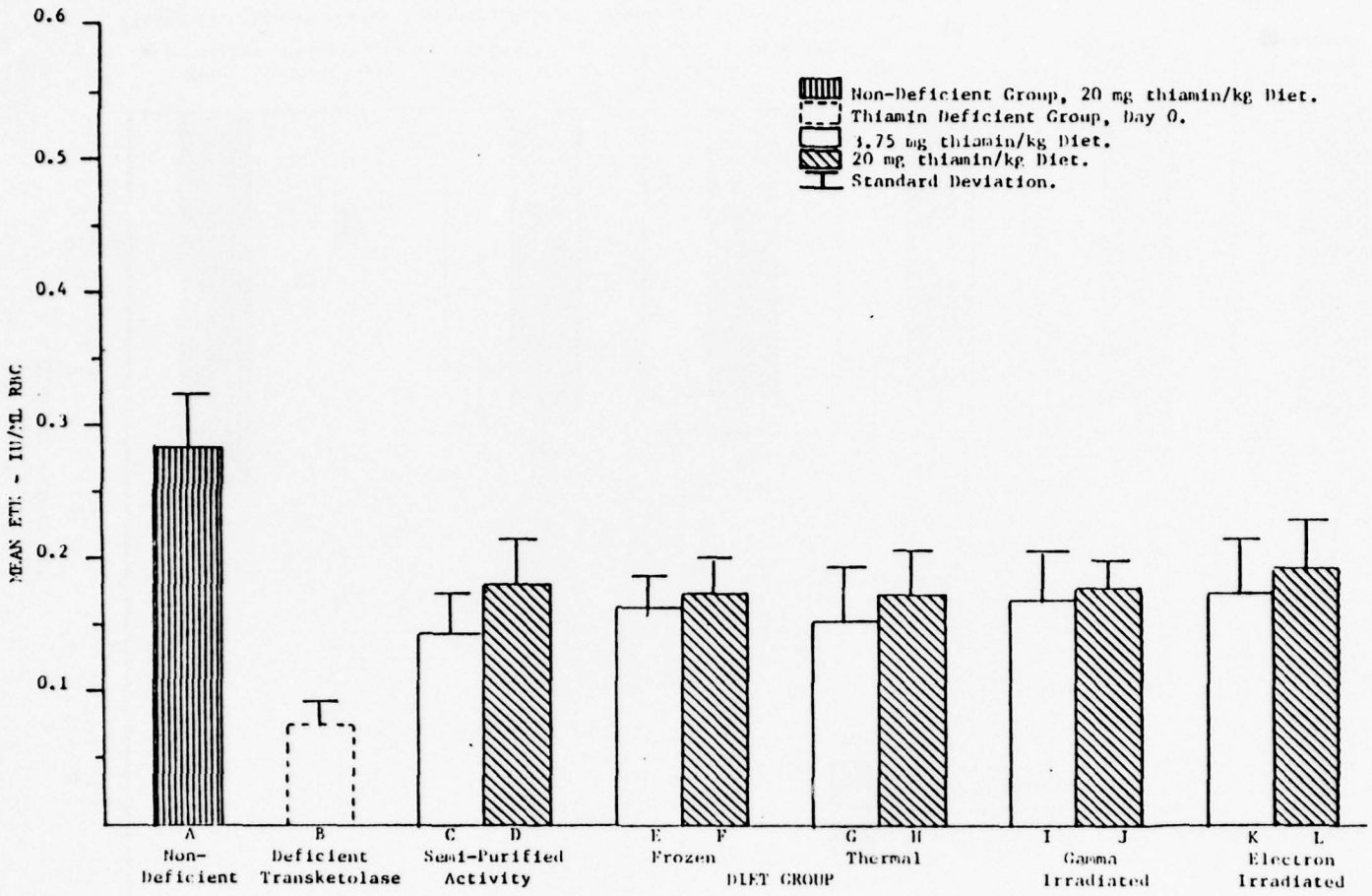

Figure 16. Transketolase Activity, Study 2 (Females), Day 7.

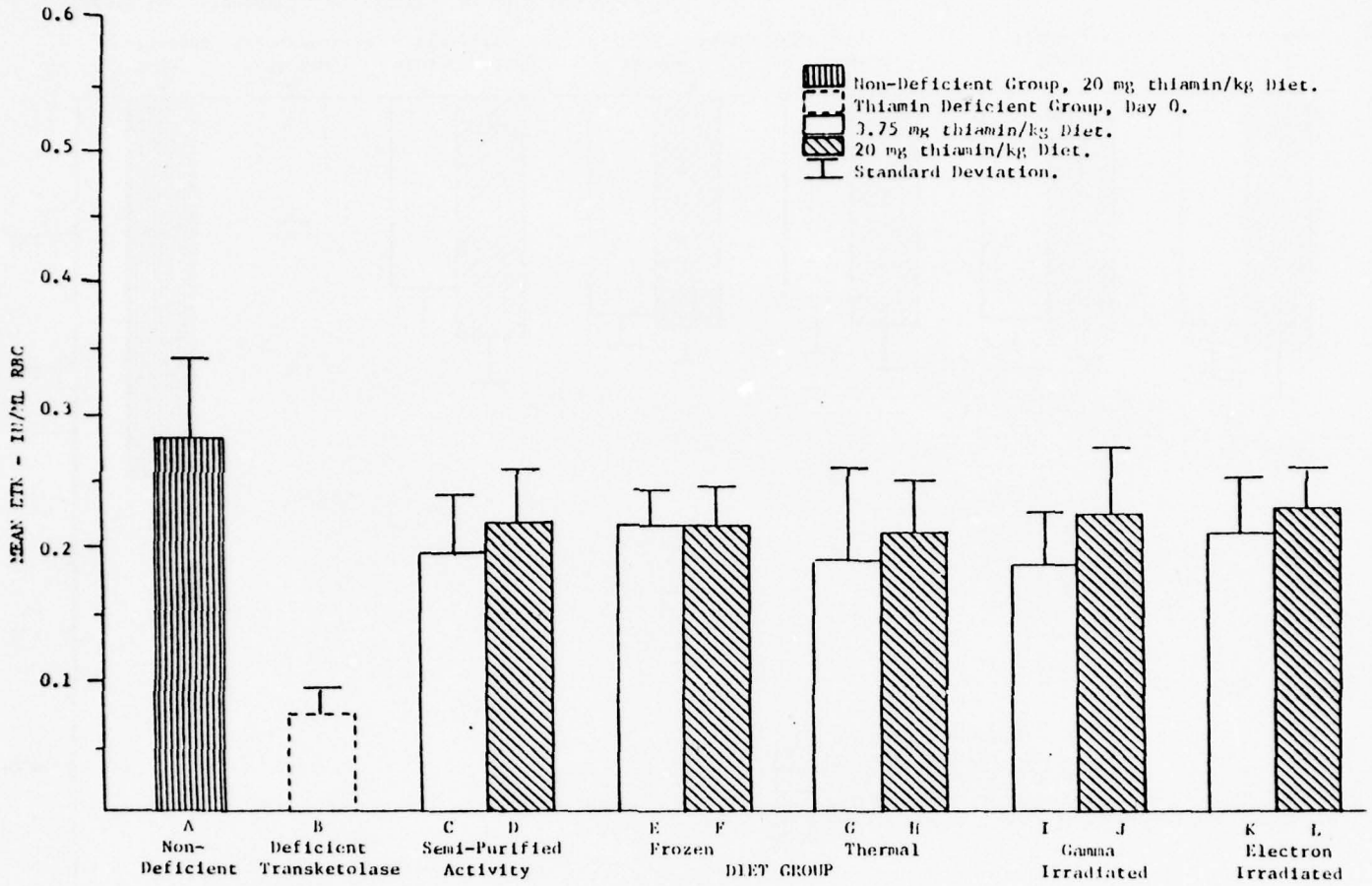

Figure 17. Transketolase Activity, Study 2 (Females), Day 14.

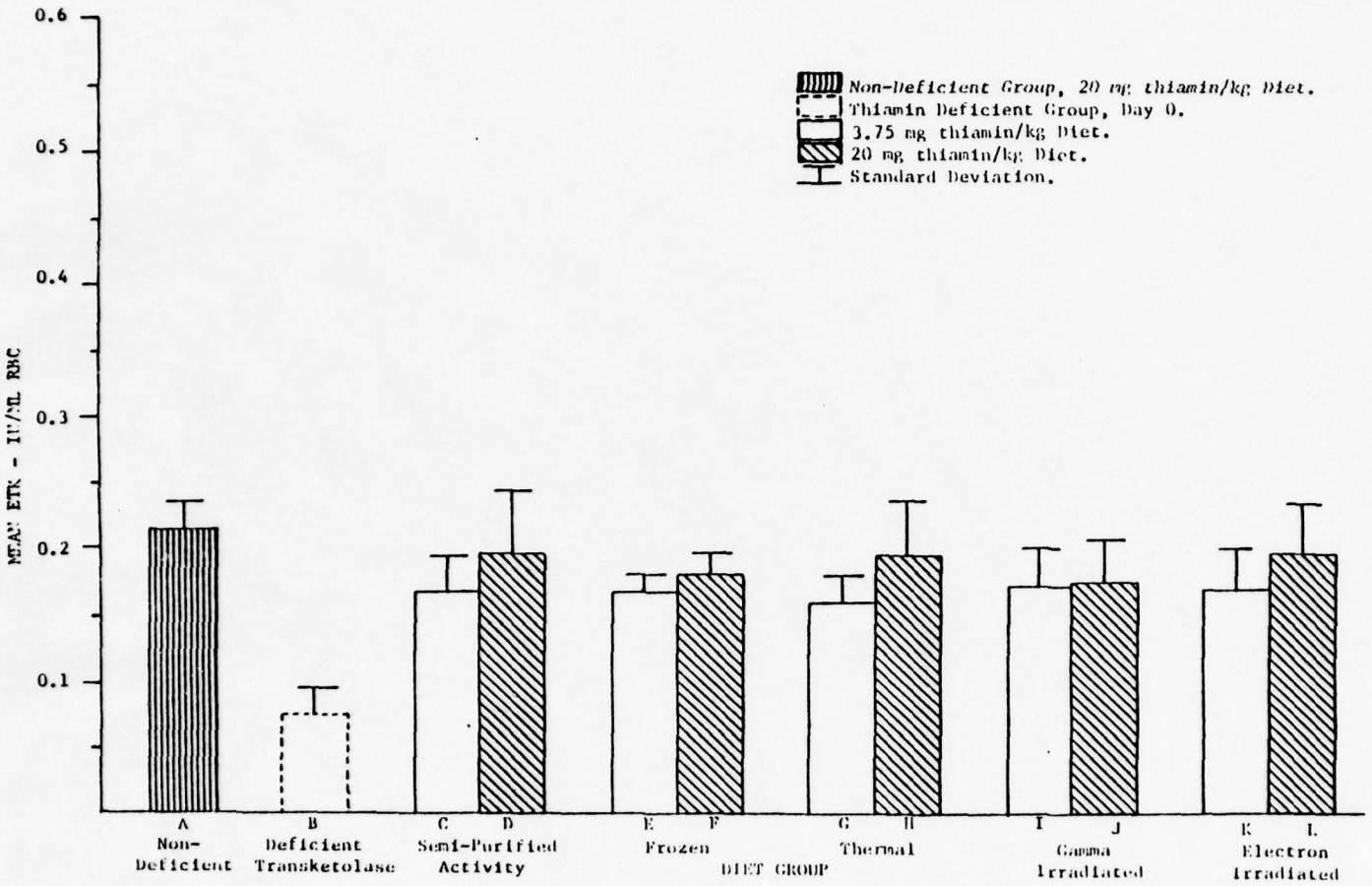

Figure 18. Transketolase Activity, Study 2 (Females), Day 28.

Page

APPENDIX B

TABLE 1. Composition of Diets

	Semipurified %	Beef %
Beef (dry weight)	-	35.0
Casein, vitamin free	20.0	-
Lard	10.0	-
Corn oil	5.0	-
L-cystine	0.2	0.2
Vitamin mix[1]	2.0	2.0
Choline chloride	0.2	0.2
Mineral mix[2]	4.0	4.0
Cerelose	58.6	58.6
	100%	100%

[1]The vitamin premix was made up in a cellulose carrier and contributed to the final diet the following vitamins in mg/kg: gelatin coated retinal (500 IU/mg) 26; Cholecalciferol (400 IU/mg), 5; DL-α-tocopheryl-acetate powder (250 IU/g), 440; Menadione - sodium bisulfite trihydrate 1.0; Riboflavin 10; Pyridoxine · HCl 20; Niacin 60; Ca - D-Pantothenate 30; Folic Acid 2.0; Biotin 1.0; B_{12}, 0.1% triturate 30. Thiamin · HCl was incorporated into a second premix and added to the diets to achieve the specified levels.

[2]The mineral mix contributed to the diet the following salts: in g/kg: $CaCO_3$, 4.78; $CaHPO_4$, 22.21; $NaHCO_3$, 1.164; NaCl, 1.494; K_2SO_4, 6.728; $MgSO_4$, 2.991; $MnSO_4.H_2O$, 0.258; in mg/kg: $ZnCO_3$, 37.6; KI, 0.337; $FeSO_4.7H_2O$, 292; $CuSO_4.5H_2O$, 33.2; Na_2SeO_3, 0.33; $Cr(Acetate)_3.H_2O$, 4.78; MoO_3, 1.51; $CoSO_4.7H_2O$, 4.79.

TABLE 2. Schedule and Diet Codes for Antithiamin Studies

PHASE	LENGTH OF PHASE	TREATMENT GROUPS
1. (Quarantine)	1 week	A
2. (Depletion)	14-16 days	A,B
3. (Repletion)	4 weeks	A, C-L

DIET CODE	DIET	THIAMIN LEVEL mg/kg dry weight
A	Semipurified	20.0 (non deficient control group)
B	Semipurified	0 (deficient diet)
C	Semipurified	3.75
D	Semipurified	20.0
Meat-Containing Diets (35% on dry weight basis)		
E	Frozen Beef	3.75
F	Frozen Beef	20.0
G	Thermally Processed	3.75
H	Thermally Processed	20.0
I	Gamma Irradiated	3.75
J	Gamma Irradiated	20.0
K	Electron Irradiated	3.75
L	Electron Irradiated	20.0

Study 1: Males

Study 2: Females

TABLE 3. Proximate Analyses, Calcium, Phosphorous and Thiamin Contents in Beef Test Meats*

TEST MEAT	MOISTURE %	PROTEIN %	FAT %	ASH %	PHOSPHOROUS %	CALCIUM %	CALORIES Kcal/g	THIAMIN mg/kg WET WT.	THIAMIN mg/kg DRY WT.
Frozen	58.4	24.0	15.4	2.14	.223	.0045	2.68	1.03	2.48
mean % SD (n=4)	2.9	2.0	2.3	0.12	.054	.0004	0.19	.07	0.18
Thermal	58.1	24.9	14.0	2.11	.243	.0046	2.56	0.22	0.52
mean % SD (n=4)	2.4	1.1	1.5	0.15	.049	.0004	0.34	0.02	0.04
Cobalt Irradiated	59.7	22.2	14.4	1.91	.197	.0050	2.74	0.24	0.59
mean % SD (n=4)	1.7	0.5	2.1	0.13	.061	.0003	0.15	0.04	0.10
Electron Irradiated	60.7	22,5	13.2	1.98	.252	.0048	2.54	0.45	1.15
mean % SD (n=4)	0.7	0.8	1.4	0.06	.035	.0006	0.05	0.04	0.11

*All meats had been heated according to procedure outlined in Diet Preparations.

TABLE 4. Thiamin Contents of Repletion Diets[1]

DIET		CALCULATED THIAMIN mg/kg (Wet Weight)	MOISTURE[2] %	CALCULATED THIAMIN mg/kg (Dry Weight)
3.75 mg/kg diet:				
Frozen (n=3)	mean	2.2	38.3	3.6
	S.D.	0.2		0.4
Thermal (n=3)	mean	2.3	38.5	3.9
	S.D.	0.1		0.6
Gamma Irradiated (n=3)	mean	2.4	40.4	3.6
	S.D.	0.3		0.4
Electron Irradiated (n=3)	mean	2.1	41.0	3.8
	S.D.	0.2		0.5
Dry Basal (n=2)	mean	3.6	----	3.6
	S.D.			
20 mg/kg diet:				
Frozen (n=3)	mean	11.3	38.6	18.4
	S.D.	0.8		1.3
Thermal (n=3)	mean	12.3	38.9	20.1
	S.D.	0.1		0.2
Gamma Irradiated (n=3)	mean	11.4	40.8	19.2
	S.D.	0.4		0.6
Electron Irradiated (n=3)	mean	11.6	40.8	19.6
	S.D.	0.5		0.9
Dry Basal (n=1)	mean	19.4	----	19.4
	S.D.			

[1] Microbiological Assay
[2] Based on mean of two samples

TABLE 5. Growth of Thiamin-Deficient Rats Repleted with Semi-Purified or Beef-Based Diets

(Study 1, Phase 3, Males)

Group	Treatment	Initial Weight (g)[1]	Final Weight (g)[1]	Average Daily Gains (g) Week 1	Week 2	Week 3	Week 4	Overall Average (g)/Day
A	Non-deficient	185.2 ± 11.7	353.2 ± 21.3	7.4	6.1	5.0	6.9	6.2
C	Dry - 3.75	147.2 ± 17.7	335.8 ± 24.9	7.8	7.3	5.6	7.8	7.0
D	Dry - 20.0	146.9 ± 16.5	326.9 ± 34.2	8.6	7.0	5.1	6.8	6.7
E	Frozen - 3.75	145.3 ± 17.7	354.9 ± 27.4	10.1	9.7	4.9	7.0	7.8
F	Frozen - 20.0	147.3 ± 14.9	346.0 ± 17.1	8.9	10.6	4.8	5.5	7.4
G	Thermal - 3.75	146.4 ± 17.3	350.8 ± 24.3	9.8	9.4	4.5	7.1	7.5
H	Thermal - 20.0	145.8 ± 20.5	340.7 ± 17.2	10.8	8.0	4.6	6.6	7.3
I	Gamma - 3.75	146.9 ± 14.6	358.7 ± 16.5	10.3	9.3	4.4	8.2	7.8
J	Gamma - 20.0	146.9 ± 16.1	349.6 ± 25.4	11.4	8.5	5.1	5.5	7.5
K	Electron - 3.75	145.8 ± 14.0	356.0 ± 19.7	11.9	7.9	5.3	6.3	7.7
L	Electron - 20.0	147.8 ± 14.8	355.0 ± 17.6	12.3	8.5	4.7	6.3	7.7

[1]Mean ± SD

TABLE 6. Growth of Thiamin-Deficient Rats Repleted With Semi-Purified or Beef-Based Diets

(Study 2, Phase 3, Females)

Group	Treatment	Initial Weight (g)[1]	Final Weight (g)[1]	Average Daily Gains (g) Week 1	Week 2	Week 3	Week 4	Overall Average (g)/Day
A	Non-deficient	133.5 ± 8.6	195.9 ± 11.9	2.8	2.7	1.2	2.6	2.3
C	Dry - 3.75	121.9 ± 12.2	188.4 ± 20.4	3.8	2.6	1.3	2.8	2.5
D	Dry - 20.0	121.2 ± 11.3	195.6 ± 26.7	4.1	2.6	1.7	2.3	2.6
E	Frozen - 3.75	121.8 ± 12.5	217.4 ± 19.8	6.0	3.7	2.5	2.3	3.5
F	Frozen - 20.0	121.2 ± 10.9	206.2 ± 21.0	5.3	3.3	2.2	1.8	3.1
G	Thermal - 3.75	121.8 ± 11.4	210.5 ± 24.4	5.4	3.1	2.3	2.8	3.3
H	Thermal - 20.0	121.5 ± 11.5	217.3 ± 21.3	5.9	3.0	2.3	3.0	3.4
I	Gamma - 3.75	120.2 ± 10.9	206.0 ± 17.0	5.0	3.3	2.4	2.5	3.2
J	Gamma - 20.0	121.5 ± 11.3	212.0 ± 15.8	5.7	3.4	2.2	2.8	3.4
K	Electron - 3.75	121.9 ± 12.7	211.2 ± 34.2	4.7	3.9	2.2	2.3	3.2
L	Electron - 20.0	121.2 ± 10.7	198.4 ± 12.2	4.5	3.0	2.2	2.1	2.9

[1]Mean ± SD

Table 7. Mean Daily Weight Gains of Male Thiamin-Deficient Rats During Repletion With Semi-Purified or Beef-Based Diets. (Study 1, Phase 3)

	Dates of Weighings									
Group	8/15-16	8/16-18	8/18-21	2/21-23	8/23-25	8/25-28	8/28-31	8/31-9/5	9/5-8	9/8-11
	Mean weight gain (g/day)[1]									
A	7.6	5.5	8.5	5.3	8.7	4.9	8.0	3.2	7.7	6.0
C	11.5	7.0	7.0	6.8	8.9	6.6	7.3	4.5	8.8	6.7
D	11.7	8.1	7.8	6.7*	8.4	6.3	8.0	3.4	8.1	5.4
E	22.9	7.5	7.5	9.9*	11.5	8.4	5.6	4.4	9.3	4.7
F	26.0	7.9	3.8	11.9	12.5	8.5	6.6	3.7	8.3	2.7*
G	24.3	8.0	6.1	11.4	7.8*	9.2	4.2	4.7	9.1	5.1
H	24.1	7.8	8.4	7.4**	9.4	7.4	6.3	3.5	9.3	3.9
I	22.5	9.3	6.9	10.6	5.5*	11.0	5.6	3.6	9.9	6.4
J	24.9	8.8	8.7	7.0	8.7*	9.4	6.8	4.0	8.5	2.4
K	23.1	9.3	9.9	7.1	5.3	10.2	5.5*	5.1*	6.8	5.8
L	23.9	10.5	9.6	6.8	9.2	9.2	7.7*	2.9	8.5	4.0

[1]Time intervals between weighings varied from 2 to 5 days. Weekly averages for Table 5 were calculated for the following periods: Week 1 (8/15-8/21), Week 2 (8/21-8/28), Week 3 (8/28-9/5), Week 4 (9/5-9/11)

*Indicates loss of one or 2 (**) animals after cardiac puncture.

Table 8. Mean Daily Weight Gains of Female Thiamin-Deficient Rats Repleted With Semi-Purified or Beef-Based Diets. (Study 2, Phase 3)

	Dates of Weighings									
Group	8/30-31	8/31-9/5	9/5-8	9/8-11	9/11-12	9/12-15	9/15-18	9/18-20	9/20-22	9/22-26
	Mean weight gain (g/day)[1]									
A	2.3	2.9	3.0***	2.5	2.2	1.2	.3	2.7	2.2	2.8
C	5.5	3.4	2.5	2.5	3.2	1.8	-.3*	2.9	3.4	2.5
D	6.3	3.7	1.9*	2.9*	3.8	2.3*	.9	2.1	2.9	2.0
E	13.6	4.5	3.9*	3.6	3.1	1.5	3.2	3.0	1.9	2.5
F	12.7	3.8	4.1	1.7	5.8	1.7*	3.2	1.6	1.3	2.1
G	12.9	3.9	3.8	2.3	3.4	1.5*	3.0	2.3	2.2	3.1
H	13.9	4.3	3.5	2.4	3.4	2.7***	2.0	2.3	3.3	2.9
I	12.5	3.5	5.0*	1.4	4.2	2.4	2.7	1.8	2.2	2.7
J	11.8	4.5	3.6**	2.9	4.6	1.8	3.4	.8	2.7	2.9
K	9.4	3.8	5.1	2.5	4.7	1.6*	3.0	1.7	1.8	2.6
L	11.2	3.1	3.2***	2.3	4.7	3.2*	1.6	1.5	1.8	2.3

[1]Time intervals between weighings varied from 2 to 5 days. Weekly averages for Table 6 were calculated for the following time periods: Week 1 (8/30-9/5), Week 2 (9/5-9/12), Week 3 (9/12-9/20), Week 4 (9/20-9/27).

*Indicates loss of one, two (**) or three (***) animals after cardiac puncture.

TABLE 9. Summary of Thiamin Pyrophosphate Stimulation (TPP Effect)*

GROUP		STUDY 1 (Males)			STUDY 2 (Females)		
		DAY 0			DAY 0		
A	mean	3.62			5.14		
	S.D.	2.92			3.93		
B	mean	23.06			29.95		
	S.D.	13.50			22.02		
		DAY 7	DAY 14	DAY 28	DAY 7	DAY 14	DAY 28
A	mean	5.46	4.87	5.57	4.87	6.71	8.32
	S.D.	3.90	4.14	2.63	3.14	2.96	5.57
C	mean	8.27	6.23	6.72	5.28	6.00	9.17
	S.D.	6.78	3.44	4.70	6.22	4.84	4.78
D	mean	6.66	3.88	5.58	6.72	6.65	8.63
	S.D.	6.79	4.06	2.35	4.60	5.52	4.85
E	mean	6.87	3.95	4.20	5.06	7.01	6.39
	S.D.	6.31	4.21	4.50	4.09	3.25	3.97
F	mean	4.65	4.28	5.76	6.17	6.59	6.67
	S.D.	4.61	3.50	2.71	3.06	3.67	5.22
G	mean	6.33	7.18	4.92	6.47	4.07	7.30
	S.D.	5.20	3.85	3.66	4.41	4.60	4.00
H	mean	3.25	5.18	6.05	5.61	6.21	7.17
	S.D.	3.83	1.91	3.45	3.59	3.49	3.77
I	mean	7.24	8.97	4.91	7.23	9.26	7.01
	S.D.	7.46	3.38	4.52	5.83	2.56	5.67
J	mean	5.67	3.06	3.59	8.17	5.48	6.69
	S.D.	5.00	2.94	3.43	5.81	3.34	4.09
K	mean	6.80	6.57	4.112	4.82	6.02	7.95
	S.D.	3.38	5.78	2.95	4.74	4.27	5.79
L	mean	5.64	6.68	4.63	3.21	7.75	6.61
	S.D.	4.25	2.13	3.71	3.40	2.37	3.23

*All Stimulation Values are given in percentages.

TABLE 10. Analysis of Variance Significance Levels - Study 1, Males[1]

	PARAMETER[2]	FOOD	VITAMIN	INTERACTION
Day 7				
	ETK	0.30(.03)	0.00(.00)	0.03(.10)
	ETK-Stimulated	0.18(.03)	0.00(.00)	0.02(.05)
	TPP Effect	0.68(.59)	0.06(.06)	0.94(.98)
Day 14				
	ETK	0.36(.05)	0.00(.00)	0.11(.02)
	ETK-Stimulated	0.16(.02)	0.00(.00)	0.22(.03)
	TPP Effect	0.11(.12)	0.02(.00)	0.02(.05)
Day 28				
	ETK	0.79(.90)	0.00(.00)	0.34(.00)
	ETK-Stimulated	0.80(.89)	0.00(.00)	0.57(.105)
	TPP Effect	0.72(.59)	0.46(.81)	0.51(.48)

[1]The first numbers in each pair represent P-values when ANOVA was performed only on meat-fed groups (E-L). The numbers in parenthesis were obtained when the two dry diet groups (C and D) were included in the ANOVA.

[2]ETK, Erythrocyte Transketolase Activity; ETK Stimulated, ETK Activity in the presence of added thiamin pyrophosphate cofactor; TPP Effect, % Increase in ETK due to added TPP.

TABLE 11. Analysis of Variance Significance Levels - Study 2, Females[1]

	PARAMETER[2]	FOOD	VITAMIN	INTERACTION
Day 7				
	ETK	.22(.20)	.05(.00)	.90(.62)
	ETK-Stimulated	.57(.49)	.02(.00)	.90(.60)
	TPP Effect	.10(.13)	.52(.41)	.89(.95)
Day 14				
	ETK	.74(.79)	.11(.04)	.43(.57)
	ETK-Stimulated	.63(.66)	.13(.04)	.66(.72)
	TPP Effect	.21(.44)	.92(.83)	.04(.13)
Day 28				
	ETK	.93(.94)	.04(.01)	.60(.65)
	ETK-Stimulated	.95(.86)	.05(.01)	.69(.70)
	TPP Effect	.89(.50)	.66(.63)	.99(1.00)

[1] The first numbers in each pair represent P-values when ANOVA was performed only on meat-fed groups(E-L). The numbers in parenthesis were obtained when the two dry diet groups (C and D) were included in the ANOVA.

[2] ETK, Erythrocyte Transketolase Activity; ETK-Stimulated, ETK Activity in the presence of added thiamin pyrophosphate cofactor; TPP Effect, % Increase in ETK due to added TPP.

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OFFICIAL COOPERATING AGENCIES

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HQDA, Office, Deputy Chief of Staff
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Washington, DC 20310

Commandant
U.S. Marine Corps
HQ, US Marine Corps, LFS-4
Washington, DC 20389

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U.S. Army Natick Res&Dev Command
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JTTS, USMC DRDNA-ZF
Natick, MA 01760

Commander
U.S. Army Natick Res&Dev Com
ATTN: Director, Food Sciences
Laboratory
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Commander
U.S. Army Natick Res&Dev Command
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Commanding Officer
Navy Food Service Systems Office
Washington Navy Yard
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Chairman, DOD Food Planning Board
Director, Supply Management Policy
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Logistics
Pentagon, Room 3B730
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Chairman, DOD Food Service Facility &
Equipment Planning Board
Office of the Chief of Engineers
Forrestal Building, Room 2-F-055
Department of the Army
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Commander
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Chairman, Joint Formulation Board
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HQ, US Marine Corps LFS-4
Washington, DC 20380

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OFFICIAL COOPERATING AGENCIES
(Continued)

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