RESEARCH and

TECHNOLOGY

DEVELOPMENT

INCORPORATED

LIFE SCIENCES DIVISION

THE METABOLISM OF INGESTED PEROXIDES

30 APRIL 1971

FINAL REPORT

BRIAN WARD
MARGARET SMITH

Contract Number NAS 9-10826

NASA Manned Spacecraft Center
Food and Nutrition Office
Houston, Texas 77058

8531 N. NEW BRAUNFELS AVE. • SAN ANTONIO, TEXAS 78217

CR-114998

TECHNOLOGY INCORPORATED

LIFE SCIENCES DIVISION

SAN ANTONIO, TEXAS 78217

FINAL REPORT

APRIL 1971

Contract Number NAS9-10826

THE METABOLISM OF INGESTED PEROXIDES

NASA Manned Spacecraft Center
Food and Nutrition Office
Houston, Texas 77058

PREPARED BY:

Brian Ward, Ph. D.
Manager, Vision/Neurosensory Group

APPROVED BY:

James V. Benedict, Ph. D.
Manager, San Antonio Branch
Life Sciences Division

FOREWORD

This report describes work performed by the Life Sciences Division of Technology Incorporated under Contract NAS 9-10826 for the Manned Spacecraft Center of the National Aeronautics and Space Administration, Houston, Texas, during the period 1 May 1970 to 30 April 1971.

SUMMARY

The Life Sciences Division of Technology Incorporated has conducted a rat feeding program to study growth patterns in albino laboratory rats having balanced diets differing by the presence of either oxidized or unoxidized fat. The control group rats of both sexes, which were fed unoxidized lard, consistently gained more weight than did the test group which ate the oxidized lipid diet. The food consumption generally correlated directly with body weight gain. The male rats, of both the test and control groups, ate more food and gained more weight than did the females. The control males, with the unoxidized fat diet, ate about 8% more food than the test males with the oxidized fat diet, and gained 18% more weight. The control females ate approximately the same amount of food as the test females and gained 9% more weight. Necropsies revealed interesting group differences. The most important were:

(1) Cardiac punctures in the test animals were virtually impossible due to excessive blood clotting.

(2) The serosa of the abdominal viscera appeared greasy in test animals, while in the control animals no such unusual observations were made.

Following dissection in a nitrogen atmosphere, samples of tissue from each rat were packaged under nitrogen and frozen for shipment to the Houston laboratory of Technology Incorporated for analysis.

TABLE OF CONTENTS

1. INTRODUCTION

A controlled feeding program was performed to determine the effects of oxidized versus unoxidized lipids, in an otherwise balanced diet, upon body weight and tissues of a colony of laboratory rats. Two hundred albino rats, of the Charles Rivers COBS-CD strain, were housed individually in the vivarium of the Life Sciences Division of Technology Incorporated, San Antonio, Texas. The colony was divided into two groups, a control group and a test group Each group contained fifty males and fifty females.

The feeding program began with 21 day old rats. The control group was fed a controlled, balanced diet containing unoxidized lipid while the test group was administered the same balanced diet using oxidized lipid rather than unoxidized lipid. The test diet differed from the control diet only in the oxidation of the lipids. Food consumption was determined for each rat twice weekly and body weight was determined once weekly. Water was available to all rats ad lib.

Thirty test and thirty control animals were sacrificed and necropsied at the end of one month. A second group of thirty test and thirty control animals was sacrificed and necropsied after three months in the program. The third group of forty test and forty control animals was sacrificed and necropsied after six months in the feeding program. Blood and tissue samples obtained from the rats were analyzed by the Analytical Services Laboratory, Life Sciences Division of Technology Incorporated, Houston, Texas.

2. PROCEDURES

2.1 Housing

The animals were housed in the environmentally controlled vivarium at Technology Incorporated, San Antonio, Texas. The temperature was maintained at 72°F and the relative humidity at 60%. Normal periods of light and dark were provided. The rats were caged individually in tough, easy-to-clean, five sided plastic boxes with wire lids. Pressed alfalfa was provided for bedding. Food loss from the dry-food feeding pots was minimized by fitting adapters to the box lids to prevent overturning. The cages were fitted with glass watering bottles. Water bottles were changed twice each week. The cages were changed and cleaned every 7-10 days.

2.2 Feeding and Weighing

Twice a week each rat of the test group was given a pre-weighed quantity of a balanced diet containing oxidized lipids. Similarly, the control group was fed with a balanced diet containing unoxidized lipids. As food consumption increased, the pre-weighed quantity was increased. The body weight of each rat was determined weekly. Table I shows the diet constituents and the amounts used in mixing one food batch. A 20 quart Hobart commercial food mixer was used for combining these ingredients. Each of the three batches of oxidized lard used throughout the study had a peroxide number of 265.

TABLE I

One Batch

Dietary Constituent	% of Diet	Amount per Batch
Lard†	15.0	1360.8 gm
Casein	18.0	1632.9 gm
Sucrose	60.0	5443.1 gm
Glycerol	1.0	90.7 gm
L-Cystine	0.02	1.81 gm
Salt Mix	4.0	362.9 gm
Vitamin Mix	1.0	13.78 gm

The vitamin mix used was not diluted with sucrose. It was calculated on the basis of the amounts of each vitamin per 100 g food mix. The actual amount used was 154.7 mg vitamin mix per 100 gm food. Each batch of food equals 8892.17 g, therefore the amount of vitamin mix required equals 13.78 g.

†Oxidized for Test Food; unoxidized for Control Food.

The vitamin and salt mixtures were prepared according to the formulae indicated in Tables II and III. The vitamin mixture was prepared in conveniently sized quantities and refrigerated until required.

2.3 Necropsy

At necropsy, each animal was anesthetized with ether and then weighed. Cardiac punctures were subsequently performed on each rat, using a 5 cc syringe and a 20 gauge needle rinsed in 5% sodium citrate. Approximately 4 ml of blood were collected from each rat in 0.3 ml of 38% sodium citrate solution. Dissection was performed in a nitrogen atmosphere to prevent oxidation of the tissue samples. Three types of tissue were collected from each rat; liver, white muscle (abdominals) and red muscle (gastrocnemius). Each sample was sealed individually in a plastic bag under nitrogen.

The bags of tissue were then frozen and shipped, in the frozen state, to the Analytical Services Laboratories of Technology Incorporated in Houston, Texas for biochemical analysis.

TABLE II

Vitamin Mix

Vitamin	Grams per 100 g food
Vitamin A 267 IU	10.68×10^{-4} g
Vitamin D_3 533 IU	13.325 μg
Vitamin E (α tocopherol)	0.006 gm
Thiamine HCL	0.0005 gm
Pyridoxine HCL	0.0005 gm
Niacin	0.004 gm
Calcium Pantothenate	0.002 gm
Choline chloride	0.1 gm
Vitamin B12	0.000003 gm
Riboflavin	0.001 gm
Folic Acid	0.00055 gm
Biotin	0.00003 gm
i-Inositol	.04 gm

TABLE III

Salt Mix

Compound	Percent Composition
Calcium carbonate $CaCO_3$	38.1400
Cupric Sulfate $CuSO_4 \cdot 5H_2O$	0.0477
Ferrous Sulfate $FeSO_4 \cdot 7H_2O$	2.7000
Magnesium Sulfate $MgSO_4$	5.7300
Manganese Sulfate $MgSO_4 \cdot 2H_2O$	0.4010
Potassium iodid KI	0.0790
Potassium phosphate Monobasic KH_2PO_4	38.9000
Sodium Chloride NaCl	13.9300
Zinc Sulfate Zn $SO_4 \cdot 7H_2O$	0.0548

Reference U.S. Pharmacopeia XVII, 1965, 862.

3. RESULTS AND DISCUSSION

Table IV shows pooled weight gains for the rats of each group during the program. The control animals, with the unoxidized fats in the diet, gained more weight than did the test animals. At the end of six months, the control males outweighed the test males by 17.8% and the control females outweighed the test females by 9.2%. Figure 1 graphically illustrates the weight gain data of Table IV. The asterisks indicate the expected body weights as published by the supplier for this particular strain of rats at age 84 days. Figure 2 illustrates the food consumption as listed in Table V. The body weights of the males and females of the test and control groups generally correlate with food consumption. The test and control females were shown to eat approximately the same amounts of food. However, it is shown that the control males had a small, but consistently higher, food intake than the test males. By the end of the program, the control males were eating more food than the test males and 18% more than the two groups of females, whose intakes were similar.

The necropsies of the rats merit special mention. The necropsies of the first two test groups of rats revealed no unusual results. However, when the final 80 rats were sacrificed, several situations were encountered which contrasted sharply with those met at the first two necropsy sessions. Blood samples were virtually impossible to obtain from cardiac punctures of the test group. The largest available needles (20 gauge) were useless due to blood clotting. Even when the thoracic cavity was opened and the exposed heart was punctured, blood collection still proved to be impossible. Attempts to drain pooled blood from the

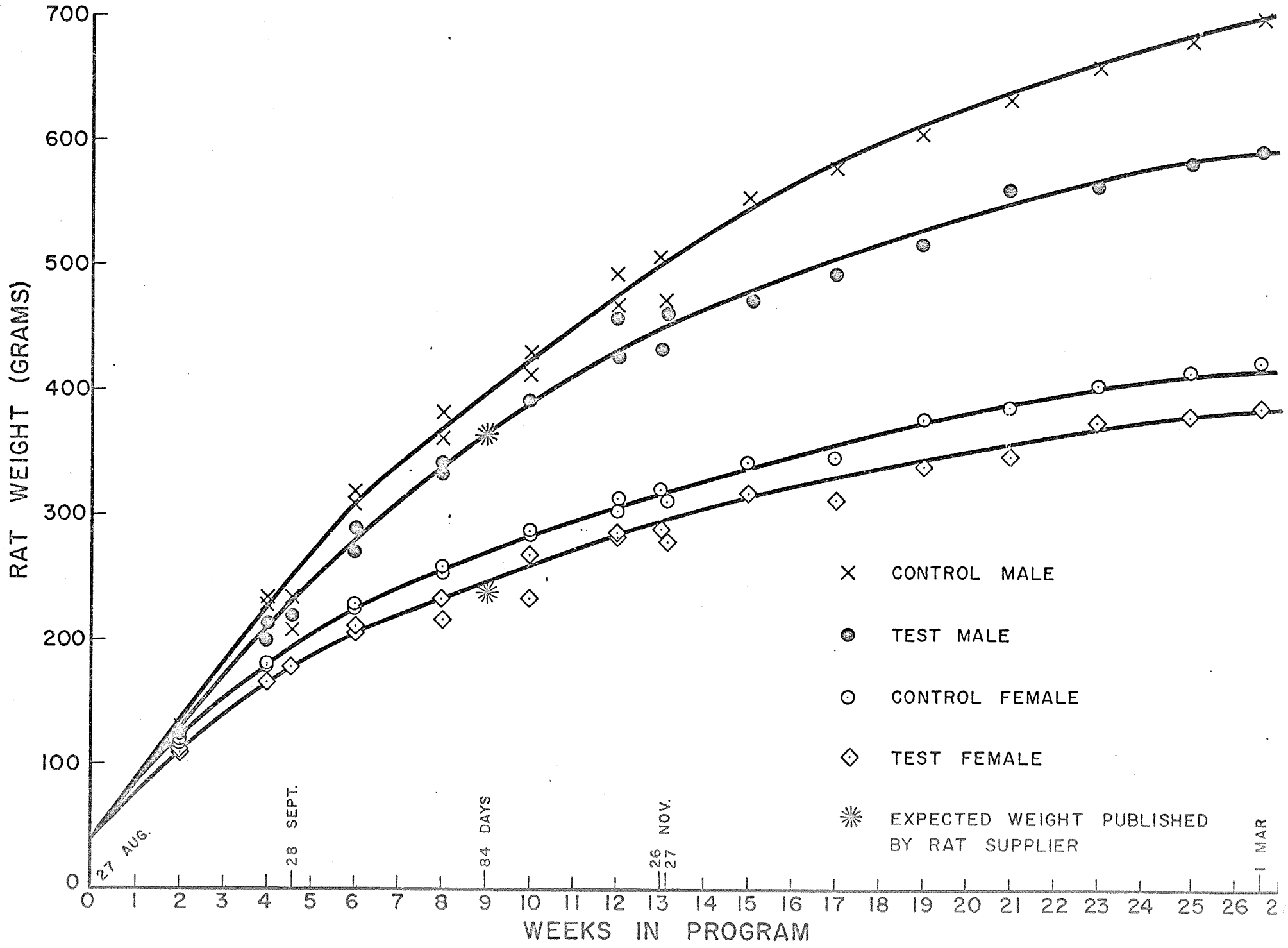

Figure 1. Rat Weight Gain

TABLE IV

Rat Weight Gain

Date	Weeks in Progress	Test Female	Control Female	Test Male	Control Male
	Initial Weight :	42.1	40.8	44.3	43.0
		41.4	44.1	43.5	45.3
		41.9	44.1	41.8	42.3
10 Sept 70	2	110.7	116.3	120.2	129.2
		109.3	124.1	124.3	120.3
		109.4	121.3	119.5	129.5
24 Sept 70	4	172.2	185.0	207.2	229.4
		167.4	179.6	213.9	229.2
		166.9	178.4	197.5	239.9
28 Sept 70 Final	Final Weight Group I	177.5	208.8	219.5	234.0
8 Oct 70	6	210.2	228.3	289.2	307.0
		205.3	224.9	268.9	318.2
22 Oct 70	8	215.2	252.2	342.6	360.4
		233.0	256.7	333.0	382.3
5 Nov 70	10	233.5	285.9	392.2	412.1
		267.3	282.3	370.1	430.1
19 Nov 70	12	283.7	303.4	458.0	467.3
		283.0	314.3	425.5	493.8
27 Nov 70 Final	Final Weight Group II	277.6	311.1	461.6	471.6
26 Nov 70	13	287.7	321.1	433.0	506.0
10 Dec 70	15	317.0	343.6	470.0	554.6
24 Dec 70	17	313.3	347.5	492.6	578.6
7 Jan 71	19	339.4	376.2	518.6	607.2
21 Jan 71	21	368.0	388.3	561.7	634.3
4 Feb 71	23	375.8	405.2	562.6	660.0
18 Feb 71	25	380.3	415.8	583.2	682.6
1 Mar 71 Final	Final Weight Group III	387.1	422.8	594.5	700.7

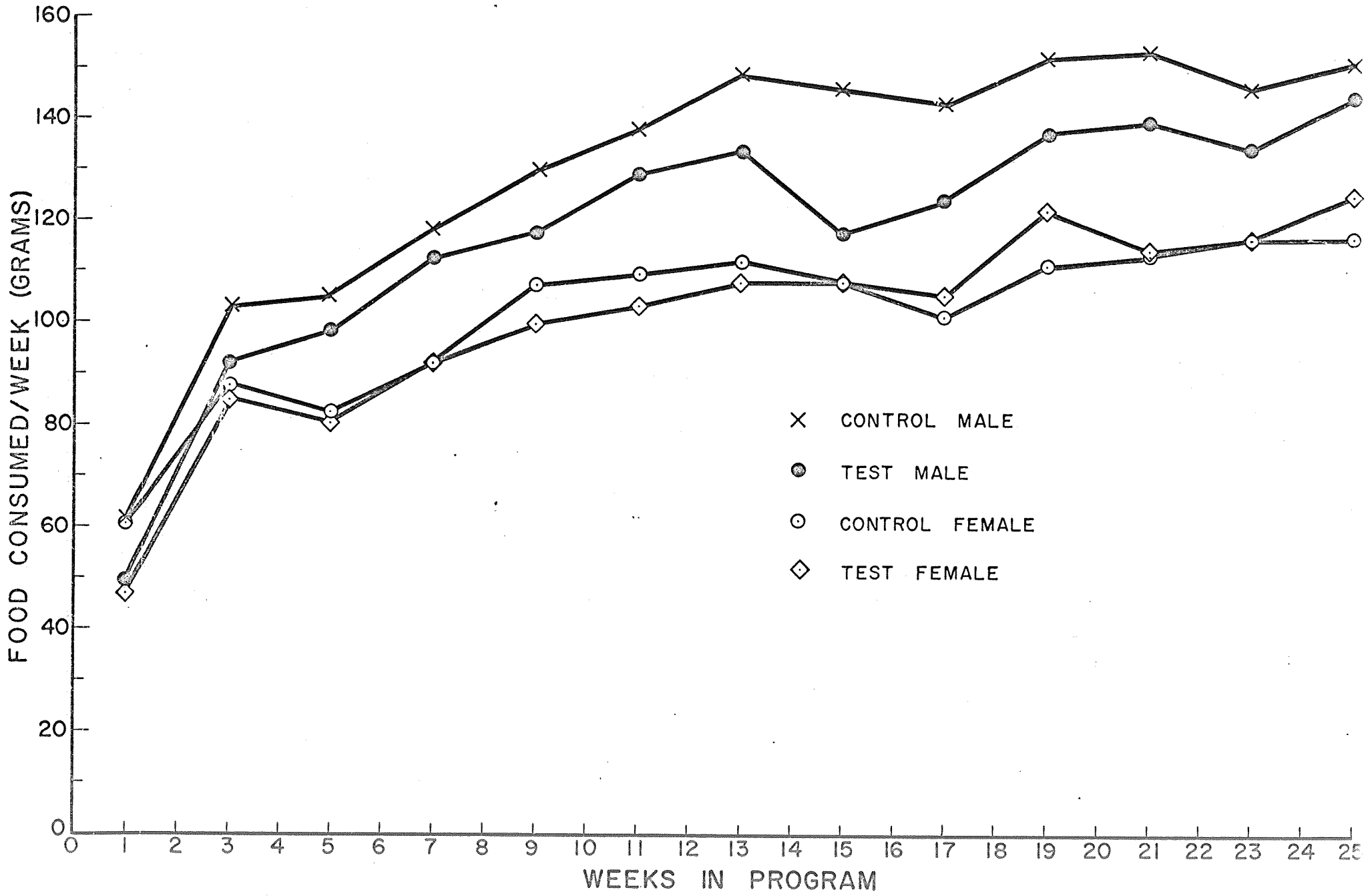

Figure 2. Rat Food Consumption

TABLE V

Food Consumption

Date	Weeks in Progress	Test Female	Control Female	Test Male	Control Male
4 Sept 70	1	47.2	60.1	49.3	61.8
18 Sept 70	3	85.1	87.5	92.2	103.7
2 Oct 70	5	81.7	82.7	98.2	105.2
16 Oct 70	7	92.0	92.0	112.6	118.7
30 Oct 70	9	99.8	107.0	117.1	130.8
13 Nov 70	11	103.5	109.2	129.7	138.8
27 Nov 70	13	108.7	112.4	133.6	149.6
11 Dec 70	15	108.6	108.1	117.5	146.8
24 Dec 70 1 day less than	17	105.5	101.4	124.5	143.5
8 Jan 71	19	122.1	111.0	137.8	152.7
22 Jan 71	21	114.0	113.0	139.4	153.3
5 Feb 71	23	116.0	116.0	134.2	146.7
19 Feb	25	125.7	117.2	144.4	151.9

thoracic cavity, with a syringe, were also futile. The pooled blood appeared to have a grease film floating on its surface, which may or may not have been due to tissue fluid contamination of the blood, from the trauma of opening the chest. The blood samples obtained were chiefly from the caudal artery, and were probably contaminated with tissue fluid. These samples were very poor. The cells apparently clotted and the serum was hemolytic. The serum, however, showed no apparent lipemia. This problem was not observed in the control animals. Heart punctures were successful in the control animals, and the blood did not appear to have any oily layer floating on its surface. The serum, however, did seem generally lipemic in this group.

When the abdominal cavities of the test animals were opened, the serosa covering the viscera appeared oily. There were visible oil droplets on the liver. Otherwise, the viscera and the liver had a normal appearance, except for gross para-renal and retroabdominal fat deposits. There were also large subcutaneous fat pads on the flanks of the animals. The males were fatter than the females in this.

The viscera of the control animals appeared normal except for an increased deposition of fat. The control animals seemed even fatter than the test animals. Among the control animals, the surfaces of a few of the livers appeared pale and mottled. Again in this group, the males were fatter than the females.