SOME EFFECTS OF MASSIVE DOSAGES OF ASCORBIC ACID AND VARYING LEVELS OF DIETARY PROTEIN ON THE GROWTH, SKELETAL DEVELOPMENT, AND NUTRITIONAL STATUS

A THESIS

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We hereby recommend that the thesis prepared under our supervision by NORMA OAKMAN SANDERS entitled SOME EFFECTS OF MASSIVE DOSAGES OF ASCORBIC ACID AND VARYING LEVELS OF DIETARY PROTEIN ON THE GROWTH, SKELETAL DEVELOPMENT, AND NUTRITIONAL STATUS be accepted as fulfilling this part of the requirements for the Degree of Master of Arts.

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—N. O. S.

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INTRODUCTION

The search for additional facts concering the functions of various nutrients in animal nutrition continues, with emphasis not only in single nutrients, but also in two or more nutrients in combination with each other.

In the laboratory in which the author of this report conducted the study upon which this thesis is based, two experimental animal studies previously had been conducted upon single nutrients, which led to the selection of the study which is reported herein. Ercelie Doss Todd (25) had worked upon different percentages of protein in an isocaloric diet constant in percentage of all nutrients except protein; and Harriet Cristeen Miller Jolly (13) had studied two extreme levels of ascorbic acid in diets similar in other respects. In the former study, vast differences in growth, skeletal status, condition of the soft tissues, relative weights of vital organs to total body weight, and other factors were found where the level of protein in the diet was altered. In the latter investigation, some differences in several of these attributes were indicated with and without a relatively large addition of ascorbic acid.

The present study was designed to ascertain possible interrelationships between protein and ascorbic acid in the diet of the albino rat.

In planning the investigation, the author desired to find whether different levels of protein as used by Todd with and without massive additions of ascorbic acid would result in differences in response by experimental rats as measured by: growth; food consumption; three blood values—blood plasma ascorbic acid, total serum protein, and serum albumin; skeletal maturation and mineralization; relationship of total skeletal weight to total body weight; and weights of certain vital organs. The study was continued for a longer period of time than that used by either of the aforementioned investigators in order to accentuate any changes for which the experimental diets may have been responsible. Moreover, the quantity of ascorbic acid employed was considerably greater than that used by Jolly (13).

It has been well established that ascorbic acid is needed for the normal metabolism of the aromatic amino acids—phenylalanine and tyrosine—and that ascorbic acid, as well as some of the proteins (glutathionone), is involved in the body's oxidation-reduction system. Other investigators have considered certain aspects of varying levels of protein, particularly in the growth and blood values of experimental animals. Weights of certain vital organs have been studied both in relation to dietary protein intake and ascorbic acid consumption under normal conditions, as well as those involving stress. Also, some of the effects of massive dosages of ascorbic acid have been investigated to some extent by previous workers.

It is hoped by the author that this study on protein and ascorbic acid may offer a new insight into dietary interrelationships in that both massive dosages of ascorbic acid and varying levels of protein were used concurrently.

EXPERIMENTAL

EXPERIMENTAL ANIMALS, THEIR CARE, AND THEIR DIETS

DESCRIPTION OF ANIMALS

Forty-eight albino rats from the animal laboratory colony of the College of Household Arts and Sciences, Texas State College for Women, were selected from five litters, weaned and placed on experiment at the age of 21 to 22 days. Three males and three females were placed on each of the eight dietary levels, as described below. Each level contained three pairs of rats; each pair consisted of one male and one female from the same litter whose combined weight was 93 to 95 grams. The weights of animals on each treatment averaged 46.8 grams per rat.

HOUSING OF EXPERIMENTAL ANIMALS

The animal laboratory in which the experiment was conducted was maintained automatically under constant conditions of temperature (between 72° and 74°). Automatically controlled lighting assured equal periods of daylight and darkness. Individual Bussey 464A60 cages, known as the Insula type, were housed in a five-deck battery containing 60 cages. These cages, 7.0 inches wide, 9.5 inches deep, and 7.5 inches high inside measurement, were closed on the back and sides with 21-gauge galvanized steel and on the front and floor with 16-gauge galvanized wire of one-half inch mesh.

DESCRIPTION OF DIETS

The experimental diets for four groups of animals were as nearly devoid of ascorbic acid as possible and contained four levels of protein. Two intermediate levels, as suggested by the Wistar Institute as the most desirable, as well as one lower and one higher level of protein were chosen. Another series of diets containing the same four levels of protein were supplemented with one per cent of ascorbic acid. All diets were made isocaloric by substitution of vitamin-test casein for cornstarch as the protein content of the diets increased. These diets were selected after consulting those directing this study and reading literature on the subject. The composition of the diets is given in Table I. Table II lists the amino acid content and Table III contains the mineral content, while the vitamin content of the diets is given in Table IV.

TABLE I

COMPOSITION OF EXPERIMENTAL DIETS[1]

PART A—INGREDIENTS IN 600-GRAM PORTION

Ingredients in 600-Gram Portion of Diet	3.2% Protein in Diet	25.2% Protein in Diet	40.2% Protein in Diet	78.2% Protein in Diet
Casein	0	132	222	450
Cornstarch	480	348	258	30
Dessicated liver	24	24	24	24
Salt mixture No. II, U.S.P. XIII	24	24	24	24
Corn oil	60	60	60	60
Cod liver oil	12	12	12	12
Gram total of ingredients	600	600	600	600

[1]Other constituents added to the 600-gram portion of diet were: 5 mg. thiamine chloride; 5 mg. riboflavin; 5.6 gm. calcium lactate; and 500 mg. alpha tocopherol.

The four diets outlined above represent those to which no ascorbic acid was added. Ascorbic acid to the extent of 1 per cent was added, respectively, to form additional identical diets, giving a total of eight distinct diets.

TABLE I

COMPOSITION OF EXPERIMENTAL DIETS

(CONTINUED)

PART B—CALORIE CONTRIBUTIONS FOR RESPECTIVE INGREDIENTS IN THE DIET

Ingredients in 600-Gram Portion of Diet	3. 2% Protein in Diet	25. 2% Protein in Diet	40. 2% Protein in Diet	78. 2% Protein in Diet
Casein	0	528	888	1,800
Cornstarch	1,920	1,392	1,032	120
Dessicated liver	384	384	384	384
Salt mixture No. II, U.S.P. XIII	0	0	0	0
Corn oil	540	540	540	540
Cod liver oil	108	108	108	108
Calorie total of ingredients in 600-gram portion of diet	2,952	2,952	2,952	2,952

TABLE II

TABLE II

ESSENTIAL AMINO ACID CONTENT OF THE DIETS ON THE BASIS OF A TEN-GRAM PORTION OF THE DIET[1]

Protein and Amino Acid Groups	Essential Amino Acids			
	Lysine	Tryptophane	Histidine	Phenylalanine
Percentage amino acids required per 10-gram portion of diet	0.10	0.02	0.04	0.07
3.2% Protein in diet A. Per cent amino acids in casein in 10-gram portion of diet	0.00	0.00	0.00	0.00
B. (A + liver.) Total per cent amino acids in 10-gram portion of diet	0.10	0.02	0.04	0.12
C. Plus or minus the requirement of amino acids	Adequate	Adequate	Adequate	+0.05

[1] Todd, Ercelle Doss (25).

TABLE II—CONTINUED

Essential Amino Acids					
Leucine	Isoleucine	Threonine	Methionine	Valine	Arginine
0.09	0.05	0.06	0.06	0.07	0.02
0.00	0.00	0.00	0.00	0.00	0.00
0.15	0.08	0.09	0.05	0.10	0.10
+0.06	+0.03	+0.03	−0.01	+0.03	+0.08

TABLE II—CONTINUED

TABLE II—CONTINUED

TABLE II—CONTINUED

Protein and Amino Acid Groups	Essential Amino Acids			
	Lysine	Tryptophane	Histidine	Phenylalanine
25. 2% Protein in diet A. Per cent amino acids in casein in 10-gram portion of diet	0.15	0.04	0.06	0.11
B. (A ∔ liver.) Total per cent amino acids in 10-gram portion of diet	0.25	0.06	0.10	0.23
C. Plus or minus the requirement of amino acids	∔0.15	∔0.04	∔0.06	∔0.16
40. 2% Protein in diet A. Per cent amino acids in casein in 10-gram portion of diet	0.26	0.07	0.10	0.20
B. (A ∔ liver.) Total per cent amino acids in 10-gram portion of diet	0.36	0.09	0.14	0.32

TABLE II—CONTINUED

Essential Amino Acids					
Leucine	Isoleucine	Threonine	Methionine	Valine	Arginine
0.27	0.14	0.09	0.08	0.15	0.08
0.42	0.22	0.18	0.13	0.25	0.18
+0.33	+0.17	+0.12	+0.07	+0.18	+0.16
0.46	0.24	0.14	0.13	0.26	0.14
0.61	0.32	0.23	0.18	0.36	0.24

TABLE II—CONTINUED

TABLE II—CONTINUED

TABLE II—CONTINUED

Protein and Amino Acid Groups	Essential Amino Acids			
	Lysine	Tryptophane	Histidine	Phenylalanine
40. 2% Protein in diet (continued) C. Plus or minus the per cent requirement of amino acids	+0.26	+0.07	+0.10	+0.25
78. 2% Protein in diet A. Per cent amino acids in casein in 10-gram portion of diet	0.52	0.14	0.20	0.39
B. (A + liver.) Total per cent amino acids in 10-gram portion of diet	0.62	0.16	0.24	0.51
C. Plus or minus the per cent requirement of amino acids	+0.52	+0.14	+0.20	+0.44

TABLE II—CONTINUED

Essential Amino Acids					
Leucine	Isoleucine	Threonine	Methionine	Valine	Arginine
+0.52	+0.27	+0.17	+0.12	+0.29	+0.22
0.93	0.49	0.29	0.26	0.53	0.29
1.08	0.57	0.38	0.31	0.63	0.39
+0.99	+0.52	+0.32	+0.25	+0.56	+0.49

END OF TABLE II

TABLE III

MINERAL CONTENT OF THE DIETS ON THE BASIS OF A TEN-GRAM PORTION OF DIET[1]

Composition of Salt Mixture No. II, U.S.P. XIII, Plus Added Calcium Lactate and Potassium Iodide			
Minerals	Mineral Requirement for 10-Gram Portion	Mineral Content of 10-Gram Portion	Relationship to the Requirement
Calcium	40-50 mg.	50 mg.	Adequate
Phosphorus	35-45 mg.	40 mg.	Adequate
Iron	0.25 mg.	2.7 mg.	+2.45 mg.
Magnesium	0.5 mg. per kilogram of body weight	11.0 mg.	+10.5 mg.
Potassium	Males, 15 mg. Females, 8 mg.	20.0 mg.	+5.0 mg. +12.0 mg.
Sodium	0.5% of diet	1.0 mg.	Adequate
Chlorine	5.0 mg.	10.0 mg.	+5.0 mg.

TABLE III—CONTINUED

Composition of Salt Mixture No. II, U. S. P. XIII, Plus Added Calcium Lactate and Potassium Iodide

Minerals	Mineral Requirement for 10-Gram Portion	Mineral Content of 10-Gram Portion	Relationship to the Requirement
Iodine	1 to 2 mcg.	800 mcg.	Adequate
Copper	0.1 mg.	0.3 mg.	+0.2 mg.
Zinc	40.0 mcg.	56.0 mcg.	+16.0 mcg.

[1]Todd, Ercelle Doss (25).

TABLE IV

VITAMIN CONTENT OF THE DIETS ON THE BASIS OF A TEN-GRAM PORTION OF DIET[1]

Composition of Liver and Cod Liver Oil Plus Added Alpha Tocopherol, Thiamine Chloride, and Riboflavin[2]			
Vitamins	Vitamin Requirement for 10-Gram Portion	Vitamin Content of 10-Gram Portion	Relationship to the Requirement
FROM LIVER			
Thiamine	10 mcg.	8,000 mcg.	Adequate
Riboflavin	40 mcg.	8,000 mcg.	Adequate
Nicotinic acid	None	0.1 mg.	
Pyridoxine	10 mcg.	13 mcg.	+3.0 mcg.
Choline	2 to 3 mg.	8 mg.	+5.0 to 6.0 mg.
Pantothenic acid	250 mg. dried liver or 100 mcg. calcium pantothenate	400 mg.	+150 mg.
Folic acid	None	3 mcg.	

TABLE IV—CONTINUED

Composition of Liver and Cod Liver Oil Plus Added Alpha Tocopherol, Thiamine Chloride, and Riboflavin[2]			
Vitamins	Vitamin Requirement for 10-Gram Portion	Vitamin Content of 10-Gram Portion	Relationship to the Requirement
B_{12}	None	0.4 mcg.	
Alpha tocopherol	1.0 mg.	1.0 mg.	Adequate
FROM COD LIVER OIL			
Vitamin A	4 mcg.	170 I. U.	Adequate
Vitamin D	Not required if calcium-phosphorus ratio is 1:1 to 2:1	17 I. U.	Adequate

[1] Todd, Ercelle Doss (25).

[2] Ascorbic acid-supplemented diets contained 1 per cent ascorbic acid.

METHOD OF FEEDING AND RECORDING FOOD CONSUMPTION

Fresh water was given to the experimental animals daily and refrigerated food was supplied as needed, both being given ad libitum. Food consumption was calculated by weighing the food and food container initially and reweighing it before fresh food was added to supplement that which had been eaten. A record of the food consumption of each animal was kept and totalled weekly.

GROWTH RECORDS

The growth records were kept in terms of the weights of the animals at the beginning of the experiment and weekly thereafter, using the same animal balances at all times.

EXTENT OF EXPERIMENT

All animals were kept on experiment for 140 days, unless death occurred before the close of this period.

BLOOD ANALYSES

Blood samples from free-flowing tail punctures were taken at specified intervals from one male and one female from each protein level except the lowest (3. 2 per cent). Their times on the experiment ranged from 89 to 94 days, with an average time on the experiment of 91 days. Using the same rats, final blood samples were collected at 140 days on the investigation.

Because of the small amount of blood obtainable from animals on the 3. 2 per cent protein levels, four extra animals, two male and two female, were placed on the experiment and sacrificed shortly before death was anticipated, as indicated by extreme deficiency signs. With the anesthetized animal still alive, an incision was made into the chest cavity; and blood was removed by cardiac puncture. These samples were taken at 45 days.

Blood samples were collected in 5-ml. oxalated centrifuge tubes and centrifuged for ten minutes at 1800 r. p. m. The serum layer was removed by means of a long-stem pipette to a small test tube. Serum samples of the male and female from each dietary group were combined so that there would be a sufficient amount for the tests.

Determination of plasma ascorbic acid was made immediately. The remainder of the serum was quick-frozen and later used for total protein and albumin determinations, insofar as the quantity of sample would permit.

DETERMINATION OF BLOOD PLASMA ASCORBIC ACID

The ascorbic acid content in the plasma was determined by an adaptation of the method used by Farmer and Abt (23).

From the sample, 0. 2 ml. of plasma was transferred to a 15-ml. centrifuge tube. With the same pipette, 0. 3 ml. of distilled water was added, thus washing out the pipette. Next, 0. 4 ml. of 5 per cent metaphosphoric acid was added; the mixture was stirred, and centrifuged for at least five minutes.

Eight-tenths milliliter of dye acetate solution was measured into a microcolorimeter tube. The supernatant fluid was removed without contamination by precipitation, and exactly 0.3 ml. was added to the dye acetate solution in the microcell. This was stirred immediately, the plunger placed in position, the cell inserted into the Evelyn microcolorimeter and the galvanometer reading taken in 30 seconds. The sample was blanked with ascorbic acid and the blank reading taken.

DETERMINATION OF TOTAL SERUM PROTEIN AND SERUM ALBUMIN

Total protein and albumin was determined on the final blood samples by the Kingsley method (9).

Each frozen sample of unhemolized serum was thawed and 0.5 ml. transferred to a graduated centrifuge tube. Twenty-three per cent sodium sulfate solution was added to the 10-ml. mark and contents of the tube mixed by repeated inversions; and a 2-ml. portion of the suspension was removed by pipette and placed in a separate small test tube. This tube was used for the determination of total protein. Three milliliters of ether were added to the remainder of the suspension in the centrifuge tube, stoppered, and shaken vigorously. This was centrifuged for about five minutes. After centrifuging, a 2-ml. portion of the clear fluid below the white layer of packed globulin precipitate was removed by means of a pipette and transferred to a second test tube to be used for albumin determination.

Four milliliters of a special biuret reagent were added to each tube, followed by 2 to 3 ml. of ether. These were stoppered, shaken vigorously, and centrifuged for five minutes. By means of a pipette, the solution under the ether layer was transferred to the cells which were used for determining density of these solutions in the Klett Electrophotometer. Readings were made within the next 10 to 20 minutes.

The electrophotometer was set at zero, using a blank made of distilled water and biuret reagent and the 540 Mu filter. Next, the two solutions for determining total protein and albumin were read. The readings on the electrophotometer were plotted on a graph, against a standard serum.

An insufficient amount of plasma from the 45-day samples taken from the animals on 3.2 per cent protein, as well as the 91-day samples from the animals on 25.2 per cent, 40.2 per cent, and 78.2 per cent protein, prevented the use of the Kingsley method for serum protein and serum albumin determinations. Instead, total protein was determined by the Falling Drop method.

By means of a Guthrie Pipette Controller, the Falling Drop technique involved releasing a drop containing 1/100 ml. of serum into a Falling Drop tube filled with xylene-bromobenzene mixture. The drop was timed as it passed between two marks, 30 cm. apart, engraved on the tube. A stop-watch calibrated in 1/10 seconds was used and the time recorded. The temperature of the water bath, which was the same as the standard solution and the serum, also was recorded. The apparent density difference of the serum and of the standard solution then was found by means of an Alignment Chart. The specific gravity of the serum was calculated; and, from charts, the grams of protein per 100 ml. of serum was found.

VITAL ORGANS

DISSECTING TECHNIQUE AND SUBSEQUENT PROCEDURE

The technique described by Donaldson (5) was used, as follows:

Lungs: The lungs were severed from the trachea and the portion of the esophagus usually taken out with them was removed.

Spleen: The vessels were cut close to the hilium.

Kidneys: All vessels were cut close to the hilium and all superficial fat removed.

Liver: The vessels were cut close to their entrance into the liver and the blood in the larger vessels gently pressed out.

Suprarenals: The suprarenals were removed from all overlying fat and the main vessel severed close to the organ.

Testes: The epididymis was removed before the testes were weighed. As much of the fat was removed as possible.

Heart: The heart was removed after cutting all of its vessels close to their proximal ends. It then was opened by a longitudinal slice through its walls and the blood clots removed from the cavities, thus exposed.

Brain: The bones from the skull were removed from above with care to preserve the paraflocculi which lie in bony pockets. The brain was severed from the cord at the level of the first cervical nerve. The brain then was removed from the floor of the cranium.

The vital organs were weighed immediately upon dissection with a Fisher Grammatic balance.

DETERMINATION OF PERCENTAGE OF BRAIN MOISTURE

To determine the water content of the brain, all brains were oven dried at 60° C. until a constant weight was obtained. The weights of the dehydrated brains as well as the percentage of water of each brain were recorded.

RECORDS OF SKELETAL STATUS

PRODUCING THE X-RAY FILMS

Periodic X-rays were taken of all of the rats in order to determine the rate of growth and the maturation of the skeleton, as well as to provide X-ray material for measuring bone density. Anteroposterior views of the left front foot were taken weekly throughout the period of the tests, or until the time of final closure of the metacarpals and phalanges. Particular care was taken in immobilizing the rat so that the carpal area, the metacarpals and phalanges, as well as the distal ends of the radius and ulna, were shown. The anteroposterior view was selected for determination of the rate of skeletal maturation in the rat, since it is comparable to the standard view of the hand used for human maturation evaluation, as presented by Todd (26), Greulich and Pyle (8). A lateral view of the right rear leg, showing the distal end of the femur, the tibia, and the fibula, as well as the os calcis, was taken at the time of the initial test and monthly thereafter for the duration of the experiment.

The film used was Eastman double-coated acetate base No-Screen X-ray film. Cardboard exposure holders were used. By means of a changeable lead-rubber protective covering for the hands of the person holding and immobilizing the animal, four exposures were made on each 8 x 10-inch film, with all quadrants of the film except that being exposed covered with a lead-rubber plate. The distance was decreased from the normal 30 inches to 15 inches in order to facilitate shorter exposures. Since the intensity of X-radiation varies with the square of the change in focal-film distance, exposures at 15 inches require only one fourth of the milliampere-seconds needed at the standard 30-inch distance. A short time was essential in the X-ray exposure of living rats. A General Electric Model F Type 4 Shockproof X-ray Unit was used, which has a power output of approximately 50 kvp. Exposures of one-eighth second were made at 15 milliamperes.

In order to provide adequate protection to laboratory personnel, the X-ray films were taken in a lead-lined protective box equipped with a lead-glass viewing window and adequate working illumination. This apparatus is the same as used by Ercelle Doss Todd (25) and is shown in Figure 1.

EVALUATING SKELETAL MATURATION

The X-rays of the left front foot of the growing animals were used to observe skeletal maturation. Three methods normally are used in the determination of skeletal maturation. Bone ossification and the degree of fusion of long bones with their ossific centers (commonly determined by examination of the hand and wrist in humans) may be observed: (a) by dissection, or (b) by inspection of the X-ray images of long bones to find the degree of fusion.

In the case of dissection there is the disadvantage that no further measurements of the same animal can be made. On the other hand, periodic X-rays can provide a series of results on the same animal.

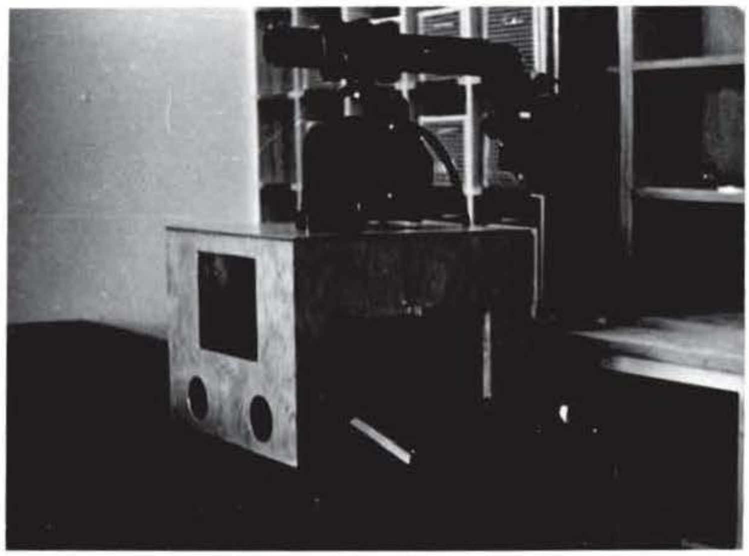

FIGURE 1

LEAD-LINED PROTECTIVE BOX

USED TO X-RAY RATS

The number of ossific centers including carpals, as well as the shape and other features of long bones, has provided a standard basis for the evaluation of skeletal age in Caucasian subjects. Skeletal age in human children may differ from the chronological age by several months or even years. Todd (26), and Greulich and Pyle (8) have used the foregoing technique. In rats, all the carpal bones are present at birth.

Inspection of the X-ray films in order to determine the degree of union of the ossific centers of the front foot was regarded as the best method of evaluating skeletal maturation in the case of rats, since the ununited centers of the metacarpals and phalanges are present at birth, as noted; and they fuse with the bone proper over a span approximately of 12 weeks, according to Dawson, as described by Donaldson (5).

ASSAYING TECHNIQUE FOR SKELETAL MATURATION

The series of X-ray films of the left front foot of each animal was examined in sequence on an illuminator, using a magnifying glass. When no evidence of epiphyseal nonunion was noted in a film, final closure was considered complete.

OBTAINING THE SKELETAL WEIGHT

After removal of the skin and major organs, the carcass of each rat was placed in a pan containing one quart of water. The carcass was allowed to simmer at 185° F. for eight hours and the soft tissue was then removed by placing the remains of the carcass in a fine sieve through which tap water was rapidly passed. The remaining skeletal material was then oven-dried overnight at 85° C. and weighed.

CRANIAL MEASUREMENTS

Calliper measurements of X-rays of all skulls were made as follows:

1. Length of cranium.
2. Zygomatic width.
3. Squamosal distance.

The foregoing measurements are diagrammed in Figure 2. Average dimensions for males and females, as well as the minimum and maximum, were compared with the findings of Hatai, as reported by Donaldson (5).

Measurements were from anteroposterior views of the skulls taken immediately after death. Exposures were made on Kodak No-Screen X-ray film with a 1/4-second exposure at 15 MaS and 50 kvp. with the apparatus already described in this section.

DETERMINATION OF BONE MINERALIZATION

Skeletal density measurements by the Mack Densitometric technique as described by Mack (14), and by Mack, Brown, and Trapp (15),

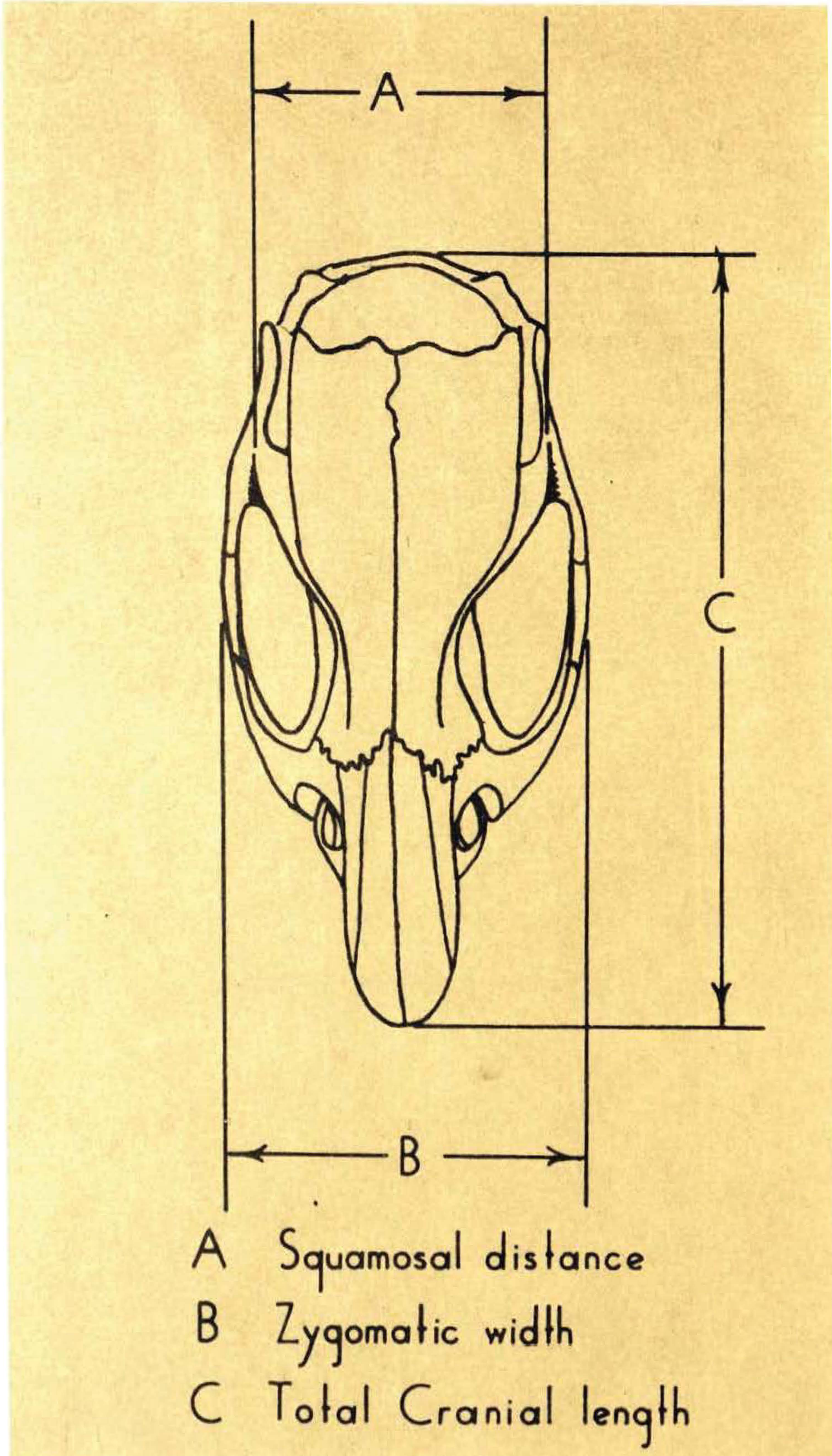

FIGURE 2

CRANIAL MEASUREMENTS

were made using Model 4 of the series of microdensitometric assemblies. Density measurements of the diaphysis of the left tibia were made in the axial direction of the bone.

PRESENTATION OF DATA

A series of tables and graphs contain the data of the study under discussion. Tables V, VI, and VII deal with food consumption of the experimental animals. Table VIII presents the growth records. Tables IX, X, and XI give the results of the ascorbic acid, total serum protein, and total serum albumin blood values.

Tables XII and XIII include the data on weights of vital organs. Table XIV gives the total and dry weights of the brains, as well as calculated percentage of total moisture.

Tables XV to XVIII, inclusive, embody the data on the several aspects of skeletal status of the animals.

TABLE V

TABLE V

FOOD CONSUMPTION OF RATS ON EACH LEVEL OF DIETARY PROTEIN

PART A—RATS ON DIETS WITH NO ADDED ASCORBIC ACID

Case Numbers of Rats	FOOD			
	1	2	3	4
3.2% Protein				
6D1-33 . . .	52	28	35	34
5D2-17 . . .	39	48	36	31
6D2-19 . . .	39	25	35	31
Average male . .	43.3	33.6	35.3	32.0
6D1-36 . . .	60	39	36	35
5D2-16 . . .	33	27	22	22
6D2-18 . . .	44	33	33	37
Average female . .	45.6	33.0	30.3	31.3
Average male and female	44.5	33.3	32.8	31.6
25.2% Protein				
6D1-46 . . .	56	58	66	86
5D2-14 . . .	47	56	66	82
6D2-20 . . .	48	58	53	72
Average male . .	50.3	57.6	61.7	80.0
6D1-35 . . .	63	70	106	75
5D2-7 . . .	64	66	61	44
6D2-17 . . .	38	61	92	80
Average female . .	55.0	65.6	86.3	66.3
Average male and female	52.6	61.6	74.0	73.2
40.2% Protein				
5D2-8 . . .	68	65	76	73
2J2-3 . . .	35	52	64	80
6D2-2 . . .	45	53	53	84
Average male . .	49.3	56.6	64.3	79.0
6D1-37 . . .	59	65	63	64
6D2-12 . . .	33	51	43	68
6D2-8 . . .	50	49	79	102
Average female . .	47.3	55.0	61.6	78.0
Average male and female	48.3	55.8	63.0	78.5
78.2% Protein				
6D1-50 . . .	33	27	57	50
5D2-12 . . .	27	43	43	46
2J2-1 . . .	23	39	56	53
Average male . .	27.6	36.3	52.0	49.6
6D1-47 . . .	46	37	46	51
5D2-10 . . .	55	26	32	28
6D2-6 . . .	33	25	39	38
Average female . .	44.6	29.3	39.0	39.0
Average male and female	36.2	32.8	45.5	44.3

TABLE V—CONTINUED

CONSUMPTION (GMS.) AT WEEKLY INTERVALS (1-10)					
5	6	7	8	9	10
37	26	24	27	26	29
31	25	22	27	22	19
33	28	34	32	26	31
33.6	26.3	26.6	28.7	24.7	26.3
26	29	20	28	27	28
23	37	31	29	27	22
36	34	34	34	34	36
28.3	33.3	28.3	30.3	29.3	28.7
31.0	29.8	27.5	29.5	27.0	27.5
78	80	73	88	71	59
107	151	133	125	119	113
78	89	103	102	98	98
87.6	106.6	103.0	105.0	96.0	90.0
61	41	59	89	64	55
78	120	94	96	94	91
69	59	85	92	92	94
69.3	73.3	79.3	92.3	83.3	80.0
78.5	90.0	91.2	98.7	89.7	85.0
103	103	120	116	117	90
67	80	78	102	92	92
72	71	77	69	83	64
80.6	84.6	91.7	95.7	97.3	82.0
55	72	81	100	79	89
69	90	117	116	121	112
89	77	84	86	87	84
71.0	79.6	94	100.7	95.7	95.0
75.8	82.2	92.8	96.7	86.5	88.5
73	81	48	68	90	93
57	57	50	56	63	69
74	65	75	72	81	92
68.0	67.6	57.7	65.3	78.0	84.7
20	51	60	49	44	45
32	39	31	42	46	38
43	43	42	42	40	48
31.6	44.3	44.3	44.3	43.3	43.7
49.8	56.0	51.0	54.8	60.7	64.2

TABLE V—CONTINUED

TABLE V—CONTINUED

TABLE V—CONTINUED

Case Number of Rats	FOOD			
	11	12	13	14
3.2% Protein				
6D1-33 . . .	29	21	22	24
5D2-17 . . .	19	16	17	17
6D2-19 . . .	26	26	25	...
Average male . .	24.7	21.0	21.3	20.5
6D1-36 . . .	23	22	20	15
5D2-16 . . .	30	29	30	31
6D2-18 . . .	32	23	26	30
Average female . .	28.3	24.7	25.3	25.3
Average male and female	26.5	22.8	23.3	23.4
25.2% Protein				
6D1-46 . . .	99	100	104	119
5D2-14 . . .	121	116	137	154
6D2-20 . . .	127	78	91	100
Average male . .	115.7	98.0	110.7	124.3
6D1-35 . . .	66	74	64	67
5D2-7 . . .	90	82	74	90
6D2-17 . . .	80	77	78	92
Average female . .	78.7	77.7	72.0	83.0
Average male and female	97.2	87.8	91.3	103.7
40.2% Protein				
5D2-8 . . .	110	95	102	109
2J2-3 . . .	81	120	169	120
6D2-2 . . .	105	121	135	128
Average male . .	98.7	112.0	135.3	119.0
6D1-37 . . .	81	84	70	82
6D2-12 . . .	144	135	126	129
6D2-8 . . .	93	81	77	89
Average female . .	106.0	100.0	91.0	100.0
Average male and female	102.3	106.0	113.2	109.5
78.2% Protein				
6D1-50 . . .	76	67	52	47
5D2-12 . . .	66	64	74	71
2J2-1 . . .	77	75	94	85
Average male . .	73.0	68.7	73.3	67.7
6D1-47 . . .	47	54	53	46
5D2-10 . . .	46	47	48	49
6D2-6 . . .	54	50	53	58
Average female . .	49.0	50.3	51.3	51.0
Average male and female	61.0	59.5	62.3	59.3

CONSUMPTION (GMS.) AT WEEKLY INTERVALS (11-20)					
15	16	17	18	19	20
18	..	..	..	..	..
..	..	..	..	..	..
..	..	..	..	..	..
18.0	..	..	..	..	..
..	..	..	..	..	..
27	..	..	..	..	..
20	33	22	21	22	19
23.5	33.0	22.0	21.0	22.0	19.0
21.7	33.0	22.0	21.0	22.0	19.0
105	99	91	85	97	132
181	229	143	119	158	171
120	132	112	119	126	111
135.3	153.3	115.3	107.7	127.0	138.0
71	80	71	72	98	120
69	85	98	70	105	68
106	107	82	87	100	74
82.0	90.7	83.7	76.3	101.0	87.3
108.7	122.0	99.5	92.0	114.0	112.7
104	115	117	98	108	114
123	172	107	72	115	101
133	194	116	166	162	146
120.0	160.3	113.3	112.0	128.3	120.3
87	73	79	86	72	82
215	134	152	114	135	162
93	103	94	86	96	109
131.7	103.3	108.3	98.7	101.0	117.7
125.8	131.8	110.8	103.7	114.7	119.0
61	59	89	58	75	59
61	82	89	72	80	79
83	89	87	87	81	83
68.3	76.7	88.3	72.3	78.7	73.7
44	46	73	54	43	60
51	54	60	56	84	64
54	44	60	65	52	61
49.3	48.0	64.3	58.3	59.7	61.7
59.0	62.3	76.3	65.3	69.2	67.7

TABLE V—CONTINUED

TABLE V—CONTINUED

TABLE V—CONTINUED

PART B—RATS ON ASCORBIC ACID SUPPLEMENTED DIETS

Case Number of Rats	FOOD			
	1	2	3	4
3. 2% Protein				
6D1-38	50	34	29	31
6D2-15	33	40	26	27
6D2-10	35	37	32	48
Average male . . .	39.3	37.0	29.0	35.3
6D1-34	46	33	27	33
5D2-15	44	24	20	22
6D2-23	46	46	43	34
Average female . . .	45.3	34.3	30.0	29.7
Average male and female .	42.3	35.7	29.5	32.5
25. 2% Protein				
5D1-9	57	68	52	85
6D2-11	42	47	82	115
6D2-1	53	50	60	92
Average male . . .	50.7	55.0	64.7	97.3
6D1-48	42	54	75	78
5D2-20	59	71	64	68
5D2-21	41	57	67	49
Average female . . .	47.3	60.7	68.7	65.0
Average male and female .	49.0	57.8	66.7	81.2
40. 2% Protein				
6D1-45	44	58	65	68
5D2-23	53	53	58	56
6D2-14	51	46	43	85
Average male . . .	49.3	52.3	55.3	69.7
6D1-39	69	87	64	85
5D2-11	56	75	65	75
6D2-4	88	56	73	91
Average female . . .	85.7	72.7	67.3	83.7
Average male and female .	67.5	62.5	61.3	76.7
78. 2% Protein				
5D2-19	52	56	30	58
6D2-16	29	39	48	55
2J2-2	22	51	67	67
Average male . . .	34.3	48.7	48.3	60.0
6D1-49	17	74	50	51
6D2-9	32	47	23	47
6D2-3	27	38	44	61
Average female . . .	25.3	53.0	39.0	53.0
Average male and female .	29.8	50.8	43.7	56.5

TABLE V—CONTINUED

CONSUMPTION (GMS.) AT WEEKLY INTERVALS (1-10)					
5	6	7	8	9	10
20	16	22	22	22	28
29	39	31	37	47	30
36	37	22	32	31	30
28.3	30.7	25.0	30.3	33.3	29.3
30	25	20	26	28	27
24	29	24	40	27	23
38	26	27	21	..	..
30.7	26.7	23.7	29.0	27.5	25.0
29.5	28.7	24.3	29.7	31.0	27.6
114	130	115	110	102	114
99	101	105	117	80	106
97	94	102	89	105	106
103.3	108.3	107.3	105.3	95.7	108.7
57	58	80	72	86	87
76	120	104	88	90	95
67	81	68	74	68	82
66.7	86.3	84.0	78.0	81.3	88.0
85.0	97.3	95.7	91.7	88.5	98.3
54	73	90	107	119	112
80	99	120	117	119	130
90	85	105	95	92	111
74.6	85.7	105.0	106.3	110.0	117.7
69	82	88	97	91	88
101	104	88	70	112	80
104	89	81	83	90	102
91.3	91.7	91.0	83.3	97.7	90.0
83.0	88.7	98.0	94.8	103.8	103.8
80	93	92	101	95	95
59	48	65	84	90	87
67	65	55	66	64	65
68.7	68.7	70.7	83.7	83.0	82.3
55	79	58	85	74	53
55	62	59	68	52	61
60	55	58	57	45	45
56.6	65.3	58.3	70.0	57.0	53.0
62.7	67.0	64.5	76.8	70.0	67.7

TABLE V—CONTINUED

TABLE V—CONTINUED

TABLE V—CONTINUED

Case Number of Rats	FOOD			
	11	12	13	14
3.2% Protein				
6D1-38	23	34	37	22
6D2-15	29	24	26	..
6D2-10	23	..	..	..
Average male	25.0	29.0	31.5	22.0
6D1-34	22	33	17	17
5D2-15	38	28	30	19
6D2-23	..	..	..	..
Average female	30.0	30.5	23.5	18.0
Average male and female .	27.0	29.7	27.5	19.3
25.2% Protein				
5D1-9	120	111	120	106
6D2-11	118	81	146	117
6D2-1	110	111	104	99
Average male	116.0	101.0	123.3	107.3
6D1-48	106	110	104	81
5D2-20	89	101	90	110
5D2-21	71	79	87	76
Average female	88.7	96.7	93.7	89.0
Average male and female .	102.3	98.8	108.5	98.2
40.2% Protein				
6D1-45	115	92	117	116
5D2-23	141	151	185	185
6D2-14	151	149	170	191
Average male	135.7	130.7	157.3	164.0
6D1-39	58	96	84	76
5D2-11	66	82	87	79
6D2-4	105	99	108	93
Average female	76.3	92.3	93.0	82.7
Average male and female .	106.0	111.5	125.2	123.3
78.2% Protein				
5D2-19	91	105	94	103
6D2-16	66	84	80	98
2J2-2	67	80	100	82
Average male	74.7	89.7	91.3	94.3
6D1-49	64	52	56	57
6D2-9	55	71	60	70
6D2-3	40	54	83	75
Average female	53.0	59.0	66.3	67.3
Average male and female .	63.8	74.3	78.3	80.8

TABLE V—CONTINUED

CONSUMPTION (GMS.) AT WEEKLY INTERVALS (11-20)					
15	16	17	18	19	20
25	21	..	..	..	..
..	..	..	..	..	..
..	..	..	..	..	..
25.0	21.0	..	..	...	..
..	..	..	..	..	..
..	..	..	..	..	..
..	..	..	..	..	..
..	..	..	..	..	..
25 0	21.0	..	..	..	..
110	119	128	116	146	127
143	149	135	85	89	107
106	120	118	108	105	110
119 7	129.3	127.0	103.0	113.3	114.7
72	67	91	60	76	85
85	75	121	108	153	88
81	98	54	99	98	73
79.3	80.0	88.7	89.0	109.0	82.0
99.5	104.7	107.8	96.0	127.8	98.3
85	135	180	162	177	205
162	204	158	209	218	172
190	203	205	194	160	108
145.7	180.7	181.0	189.3	185.0	161.7
87	83	71	78	87	66
78	86	66	90	81	76
118	105	107	119	91	140
94.3	91.3	81.3	95 7	86.3	94.0
120.0	136.0	131.2	141.7	135.7	127.8
129	123	117	98	113	102
104	96	116	95	98	103
116	100	105	124	106	91
116.3	106.3	112.7	105.7	105.7	98.7
65	82	65	59	73	60
58	54	62	56	67	59
78	72	82	77	74	79
67.0	69.3	69.7	64.0	68.0	66.0
91.7	87.8	91.2	84.8	86.8	82.3

END OF TABLE V

TABLE VI

TABLE VI

AVERAGE DAILY FOOD CONSUMPTION IN GRAMS PER GRAM OF EXPERIMENTAL ANIMAL

PART A—GROUP WITH NO ADDED ASCORBIC ACID

Rat Number	WEEK ON EXPERIMENT			
	1	2	3	4
3.2% Protein				
6D1-33	0.165	0.100	0.135	0.118
5D2-17	0.136	0.184	0.146	0.123
6D2-19	0.133	0.087	0.125	0.114
Average male	0.145	0.124	0.135	0.118
6D1-36	0.190	0.129	0.119	0.125
5D2-16	0.109	0.086	0.085	0.087
6D2-18	0.139	0.109	0.117	0.132
Average female	0.146	0.111	0.107	0.115
Average male and female .	0.146	0.118	0.121	0.116
25.2% Protein				
6D1-46	0.116	0.081	0.077	0.077
5D2-14	0.110	0.101	0.094	0.098
6D2-20	0.109	0.090	0.069	0.082
Average male	0.112	0.091	0.080	0.086
6D1-35	0.125	0.102	0.136	0.087
5D2-7	0.122	0.094	0.079	0.052
6D2-17	0.086	0.106	0.129	0.089
Average female	0.090	0.101	0.115	0.076
Average male and female .	0.101	0.097	0.097	0.081
40.2% Protein				
5D2-8	0.144	0.095	0.085	0.072
2J2-3	0.084	0.088	0.085	0.088
6D2-2	0.096	0.088	0.072	0.098
Average male	0.108	0.090	0.087	0.086
6D1-37	0.124	0.098	0.080	0.071
6D2-12	0.086	0.090	0.060	0.082
6D2-8	0.101	0.078	0.099	0.113
Average female	0.104	0.089	0.080	0.089
Average male and female .	0.106	0.090	0.080	0.088
78.2% Protein				
6D1-50	0.094	0.060	0.104	0.077
5D2-12	0.071	0.090	0.072	0.069
2J2-1	0.061	0.083	0.094	0.070
Average male	0.075	0.078	0.090	0.072
6D1-47	0.122	0.081	0.085	0.089
5D2-10	0.149	0.066	0.071	0.062
6D2-6	0.084	0.065	0.077	0.068
Average female	0.118	0.071	0.078	0.075
Average male and female .	0.097	0.074	0.084	0.074

TABLE VI—CONTINUED

WEEK ON EXPERIMENT					
5	6	7	8	9	10
0.132	0.088	0.084	0.089	0.091	0.099
0.123	0.102	0.092	0.110	0.098	0.080
0.118	0.103	0.115	0.111	0.091	0.111
0.124	0.098	0.097	0.103	0.093	0.097
0.092	0.104	0.071	0.100	0.096	0.105
0.088	0.154	0.123	0.115	0.116	0.092
0.128	0.124	0.124	0.121	0.124	0.132
0.103	0.127	0.106	0.112	0.112	0.110
0.114	0.112	0.102	0.108	0.102	0.103
0.064	0.061	0.054	0.064	0.052	0.048
0.107	0.129	0.094	0.085	0.081	0.074
0.074	0.083	0.082	0.075	0.066	0.065
0.082	0.091	0.077	0.075	0.066	0.062
0.071	0.047	0.071	0.111	0.070	0.063
0.091	0.125	0.089	0.092	0.088	0.079
0.069	0.061	0.082	0.084	0.078	0.077
0.077	0.078	0.081	0.096	0.079	0.072
0.080	0.084	0.079	0.086	0.072	0.067
0.082	0.072	0.197	0.070	0.066	0.050
0.073	0.078	0.071	0.085	0.072	0.069
0.084	0.079	0.077	0.066	0.077	0.053
0.080	0.076	0.115	0.074	0.072	0.057
0.061	0.073	0.072	0.083	0.060	0.066
0.072	0.089	0.109	0.109	0.109	0.101
0.088	0.073	0.078	0.071	0.074	0.068
0.074	0.076	0.086	0.088	0.081	0.078
0.077	0.077	0.101	0.081	0.076	0.068
0.102	0.105	0.060	0.075	0.097	0.098
0.076	0.070	0.053	0.058	0.057	0.059
0.113	0.069	0.074	0.065	0.067	0.067
0.097	0.081	0.062	0.066	0.074	0.075
0.035	0.086	0.096	0.075	0.063	0.064
0.069	0.073	0.051	0.063	0.064	0.056
0.065	0.064	0.060	0.056	0.055	0.056
0.056	0.074	0.069	0.065	0.061	0.059
0.076	0.078	0.066	0.066	0.068	0.067

TABLE VI—CONTINUED

TABLE VI—CONTINUED

TABLE VI—CONTINUED

Rat Number	WEEK ON EXPERIMENT			
	11	12	13	14
3.2% Protein				
6D1-33 . . .	0.109	0.081	0.092	0.104
5D2-17 . . .	0.085	0.076	0.076	0.081
6D2-19 . . .	0.086	0.095	0.092	...
Average male . .	0.093	0.084	0.087	0.092
6D1-36 . . .	0.091	0.085	0.082	0.074
5D2-16 . . .	0.126	0.122	0.122	0.126
6D2-18 . . .	0.117	0.086	0.100	0.119
Average female . .	0.111	0.098	0.101	0.106
Average male and female	0.102	0.091	0.094	0.101
25.2% Protein				
6D1-46 . . .	0.071	0.069	0.066	0.070
5D2-14 . . .	0.078	0.072	0.083	0.088
6D2-20 . . .	0.079	0.048	0.058	0.058
Average male . .	0.076	0.063	0.069	0.072
6D1-35 . . .	0.074	0.074	0.068	0.073
5D2-7 . . .	0.075	0.066	0.061	0.068
6D2-17 . . .	0.063	0.061	0.065	0.065
Average female . .	0.071	0.067	0.065	0.069
Average male and female	0.074	0.065	0.067	0.070
40.2% Protein				
5D2-8 . . .	0.059	0.050	0.052	0.053
2J2-3 . . .	0.058	0.058	0.109	0.075
6D2-2 . . .	0.075	0.078	0.083	0.073
Average male . .	0.064	0.062	0.081	0.067
6D1-37 . . .	0.062	0.087	0.051	0.057
6D2-12 . . .	0.123	0.107	0.103	0.101
6D2-8 . . .	0.071	0.061	0.060	0.067
Average female . .	0.085	0.085	0.073	0.075
Average male and female	0.074	0.074	0.077	0.071
78.2% Protein				
6D1-50 . . .	0.079	0.095	0.049	0.043
5D2-12 . . .	0.056	0.054	0.059	0.052
2J2-1 . . .	0.053	0.052	0.060	0.074
Average male . .	0.063	0.067	0.056	0.056
6D1-47 . . .	0.067	0.079	0.075	0.060
5D2-10 . . .	0.057	0.060	0.061	0.057
6D2-6 . . .	0.064	0.058	0.058	0.060
Average female . .	0.063	0.066	0.065	0.059
Average male and female	0.063	0.066	0.060	0.058

TABLE VI—CONTINUED

WEEK ON EXPERIMENT					
15	16	17	18	19	20
0.080	...	...	...	...	...
...	...	...	...	...	...
...	...	...	...	...	...
0.080	...	...	...	...	...
...	...	...	...	...	...
0.117	...	...	...	...	...
0.079	0.131	0.090	0.083	0.090	...
0.098	0.131	0.090	0.083	0.090	...
0.092	0.131	0.090	0.033	0.090	...
0.061	0.057	0.051	0.046	0.054	0.070
0.102	0.125	0.076	0.064	0.085	0.089
0.067	0.071	0.060	0.082	0.063	0.052
0.077	0.084	0.062	0.064	0.067	0.070
0.072	0.078	0.064	0.065	0.080	0.090
0.054	0.064	0.071	0.052	0.071	0.047
0.080	0.080	0.058	0.061	0.066	0.048
0.069	0.074	0.064	0.059	0.072	0.062
0.073	0.079	0.063	0.062	0.070	0.066
0.052	0.053	0.054	0.043	0.046	0.048
0.074	0.099	0.107	0.040	0.061	0.052
0.072	0.100	0.058	0.082	0.052	0.065
0.066	0.084	0.073	0.055	0.053	0.055
0.061	0.051	0.053	0.058	0.049	0.054
0.167	0.104	0.115	0.086	0.102	0.121
0.065	0.069	0.060	0.057	0.059	0.066
0.098	0.075	0.076	0.067	0.070	0.080
0.082	0.080	0.074	0.061	0.062	0.068
0.055	0.052	0.079	0.050	0.064	0.051
0.046	0.057	0.060	0.047	0.049	0.048
0.049	0.050	0.048	0.048	0.044	0.043
0.050	0.053	0.062	0.048	0.052	0.047
0.059	0.057	0.088	0.064	0.050	0.064
0.058	0.058	0.060	0.054	0.078	0.054
0.052	0.042	0.056	0.058	0.045	0.051
0.056	0.052	0.068	0.059	0.058	0.056
0.053	0.052	0.065	0.054	0.055	0.052

TABLE VI—CONTINUED

TABLE VI—CONTINUED

TABLE VI—CONTINUED

PART B—GROUP WITH ADDED ASCORBIC ACID

Rat Number	WEEK ON EXPERIMENT			
	1	2	3	4
3.2% Protein				
6D1-38 . . .	0.166	0.121	0.104	0.108
6D2-15 . . .	0.107	0.133	0.086	0.088
6D2-10 . . .	0.125	0.147	0.131	0.201
Average male . .	0.133	0.134	0.107	0.132
6D1-34 . . .	0.153	0.118	0.099	0.121
5D2-15 . . .	0.157	0.098	0.082	0.095
6D2-23 . . .	0.137	0.143	0.146	0.116
Average female . .	0.149	0.120	0.109	0.111
Average male and female	0.141	0.127	0.108	0.121
25.2% Protein				
5D2-9 . . .	0.166	0.099	0.069	0.088
6D2-11 . . .	0.086	0.069	0.100	0.110
6D2-1 . . .	0.098	0.069	0.073	0.089
Average male . .	0.117	0.079	0.081	0.097
6D1-48 . . .	0.100	0.091	0.098	0.096
5D2-20 . . .	0.117	0.103	0.081	0.079
5D2-21 . . .	0.094	0.096	0.096	0.060
Average female . .	0.104	0.097	0.092	0.078
Average male and female	0.110	0.088	0.086	0.088
40.2% Protein				
6D1-45 . . .	0.098	0.120	0.074	0.066
5D2-23 . . .	0.124	0.098	0.089	0.069
6D2-14 . . .	0.112	0.077	0.057	0.087
Average male . .	0.111	0.098	0.073	0.074
6D1-39 . . .	0.144	0.132	0.076	0.087
5D2-11 . . .	0.132	0.100	0.075	0.079
5D2-4 . . .	0.180	0.095	0.097	0.100
Average female . .	0.152	0.109	0.083	0.089
Average male and female	0.132	0.104	0.078	0.081
78.2% Protein				
5D2-19 . . .	0.126	0.092	0.044	0.075
6D2-16 . . .	0.068	0.072	0.058	0.079
2J2-2 . . .	0.058	0.098	0.103	0.083
Average male . .	0.084	0.087	0.075	0.079
6D1-49 . . .	0.050	0.166	0.084	0.079
6D2-9 . . .	0.083	0.096	0.037	0.066
6D2-3 . . .	0.071	0.086	0.081	0.093
Average female . .	0.068	0.116	0.071	0.079
Average male and female	0.076	0.102	0.073	0.079

TABLE VI—CONTINUED

WEEK ON EXPERIMENT					
5	6	7	8	9	10
0.077	0.063	0.085	0.083	0.092	0.114
0.104	0.139	0.108	0.132	0.156	0.102
0.146	0.170	0.134	0.142	0.142	0.138
0.109	0.124	0.109	0.119	0.130	0.118
0.119	0.096	0.077	0.103	0.118	0.110
0.098	0.126	0.101	0.179	0.124	0.106
0.136	0.100	0.092	0.077	...	...
0.118	0.107	0.090	0.120	0.121	0.108
0.113	0.116	0.100	0.120	0.126	0.113
0.090	0.086	0.070	0.064	0.057	0.061
0.069	0.069	0.069	0.069	0.046	0.060
0.076	0.073	0.071	0.059	0.068	0.062
0.078	0.076	0.070	0.064	0.057	0.061
0.056	0.057	0.071	0.061	0.069	0.074
0.082	0.108	0.083	0.074	0.071	0.075
0.100	0.075	0.057	0.064	0.056	0.067
0.079	0.080	0.070	0.066	0.065	0.072
0.078	0.078	0.070	0.065	0.061	0.066
0.050	0.063	0.068	0.071	0.075	0.069
0.078	0.084	0.082	0.076	0.076	0.077
0.076	0.066	0.075	0.064	0.057	0.069
0.068	0.071	0.075	0.070	0.069	0.072
0.064	0.067	0.068	0.072	0.062	0.060
0.089	0.082	0.063	0.052	0.081	0.056
0.102	0.089	0.078	0.075	0.078	0.084
0.085	0.079	0.070	0.066	0.074	0.067
0.076	0.075	0.072	0.068	0.071	0.070
0.083	0.082	0.069	0.072	0.065	0.062
0.077	0.062	0.083	0.099	0.097	0.086
0.078	0.071	0.058	0.069	0.058	0.057
0.079	0.072	0.070	0.080	0.075	0.068
0.116	0.111	0.077	0.105	0.087	0.063
0.069	0.069	0.063	0.068	0.053	0.059
0.077	0.068	0.073	0.077	0.059	0.057
0.087	0.083	0.071	0.083	0.066	0.060
0.083	0.078	0.070	0.082	0.070	0.064

TABLE VI--CONTINUED

TABLE VI—CONTINUED

TABLE VI—CONTINUED

Rat Number	WEEK ON EXPERIMENT			
	11	12	13	14
3.2% Protein				
6D1-38 . . .	0.099	0.148	0.090	0.095
6D2-15 . . .	0.106	0.090	0.090	...
6D2-10 . . .	0.117	...	...	...
Average male . .	0.107	0.119	0.090	0.095
6D1-34 . . .	0.095	0.142	0.076	0.081
5D2-15 . . .	0.180	0.129	0.138	0.101
6D2-23 . . .	...	...	...	...
Average female . .	0.137	0.135	0.107	0.091
Average male and female	0.120	0.127	0.098	0.092
25.2% Protein				
5D2-9 . . .	0.064	0.056	0.059	0.050
6D2-11 . . .	0.065	0.043	0.078	0.061
6D2-1 . . .	0.062	0.059	0.054	0.051
Average male . .	0.064	0.053	0.064	0.054
6D1-48 . . .	0.086	0.084	0.077	0.059
5D2-20 . . .	0.068	0.075	0.070	0.077
5D2-21 . . .	0.056	0.061	0.066	0.057
Average female . .	0.070	0.073	0.071	0.064
Average male and female	0.067	0.063	0.067	0.059
40.2% Protein				
6D1-45 . . .	0.069	0.053	0.066	0.064
5D2-23 . . .	0.085	0.089	0.104	0.100
6D2-14 . . .	0.089	0.082	0.090	0.098
Average male . .	0.081	0.075	0.087	0.087
6D1-39 . . .	0.041	0.064	0.055	0.048
5D2-11 . . .	0.045	0.056	0.053	0.052
5D2-4 . . .	0.083	0.073	0.074	0.063
Average female . .	0.056	0.064	0.062	0.054
Average male and female	0.068	0.070	0.074	0.070
78.2% Protein				
5D2-19 . . .	0.058	0.062	0.055	0.056
6D2-16 . . .	0.062	0.074	0.067	0.074
2J2-2 . . .	0.055	0.063	0.075	0.059
Average male . .	0.058	0.066	0.066	0.063
6D1-49 . . .	0.077	0.063	0.055	0.066
6D2-9 . . .	0.053	0.065	0.039	0.063
6D2-3 . . .	0.056	0.069	0.100	0.082
Average female . .	0.062	0.066	0.065	0.070
Average male and female	0.060	0.066	0.066	0.066

TABLE VI--CONTINUED

WEEK ON EXPERIMENT					
15	16	17	18	19	20
0.119	0.091	...	...	...	...
...	...	...	...	...	...
...	...	...	...	...	...
0.119	0.091	...	...	...	...
...	...	...	...	...	...
...	...	...	...	...	...
...	...	...	...	...	...
...	...	...	...	...	...
0.119	0.091	...	...	...	...
0.050	0.052	0.056	0.050	0.059	0.051
0.072	0.074	0.066	0.044	0.072	0.051
0.052	0.056	0.054	0.048	0.046	0.046
0.053	0.061	0.059	0.047	0.059	0.049
0.054	0.049	0.070	0.044	0.053	0.058
0.059	0.052	0.083	0.072	0.102	0.057
0.063	0.073	0.039	0.073	0.071	0.052
0.059	0.058	0.064	0.063	0.075	0.056
0.058	0.060	0.061	0.055	0.067	0.052
0.046	0.070	0.090	0.082	0.092	0.105
0.086	0.108	0.136	0.109	0.112	0.088
0.093	0.094	0.094	0.087	0.067	0.044
0.075	0.091	0.107	0.093	0.090	0.079
0.053	0.050	0.043	0.048	0.052	0.040
0.051	0.054	0.042	0.055	0.049	0.045
0.073	0.069	0.071	0.077	0.058	0.068
0.061	0.058	0.052	0.060	0.053	0.048
0.068	0.074	0.080	0.076	0.072	0.064
0.069	0.064	0.058	0.050	0.054	0.047
0.078	0.068	0.080	0.065	0.062	0.060
0.080	0.066	0.066	0.075	0.061	0.048
0.076	0.066	0.063	0.063	0.059	0.052
0.070	0.085	0.065	0.056	0.064	0.052
0.051	0.047	0.051	0.047	0.057	0.049
0.082	0.069	0.075	0.067	0.063	0.065
0.068	0.067	0.064	0.057	0.061	0.055
0.072	0.066	0.066	0.060	0.060	0.054

END OF TABLE VI

TABLE VII

ADEQUACY OF ESSENTIAL NUTRIENTS IN 3.2 PER CENT PROTEIN DIETS PER GRAM OF FOOD CONSUMED

PART A— ESSENTIAL AMINO ACIDS

Essential Amino Acids	Grams of Food Consumed				Unit	Daily Re-quire-ment	Adequacy
	3	4	5	6			
Lysine	0.030	0.040	0.050	0.060	Grams	0.100	Inadequate
Tryptophan	0.006	0.008	0.010	0.012	Grams	0.020	Inadequate
Histidine	0.012	0.016	0.020	0.024	Grams	0.040	Inadequate
Phenylala-nine	0.036	0.048	0.060	0.072	Grams	0.070	Adequate at 6 gm. con-sumption
Leucine	0.045	0.060	0.075	0.090	Grams	0.090	Adequate at 6 gm. con-sumption
Isoleucine	0.024	0.032	0.040	0.048	Grams	0.050	Inadequate
Threonine	0.027	0.036	0.045	0.054	Grams	0.060	Inadequate
Methionine	0.015	0.020	0.025	0.030	Grams	0.060	Inadequate
Valine	0.030	0.040	0.050	0.060	Grams	0.070	Inadequate
Arginine	0.030	0.040	0.050	0.060	Grams	0.020	Adequate

TABLE VII

ADEQUACY OF ESSENTIAL NUTRIENTS IN 3.2 PER CENT PROTEIN DIETS PER GRAM OF FOOD CONSUMED (CONTINUED)

PART B—MINERALS

Minerals	Grams of Food Consumed				Unit	Daily Re-quire-ment	Adequacy
	3	4	5	6			
Calcium	15	20	25	30	Mg.	40-50	Inadequate
Phosphorus	12	16	20	24	Mg.	35-45	Inadequate
Iron	0.81	1.08	1.35	1.62	Mg.	0.25	Adequate
Magnesium	3.3	4.4	5.5	6.6	Mg.	0.50 Mg/Kg body wt.	Adequate
Potassium	6	8	10	12	Mg.	Males, 15 mg.; females, 8 mg.	Adequate for males; ade-quate above 3 gm. con-sumption for females
Sodium	4.2	5.6	7.0	8.4	Mg.	0.5% of diet	Adequate
Chlorine	3	4	5	6	Mg.	5	Adequate a-bove 4 gm. consumption
Iodine	240	320	400	480	Mcg.	1-2	Adequate
Copper	0.09	0.12	0.15	0.18	Mg.	0.1	Adequate over 3 gm. cons.
Zinc	16.8	22.4	28.0	33.6	Mcg.	40	Inadequate

TABLE VII

ADEQUACY OF ESSENTIAL NUTRIENTS IN 3.2 PER CENT PROTEIN DIETS PER GRAM OF FOOD CONSUMED (CONTINUED)

PART C—VITAMINS

Vitamins	Grams of Food Consumed				Unit	Daily Re-quire-ment	Adequacy
	3	4	5	6			
Thiamine	2400	3200	4000	5600	Mcg.	10	Adequate
Riboflavin	2400	3200	4000	5600	Mcg.	40	Adequate
Nicotinic acid	0.03	0.04	0.05	0.06	Mg.	None	Adequate
Pyridoxine	3.9	5.2	6.5	7.8	Mcg.	10	Inadequate
Choline	2.4	3.2	4.0	4.8	Mg.	2-3	Adequate
Pantothenic acid	120	160	200	240	Mg.	250 mg. dried liver	Inadequate
Folic acid	0.9	1.2	1.5	1.8	Mcg.	None	Adequate
B_{12}	0.12	0.16	0.20	0.24	Mcg.	None	Adequate
Vitamin A	51	68	85	102	I. U.	4 mcg.	Adequate
Vitamin D	5.1	6.8	8.5	10.2	I. U.	*	Adequate
Alpha tocopherol	0.3	0.4	0.5	0.6	Mg.	1.0	Inadequate

*Vitamin D not required if Ca : P ratio is 1 : 1 to 2 : 1.

TABLE VIII

TABLE VIII

WEEKLY WEIGHTS OF RATS ON EACH LEVEL OF DIETARY PROTEIN

PART A—RATS ON DIETS WITH NO ADDED ASCORBIC ACID

Case Numbers of Rats	Initial Wt. (Gms.)	WEIGHTS			
		1	2	3	4
3.2% Protein					
6D1-33	49	45	40	44	41
5D2-17	45	41	37	35	36
6D2-19	46	42	41	40	39
Average male . . .	46.7	42.6	39.3	39.7	38.7
6D1-36	46	45	43	43	40
5D2-16	48	43	40	37	36
6D2-18	47	45	43	40	40
Average female . . .	47.0	44.3	42.0	40.0	38.7
Average male and female .	46.8	43.5	40.7	39.8	38.7
25.2% Protein					
6D1-46	46	69	102	122	159
5D2-14	40	61	79	100	119
6D2-20	48	63	92	109	126
Average male . . .	44.7	64.3	91.0	110.3	134.7
6D1-35	48	72	88	111	123
5D2-7	53	75	100	109	121
6D2-17	45	63	82	102	127
Average female . . .	48.7	70.0	93.3	107.3	125.7
Average male and female .	46.7	67.2	92.2	108.8	129.2
40.2% Protein					
5D1-8	47	67	97	127	144
2J2-3	49	59	84	107	129
6D2-2	47	67	86	105	122
Average male . . .	47.7	64.3	89.0	113.0	131.7
6D1-37	46	68	95	113	128
6D2-12	44	55	81	103	118
6D2-8	47	71	91	113	128
Average female . . .	45.7	64.7	88.7	109.7	124.7
Average male and female .	46.7	64.5	88.8	111.3	128.1
78.2% Protein					
6D1-50	47	50	64	78	93
5D1-12	46	54	68	85	95
2J2-1	46	54	67	85	108
Average male . . .	46.3	52.7	66.3	82.7	96.7
6D1-47	46	54	65	77	82
5D2-10	47	53	56	64	65
6D2-6	47	56	55	72	80
Average female . . .	46.7	54.3	58.7	71.0	75.7
Average male and female .	46.5	53.5	62.5	76.8	87.2

TABLE VIII-CONTINUED

AT WEEKLY INTERVALS (GMS.) (1-10)					
5	6	7	8	9	10
40	42	41	43	41	42
36	35	34	35	32	34
40	39	41	41	41	40
38.7	38.7	38.7	39.7	38.0	38.7
40	40	40	40	40	38
37	34	36	36	33	34
40	39	39	40	39	39
39.0	37.7	38.3	38.7	37.3	37.0
38.8	38.2	38.5	39.2	37.7	37.8
175	187	194	196	195	175
143	167	202	209	210	218
150	153	179	195	210	217
156.0	169.0	191.6	200.0	205.0	203.3
123	124	119	114	130	124
123	137	154	149	153	168
143	138	148	157	167	173
129.6	133.0	140.3	140.0	150.0	155.0
142.8	151.0	166.0	170.0	177.5	179.2
179	203	226	235	252	256
132	147	157	171	182	189
123	129	142	149	154	171
144.7	159.7	175.0	185.0	196.0	205.3
128	140	161	172	189	192
137	144	153	152	158	159
145	150	153	174	169	177
136.7	144.7	155.7	159.3	172.0	176.0
140.7	152.2	165.3	175.5	184.0	190.7
102	110	115	129	132	136
107	116	136	137	150	168
119	135	144	158	173	195
109.3	120.3	131.7	141.3	151.7	166.3
81	85	89	93	99	100
66	76	87	95	102	97
94	96	100	107	104	113
80.3	85.7	92.0	99.3	101.7	103.3
94.8	103.0	111.8	119.8	126.7	134.8

TABLE VIII—CONTINUED

TABLE VIII—CONTINUED

TABLE VIII-CONTINUED

Case Numbers of Rats	WEIGHTS			
	11	12	13	14
3.2% Protein				
6D1-33	38	37	34	33
5D2-17	32	30	32	30
6D2-19	43	39	39	..
Average male	37.7	35.3	35.0	31.5
6D1-36	36	37	35	29
5D2-16	34	34	35	35
6D2-18	39	38	37	36
Average female . . .	36.3	36.3	35.7	33.3
Average male and female .	37.0	35.8	35.3	32.6
25.2% Protein				
6D1-46	198	206	226	241
5D2-14	222	230	236	250
6D2-20	228	234	226	246
Average male	216.0	223.3	229.3	245.7
6D1-35	128	142	135	132
5D2-7	170	178	173	189
6D2-17	182	180	172	202
Average female . . .	160.0	166.7	160.0	174.3
Average male and female .	188.0	195.0	194.7	210.0
40.2% Protein				
5D1-8	265	272	280	291
2J2-3	198	206	221	230
6D2-2	199	222	231	250
Average male	220.7	233.3	244.0	257.0
6D1-37	188	197	196	204
6D2-12	167	180	175	183
6D2-8	187	188	182	190
Average female . . .	180.7	188.3	184.3	192.3
Average male and female .	200.7	210.8	214.2	224.7
78.2% Protein				
6D1-50	137	144	153	155
5D1-12	168	168	179	196
2J2-1	208	208	225	233
Average male	171.0	173.3	185.7	194.7
6D1-47	100	98	101	110
5D2-10	115	111	113	123
6D2-6	120	123	131	139
Average female . . .	111.7	110.7	115.0	118.0
Average male and female .	141.3	142.0	150.3	156.3

TABLE VIII-CONTINUED

(GMS.) AT WEEKLY INTERVALS (11-20)					
15	16	17	18	19	20
32	..	..	..	..	..
..	..	..	..	..	..
..	..	..	..	..	..
32.0	..	..	..	..	..
..	..	..	..	..	..
33	..	..	..	..	..
36	36	35	36	35	34
34.5	36.0	35.0	36.0	35.0	34.0
33.7	36.0	35.0	36.0	35.0	34.0
245	250	255	262	257	270
253	262	268	267	273	276
254	264	268	274	287	303
250.7	258.7	263.7	267.7	272.3	283.0
140	146	158	159	176	191
182	189	198	192	212	208
191	192	203	204	217	218
171.0	175.7	186.3	185.0	201.7	205.7
210.8	217.2	225.0	226.3	238.7	244.3
292	308	312	325	332	340
233	248	255	256	268	275
264	278	286	288	312	320
263.0	278.0	284.3	289.7	304.0	311.7
202	203	211	213	208	217
184	184	188	189	189	192
203	213	222	214	233	235
196.3	200.0	207.0	205.3	210.0	214.7
229.7	239.0	245.7	247.5	257.0	263.2
158	163	160	166	167	165
191	206	212	219	233	234
243	255	260	254	265	276
197.3	208.0	210.7	213.0	221.7	225.0
107	116	118	120	122	133
125	132	142	149	154	168
149	148	154	160	164	170
127.0	132.0	138.0	143.0	146.7	157.0
162.2	170.0	174.3	178.0	184.2	191.0

TABLE VIII—CONTINUED

TABLE VIII—CONTINUED

TABLE VIII-CONTINUED

PART B—RATS ON ASCORBIC ACID SUPPLEMENTED DIETS

Case Numbers of Rats	Initial Wt. (Gms.)	WEIGHTS			
		1	2	3	4
3.2% Protein					
6D1-38 . . .	46	43	40	40	41
6D2-15 . . .	48	44	43	43	44
6D2-10 . . .	45	40	36	35	34
Average male . .	46.3	42.3	39.7	39.3	39.7
6D1-34 . . .	49	43	40	39	39
5D2-15 . . .	45	40	35	35	33
6D2-23 . . .	48	48	46	42	42
Average female . .	47.3	43.7	40.3	38.7	38.0
Average male and female	47.2	43.0	40.0	39.0	39.0
25.2% Protein					
5D2-9 . . .	46	49	98	108	137
6D2-11 . . .	46	70	97	117	149
6D2-1 . . .	50	77	103	118	148
Average male . .	47.3	65.3	99.3	114.3	144.7
6D1-48 . . .	49	60	85	109	134
5D2-20 . . .	49	72	98	113	121
5D2-21 . . .	43	62	85	100	116
Average female . .	47.0	64.7	89.3	107.3	123.7
Average male and female	47.2	65.0	94.3	110.8	134.2
40.2% Protein					
6D1-45 . . .	47	64	99	126	148
5D2-23 . . .	42	61	77	93	116
6D2-14 . . .	47	65	85	108	140
Average male . .	45.3	63.3	87.0	109.0	134.7
6D1-39 . . .	47	68	94	121	140
5D2-11 . . .	51	73	107	124	136
6D2-4 . . .	47	70	84	107	130
Average female . .	48.3	70.3	95.0	117.3	135.3
Average male and female	46.8	66.8	91.0	113.2	135.0
78.2% Protein					
5D2-19 . . .	48	59	87	98	110
6D2-16 . . .	49	61	77	88	100
2J2-2 . . .	50	54	74	93	115
Average male . .	49.0	58.0	79.3	93.0	108.3
6D1-49 . . .	46	49	64	76	92
6D2-9 . . .	44	55	70	89	102
6D2-3 . . .	45	54	63	78	94
Average female . .	45.0	52.7	65.7	81.0	96.0
Average male and female	47.0	55.3	72.5	87.0	102.2

TABLE VIII-CONTINUED

(GMS.) AT WEEKLY INTERVALS (1-10)					
5	6	7	8	9	10
37	36	37	38	34	35
40	40	41	40	43	42
35	31	34	32	31	31
37.3	35.7	37.3	36.7	39.3	36.0
36	37	37	36	34	35
35	33	34	32	31	31
40	37	42	39	..	..
37.0	35.7	37.7	35.7	32.5	33.0
37.2	35.7	37.5	36.2	34.6	34.8
181	217	236	244	255	264
185	208	218	243	247	251
182	183	206	216	220	245
182.7	202.7	220.0	234.3	240.7	253.3
145	145	160	169	177	167
133	159	178	170	180	181
137	155	170	166	173	175
138.3	153.0	169.3	168.3	176.7	174.3
160.5	177.8	194.6	201.3	208.7	215.3
154	165	188	214	228	233
147	169	210	220	225	242
169	185	200	212	230	230
156.7	173.0	199.3	215.3	227.7	235.0
153	174	186	192	210	210
162	182	198	192	198	204
146	143	149	158	164	173
153.7	166.3	177.7	180.7	190.7	195.7
155.0	169.7	188.5	198.0	209.2	198.7
137	162	190	200	209	218
109	110	112	121	132	144
122	130	135	137	149	162
122.7	134.0	145.7	152.7	163.3	174.7
97	102	107	116	122	120
116	128	134	142	140	146
112	115	114	105	109	113
108.3	115.0	118.3	121.0	123.7	126.3
115.5	124.5	132.0	136.8	143.5	150.5

TABLE VIII—CONTINUED

TABLE VIII--CONTINUED

TABLE VIII-CONTINUED

Case Numbers of Rats	WEIGHTS			
	11	12	13	14
3.2% Protein				
6D1-38	33	33	34	33
6D2-15	39	38	41	..
6D2-10	28	..	..	..
Average male	33.3	35.5	37.5	33.0
6D1-34	33	33	32	30
5D2-15	30	31	31	27
6D2-23	..	..	..	..
Average female	31.3	32.0	31.3	28.5
Average male and female .	32.6	33.7	34.5	30.0
25.2% Protein				
5D2-9	269	282	292	301
6D2-11	261	266	269	274
6D2-1	253	270	275	278
Average male	261.0	272.7	278.7	284.3
6D1-48	177	187	193	197
5D2-20	187	192	184	203
5D2-21	180	186	189	192
Average female	181.3	188.3	188.7	197.3
Average male and female .	221.2	230.5	233.7	240.8
40.2% Protein				
6D1-45	238	248	253	259
5D2-23	238	241	255	262
6D2-14	241	258	271	278
Average male	239.0	249.0	259.7	266.3
6D1-39	204	214	217	226
5D2-11	209	210	214	217
6D2-4	181	194	209	212
Average female	198.0	206.0	213.3	218.3
Average male and female .	218.5	227.5	236.5	242.3
78.2% Protein				
5D2-19	225	241	243	260
6D2-16	153	163	171	190
2J2-2	174	182	190	199
Average male	184.0	195.3	201.3	216.3
6D1-49	118	117	124	124
6D2-9	149	155	154	158
6D2-3	102	112	119	130
Average female	123.0	128.0	132.3	137.3
Average male and female .	153.5	161.7	166.8	176.8

TABLE VIII-CONTINUED

(GMS.) AT WEEKLY INTERVALS (11-20)					
15	16	17	18	19	20
30	33	..	..	..	..
..	..	..	..	..	..
..	..	..	..	..	..
30.0	33.0	..	..	..	..
..	..	..	..	..	..
..	..	..	..	..	..
..	..	..	..	..	..
..	..	..	..	..	..
30.0	33.0	..	..	..	..
314	325	328	333	353	356
282	286	292	275	291	302
292	307	314	321	327	338
296.0	306.0	311.3	309.7	323.7	332.0
191	194	187	196	203	210
205	206	208	213	214	221
185	192	198	194	198	202
193.7	197.3	197.7	201.0	205.0	211.0
244.8	251.7	254.5	255.3	247.7	271.5
263	275	287	283	275	280
260	271	271	275	277	278
292	309	313	318	340	354
274.3	285.0	293.0	292.0	297.3	304.4
234	234	234	231	237	235
220	227	223	233	237	242
217	218	216	220	224	232
223.7	226.3	224.3	228.0	232.7	236.3
249.0	255.7	257.3	260.0	265.0	270.2
266	275	287	280	301	307
190	203	208	208	224	244
208	218	228	237	248	270
221.3	232.0	241.0	241.7	257.7	273.7
133	138	143	151	164	164
161	163	173	169	167	172
136	150	157	164	167	174
143.3	150.3	157.7	161.3	166.0	170.0
182.3	191.2	199.3	201.5	211.8	221.8

END OF TABLE VIII

TABLE IX

AVERAGE PLASMA ASCORBIC ACID VALUES OF RATS ON THE NINETY-FIRST AND ONE HUNDRED FORTIETH DAYS OF EXPERIMENT

Dietary Levels	Mg. Ascorbic Acid/100 Ml. at 91st Day		Mg. Ascorbic Acid/100 Ml. at 140th Day	
	Group with No Added Ascorbic Acid	Ascorbic Acid Group	Group with No Added Ascorbic Acid	Ascorbic Acid Group
3.2% Protein Average male and female[1]	0.05	1.00	....	....
25.2% Protein Average male and female	1.91	1.88	0.81	1.56
40.2% Protein Average male and female	0.80	1.89	2.37	1.96
78.2% Protein Average male and female	0.88	0.68	0.91	2.50

[1]Ascorbic acid values were ascertained from blood samples taken when rats had been on the experiment 45 days.

TABLE X

AVERAGE TOTAL SERUM PROTEIN VALUES OF RATS ON THE NINETY-FIRST AND ONE HUNDRED FORTIETH DAYS OF EXPERIMENT

Dietary Levels	Falling Drop Test on 91st Day; Per Cent Protein/100 Ml.		Kingsley Test on 140th Day; Gms. Protein/100 Ml.	
	Group with No Added Ascorbic Acid	Ascorbic Acid Group	Group with No Added Ascorbic Acid	Ascorbic Acid Group
3.2% Protein Average male and female				3.80[1]
25.2% Protein Average male and female	6.40	8.13	6.00	4.93
40.2% Protein Average male and female	6.12	6.80	4.07	3.93
78.2% Protein Average male and female	8.68	4.86	5.10	4.50

[1]Serum from rats on 3.2 per cent protein diets, both with and without ascorbic acid supplement, was combined from 45 day samples in order to make this test.

TABLE XI

AVERAGE SERUM ALBUMIN VALUES OF RATS ON THE ONE HUNDRED FORTIETH DAY OF EXPERIMENT

Dietary Levels	Albumin Values (Gms. /100 Ml.)	
	Group with No Added Ascorbic Acid	Group with Added Ascorbic Acid
25. 2% Protein Average male and female	3. 93	3. 33
40. 2% Protein Average male and female	3. 43	3. 20
78. 2% Protein Average male and female	3. 87	3. 53

TABLE XII

AVERAGE WEIGHTS IN GRAMS OF VITAL ORGANS OF RATS ON EACH LEVEL OF DIETARY PROTEIN

PART A—GROUP WITH NO ADDED ASCORBIC ACID

Various Vital Organs	3.2% Protein		25.2% Protein		40.2% Protein		78.2% Protein	
	Male	Female	Male	Female	Male	Female	Male	Female
Lungs	0.5723	0.6726	2.9065	2.3430	3.2085	2.1750	2.2130	1.6294
Spleen	0.0384	0.0558	0.4580	0.3987	0.4674	0.4325	0.4576	0.3921
Kidneys	0.4652	0.4779	2.2980	1.7009	2.8348	1.8890	2.8009	2.0958
Liver	1.1506	1.2563	13.6008	10.1166	16.2244	11.4307	14.2629	12.1071
Suprarenals	0.0127	0.0158	0.0749	0.0695	0.0677	0.0812	0.0600	0.0562
Testicles in males	0.0878	...	2.9104	...	3.3764	...	3.0469	...
Heart	0.2606	0.2839	1.0674	0.7796	1.1424	0.8806	0.9144	0.7291
Brain	1.1164	1.1693	1.6971	1.6182	1.7469	1.5696	1.4844	1.5830

TABLE XII

AVERAGE WEIGHTS IN GRAMS OF VITAL ORGANS OF RATS ON EACH LEVEL OF DIETARY PROTEIN

(CONTINUED)

PART B—GROUP WITH ADDED ASCORBIC ACID

Various Vital Organs	3.2% Protein		25.2% Protein		40.2% Protein		78.2% Protein	
	Male	Female	Male	Female	Male	Female	Male	Female
Lungs	0.6273	0.5772	3.4946	2.0417	2.4420	2.1852	2.8898	1.8906
Spleen	0.0294	0.0386	0.4828	0.3879	0.4510	0.4656	0.5841	0.4480
Kidneys	0.5150	0.5107	2.6946	1.6583	2.5273	2.2729	3.4856	2.1444
Liver	1.2564	1.5063	16.1671	9.3329	14.7909	12.5865	19.1023	11.9048
Suprarenals	0.0128	0.0113	0.0641	0.0642	0.0639	0.0890	0.0514	0.0664
Testicles in males	0.0865	...	3.2603	...	3.1399	...	3.3339	...
Heart	0.2246	0.2175	1.1587	0.7814	1.0761	0.9312	1.1940	0.7383
Brain	1.2780	1.1718	1.6084	1.5678	1.6475	1.6230	1.7087	1.4774

TABLE XIII

THE RATIO OF AVERAGE ORGAN WEIGHTS TO AVERAGE BODY WEIGHT (x 100)

PART A—GROUP WITH NO ADDED ASCORBIC ACID

Various Vital Organs	3.2% Protein		25.2% Protein		40.2% Protein		78.2% Protein	
	Male	Female	Male	Female	Male	Female	Male	Female
Lungs	1.70	2.1	1.03	1.14	1.03	1.01	0.98	1.04
Spleen	0.11	0.17	0.16	0.19	0.15	0.20	0.18	0.25
Kidneys	1.38	1.49	0.81	0.83	0.91	0.88	1.10	1.33
Liver	3.41	3.93	4.81	4.92	5.20	5.32	5.59	7.71
Supra-renals	0.038	0.049	0.056	0.034	0.022	0.038	0.027	0.036
Testicles in males	0.26	...	1.03	...	1.08	...	1.19	...
Heart	0.77	0.89	0.38	0.38	0.37	0.41	0.36	0.46
Brain	3.31	3.65	0.60	0.79	0.56	0.73	0.58	1.01

TABLE XIII

THE RATIO OF AVERAGE ORGAN WEIGHTS TO AVERAGE BODY WEIGHT (x 100)

(CONTINUED)

PART B—GROUP WITH ADDED ASCORBIC ACID

Various Vital Organs	3.2% Protein		25.2% Protein		40.2% Protein		78.2% Protein	
	Male	Female	Male	Female	Male	Female	Male	Female
Lungs	1.84	1.80	1.05	0.97	0.80	0.92	1.06	1.11
Spleen	0.09	0.12	0.14	0.18	0.15	0.20	0.21	0.26
Kidneys	1.51	1.60	0.81	0.79	0.83	0.96	1.27	1.26
Liver	3.69	4.71	4.87	4.42	4.86	5.33	6.98	7.00
Supra-renals	0.038	0.035	0.019	0.030	0.021	0.038	0.019	0.039
Testicles in males	0.25	...	0.98	...	1.03	...	1.22	...
Heart	0.66	0.68	0.35	0.37	0.35	0.39	0.44	0.43
Brain	3.45	3.66	0.48	0.74	0.54	0.69	0.62	0.87

TABLE XIV

TOTAL WEIGHTS AND MOISTURE CONTENT OF BRAINS OF EXPERIMENTAL ANIMALS

PART A—GROUP WITH NO ADDED ASCORBIC ACID

Rat Number	Total Weights of Brains (Gms.)	Dry Weights of Brains (Gms.)	Percentage of Total Moisture
3.2% Protein			
6D1-33 . . .	1.2641	0.2670	78.9
5D2-17 . . .	0.9902	0.2058	79.2
6D2-19 . . .	1.0948	0.2423	77.9
Average male . .	1.1164	0.2384	78.7
6D1-36 . . .	0.9302	0.2026	78.2
5D2-16 . . .	1.1940	0.2627	78.0
6D2-18 . . .	1.3838	0.3068	77.8
Average female . .	1.1643	0.2574	78.0
Average male & female	1.1428	0.2479	78.3
25.2% Protein			
6D1-46 . . .	1.5639	0.3339	78.6
5D2-14 . . .	1.6583	0.3328	79.9
6D2-20 . . .	1.8691	0.4762	74.5
Average male . .	1.6971	0.3810	77.7
6D1-35 . . .	1.5180	0.3333	78.0
5D2-7 . . .	1.5528	0.3527	77.3
6D2-17 . . .	1.7838	0.3966	77.8
Average female . .	1.6182	0.3609	77.7
Average male & female	1.6576	0.3709	77.7

TABLE XIV—CONTINUED

Rat Number	Total Weights of Brains (Gms.)	Dry Weights of Brains (Gms.)	Percentage of Total Moisture
40. 2% Protein			
5D2-8 . . .	1.7962	0.3998	77.7
2J2-3 . . .	1.8039	0.4102	77.3
6D2-2 . . .	1.6405	0.3601	78.0
Average male . .	1.7469	0.3900	77.7
6D1-37 . . .	1.4755	0.3248	78.0
6D2-12 . . .	1.6296	0.3361	79.4
6D2-8 . . .	1.6037	....	...
Average female . .	1.5696	0.3304	78.7
Average male & female	1.6582	0.3602	78.2
78. 2% Protein			
6D1-50 . . .	1.4830	0.3116	79.0
5D2-12 . . .	1.4873	0.3175	78.6
2J2-1 . . .	2.0679	0.3840	81.4
Average male . .	1.6794	0.3377	79.7
6D1-47 . . .	1.4844	0.3294	77.8
5D2-10 . . .	1.6258	0.3636	77.2
6D2-6 . . .	1.6387	0.3661	77.7
Average female . .	1.5830	0.3530	77.6
Average male & female	1.6312	0.3453	78.6

TABLE XIV

TOTAL WEIGHTS AND MOISTURE CONTENT OF BRAINS OF EXPERIMENTAL ANIMALS (CONTINUED)

PART B—GROUP WITH ADDED ASCORBIC ACID

Rat Number	Total Weights of Brains (Gms.)	Dry Weights of Brains (Gms.)	Percentage of Total Moisture
3.2% Protein			
6D1-38 . . .	1.2230	0.2537	79.2
6D2-15 . . .	1.2998	0.2927	77.5
6D2-10 . . .	1.3111	0.2704	79.4
Average male . .	1.2780	0.2723	78.7
6D1-34 . . .	1.0860	0.2301	78.8
5D2-15 . . .	1.1639	0.2475	78.7
6D2-23 . . .	1.2654	0.2705	78.6
Average female . .	1.1718	0.2494	78.7
Average male & female	1.2249	0.2608	78.7
25.2% Protein			
5D2-9 . . .	1.4778	0.3188	78.4
6D2-11 . . .	1.7197	0.3554	79.3
6D2-1 . . .	1.6276	0.3585	78.0
Average male . .	1.6084	0.3442	78.6
6D1-48 . . .	1.6193	0.3598	77.8
5D2-20 . . .	1.5844	0.3571	77.4
5D2-21 . . .	1.4998	0.3144	79.0
Average female . .	1.5678	0.3438	78.1
Average male & female	1.5881	0.3440	78.3

TABLE XIV—CONTINUED

Rat Number	Total Weights of Brains (Gms.)	Dry Weights of Brains (Gms.)	Percentage of Total Moisture
40.2% Protein			
6D1-45 . . .	1.6426	0.3603	78.1
5D2-23 . . .	1.5735	0.3294	79.1
6D2-14 . . .	1.7263	0.4019	76.7
Average male . .	1.6475	0.3639	78.0
6D1-39 . . .	1.6156	0.3354	79.2
5D2-11 . . .	1.5383	0.3393	77.9
6D2-4 . . .	1.7152	...	...
Average female . .	1.6230	0.3373	78.5
Average male & female	1.6352	0.3506	78.2
78.2% Protein			
5D2-19 . . .	1.4981	0.3319	77.8
6D2-16 . . .	1.7396	0.4178	76.0
2J2-2 . . .	1.8884	0.4573	75.8
Average male . .	1.7087	0.4023	76.5
6D1-49 . . .	1.4557	0.3105	78.7
6D2-9 . . .	1.3926	0.2910	78.0
6D2-3 . . .	1.5839	0.3549	77.6
Average female . .	1.4774	0.3188	78.1
Average male & female	1.5930	0.3605	77.3

TABLE XV

DATA ON CLOSURE OF METACARPALS AND PHALANGES AND ADJOINING OSSIFIC CENTERS

PART A—RATS ON DIETS CONTAINING 3.2% PROTEIN

Bones	Average Week of Closure for Group on No Added Ascorbic Acid								
	Rat Number								
	6D1-33	5D2-17	6D2-19	Average Male	6D1-36	5D2-16	6D2-18	Average Female	Average Male, Fem.
First metacarpal	7	7	8	7.3	6	8	6	6.7	7.0
Second metacarpal	8	8	9	8.3	7	9	7	7.7	8.0
Third metacarpal	8	8	9	8.3	7	9	7	7.7	8.0
Fourth metacarpal	7	7	7	7.0	4	6	7	5.7	6.4
Average metacarpals	7.5	7.5	8.2	7.7	6.0	8.0	6.8	7.0	7.4
First proximal phalanx	7	7	9	7.7	9	6	8	7.7	7.7
Second proximal phalanx	9	8	9	8.7	9	7	8	8.0	8.4
Third proximal phalanx	9	8	9	8.7	9	7	8	8.0	8.4
Fourth proximal phalanx	6	7	7	6.7	8	5	8	7.0	6.8
Average of phalanges	7.8	7.5	8.5	8.0	8.8	6.2	8.0	7.7	7.8
Average of all bones	7.6	7.5	8.4	7.8	7.4	7.1	7.4	7.3	7.6

TABLE XV—CONTINUED

Bones	Average Week of Closure for Group with Added Ascorbic Acid								
	Rat Number								
	6D1-38	6D2-15	6D2-10	Average Male	6D1-34	5D2-15	6D2-23	Average Female	Average Male, Fem.
First metacarpal	9	6	8	7.7	7	9	6	7.3	7.5
Second metacarpal	10	7	9	8.7	8	11	7	8.7	8.7
Third metacarpal	10	7	9	8.7	8	11	7	8.7	8.7
Fourth metacarpal	6	5	7	6.0	6	6	4	5.3	5.8
Average of metacarpals	8.8	6.2	8.2	7.8	7.2	9.2	6.0	7.5	7.6
First proximal phalanx	9	9	9	9.0	8	10	5	7.7	8.4
Second proximal phalanx	10	9	10	9.7	11	11	6	9.3	9.5
Third proximal phalanx	10	9	10	9.7	11	11	6	9.3	9.5
Fourth proximal phalanx	6	8	8	7.3	8	4	4	5.3	6.3
Average of phalanges	8.8	8.8	9.2	8.9	9.5	9.0	5.2	7.9	8.4
Average of all bones	8.8	7.5	8.7	8.4	8.4	9.1	5.6	7.7	8.0

TABLE XV

DATA ON CLOSURE OF METACARPALS AND PHALANGES AND ADJOINING OSSIFIC CENTERS (CONTINUED)

PART B—RATS ON DIETS CONTAINING 25.2% PROTEIN

Bones	Average Week of Closure for Group on No Added Ascorbic Acid								
	Rat Number								
	6D1-46	5D2-14	6D2-20	Average Male	6D1-35	5D2-7	6D2-17	Average Female	Average Male, Fem.
First metacarpal	8	10	9	9.0	9	10	9	9.3	9.2
Second metacarpal	11	11	11	11.0	11	12	12	11.7	11.3
Third metacarpal	11	11	11	11.0	11	12	12	11.7	11.3
Fourth metacarpal	8	8	10	8.7	8	10	9	9.0	9.8
Average of metacarpals	9.5	10.0	10.0	9.9	9.8	11.0	10.5	10.4	10.2
First proximal phalanx	8	9	9	8.7	10	10	7	9.0	8.8
Second proximal phalanx	10	9	10	9.7	11	10	9	10.0	9.8
Third proximal phalanx	10	9	10	9.7	11	10	9	10.0	9.8
Fourth proximal phalanx	7	9	9	8.3	7	10	7	8.0	8.2
Average of phalanges	8.8	9.0	9.5	9.1	9.8	10.0	8.0	9.2	9.2
Average of all bones	9.2	9.5	9.8	9.5	9.8	10.5	9.2	9.8	9.7

TABLE XV—CONTINUED

Bones	Average Week of Closure for Group with Added Ascorbic Acid								
	Rat Number								
	5D2-9	6D2-11	6D2-1	Average Male	6D1-48	5D2-20	5D2-21	Average Female	Average Male, Fem.
First metacarpal	9	10	10	9.7	14	13	11	12.7	11.2
Second metacarpal	10	13	11	11.3	16	15	15	15.3	13.3
Third metacarpal	10	13	11	11.3	16	15	15	15.3	13.3
Fourth metacarpal	7	7	7	7.0	7	9	6	7.3	7.2
Average of metacarpals	9.0	10.8	9.8	9.9	13.2	13.0	11.8	10.4	10.2
First proximal phalanx	7	8	7	7.3	7	7	8	7.3	7.3
Second proximal phalanx	7	9	8	8.0	7	9	9	8.3	8.2
Third proximal phalanx	7	10	8	8.3	7	9	9	8.3	8.3
Fourth proximal phalanx	5	7	6	6.0	6	6	8	6.7	6.3
Average of phalanges	6.5	8.5	7.2	9.1	6.8	7.8	8.5	9.2	9.2
Average of all bones	7.8	9.6	8.5	9.5	10.0	10.4	10.2	9.8	9.7

TABLE XV

DATA ON CLOSURE OF METACARPALS AND PHALANGES AND ADJOINING OSSIFIC CENTERS (CONTINUED)

PART C—RATS ON DIETS CONTAINING 40.2% PROTEIN

Bones	Average Week of Closure for Group on No Added Ascorbic Acid								
	Rat Number								
	5D2-8	2J2-3	6D2-2	Average Male	6D1-37	6D2-12	6D2-8	Average Female	Average Male, Fem.
First metacarpal	10	11	10	10.3	11	11	12	11.2	10.8
Second metacarpal	11	12	13	12.0	12	13	13	12.7	12.3
Third metacarpal	11	12	13	12.0	12	13	13	12.7	12.3
Fourth metacarpal	8	10	9	9.0	10	8	11	9.7	9.3
Average of metacarpals	10.0	11.2	11.2	10.8	11.2	11.2	12.2	11.6	11.2
First proximal phalanx	8	9	8	8.3	8	7	7	7.3	7.8
Second proximal phalanx	9	11	9	9.7	9	8	8	8.3	9.0
Third proximal phalanx	9	11	9	9.7	9	8	8	8.3	9.0
Fourth proximal phalanx	8	9	8	8.3	7	7	7	7.0	7.7
Average of phalanges	8.5	10.0	8.5	9.0	8.2	7.5	7.5	7.7	8.4
Average of all bones	9.2	10.6	9.8	9.9	9.7	9.4	9.8	9.6	9.7

TABLE XV—CONTINUED

Bones	Average Week of Closure for Group with Added Ascorbic Acid								
	Rat Number								
	6D1-45	5D2-23	6D2-14	Average Male	6D1-39	5D2-11	6D2-4	Average Female	Average Male, Fem.
First metacarpal	12	9	10	10.3	13	9	11	11.0	10.7
Second metacarpal	14	11	12	12.0	15	11	13	13.0	12.7
Third metacarpal	16	11	12	13.0	15	11	13	13.0	13.0
Fourth metacarpal	6	8	7	7.0	7	6	8	7.0	7.0
Average of metacarpals	12.0	9.8	10.2	10.6	12.5	9.2	11.2	11.0	10.8
First proximal phalanx	11	9	8	9.3	7	6	7	6.7	8.0
Second proximal phalanx	11	9	8	9.3	7	8	8	7.7	8.5
Third proximal phalanx	11	9	8	9.3	7	8	8	7.7	8.5
Fourth proximal phalanx	6	8	7	7.0	6	5	7	6.0	6.5
Average of phalanges	9.8	8.8	7.8	8.7	6.8	6.8	7.5	7.0	7.9
Average of all bones	10.9	9.3	9.0	9.7	9.6	8.0	9.4	9.0	9.4

TABLE XV

DATA ON CLOSURE OF METACARPALS AND PHALANGES AND ADJOINING OSSIFIC CENTERS (CONTINUED)

PART D—RATS ON DIETS CONTAINING 78.2% PROTEIN

Bones	Average Week of Closure for Group on No Added Ascorbic Acid								
	Rat Number								
	6D1-50	5D2-12	2J2-1	Average Male	6D1-47	5D2-10	6D2-6	Average Female	Average Male, Fem.
First metacarpal	13	13	12	12.7	12	13	12	12.3	12.5
Second metacarpal	15	16	13	14.7	15	15	14	14.7	14.7
Third metacarpal	15	16	13	14.7	15	15	14	14.7	14.7
Fourth metacarpal	10	12	9	10.3	12	12	9	11.0	10.7
Average of metacarpals	13.2	14.2	11.8	13.1	13.5	13.8	12.2	13.1	13.2
First proximal phalanx	10	10	9	9.7	13	13	12	12.7	11.2
Second proximal phalanx	12	11	10	11.0	14	14	13	13.7	12.3
Third proximal phalanx	12	11	10	11.0	14	14	13	13.7	12.3
Fourth proximal phalanx	12	10	9	10.3	12	12	11	11.7	11.0
Average of phalanges	11.5	10.5	9.5	10.5	13.2	13.2	12.2	13.0	11.7
Average of all bones	12.4	12.4	10.6	11.8	13.4	13.5	12.2	13.1	12.4

TABLE XV—CONTINUED

Bones	Average Week of Closure for Group with Added Ascorbic Acid								
	Rat Number								
	5D2-19	6D2-16	2J2-2	Average Male	6D1-49	6D2-9	6D2-3	Average Female	Average Male, Fem.
First metacarpal	14	14	13	13.7	15	11	12	12.7	13.2
Second metacarpal	16	16	15	15.7	16	13	13	14.0	14.8
Third metacarpal	16	16	15	15.7	17	13	13	14.3	15.0
Fourth metacarpal	11	12	10	11.0	13	10	9	10.7	10.8
Average of metacarpals	14.2	14.5	13.2	14.0	15.2	11.8	11.8	12.9	13.4
First proximal phalanx	8	10	8	8.7	10	8	9	9.0	8.8
Second proximal phalanx	11	12	10	11.0	12	8	9	9.7	10.3
Third proximal phalanx	11	12	10	11.0	12	8	9	9.7	10.3
Fourth proximal phalanx	8	9	8	8.7	8	7	9	8.0	8.2
Average of phalanges	9.0	10.8	9.0	9.8	10.5	7.8	9.0	9.1	9.4
Average of all bones	11.6	12.6	11.1	11.9	12.8	9.8	10.4	11.0	11.4

TABLE XVI

SKELETAL MINERALIZATION OF RATS ON EACH DIETARY LEVEL: EQUIVALENT GRAMS OF IVORY PER CUBIC CENTIMETER OF BONE (B-INDEX) OF DIAPHYSIS OF RIGHT TIBIA

Dietary Protein	Sex	Group with No Added Ascorbic Acid		Group with Added Ascorbic Acid	
		Rat Number	B-Index	Rat Number	B-Index
3.2%	Males	6D1-33 5D2-17 6D2-19	0.958 0.776 0.595	6D1-38 6D2-15 6D2-10	0.579 0.657 0.736
		Average	0.656	Average	0.657
	Females	6D1-36 5D2-16 6D2-18	0.873 0.678 0.595	6D1-34 5D2-15 6D2-23	0.699 0.874 0.657
		Average	0.858	Average	0.743
	Average male and female		0.757	...	0.700

TABLE XVI—CONTINUED

Dietary Protein	Sex	Group with No Added Ascorbic Acid		Group with Added Ascorbic Acid	
		Rat Number	B-Index	Rat Number	B-Index
25.2%	Males	6D1-46 5D2-14 6D2-20	1.134 1.099 1.173	5D1-9 6D2-11 6D2-1	1.030 1.134 1.005
		Average	1.135	Average	1.056
	Females	6D1-35 5D2-7 6D2-17	1.147 1.060 0.973	6D1-48 5D2-20 5D2-21	1.415 1.360 1.471
		Average	1.060	Average	1.415
	Average male and female		1.097	....	1.236
40.2%	Males	5D2-8 2J2-3 6D2-2	1.088 1.211 0.845	6D1-45 5D2-23 6D2-14	1.222 0.997 1.451
		Average	1.048	Average	1.223
	Females	6D1-37 6D2-12 6D2-8	0.942 0.797 1.088	6D1-39 5D2-11 6D2-4	1.165 0.990 0.944
		Average	0.942	Average	1.033
	Average male and female		0.995	....	1.128

TABLE XVI—CONTINUED

Dietary Protein	Sex	Group with No Added Ascorbic Acid		Group with Added Ascorbic Acid	
		Rat Number	B-Index	Rat Number	B-Index
78.2%	Males	6D1-50 5D2-12 2J1-1	1.092 1.184 0.999	5D2-19 6D2-16 2J2-2	1.310 0.929 0.964
		Average	1.091	Average	1.067
	Females	6D1-47 5D2-10 6D2-6	1.064 1.294 0.834	6D1-49 6D2-9 6D2-3	1.106 1.153 1.060
		Average	1.064	Average	1.106
	Average male and female		1.078	...	1.086

TABLE XVII

RATIO OF TOTAL BODY WEIGHTS TO TOTAL SKELETAL WEIGHTS OF EXPERIMENTAL ANIMALS

PART A—GROUP WITH NO ADDED ASCORBIC ACID

Rat Number	Total Skeletal Weights (Gms.)	Total Body Weights (Gms.)	Ratio of Total Body Weights to Skeletal Weights
3.2% Protein			
6D1-33 . . .	2.3393	32	13.9
5D2-17 . . .	1.9800	30	15.0
6D2-19 . . .	2.6532	39	15.0
Average male . .	2.3242	33.7	14.6
6D1-36 . . .	2.3889	29	12.1
5D2-16 . . .	2.6000	33	12.7
6D2-18 . . .	2.6224	34	13.1
Average female . .	2.5371	32.0	12.6
Average male and female	2.4306	32.8	13.6
25.2% Protein			
6D1-46 . . .	9.5289	270	28.4
5D2-14 . . .	11.2104	276	14.6
6D2-20 . . .	9.4131	303	32.2
Average male . .	10.0508	283.0	25.1

TABLE XVII—CONTINUED

Rat Number	Total Skeletal Weights (Gms.)	Total Body Weights (Gms.)	Ratio of Total Body Weights to Skeletal Weights
6D1-35 . . .	6.8396	191	28.1
5D2-7 . . .	8.8019	208	23.6
6D2-17 . . .	8.9756	218	24.2
Average female . .	8.2056	205.7	25.3
Average male and female	9.1282	244.3	25.2
40.2% Protein			
5D2-8 . . .	12.8051	340	26.6
2J2-3 . . .	10.1217	275	27.2
6D2-2 . . .	11.6960	320	27.3
Average male . .	11.5409	311.7	27.0
6D1-37 . . .	10.0021	217	21.7
6D2-12 . . .	6.7158	192	28.7
6D2-8 . . .	9.4106	235	25.8
Average female . .	8.7095	214.7	25.4
Average male and female	10.1252	263.2	26.2
78.2% Protein			
6D1-50 . . .	7.2346	165	22.9
5D2-12 . . .	8.4413	234	27.8
2J2-1 . . .	9.3661	276	29.4
Average male . .	8.3473	225.0	26.7

TABLE XVII—CONTINUED

Rat Number	Total Skeletal Weights (Gms.)	Total Body Weights (Gms.)	Ratio of Total Body Weights to Skeletal Weights
6D1-47 . . .	5.8294	133	22.9
5D2-10 . . .	7.6631	168	21.8
6D2-6 . . .	7.0644	170	23.9
Average female . .	6.8523	157.0	22.9
Average male and female	7.5998	191.0	24.8

TABLE XVII

RATIO OF TOTAL BODY WEIGHTS TO TOTAL SKELETAL WEIGHTS OF EXPERIMENTAL ANIMALS (CONTINUED)

PART B—GROUP WITH ADDED ASCORBIC ACID

Rat Number	Total Skeletal Weights (Gms.)	Total Body Weights (Gms.)	Ratio of Total Body Weights to Skeletal Weights
3.2% Protein			
6D1-38 . . .	2.3004	33	14.3
6D2-15 . . .	2.8095	41	14.6
6D2-10 . . .	2.1236	28	13.3
Average male . .	2.4112	34.0	14.1
6D1-34 . . .	2.0414	30	15.0
5D2-15 . . .	2.8095	27	9.6
6D2-23 . . .	2.6513	39	15.0
Average female . .	2.5007	32.0	13.2
Average male and female	2.4559	33.0	13.6
25.2% Protein			
5D2-9 . . .	13.9035	356	25.6
6D2-11 . . .	13.6252	302	22.2
6D2-1 . . .	13.1384	338	25.8
Average male . .	13.5557	332.0	24.5

TABLE XVII—CONTINUED

Rat Number	Total Skeletal Weights (Gms.)	Total Body Weights (Gms.)	Ratio of Total Body Weights to Skeletal Weights
6D1-48 . . .	9.4113	210	22.3
5D2-20 . . .	9.4131	221	23.5
5D2-21 . . .	9.5707	202	21.0
Average female . .	9.4650	211.0	22.3
Average male and female	11.5103	271.5	23.4
40.2% Protein			
6D1-45 . . .	10.4494	280	26.9
5D2-23 . . .	12.4284	278	22.4
6D2-14 . . .	9.0031	354	39.3
Average male . .	10.6270	304.0	29.5
6D1-39 . . .	10.9231	235	21.6
5D2-11 . . .	10.5747	242	22.9
6D2-4 . . .	9.5558	232	24.2
Average female . .	10.3512	236.3	22.9
Average male and female	10.4891	270.2	26.2
78.2% Protein			
5D2-19 . . .	11.1251	307	27.7
6D2-16 . . .	12.4867	244	19.5
2J2-2 . . .	10.3389	270	26.2
Average male . .	11.3169	273.7	24.5

TABLE XVII—CONTINUED

Rat Number	Total Skeletal Weights (Gms.)	Total Body Weights (Gms.)	Ratio of Total Body Weights to Skeletal Weights
6D1-49 . . .	6.0314	164	27.3
6D2-9 . . .	6.9156	172	24.9
6D2-3 . . .	7.4593	174	23.2
Average female . .	6.8021	170.0	25.1
Average male and female	9.0595	218.5	24.8

TABLE XVIII

AVERAGE CRANIAL MEASUREMENTS OF RATS AND RATIO OF ZYGOMATIC TO TOTAL CRANIAL MEASUREMENTS

PART A—GROUP WITH NO ADDED ASCORBIC ACID

Rats on Various Levels of Dietary Protein	Squamosal Measurements (Cm.)	Zygomatic Measurements (Cm.)	Total Cranial Measurements (Cm.)	Ratio of Zygomatic to Cranial Measurements
3.2% Protein				
Average male	1.52	1.52	3.54	2.33
Average female	1.56	1.52	3.57	2.35
Average male and female	1.54	1.52	3.56	2.34
25.2% Protein				
Average male	1.95	2.43	4.44	1.83
Average female	1.87	2.30	4.24	1.84
Average male and female	1.91	2.36	4.34	1.84

TABLE XVIII—CONTINUED

Rats on Various Levels of Dietary Protein	Squamosal Measurements (Cm.)	Zygomatic Measurements (Cm.)	Total Cranial Measurements (Cm.)	Ratio of Zygomatic to Cranial Measurements
40.2% Protein				
Average male	1.95	2.38	4.61	1.94
Average female	1.90	2.28	4.24	1.86
Average male and female	1.92	2.34	4.42	1.89
78.2% Protein				
Average male	1.84	2.35	4.33	1.84
Average female	1.76	2.16	4.09	1.89
Average male and female	1.80	2.26	4.21	1.86

TABLE XVIII

AVERAGE CRANIAL MEASUREMENTS OF RATS AND RATIO OF ZYGOMATIC TO TOTAL CRANIAL MEASUREMENTS (CONTINUED)

PART B—GROUP WITH ADDED ASCORBIC ACID

Rats on Various Levels of Dietary Protein	Squamosal Measurements (Cm.)	Zygomatic Measurements (Cm.)	Total Cranial Measurements (Cm.)	Ratio of Zygomatic to Cranial Measurements
3.2% Protein				
Average male	1.56	1.55	3.50	2.26
Average female	1.54	1.50	3.55	2.37
Average male and female	1.56	1.52	3.52	2.32
25.2% Protein				
Average male	1.99	2.44	4.56	1.87
Average female	1.91	2.31	4.49	1.94
Average male and female	1.94	2.37	4.52	1.91

TABLE XVIII—CONTINUED

Rats on Various Levels of Dietary Protein	Squamosal Measurements (Cm.)	Zygomatic Measurements (Cm.)	Total Cranial Measurements (Cm.)	Ratio of Zygomatic to Cranial Measurements
40.2% Protein				
Average male	1.93	2.38	4.44	1.86
Average female	1.94	2.28	4.49	1.97
Average male and female	1.94	2.33	4.46	1.91
78.2% Protein				
Average male	1.94	2.38	4.64	1.95
Average female	1.85	2.18	4.14	1.90
Average male and female	1.84	2.28	4.38	1.92

DISCUSSION OF FINDINGS

FOOD CONSUMPTION OF EXPERIMENTAL ANIMALS

Table II gives the 10 essential amino acids needed for optimum growth and development in the albino rat, and the amount of these nutrients present in a 10-gram portion of the diet. Also, in Table III, there is a listing of the mineral content of a 10-gram portion of the diet, as well as the daily requirement for these minerals by the rat.

Table IV indicates the vitamins necessary for optimum health of the rat according to present standards, in addition to the quantity of the vitamins found in a 10-gram portion of the diet. According to the present status of the science of nutrition, if the experimental rats in the study had eaten 10 grams of food daily, they would have received an adequate supply of the 10 essential amino acids, as well as the minerals and the vitamins, with the exception of the 3.2 per cent group. The diet containing this level of dietary protein was deficient in methionine on a 10-gram basis. The rats on this protein level, however, consumed an average of only three to six grams of food daily. Table VII gives the adequacy of the essential nutrients for the rats on this 3.2 per cent protein level, and thus shows that these animals suffered numerous dietary deficiencies in addition to the protein deficiency.

The total food consumption of the individual rats on all dietary levels at weekly intervals is given in Table V.

FOOD CONSUMPTION OF ANIMALS ON 3.2 PER CENT LEVEL OF DIETARY PROTEIN

As discussed previously, the experimental animals on this low protein diet consumed less than one-half the amount necessary to furnish the essential nutrients. With the exception of the second, third, fourth, and fifth weeks, the females ate slightly more food each week than did the males on the diet without added ascorbic acid. The average weekly consumption for males was 27.3 gms. as compared to 27.7 gms. for females, with the average male and female consumption being 27.5 gms.

For the ascorbic acid supplemented group on the 3.2 per cent protein diet, the food consumption was slightly higher both for males and females, while in this group the males on an average ate slightly more than did the females. The higher consumption each week alternated from male to female in no definite pattern. The total average food consumption for males was 29.4 gms. a week and for the females it was 28.8 gms. The average male and female weekly food consumption was 29.1 gms.

The average male weekly consumption of those with added ascorbic acid exceeded the group with no ascorbic acid supplement by 2. 1 gms. Females in the former group exceeded those on the latter, on the average, by 1. 1 gms. When both sexes were averaged together, the former group averaged 1. 6 gms. in food consumption more than the latter, in this lowest protein group.

FOOD CONSUMPTION OF ANIMALS ON 25. 2 PER CENT LEVEL OF DIETARY PROTEIN

The diet with the second level of protein (25. 2 per cent) caused a marked increase in total intake by the experimental rats. In the group to which no ascorbic acid was added, the males averaged 75. 8 gms. greater in intake than did the males on the 3. 2 per cent protein level. The females on the 25. 2 per cent protein level ate, on the average, 51. 4 gms. more than those on the lowest protein level.

Those on the 25. 2 per cent protein diet with supplementary ascorbic acid exceeded the lowest protein group by amounts practically the same as those with no added vitamin C (74. 7 gms. for males and 52. 3 gms. for females).

During the first week of this study, the male rats receiving the 25. 2 per cent protein diet without added ascorbic acid consumed an average of 50. 3 gms. of food, with the consumption increasing gradually until the sixth week, when the average amount of food eaten was 106.6 gms. From the sixth to the twentieth week, which ended the experiment, the animals ate a weekly average ranging between 90.0 and 153.3 gms. The over-all weekly average for males was 103.1 gms.

After the third week, the females consistently consumed less food than did the males. For the first week of the experiment, the average female consumption was 55.0 gms, which increased steadily until the sixth week, when the consumption was 90.0 gms. The weekly food eaten thereafter ranged between 90.0 and 122.0 gms,, with an over-all average of 79.1 gms.

The average male and female weekly consumption of food was 91.2 gms. on the 25.2 per cent protein diet.

The males on the high ascorbic acid treatment ate an average of only one gram of food more than the males on the same level of protein without the additional ascorbic acid. An average of 50.7 gms. of food was eaten by the males during the first week of the test. Following the same pattern as the group without added ascorbic acid, the consumption increased until the sixth week before tapering off at 108.3 gms. Weekly consumption until the end of the 140-day period averaged from 95.7 to 129.3 gms., with an average of 104.1 gms.

The females on this same dietary level ate 47.3 gms. of food the first week, and from the sixth to the twentieth week, females had an average weekly consumption ranging from 88.5 to 127.8 gms. With the exception of the second and third weeks, the females ate less food each week than did the males. The average weekly consumption for the females was 81.1 gms., which was 2.0 gms. greater than that of the group with no added ascorbic acid.

The weekly average male and female consumption of food on the 25.2 per cent level of dietary protein with added ascorbic acid was 92.6 gms. This was 1.4 gms. weekly greater than that of the group without added ascorbic acid.

The food supply for both parallel groups (with and without added ascorbic acid) for both sexes was sufficiently high to insure an adequate all-around diet with respect to the essential amino acids, vitamins, and minerals, according to present standards.

FOOD CONSUMPTION OF ANIMALS ON 40.2 PER CENT LEVEL OF DIETARY PROTEIN

The males on the 40.2 per cent protein diet without supplementary ascorbic acid averaged 4.3 gms. weekly less than did their counterpart on the 25.2 per cent diets, whereas the females of this higher protein group averaged 12.7 gms. a week more than did the next lower protein group.

For those receiving massive dosages of ascorbic acid, the males consumed 18.7 gms. more, on the average, than did the rats on the 25.2 per cent protein level, while the females' food consumption increased only 6.0 gms.

The first week's food consumption for the males on this level of dietary protein without added ascorbic acid was 49.3 gms. This amount increased gradually until the seventh week, when consumption reached 91.7 gms. From the eighth to the final week, food consumption of the males leveled off with a range of 82.0 to 160.3 gms. The total average weekly consumption for males was 98.8 gms.

The females on this dietary level ate 47.3 gms. the first week with an increase until the eighth week, with a slight drop on the fourth week of the experiment. At the eighth week, consumption reached 100.7 gms; and it fluctuated between 91.0 and 131.7 gms. until the end of the experimental period. The total weekly average for females was 91.8 gms., which was 7.0 gms. a week lower than for the males.

The average male and female weekly consumption was calculated at 95.2 gms. for this group without added ascorbic acid, while the ascorbic acid supplemented group ate 104.9 gms. a week.

The males on the 40.2 per cent level of dietary protein receiving 1 per cent ascorbic acid ate the same amount during the first week of the experiment as the males not receiving an additional supply of vitamin C. The animals on the high ascorbic acid diet continued to eat more each week until the eleventh week, when they consumed an average of 135.7 gms. From then until the twentieth week, the range of consumption fell between 130.7 and 189.3 gms. The greatest single weekly food consumption for any dietary level occurred for the males receiving 40.2 per cent protein, with added ascorbic acid at the eighteenth week, with an average intake of 189.3 gms. The total weekly average for males was 122.8 gms. This was 24.0 gms. more than was eaten by the group not receiving additional vitamin C.

The females, in contrast, ate 4.7 gms. a week less than did those in the group without added ascorbic acid. This was the only instance where food consumption for the group without added ascorbic acid exceeded that of the comparable group with supplementary vitamin C. The total average consumption for the females of this group averaged 81.7 gms., while consumption from the first to the last week was fairly consistent, showing no increase in the early groups, as was the case with both sexes for all other protein level groups. These animals averaged 85.7 gms. of food during the first week, with the weekly average throughout ranging from 67.3 to 97.7 gms. The females in this dietary group ate more than did the males up to the seventh week, whereas the males far exceeded the females in food consumption from then until the end of the experiment.

The males ate a total weekly average of 35.7 gms. more than the females.

The essential nutrients received by the animals on this dietary protein level, both with and without added ascorbic acid, was adequate in all considered nutrients according to present scientific knowledge of the subject.

FOOD CONSUMPTION OF ANIMALS ON 78.2 PER CENT LEVEL OF DIETARY PROTEIN

The average weekly food consumption of the males on this higher level of protein was 32.4 gms. less, while the females ate 44.5 gms. less than did the rats on the previous protein dietary level without added ascorbic acid. The average total male and female consumption was 38.3 gms. less than was that of both sexes on the next lower protein level. Neither the males nor the females ate an adequate amount of food of this protein level to supply the essential vitamins and minerals, except the males receiving added vitamin C. Because of the high protein content of these diets, sufficient of the essential amino acids was furnished.

The males on this protein level without added ascorbic acid had a weekly average intake of 66.4 gms., with food consumption rising from 27.6 gms. for the first week to 52.0 gms. for the third week. The weekly average thereafter ranged from 49.6 to 88.3 gms.

The females ate a weekly average of 47.3 gms., which was 19.1 gms. less than that eaten by the males on this diet. There was no gradual rise in food intake; weekly fluctuation ranged from 29.3 to 64.3 gms.

The group receiving added ascorbic acid showed a much higher over-all food consumption. The males ate 15.8 gms. more per week than those with no added ascorbic acid, but with the same diet otherwise. The females, on the other hand, consumed 11.7 gms. more than the experimental rats on the 78.2 per cent protein diet with no added ascorbic acid. The males received an adequate amount of the essential nutrients, since their weekly intake averaged 82.2 gms. The females, however, were lacking in vitamins and minerals, because the average weekly intake was 59.0 gms., although they received an adequate supply of amino acids.

Neither the males nor the females showed a gradual rise in food intake. For the males, the first week's consumption was 34.3 gms., while the greatest amount eaten was 112.7 gms. The females consumed between 25.3 gms. (the first week) and 69.7 gms. (the seventeenth week).

The total male and female over-all average weekly food consumption was 70.6 gms. These experimental animals ate 40.6 gms. less for the males and 23.1 gms. less for the females than did the 40.2 per cent group with added ascorbic acid.

SUMMARY OF FOOD CONSUMPTION ON ALL LEVELS OF DIETARY PROTEIN

Figure 3 gives a graphic comparison of the average daily food intake of males on each level of dietary protein with and without added ascorbic acid, as well as comparison of the average daily food intake for the females on these dietary levels.

On all dietary levels the males ate more than did the females, except on the 3.2 per cent protein level. With the exception of the females receiving 40.2 per cent protein, the groups receiving massive dosages of ascorbic acid consumed more food than did those without added ascorbic acid. The most food was consumed by the males on 40.2 per cent protein with added ascorbic acid (a weekly average of 122.8 gms.). In the group receiving no added ascorbic acid, the males ate more on the 25.2 per cent protein level. In descending order came the following groups: 40.2 per cent protein with added ascorbic acid, 25.2 per cent protein with added ascorbic acid, 25.2 per cent protein without added ascorbic acid, 40.2 per cent protein without added ascorbic acid, 78.2 per cent protein with added ascorbic acid, 78.2 per cent protein without added ascorbic acid, 3.2 per cent protein with added ascorbic acid, and 3.2 per cent protein without added ascorbic acid.

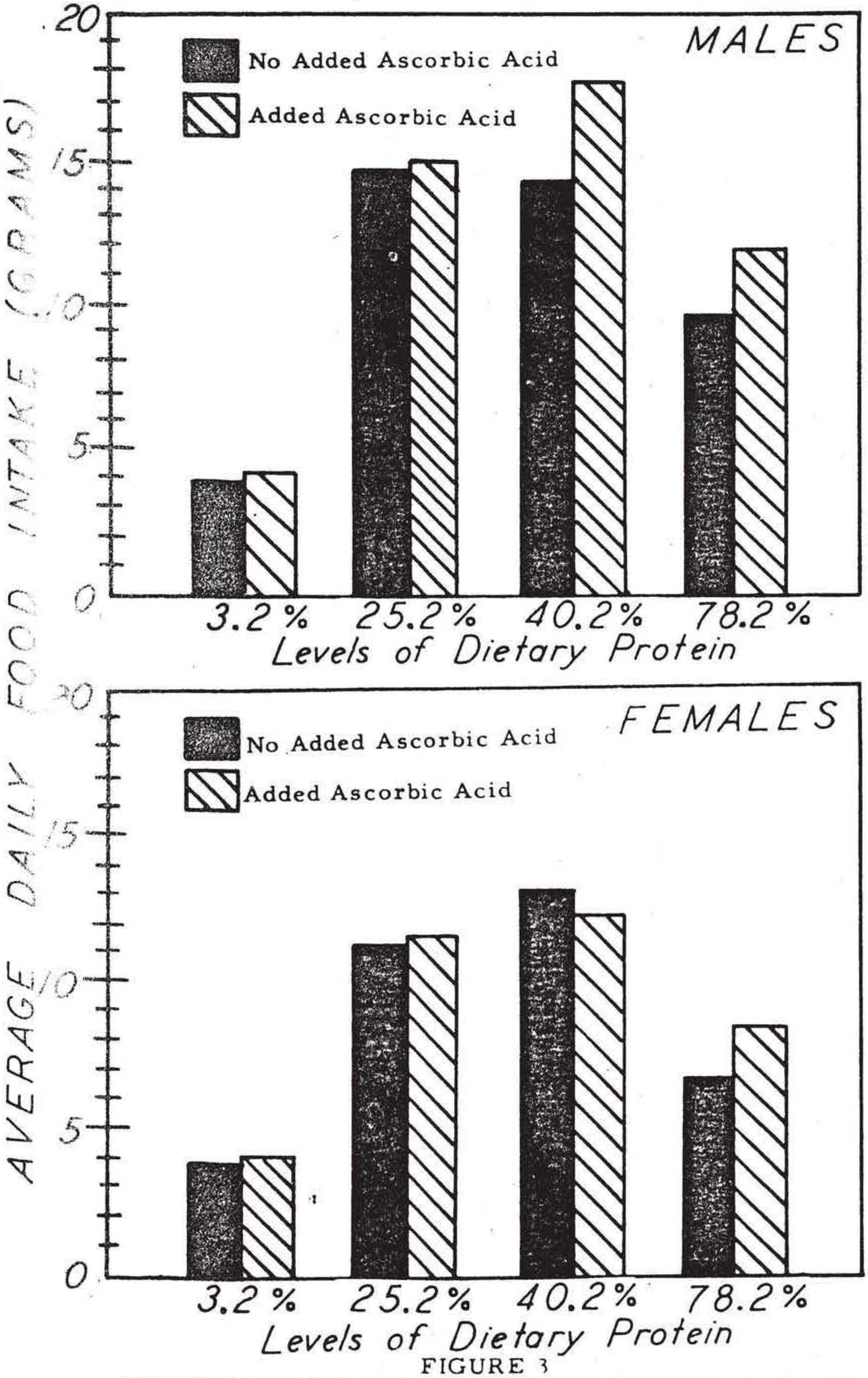

FIGURE 3
COMPARISON OF AVERAGE WEEKLY FOOD CONSUMPTION OF RATS ON THE EIGHT PROTEIN DIETARY LEVELS, WITH AND WITHOUT SUPPLEMENTARY ASCORBIC ACID

The most food consumption for the females was on the 40.2 per cent protein group without added ascorbic acid, at 91.8 gms. In the ascorbic acid supplemented group the females on the 40.2 per cent protein diet again ate the most. The other groups, in descending order, were: 40.2 per cent protein without added ascorbic acid, 40.2 per cent protein with added ascorbic acid, 25.2 per cent protein without added ascorbic acid, 78.2 per cent protein with added ascorbic acid, 78.2 per cent protein without added ascorbic acid, 3.2 per cent protein group with added ascorbic acid, and 3.2 per cent protein group without added ascorbic acid.

On an average, the rats on the ascorbic acid supplemented diets reached a peak of food consumption slightly earlier (77 days) than did those rats not receiving the added ascorbic acid (80 days). Those animals receiving no added ascorbic acid reached a peak of food consumption as follows: 3.2 per cent protein without added ascorbic acid, males and females (first week); 25.2 per cent protein without added ascorbic acid, males (sixteenth week), females (eighth week); 40.2 per cent protein without added ascorbic acid, males (sixteenth week), females (fifteenth week); 78.2 per cent protein, males and females (seventeenth week). The ascorbic acid supplemented groups ate the most food during the following weeks: 3.2 per cent protein, males and females (first week); 25.2 per cent protein, males (sixteenth week), females (twelfth week); 40. 2 per cent protein, males (eighteenth week), females (eighth week); 78. 2 per cent protein, males (fifteenth week), females (seventeenth week).

In summary, all groups with the added ascorbic acid, except one (females at the 40. 2 per cent protein level) ate more total food than did the parallel protein level group without added ascorbic acid.

Those on the 40. 2 per cent protein level, regardless of sex or of ascorbic acid supplement, averaged the highest amount of total food of any of the protein level groups, as has been indicated. The total food consumption increased steadily from the 3. 2 per cent to the 40. 2 per cent protein groups, and decreased with the 78. 2 per cent group. The highest protein group fell between the 3. 2 and the 25. 2 per cent groups, whether supplementary ascorbic acid was added or not.

GROWTH CHARACTERISTICS OF EXPERIMENTAL ANIMALS

Table VIII gives the weekly weights of the individual animals, as well as average weights of males and females on all levels of dietary protein, both with and without added ascorbic acid.

GROWTH CHARACTERISTICS OF THE 3. 2 PER CENT PROTEIN GROUP

The animals on the 3. 2 per cent level of dietary protein, both with and without added ascorbic acid, showed no growth whatsoever during the period of this experiment.

The initial weight of the males not receiving added ascorbic acid averaged 46.7 gms. A gradual decline in weight occurred until the fifteenth week, when the average weight for the males was 32.0 gms. The fluctuations ranged between 31.5 gms. and 42.6 gms. The total average weekly weight for males was 37.6 gms.

The females initially averaged 47.0 gms. in weight; and, at the end of 20 weeks, they averaged 34.0 gms., with a fluctuation ranging between 44.3 gms. and 33.3 gms. The female weights averaged 37.2 gms., which was 0.4 gm. lower than the males on the same diet, which was not notably different. The females lived longer than did the males. They lived an average of 16 weeks on the experiment, while the males lived only 14 weeks. One female on this diet lived the full term of the experiment, which was 140 days.

The total male and female weight averaged 37.4 gms. per week for this lowest protein group without ascorbic acid added to their diet, while those receiving added ascorbic acid averaged 36.0 gms. per week. The animals on the lowest protein level were not notably different in weight, whether they had supplementary ascorbic acid or not.

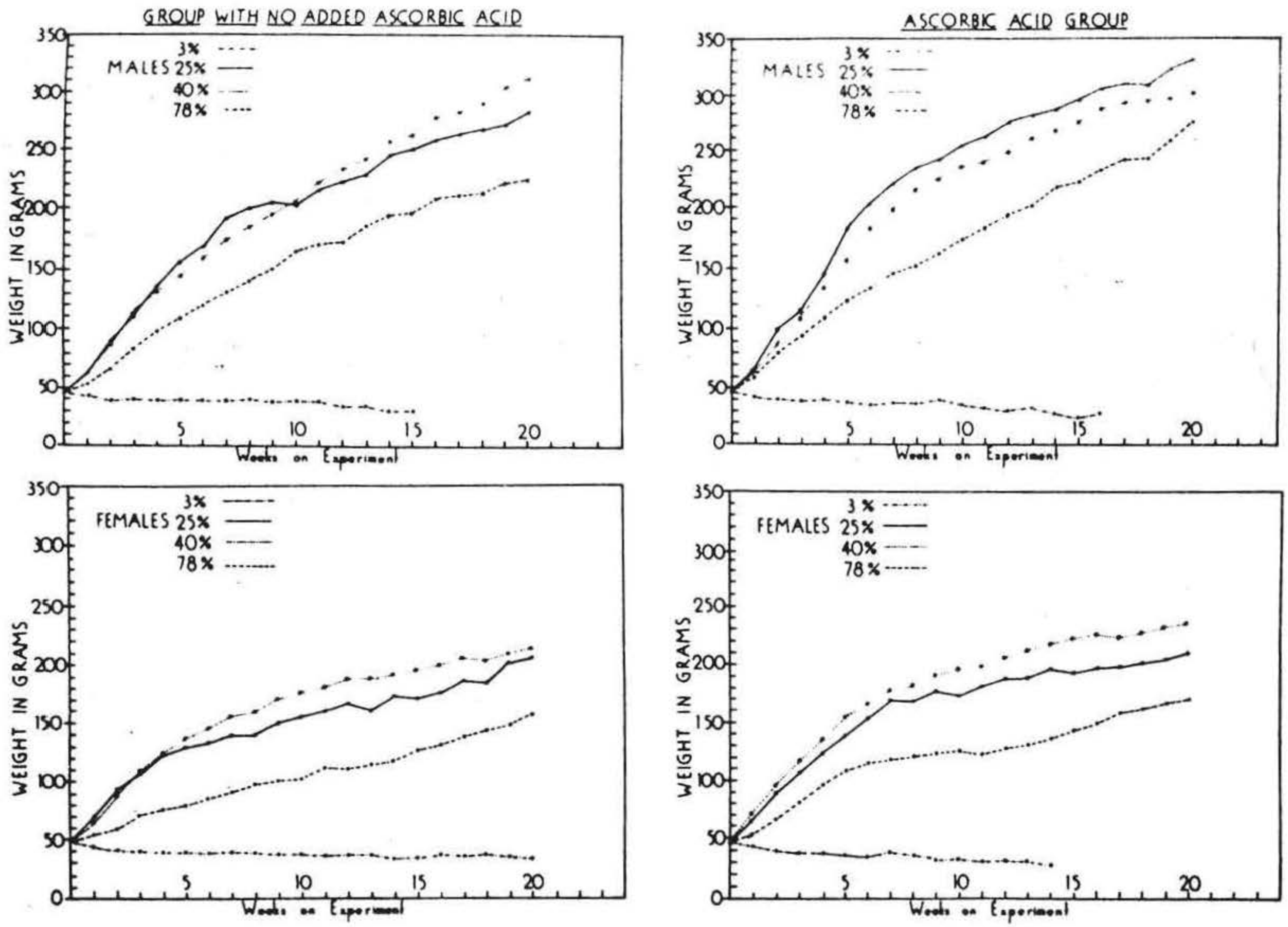

FIGURE 4

COMPARISON OF GROWTH OF RATS ON VARYING LEVELS OF DIETARY PROTEIN, WITH AND WITHOUT SUPPLEMENTARY

This constitutes the only instance in which the animals with ascorbic acid did not out-weigh the parallel group without the added vitamin C.

In the ascorbic acid supplemented group, the males again weighed more than did the females (36.6 gms. and 35.4 gms., respectively). The difference between the weights of the males and females, however, was greater in this group (1.2 gms. as compared to 0.4 gm. for the group without added ascorbic acid). The initial weight of the males averaged 46.3 gms., with a final average weight of 33.0 gms. The lowest weight reached by animals on this diet was 30.0 gms.

In the opposite direction, males on the 3.2 per cent protein diet receiving added ascorbic acid lived one week longer than did females on the same dietary. The males averaged 13 weeks on the experiment, as compared with 12 weeks for the females. This was one and four weeks, respectively, lower than the males and females on this low protein diet without added ascorbic acid.

The females consuming the low protein diet, with massive dosages of ascorbic acid added, followed the same pattern of decline in weight as was found in all other groups on the 3.2 per cent protein level. The initial weight averaged 47.3 gms.; and they had declined to 28.5 gms. at the fourteenth week, which was the lowest average weight found during the experiment.

GROWTH CHARACTERISTICS OF THE 25.2 PER CENT PROTEIN GROUP

The experimental animals on this second level of dietary protein showed a variable growth pattern. Generally speaking, the growth was not quite as outstanding as for the 40.2 per cent group of rats, except for the fact that the males in the ascorbic acid supplemented group demonstrated growth progress which was consistently higher than that of any other of the dietary groups.

The males receiving no additional ascorbic acid in their diets, on the other hand, gained rapidly until the tenth week, when there was a slight drop, followed by a gradual increase in growth ending on the one hundred and fortieth day (28.7 gms. lower than the 40.2 per cent males without added ascorbic acid).

The males on the second dietary level of protein without additional ascorbic acid averaged 44.7 gms. at the beginning of the experiment; and, except for a slight drop at the tenth week, they gained steadily until they had reached a weight of 283.0 gms. by the end of the test period. The average weekly weight for these males was 201.8 gms. The greatest weight gain was recorded for the second week, with an average gain of 26.7 gms. After the seventh week, the weight gain tapered off to an average of 6.0 gms. per week. At the tenth week, however, there was an average loss of weight of 1.7 gms.

The growth of the females, with and without added ascorbic acid, was consistently lower than that of the males, on the 25.2 per cent protein level. On this protein level, the females with added ascorbic acid grew faster than did any of the other females of any other protein level, until the middle of the third week. At this point, they dropped below the females on the 40.2 per cent protein diet, and remained in this relative position until the end of the experiment, when they were 11.0 gms. lower in weight than the latter. The initial weight of this group was 48.7 gms.; this rose steadily until the eighth week, when there was no notable change. Again, there was a rise until the thirteenth week, when a slight drop in growth appeared. From the thirteenth week until the twentieth week, there was an inconsistent pattern of growth, followed by decreases in weight until the end of the experiment.

This group showed the most erratic growth curve of any of the dietary groups, male or female.

The greatest weekly weight gain in this protein level again appeared during the second week, when the weight gain of the females on this second level of protein, without added ascorbic acid, averaged 25.0 gms. The total average weekly weight for these rats was 151.5 gms.; and the total male and female weekly weight averaged 176.6 gms. (9.8 gms. less than did the next dietary protein level without added ascorbic acid).

The males receiving massive dosages of ascorbic acid averaged 34.8 gms. more weekly than did those not on ascorbic acid supplemented diets. The growth of females was higher by an average of 14.8 gms. than that of the parallel females without ascorbic acid supplements.

The males (25.2 per cent dietary protein group with added ascorbic acid) gained 70.3 gms. more on a weekly average than did the females receiving the same food. The total male and female weekly weight gain averaged 201.4 gms. (24.8 gms. more than the males and females consuming no added ascorbic acid).

The initial weight of the males was 47.3 gms.; and this increased sharply until the end of the experiment, when it reached 332.0 gms. — the greatest amount of weight achieved by any animal on any of the experimental diets. The total weekly average was 236.6 gms., with the greatest increase being 38.0 gms. during the fifth week on the experiment.

For the females on this same diet, the initial weight was 47.0 gms. This had reached 211.0 gms. by the end of this study. During three of the weeks there was a slight decrease in weight. The greatest increase occurred during the second week, with 24.7 gms. being recorded. The growth of the females on the 25.2 per cent protein diet receiving added ascorbic acid fell consistently lower than did that of the females on the next highest level of protein.

GROWTH CHARACTERISTICS OF THE 40.2 PER CENT PROTEIN GROUP

The rats receiving 40.2 per cent dietary protein, both with and without added ascorbic acid, showed a higher growth pattern than did any of the other dietary groups, with the exception of the males in the 25.2 per cent protein group with added ascorbic acid. The growth of the males was consistently higher than that of the females on this same diet. The weekly weights averaged higher for those animals receiving added ascorbic acid than did those without an ascorbic acid supplement.

The initial weight of the males on 40.2 per cent protein without added ascorbic acid was 47.7 gms.; and this increased steadily until it had reached 311.7 gms. by the end of the test period. The average weekly weight was 207.4 gms. (41.9 gms. higher than the females receiving the same diet). The greatest weight gain occurred during the second week, with a gain of 24.7 gms. being recorded.

The females began the experiment averaging 45.7 gms. in weight; and they concluded the one hundred and forty day period weighing an average of 214.7 gms. Again, it was during the second week that the females gained the most weight (24.0 gms.). The total weekly weight averaged 165.5 gms. (18.9 gms. less than the corresponding animals receiving massive dosages of vitamin C).

The males and females on this same diet weighed an average of 14.8 gms. less than did the males and females consuming added ascorbic acid (186.4 gms. and 201.2 gms., respectively).

The males receiving this third level of protein (40.2 per cent) with 1 per cent of ascorbic acid added, ended the experiment weighing 304.0 gms. (from an initial 45.3 gms.), with an average weekly weight of 217.9 gms.

The females, however, averaged 184.4 gms. weekly, with an initial weight of 48.3 gms., then rising to 236.3 gms. by the twentieth week of the study. The highest weekly weight gain appeared during the second week, with an increase of 23.7 gms. for the males and 24.7 gms. for the females.

GROWTH CHARACTERISTICS OF THE 78.2 PER CENT PROTEIN GROUP

In all cases, the experimental animals receiving 78.2 per cent protein, ranked below those on the 25.2 and the 40.2 per cent protein-containing diets.

The males gained more weight than did the females; and those rats receiving added ascorbic acid grew more than did those not receiving ascorbic acid supplements.

The males averaged an initial weight of 49.0 gms. and 46.3 gms., respectively, for those consuming a diet with and without vitamin C supplements. This initial weight rose to 273.7 gms. for the group without added ascorbic acid and 225.0 gms. for the group with supplementary ascorbic acid. For the latter group, the greatest amount of growth occurred during the second week, with a gain of 21.3 gms.

The males on this highest level of protein without added ascorbic acid averaged 156.1 gms. for all weekly weights, while the males on the same diet but receiving massive dosages of ascorbic acid averaged 18.7 gms more, or a total of 174.8 gms.

The females not receiving added ascorbic acid averaged 50.1 gms. less than did the males on the same diet (106.1 gms. weekly average). The initial weight of 46.7 gms. rose to 157.0 gms. by the end of the experiment; and the highest weekly weight gain was 12.3 gms. during the third week.

For the corresponding group of animals receiving added ascorbic acid, the highest weight gain was 15.3 gms., which was recorded identically during the third and fourth weeks of the experiment. The initial weight of this group averaged 45.0 gms. This increased to 170.0 gms. by the conclusion of the experiment, with a total weekly average of 121.7 gms. (15.6 gms. more than the females on this high protein diet without added ascorbic acid).

The males and females on the 78.2 per cent protein diet without added ascorbic acid averaged 131.1 gms. weekly, as compared with 148.2 gms. for those on the contrasting diet (78.2 per cent protein diet with added ascorbic acid).

<u>SUMMARY OF CHARACTERISTIC GROWTH PERFORMANCE OF ALL DIETARY GROUPS</u>

The animals on the lowest protein diet (3.2 per cent) showed no growth during the experimental period. Moreover, in all cases, with the exception of one female on this protein level without added ascorbic acid, the animals on this diet died before the end of this investigation.

Irrespective of whether or not ascorbic acid was added to the diet, males tended to exceed females on the same diet insofar as growth progress was concerned.

As the weeks of the study advanced, growth progress of the rats with supplementary ascorbic acid exceeded that of each corresponding sex-protein level group with no supplementary vitamin C, with the exception of the animals on the lowest protein level. As noted, these animals exhibited no growth throughout, with most of them dying before the close of the study.

In ascending order, the ranking of the various groups of experimental animals with respect to growth progress was the following:

ANIMALS WITH SUPPLEMENTARY ASCORBIC ACID

MALES	FEMALES
Dietary Protein Levels	Dietary Protein Levels
3.2%	3.2%
78.2	78.2
40.2	25.2
25.2	40.2

ANIMALS WITH NO SUPPLEMENTARY ASCORBIC ACID

MALES	FEMALES
Dietary Protein Levels	Dietary Protein Levels
3.2%	3.2%
78.2	78.2
25.2	25.2
40.2	40.2

Except for the one case of the males with added ascorbic acid which were on the 25.2 per cent protein diet slightly exceeding those on the 40.2 per cent protein level, the rats with 40.2 per cent protein surpassed the other three groups in growth. The rats on 3.2 per cent protein uniformly made the poorest growth progress, whereas those on the highest protein level always ranked next to the poorest.

FOOD CONSUMPTION PER GRAM OF BODY WEIGHT OF EXPERIMENTAL ANIMALS

The rats on the lowest protein dietary level consumed the most food per gram of body weight. The males and females are relatively the same amount per gram of body weight on each of the diets, with and without added ascorbic acid. Food consumption per gram of body weight followed a general pattern. It was highest for the first week of the experiment, and declined gradually thereafter.

For the males on the lowest protein diet with no added ascorbic acid, the values began at 0.145 gm. per gram of body weight for the first week; these fluctuated slightly for the next seven weeks, with an average of 0.114 gm.; they declined gradually thereafter until the end of the experiment, seven weeks later, with a weekly average of 0.089 gm. of food per gram of body weight.

The females had a similar initial average, with 0.149 gm. for the first week of the experiment, and with minor fluctuations until

0.091 gm. was reached when the test period was ended for these animals at the fourteenth week. These animals had a slightly higher over-all weekly average intake per gram of body weight than did the males (0.116 gm. of food per gram of body weight).

The group of animals on this lowest protein level receiving added ascorbic acid followed somewhat the same general pattern as those with no supplementary vitamin C. The males consumed 0.133 gm. of food during the first week of the experiment, fluctuating somewhat throughout nine weeks, with an over-all weekly average of 0.12 gm. of intake per gram of body weight for this period. From the tenth through the sixteenth week, when the study closed for these animals because of death, the average was 0.105 gm., with a final consumption of 0.091 gm. of food per gram of body weight.

The females on the 3.2 per cent protein level with supplementary ascorbic acid consumed 0.149 gm. of food per gram of body weight during the first week of the study. There followed a gradual decline in this factor with fluctuations until the fourteenth week, when the average was 0.091 gm., and when the experiment ended for this group of animals. The over-all average for these females was 0.113 gm. of food consumed per gram of body weight.

The male rats on the 25.2 per cent protein level without added ascorbic acid consumed 0.112 gm. of food per gram of rat weight during the first week of the experiment. A sharp drop ensued during the second week (0.091 gm.), with less pronounced fluctuations thereafter than were noted for the animals on the 3.2 per cent protein diet. At the close of the study the weekly average intake was 0.070 gm. of food per gram of body weight, with an over-all weekly average of 0.076 gm.

The females on the same diet (25.2 per cent protein with no added ascorbic acid) consumed an average of 0.090 gm. of food per gram of rat weight during the first week, with the amount increasing steadily during the next two weeks (0.101 gm. and 0.115 gm., respectively). This dropped to 0.076 gm. for the fourth week, when a plateau was reached and maintained until the eleventh week, with a weekly average of 0.079 gm. A gradual decline from the twelfth to the twentieth week (averaging 0.067 gm. of food per gram of body weight) ended with 0.062 gm. being recorded for the final week.

The males on the same diet (25.2 per cent protein with massive dosages of vitamin C) showed a sharp drop in food intake per gram of body weight during the second week of the experiment—from 0.117 gm. to 0.079 gm. of food per gram of body weight. Then followed a gradual decline, with only minor deviations until the termination of this project. The final week's food consumption per gram of body weight was 0.049 gm. The total weekly food consumption per gram of body weight after the decline during the second week, averaged 0.065 gm.

Showing a smoother decline from the beginning of the study (0.104 gm.) to the final week (0.056 gm.), the females on a corresponding dietary averaged 0.073 gm. weekly during the full period of the investigation.

The 40.2 per cent protein groups followed much the same pattern and the same level of consumption of food per gram of weight of experimental animal as did the animals on 25.2 per cent protein. A sharper decline was exhibited by the males on the 40.2 per cent protein dietary with no added ascorbic acid than by the females receiving the same type of food. The food consumption per gram of body weight averaged 0.108 gm. for the first week for these animals, dropping to 0.090 gm. the second week. For the next four weeks, these animals averaged 0.081 gm. At the seventh week, however, this rose to 0.115 gm., only to drop again during the following week. From the eighth to the seventeenth week, a gradual decline occurred with a weekly average of 0.070 gm. Another sharp drop brought the average weekly food consumption per gram of body weight to 0.054 gm. for the remaining three weeks of the experiment.

The females on the same diet averaged 0.104 gm. of food per gram of body weight during the first week of the study. From the second to the final test week, the consumption of food per gram of body weight averaged 0.079 gm., with the exception of the fifteenth week, when the intake suddenly rose to 0.098 gm., giving an over-all average of 0.080 gm.

The males on this third level of dietary protein (40.2 per cent) with added ascorbic acid consumed 0.111 gm. of food per gram of body weight for the first week of the study; then they dropped to an average of 0.098 gm. during the second week. From the third to the tenth week these animals averaged 0.071 gm. of food intake per gram of body weight. This rose to form a plateau from the eleventh to the nineteenth week, with an average during this period of 0.087 gm. weekly. The final week showed an average intake of 0.079 gm.

The females receiving an identical diet (40.2 per cent protein with added vitamin C) consumed the highest amount of food per gram of body weight for any of the groups during the first week (0.152 gm.). This was followed by two sharp declines in the second and in the third weeks (0.109 gm. and 0.083 gm., respectively). There was a very gradual decline thereafter to 0.067 gm. by the tenth week. Another plateau was recorded from the eleventh to the nineteenth week, with a weekly over-all average of 0.058 gm. During the final week, these females consumed only 0.048 gm. of food per gram of body weight.

Falling somewhat below these two middle groups, the 78.2 per cent protein groups showed an erratic pattern of food consumption per gram of body weight as the weeks of this study progressed. Those males receiving no added ascorbic acid averaged 0.076 gm. for the first two weeks. There were sharp fluctuations during the third, fourth, fifth, sixth, and seventh weeks (0.090 gm., 0.072 gm., 0.097 gm., 0.081 gm., and 0.062 gm., respectively). The weekly average from the seventh to the twelfth week was 0.068 gm., followed by a slight decline from the thirteenth to the seventeenth week, with an average for this period of 0.055 gm. The remaining three weeks averaged 0.049 gm.

The females in the 78.2 per cent group without added ascorbic acid consumed an average of 0.118 gm. of food per gram of body weight during the initial week. This declined during the next three weeks, with an average for this period of 0.075 gm. This fell again the fifth week to 0.056 gm., and then rose slightly to follow a more gradual pattern of decline from the sixth week (0.074 gm.) to the twentieth week (0.056 gm.).

The males on the highest level of dietary protein (78.2 per cent) receiving massive dosages of ascorbic acid differed from the other dietary groups in that the initial food consumption per gram of body weight (0.084 gm.) showed only a slight decline until the ninth week, with an average weekly intake of 0.079 gm. during this period. A plateau from the tenth to the eighteenth week averaged 0.066 gm., while the remaining two weeks averaged 0.055 gm. of food consumption per gram of body weight.

Following the more general pattern set by the other dietary groups, the females receiving the diet with 78.2 per cent of protein showed sharp changes during the first few weeks of the experiment, with 0.068 gm., 0.116 gm., and 0.071 gm. being recorded for the first, second, and third weeks, respectively. From the third to the eighth week, the food consumption per gram of body weight averaged 0.079 gm. weekly, while the average for the ninth to the seventeenth week was 0.065 gm. Again, there was a decline, and the food consumption per gram of body weight fell to an average of 0.058 gm. for the final three weeks of the study.

The food consumption per gram of body weight for the males and females on varying levels of protein, both with and without added ascorbic acid, will be summarized under the next section of this report, on a Comparison of Food Consumption and Growth of Animals on All Dietary Levels.

COMPARISON OF FOOD CONSUMPTION AND GROWTH OF ANIMALS ON ALL DIETARY LEVELS

The experimental animals on the lowest level of dietary protein, both with and without added ascorbic acid, consistently ate less food than any of the other dietary groups and showed a gradual decline in weight during the entire test. Higher food consumption per gram of body weight was recorded for these animals as compared to the other rats on various dietaries. There was no notable difference noted either between the males and females or between the ascorbic acid supplemented group and those with no added vitamin C on growth, food consumption, or grams of food consumed per gram of body weight.

The experimental animals on 78.2 per cent protein exhibited next to the lowest growth accomplishment of any of the series, and next to the lowest total food consumption. There was a sharp difference, both in food consumption and in growth, however, between the animals on 3.2 and on 78.2 per cent protein.

For grams of food consumed per gram of body weight, moreover, the group on the highest protein-containing diet ate less than did the rats in any of the other protein groups, whether ascorbic acid was added to the diet or not. In growth and total food consumption, on the other hand, the animals with the 78.2 per cent protein diet and added ascorbic acid surpassed those with no added ascorbic acid. The males uniformly showed higher values than did the females of comparable groups in these two latter values.

In growth and in total food consumed, the animals receiving 40.2 per cent of their basic diet as protein tended to surpass all other groups, whether ascorbic acid was added to the diet or not, with two exceptions as shown below when the rank order of performance of the specified groups appears in ascending order.

RANK ORDER

GROWTH	TOTAL FOOD CONSUMPTION
MALES WITH NO ADDED ASCORBIC ACID	
Dietary Protein Levels	Dietary Protein Levels
3.2%	3.2%
78.2	78.2
25.2	40.2
40.2	25.2
MALES WITH ADDED ASCORBIC ACID	
3.2%	3.2%
78.2	78.2
40.2	25.2
25.2	40.2

GROWTH	TOTAL FOOD CONSUMPTION
FEMALES WITH NO ADDED ASCORBIC ACID	
Dietary Protein Levels	Dietary Protein Levels
3.2%	3.2%
78.2	78.2
25.2	25.2
40.2	40.2
FEMALES WITH ADDED ASCORBIC ACID	
3.2%	3.2%
78.2	78.2
25.2	25.2
40.2	40.2

The above rank order summary of growth and total food intake also is seen to run in the same order except in the one case in which food consumption by the rats on 25.2 per cent protein exceeded those on 40.2 per cent, and in the other instance in which growth of the 25.2 per cent protein group somewhat exceeded those on 40.2 per cent protein. These two superior groups tended to be relatively close in their food consumption throughout.

Among the four sex-ascorbic acid supplemented groups, the females receiving 40.2 per cent protein with added ascorbic acid consistently consumed more food and exhibited greater growth than did those with no supplementary vitamin C. The reverse was true with the males, although the differences were small.

The experimental animals receiving 25.2 per cent protein generally ranked second to the 40.2 per cent groups with the exceptions noted.

Growth and food consumption fell consistently higher for males than for females.

PHYSICAL APPEARANCE OF EXPERIMENTAL ANIMALS

The physical appearance of the experimental animals on the low protein diet (3.2 per cent protein) markedly differed from the rats on the three higher levels of protein. Rats on 25.2 per cent and 40.2 per cent protein, both with and without added ascorbic acid, had smooth coats and an alert appearance, while those receiving 78.2 per cent protein exhibited somewhat uneven, rough coats of hair, with no other marked gross appearance of dietary deficiencies. The size of the animals on the three higher levels of protein, both with and without added ascorbic acid, generally followed the same pattern as that indicated in the recorded weights, as discussed previously. The sizes of the animals on the lowest protein level were markedly stunted.

The rats receiving the 3.2 per cent protein dietary, both with and without the ascorbic acid supplement, showed varied symptoms of protein, mineral, and vitamin deficiencies. The rats reacted individually, showing no definite pattern for any one of the deficiencies. One male on this low protein diet with massive dosages of ascorbic acid, for example, died with no apparent abnormalities except extreme stunting, after 76 days on the experiment. The length of time of these rats on the experiment ranged from 60 days to the full 140 days, with an over-all average of 14 weeks before death occurred.

The experimental animals exhibited multi-deficiencies, difficult to attribute to any one nutrient. Some of the symptoms observed were the following:

1). Brown, greasy lesions appeared around the mouths of several of the rats.

2). Alopecia occurred in such places as the mouth, the front foot, and the abdominal genitalia area.

3). As the experiment progressed, the animals, generally speaking, moved more slowly and tended to walk stiffly on their toes.

4). They seemed to have somewhat defective vision, reacting slowly to light, walking off tables and into solid objects.

5). Several animals exhibited poor muscular co-ordination, particularly in washing their faces.

6). On several occasions, an animal was observed with a swollen face (either on one or both sides), and with a soft, pliable lump in the chest area which sometimes hung down between the front legs of the animal as much as one-half of an inch. This lump disappeared and reappeared at various times, but did not seem to cause the animal particular pain. Upon dissection, one female who had been on experiment only 60 days and had developed a large lump, was found to have fluid in the chest area.

The hai r of these low protein rats was thin, somewhat oily in many instances, and coarse in texture.

The most marked difference in the general appearance of the animals resulted from the various levels of protein. No notable gross differences between the animals with and without supplementary ascorbic acid were noted in the parallel groups.

BLOOD VALUES OF EXPERIMENTAL ANIMALS

BLOOD PLASMA ASCORBIC ACID

Table IX has shown the blood plasma ascorbic acid values of the experimental animals, determined on samples of blood taken on the ninety-first and the one hundred fortieth day of the study.

The 3.2 per cent protein group without added ascorbic acid, males and females together, averaged 0.05 mg. of ascorbic acid per 100 ml. of blood, as compared with 1.00 mg. per 100 ml. of blood for the group with massive dosages of ascorbic acid. Because of the shorter life span of these lowest protein consuming animals, only one blood test was made on this group, and this was done on the forty-fifth day of the experiment.

The three highest levels of protein showed no definite pattern of ascorbic acid content in the blood, but instead they gave erratic results. Neither did the ascorbic acid supplemented groups consistently show a greater ascorbic acid content of the blood than did those groups not receiving added ascorbic acid at either of the test periods. When the values for all animals in the two respective groups—those having added ascorbic acid and those with none of this supplementary vitamin—were compared, however, the average was higher for those on the ascorbic acid supplemented diets.

Aside from the animals on the lowest protein level, the lowest average value found was 0.80 mg. per 100 ml. of blood for the animals on the 40.2 per cent protein diet without added vitamin C. The highest average was 2.5 mg. of ascorbic acid for the animals on 78.2 per cent protein with added ascorbic acid (2.5 mg. per 100 ml. of blood).

BLOOD SERUM PROTEIN

The average total serum protein values for the experimental animals have been given in Table X.

The blood serum from all rats on the 3.2 per cent protein diets, both with and without the ascorbic acid supplement, was combined from the 75-day samples in order to provide the amounts needed to make this test. The lowest serum protein value (3.80 gms. per 100 ml. of blood) found for any of the dietary groups was obtained for these lowest protein consuming animals.

For all remaining groups, there was no pattern established for the total serum protein in the blood. The values ranged from 3.93 to 8.68 mg. per 100 ml., with no trend of differences between those on different protein levels, or those with or without supplementary ascorbic acid, with one exception. The groups receiving supplementary ascorbic acid were consistently lower in total serum protein than were the corresponding protein groups with no added ascorbic acid at the final test period.

BLOOD SERUM ALBUMIN

As shown in Table XI, the average values for blood serum albumin for the animals on the 25.2, 40.2, and 78.2 per cent protein diets were closely similar (range 3.20 to 3.93 mg. per 100 ml.). Within this narrow range, however, the animals with added ascorbic acid fell consistently lower at the final test period than did those with no ascorbic acid supplement.

These lower blood total protein and albumin values in the blood which were found at the last test period for the animals with added ascorbic acid could possibly be explained on the basis of the greater tendency toward growth progress with these animals.

VITAL ORGANS OF EXPERIMENTAL ANIMALS

WEIGHTS OF ORGANS OF ANIMALS ON ALL DIETARY LEVELS

Table XII has given the weights of the lungs, heart, liver, spleen, brain, suprarenals, and kidneys of both sexes of the experimental animals, as well as the testicles of the males, on each level of dietary protein, both with and without added ascorbic acid.

For the animals on the 3.2 per cent protein diet, the weights of the organs were consistently lower than those of the animals on the three higher levels of protein. There appeared to be no notable difference between the groups with and without added ascorbic acid. The females in every case showed greater organ weights than did the males on the low protein diet without added ascorbic acid. Those animals receiving ascorbic acid supplement had less marked sex differences (with the male in most cases having higher organ weights).

In the three highest levels of dietary protein (25. 2 per cent, 40. 2 per cent, and 78. 2 per cent), the vital organs of the males (with only a few exceptions) weighed more than did those of the females.

The females consuming 40. 2 per cent protein and the males and females on 78. 2 per cent protein receiving ascorbic acid supplements had vital organs weighing more than did those without added ascorbic acid. The remaining groups showed an erratic pattern for ascorbic acid differences, except for the fact that males on 40. 2 per cent protein without added ascorbic acid consistently showed greater organ weights than those with ascorbic acid supplements.

As the protein content of the diet rose, there was a corresponding rise in the weights of all the vital organs of the males receiving up to the 40. 2 per cent level of protein, followed by a slight drop with the 78. 2 per cent group, all with no added ascorbic acid, with the one exception of the suprarenals, which weighed most at the 25. 2 per cent protein level, showing a gradual decline for the remaining two groups.

The females, both with and without ascorbic acid supplements, followed this same pattern, generally speaking. The females on a dietary without added ascorbic acid showed the highest weights for lungs and brains at the 25.2 per cent protein level, but the highest weights for liver and kidneys occurred at the 78.2 per cent protein level.

The males receiving massive dosages of ascorbic acid in their food showed a general decline in vital organ weights at the 40.2 per cent protein level, with the highest weights of each organ showing only an irregular pattern among the three higher levels of protein.

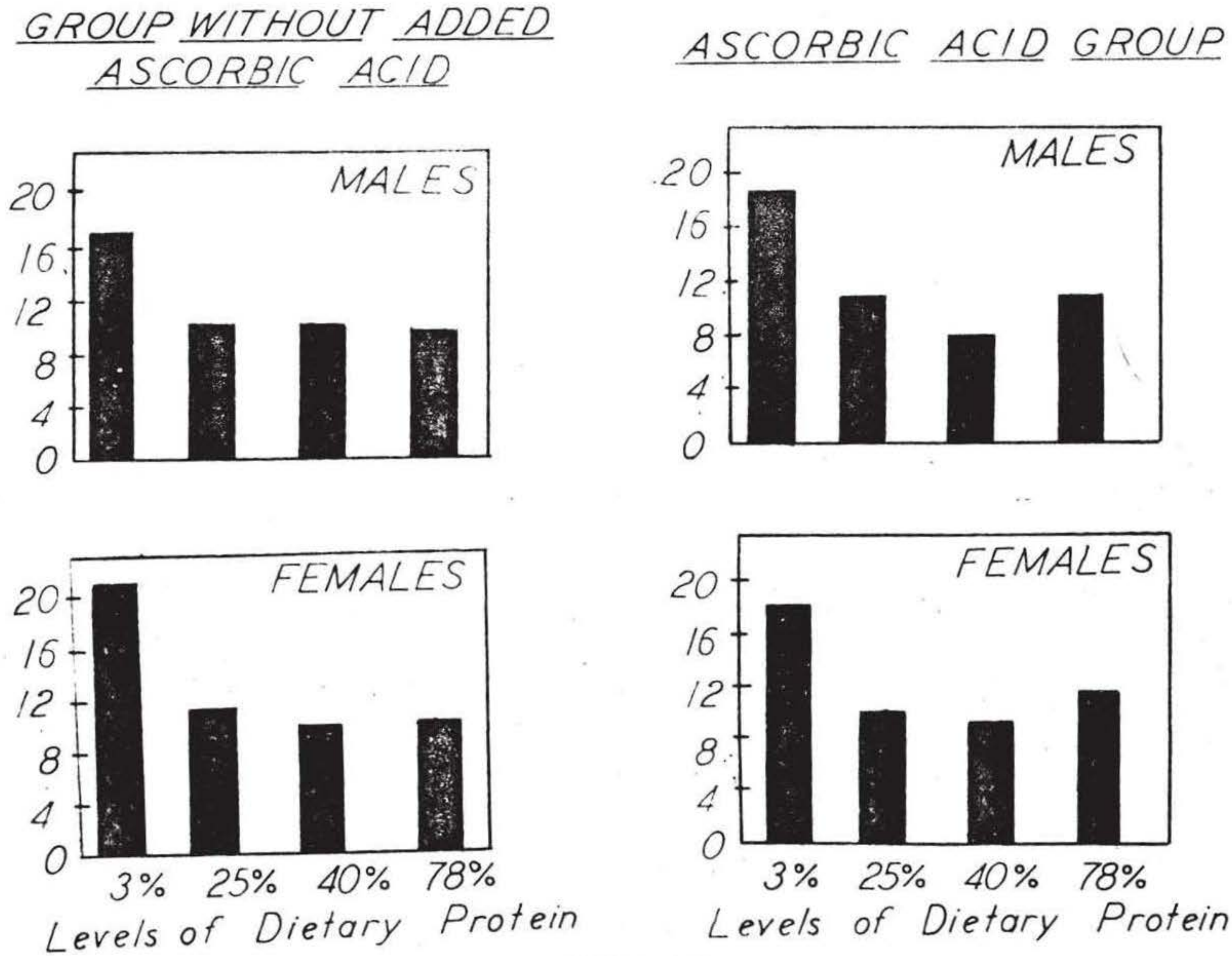

FIGURE 5

RATIO OF THE WEIGHT OF THE VITAL ORGANS
TO TOTAL BODY WEIGHT

PART A—LUNGS

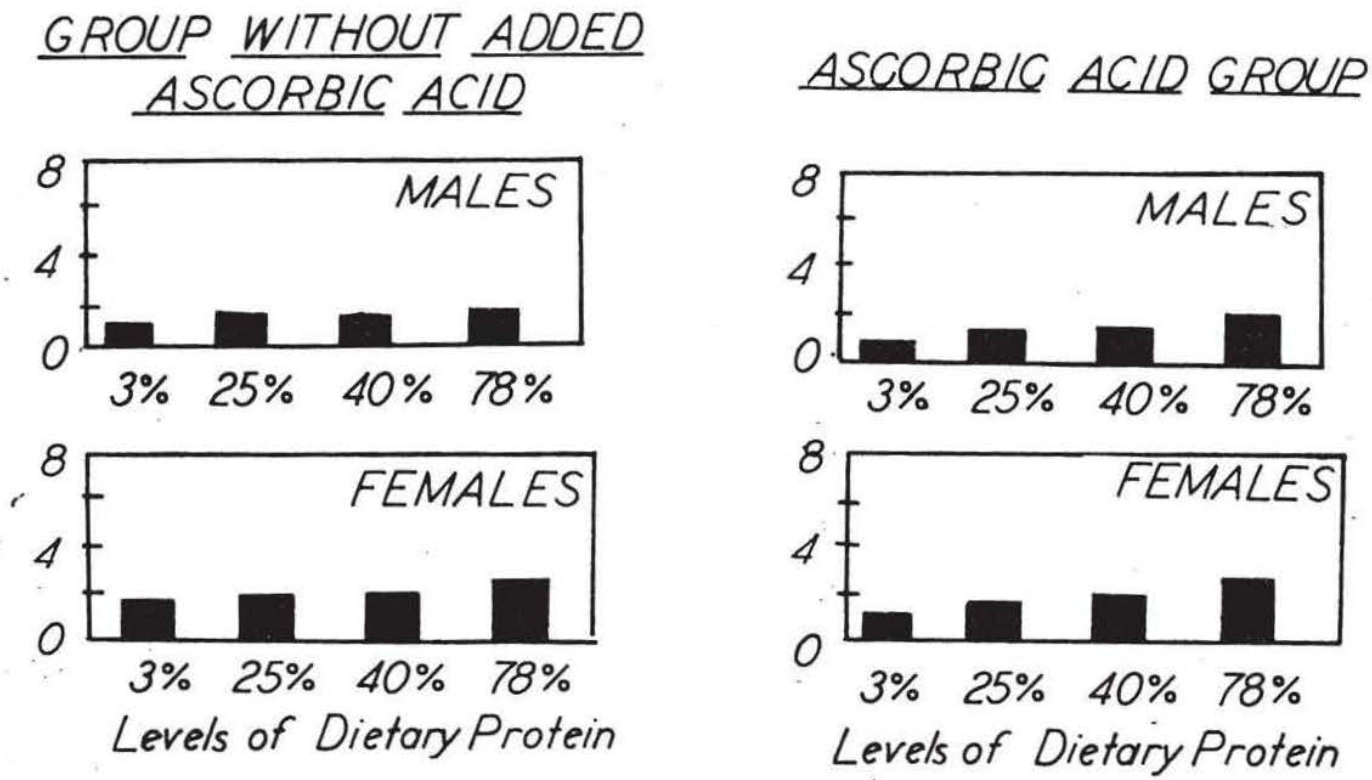

FIGURE 5

RATIO OF THE WEIGHT OF THE VITAL ORGANS
TO TOTAL BODY WEIGHT

PART B—SPLEEN

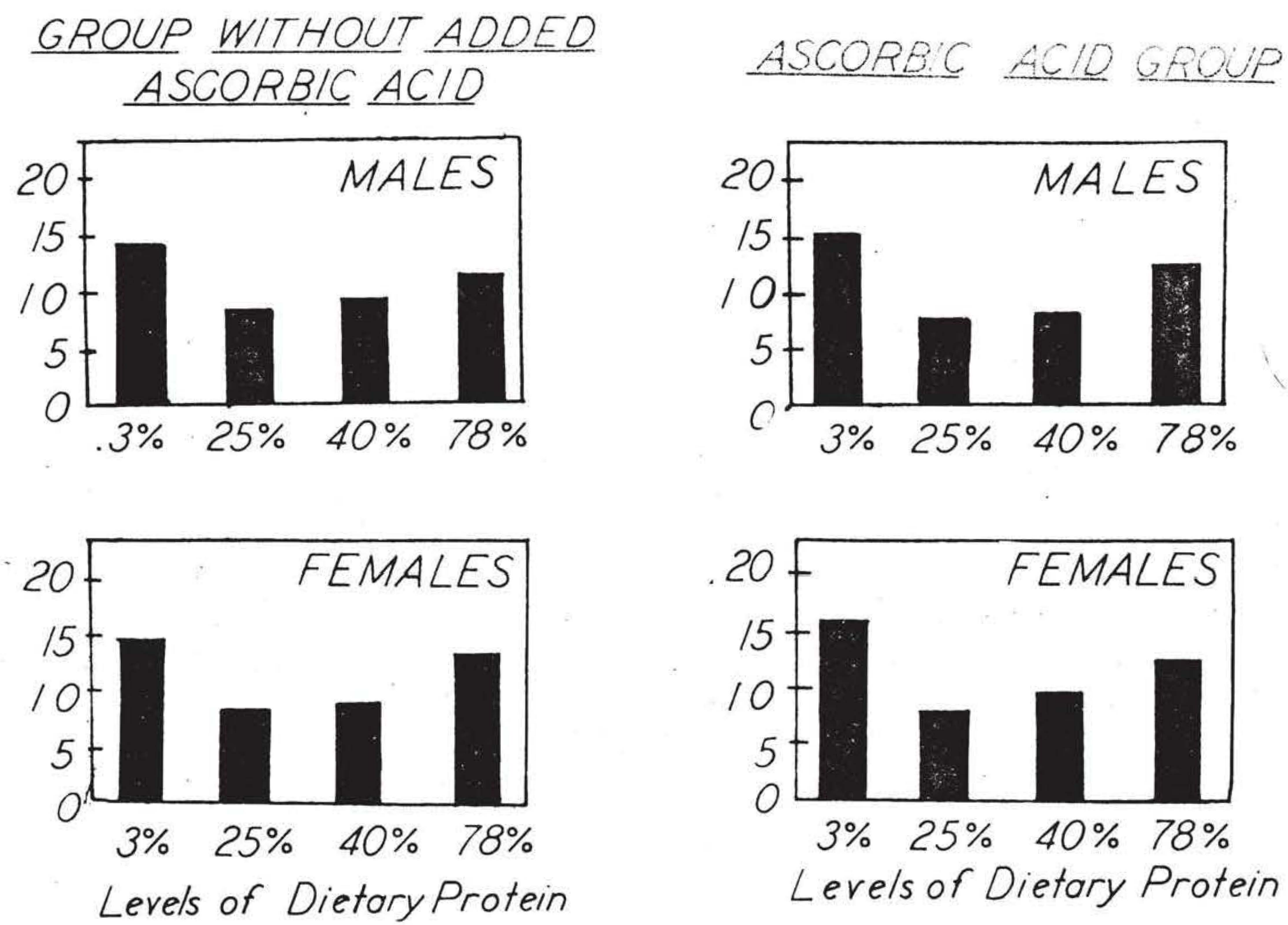

FIGURE 5

RATIO OF THE WEIGHT OF THE VITAL ORGANS
TO TOTAL BODY WEIGHT

PART C—KIDNEYS

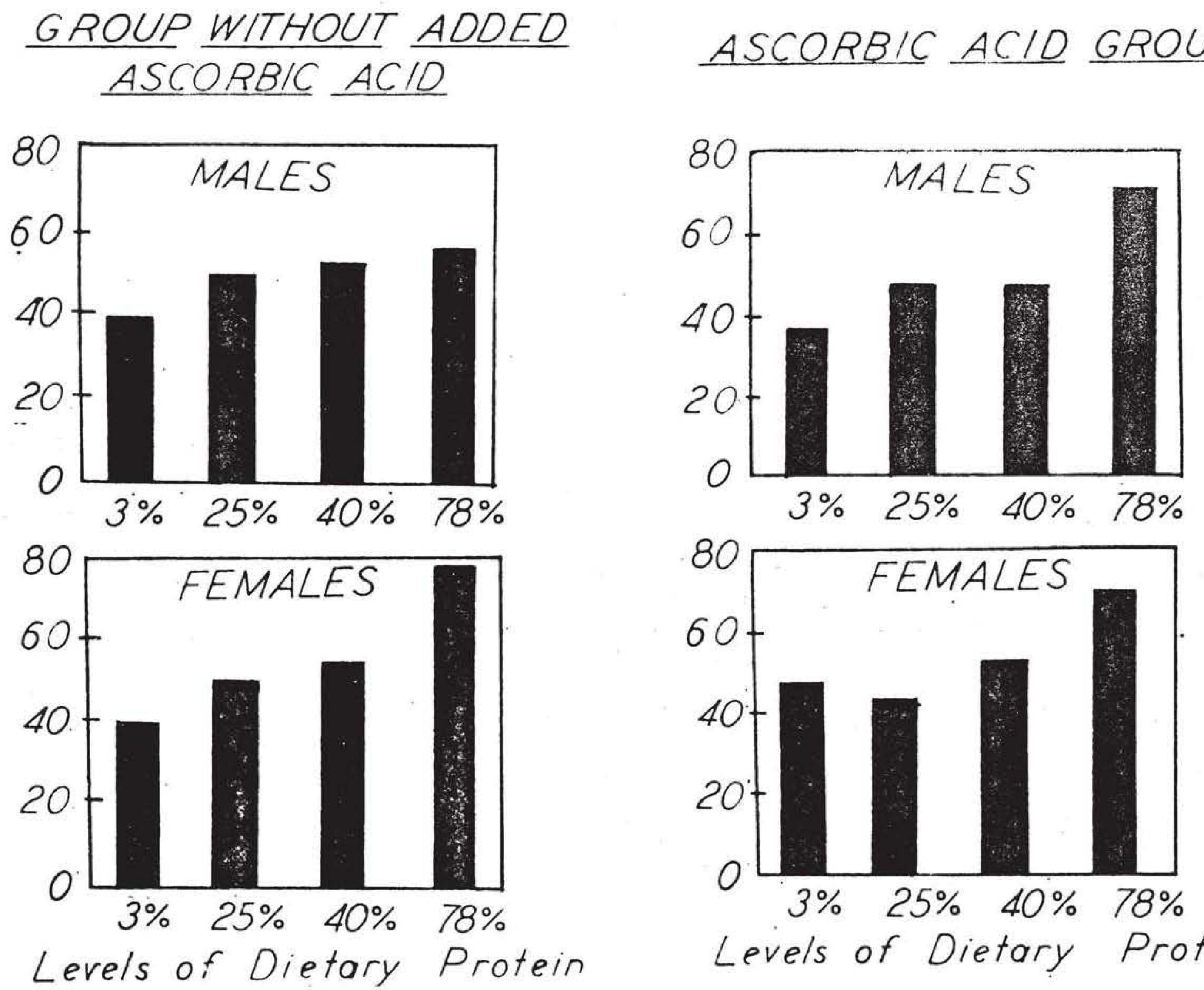

FIGURE 5

RATIO OF THE WEIGHT OF THE VITAL ORGANS
TO TOTAL BODY WEIGHT

PART D—LIVER

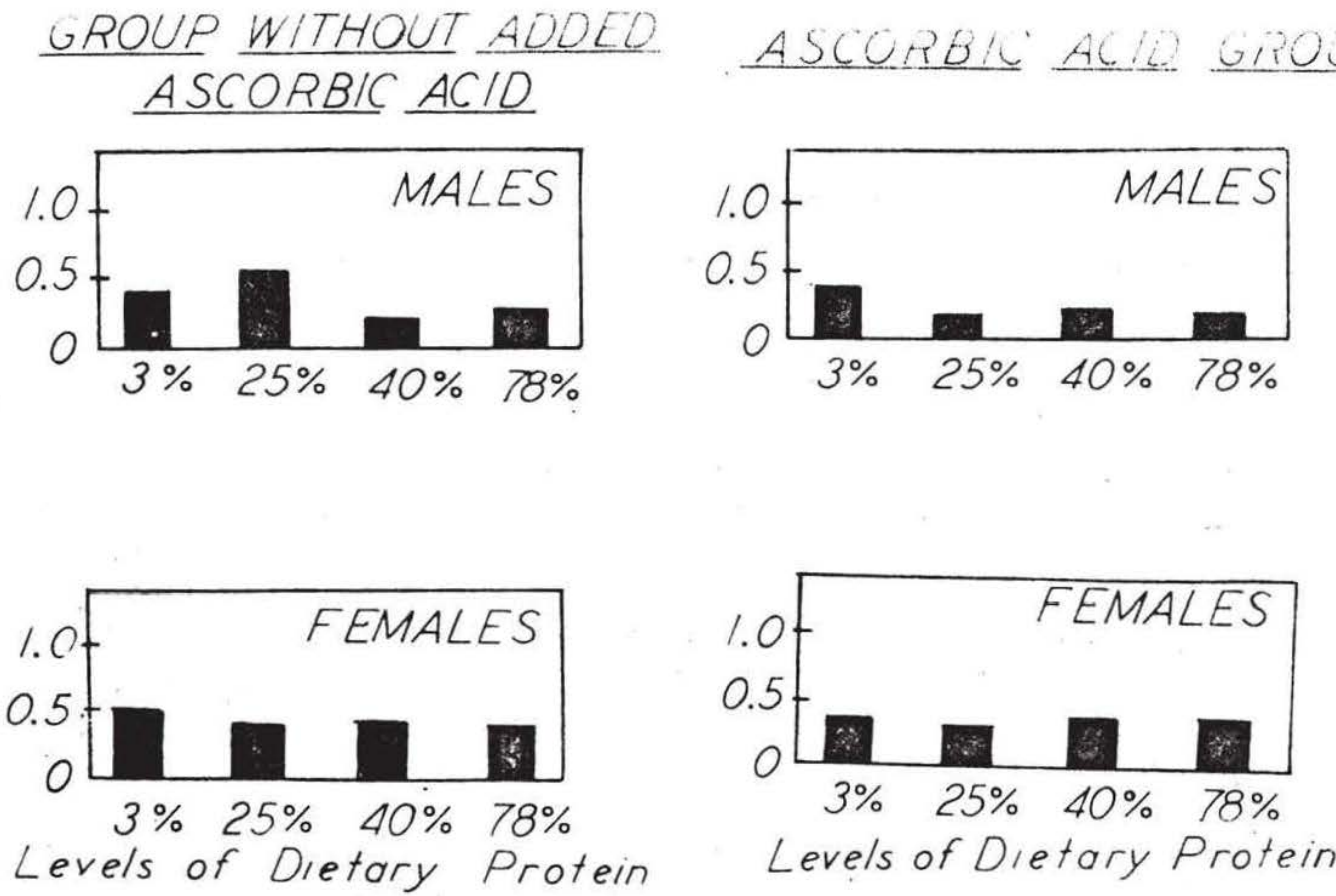

FIGURE 5

RATIO OF THE WEIGHT OF THE VITAL ORGANS
TO TOTAL BODY WEIGHT

PART E—SUPRARENALS

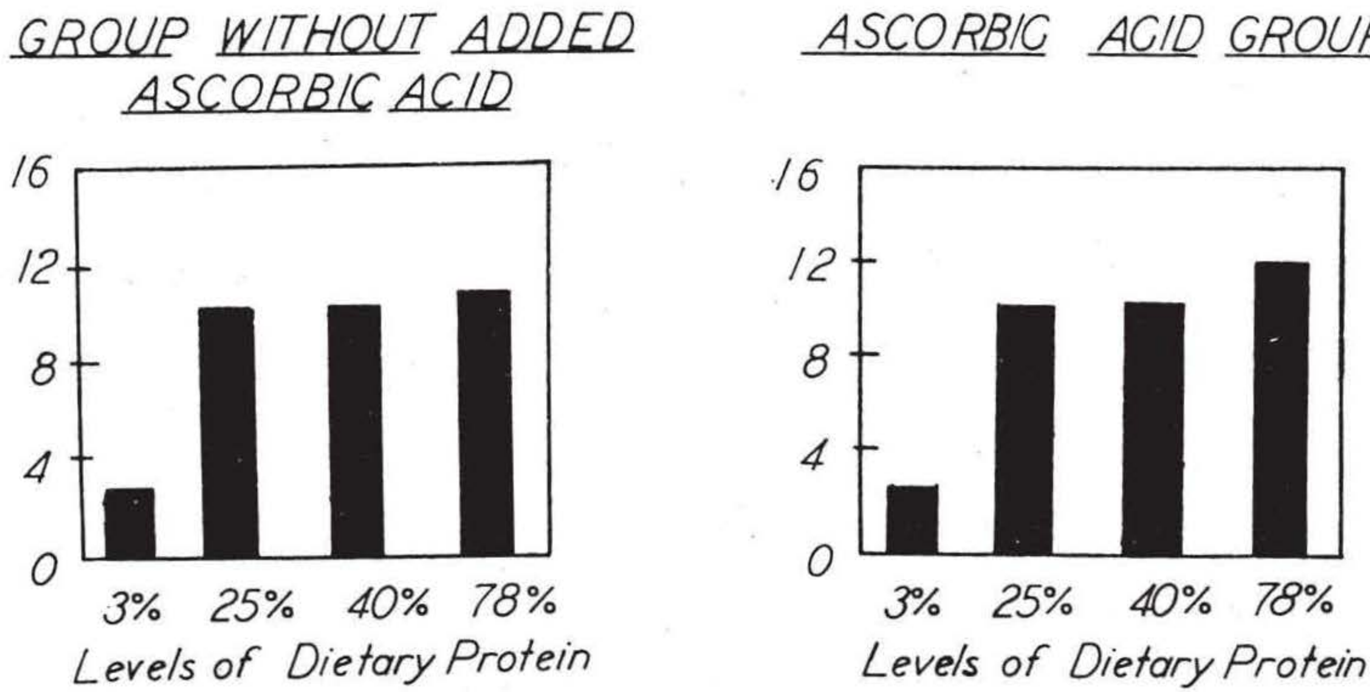

FIGURE 5

RATIO OF THE WEIGHT OF THE VITAL ORGANS
TO TOTAL BODY WEIGHT

PART F—TESTICLES

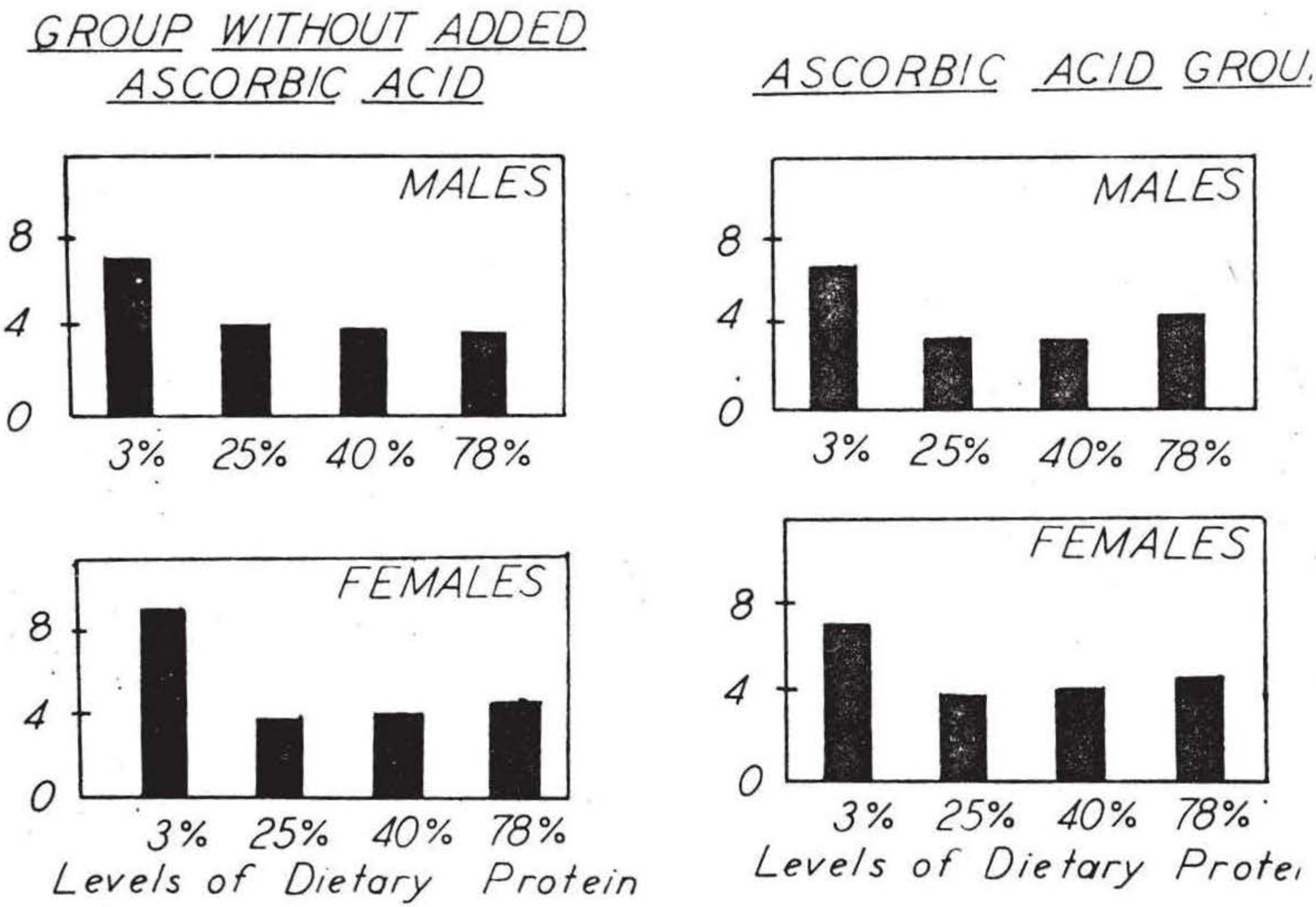

FIGURE 5

RATIO OF THE WEIGHT OF THE VITAL ORGANS
TO TOTAL BODY WEIGHT

PART G—HEART

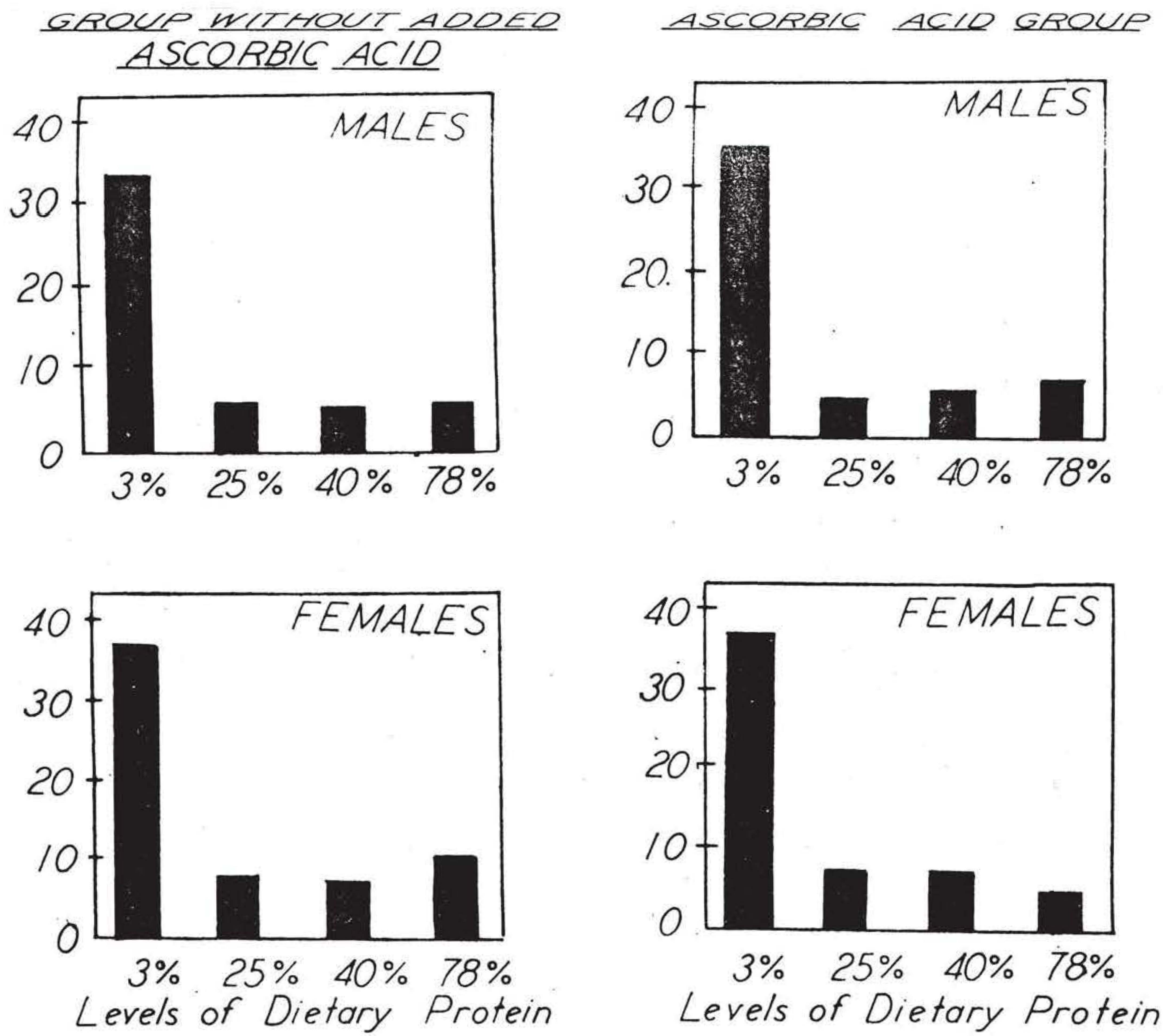

FIGURE 5

RATIO OF THE WEIGHT OF THE VITAL ORGANS
TO TOTAL BODY WEIGHT

PART H—BRAIN

THE RATIO OF THE WEIGHTS OF THE VITAL ORGANS TO TOTAL BODY WEIGHT

The data on the ratio of average weights of vital organs to average body weights of the experimental animals in the various groups (x 100) have been given in Table XIII. Figure 5 also shows these data in graphic form. In this case, in order to place two ordinates in a proper scale, the organ weights have been multiplied by 100 before dividing by the total body weight in each case.

The ratio of the weight of the lungs to the body weight was greatest for the experimental animals on the 3.2 per cent protein level, whether they had received supplementary ascorbic acid or not. (See Figure 5, Part A). The ratio for the males was 1.70, and for the females, 2.10, without added ascorbic acid, at this protein level. When ascorbic acid was added, the ratio was 1.84 and 1.80 for males and females, respectively.

The animals on the three highest protein dietaries showed approximately the same results, except for a slight drop in ratio for the males receiving 40.2 per cent protein. There was little sex or ascorbic acid differences other than the one previously mentioned.

The ratio of the spleen to total body weight (Figure 5, Part B) was lowest for the four protein groups among the males with added ascorbic acid in their diet. These aforementioned ratios were comparatively the same. For the remaining groups, there was a gradual increase in this ratio as the protein content of the diet increased, with the two intermediary groups showing practically the same values. The females showed an over-all higher ratio than did the males. There were, however, negligible differences between the groups with and without supplementary ascorbic acid.

The 3.2 per cent protein group had the highest ratio of weight of kidneys to total body weight of any of the other dietary groups (see Figure 5, Part C). The three higher dietary protein levels showed an increase in ratio with the protein increase, with the ratio of the 25.2 per cent group of animals being approximately one-half that of the lowest protein group. There were little sex or ascorbic acid differences.

The ratio of the weight of the liver (Figure 5, Part D) to the weight of the experimental animal was consistently higher for the 78.2 per cent protein group, although there was less marked difference for the males receiving no added ascorbic acid than for the males with the ascorbic acid supplement.

In general, there was an increase in the ratio of liver weight to body weight with an increase in the protein content of the diet, with but one exception, that of the animals on 25.2 per cent protein with added ascorbic acid being slightly lower in the ratio than those on the lowest protein level.

There was no pattern of sex or ascorbic acid difference in the liver to body weight ratio.

The suprarenals (Figure 5, Part E) showed the lowest ratio to total body weight of any of the vital organs. The groups without added ascorbic acid consistently showed greater ratios than the parallel groups with massive dosages of ascorbic acid.

The males had lower ratios than the females, except at the 25.2 per cent protein level without added ascorbic acid (females, 0.034; males, 0.056). On an average, the 3.2 per cent protein group, both male and female, with and without added ascorbic acid, had a higher ratio of suprerenal weight to total body weight than did the other dietary protein groups.

The 3.2 per cent protein group of males showed the lowest ratio of weight of testicles to total body weight of any of the other groups (see Figure 5, Part F). The three higher protein groups were very close in these values, with the 78.2 per cent protein group being the highest, whether ascorbic acid was added to the diet or not. The ratios for the rats receiving the three highest levels of protein, both with and without added ascorbic acid, ranged between 0.98 and 1.22.

These higher protein level groups had ratios from four to five times greater than the 3.2 per cent protein groups. There was no notable difference between those animals without added ascorbic acid in their diets and those receiving massive dosages of this vitamin.

The ratio of the weight of the heart to the total body weight of the experimental animal was greatest at the 3.2 per cent protein level (almost twice as great as any of the other dietary protein levels), both with and without added ascorbic acid. The three highest levels of protein groups had ratios within a narrow range (0.35 to 0.46). An ascorbic acid deviation was noted for the females on the 3.2 per cent protein dietary. Those animals receiving added ascorbic acid had an average ratio for the heart to the total body weight of 0.68, while the females without added ascorbic acid showed an average ratio of 0.89.

The only sex difference with respect to the heart ratios was noted in this same area, with the females on 3.2 per cent protein dietary without added ascorbic acid having the ratio of heart to body weight of 0.89, as compared with the ratio of 0.77 for the males on this same diet.

The last of the vital organs evaluated in this test was the brain (Figure 5, Part H). As in the case of the lungs, kidneys, suprarenals, and heart, the weights of the brains of the 3.2 per cent protein animals showed a higher ratio to total body weights than did that of the remaining protein groups. The contrast was more outstanding for these organs than for any of the others studied. For this protein level, the ratio of the brain to the total body weight was six times that of any for the other protein groups. When one considers that these animals are but one-tenth the size of some of those with a more favorable protein diet, the question arises as to whether the brain is proportionately larger because of need, or whether stunting of the skeletal structure simply is not accompanied by a concurrent stunting of the brain itself. This requires extensive further study.

The females on the 78.2 per cent protein diet with no ascorbic acid supplement had an average ratio of brain weight to total body weight of 1.01 as compared to the corresponding group with added ascorbic acid, which had a ratio which averaged only 0.87. In this same protein group, females exceeded males in the sub-group with no ascorbic acid supplement with a ratio of 1.01 for the former as compared with 0.58 for the latter. Other than these, there were no notable sex differences or differences between those receiving or not receiving ascorbic acid supplements.

In summary, the experimental animals receiving the lowest protein dietary showed the lowest vital organ ratio of any of the protein groups for the following cases:

Spleen.

Liver (with one exception— the 25.2 per cent protein group for the females with the ascorbic acid supplement).

Testicles.

The ratio of organ weight to total body weight was highest for the lowest protein levels for the following organs:

Lungs.

Kidneys.

Heart.

Brain.

No distinct order was found for the suprarenals.

The three higher protein groups showed relatively similar ratios for the individual vital organs, in most cases. In the case of the kidneys and the liver, however, there was an increase in the ratio with an increase in dietary protein.

There were no definite patterns of sex or ascorbic acid differences in any of the ratios. Individual group differences have been discussed above.

BRAIN WEIGHTS AND BRAIN MOISTURE

The total weights and the moisture content of the brains of the experimental animals have been listed in Table XIV.

Part A of this table has shown that the average total weights of the brains of the animals on the lowest protein level tended to be slightly lower than those of the animals on the three higher levels, with no marked differences in the latter three, and no distinct trends among the various other experimental groups.

The percentage of total moisture of the brains ranged between 74.5 per cent and 81.4 per cent, with an average of 78.2 per cent. There were no noteworthy differences between any of the groups, either male or female; and neither the percentage of dietary protein nor the addition of ascorbic acid seemed to influence markedly the percentage of moisture in the brains of the experimental animals.

SKELETAL STATUS OF EXPERIMENTAL ANIMALS

The skeletal status of an experimental animal cannot be evaluated by one criterion alone. In order to achieve an adult status, the skeleton of the young animal must increase in size while maturing in the sense of achieving the skeletal pattern characteristic of an animal which has arrived at the adult stage. In addition, the skeletal density may suffer at the expense of growth and maturation—if the diet is not optimum; or good skeletal density may be maintained throughout the growing period.

In this study, the following aspects of skeletal status have been evaluated: skeletal maturation; skeletal density; ratio of total body weight to skeletal weight (final); and certain cranial measurements (final). The growth progress of the animal has been followed by means of overall body weight, although this does not specifically represent skeletal growth per se.

SKELETAL MATURATION

Whereas the human being develops many ossific centers after birth, both the time of appearance of new ossific centers as well as the time of closure of the space between certain long bones and the adjoining ossific centers may be used as criteria of maturation progress. In the rat, on the other hand, all ossific centers appear to be present at birth. Therefore, in this study, the period at which the metacarpals and the phalanges fused with the adjoining ossific centers was used to evaluate maturation from the paws of the experimental animals.

Table XV has given the data on this part of the study. Figure 6 shows the same data in graphic form.

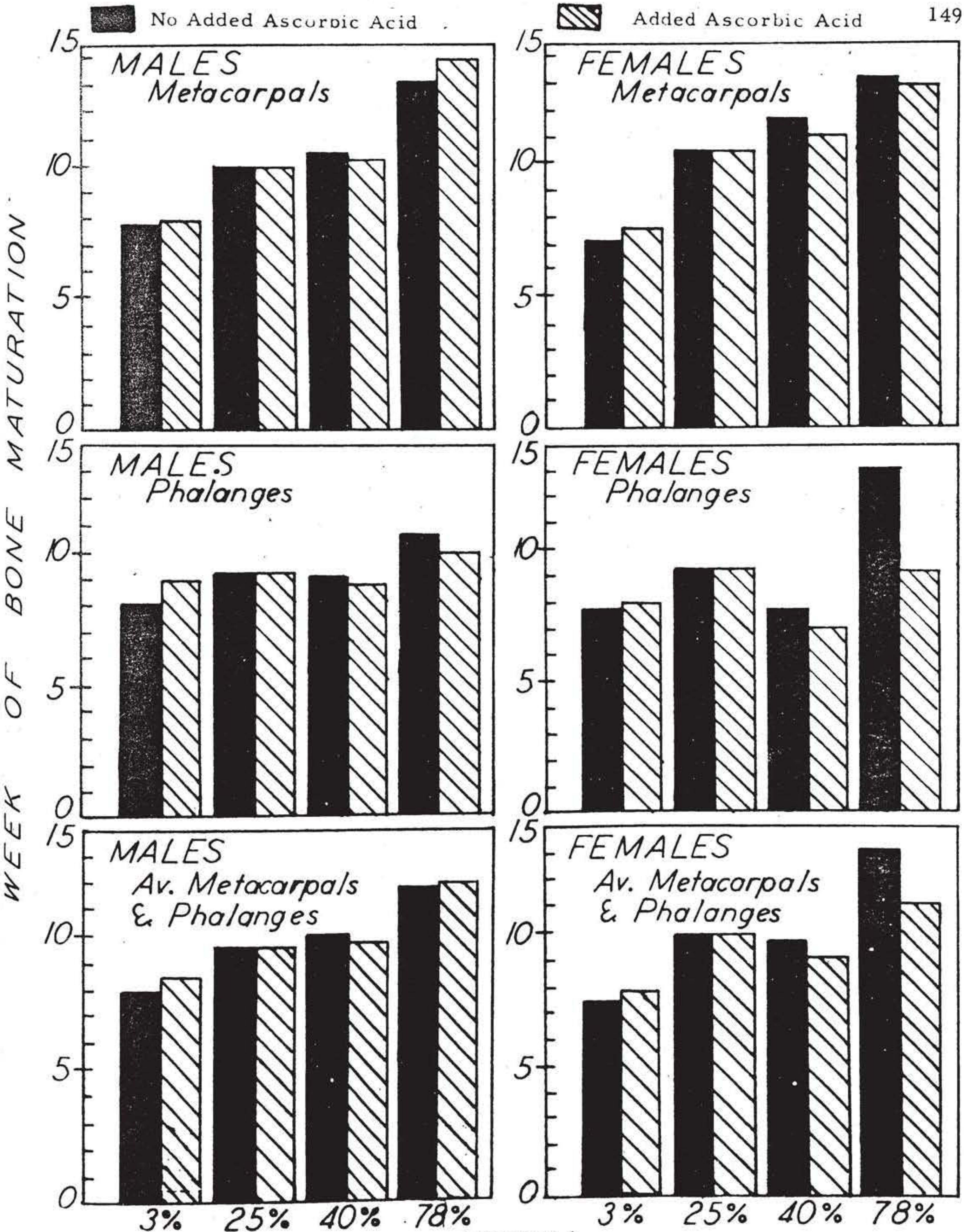

FIGURE 6
COMPARISON OF THE TIME OF COMPLETED MATURATION OF METACARPALS, PHALANGES, AND BOTH METACARPALS AND PHALANGES OF RATS ON ALL EXPERIMENTAL DIETARY LEVELS

Maturation of Metacarpals

In the case of the metacarpals, the higher the level of dietary protein, the longer the time required for bone closure. For both males and females, the 3.2 per cent protein consuming groups averaged 7.5 weeks; the 25.2 per cent protein groups averaged 10.2 weeks; the 40.2 per cent protein groups required an average of 11.0 weeks; and the 78.2 per cent protein groups averaged 13.3 weeks on the experiment before the metacarpals and the adjoining ossific centers had completely fused.

There were but slight differences between the metacarpal-ossific center closure time of the rats without added ascorbic acid and of those with ascorbic acid added to their diet. The time differences ranged from two to 10 per cent, with no definite pattern established.

Maturation of Phalanges

The phalanges of the experimental animals did not show as consistent a rate of maturation as did the metacarpals. As in the case of the metacarpals, those on the lowest protein level matured earliest and those on the highest levels matured latest, for the males. In the case of the females, however, the rats on 40.2 per cent protein matured at about the same time as the 3.2 per cent protein group. Aside from this, the 25.2 and the 40.2 per cent rats performed similarly in the rate of maturation. The phalanges of the males on 25.2 per cent protein had an average closure time of 9. 1 weeks, while the females on the same dietary had an average closure of 9. 2 weeks. The phalanges of the males on the next higher level of protein (40. 2 per cent) united at an average of 8. 8 weeks, while the females on corresponding diets, both with and without added ascorbic acid, averaged 7. 4 weeks.

The most notable difference in maturation of the phalanges between the groups with and without supplementary ascorbic acid appeared in the case of the females receiving 78. 2 per cent protein. With these animals, the ascorbic group matured in 9. 1 weeks, and the group without added ascorbic acid in 13. 0 weeks.

Summary of Skeletal Maturation

When considering the average closure time both of the metacarpals and the phalanges, the same general pattern was noted as that found when these two areas were considered separately. In short, an increase of protein in the diet appears to cause a slower rate of bone closure. This fact was consistently noted in the case of the male rats of both ascorbic acid groups. There was a deviation from this observed pattern, however, in the case of the females in which the 40. 2 per cent level showed a faster rate of closure than did the 25. 2 per cent protein group, whether vitamin C was added to the diet or not.

No consistent differences in the rate of bone closure were observed between the parallel groups of rats receiving diets with added ascorbic acid and those without this added vitamin.

SKELETAL MINERALIZATION

The degree of skeletal mineralization of the experimental animals was determined from final X-rays of the left tibia of all rats, as noted. These values have been shown in Table XVI. The mineralization values are expressed in terms of equivalent grams of ivory per cubic centimeter of bone.

The lowest mineralization values were found in the rats on the 3.2 per cent level of dietary protein. The average mineralization value of the rats on this level which had been fed the ascorbic acid-supplemented diet was 0.700 gm. of ivory per cc. of bone. Those without added ascorbic acid had a higher final value (0.757 gm. of ivory per cc. of bone). The rats on this lowest protein level were the only ones in which the ascorbic acid-supplemented group had a lower degree of skeletal mineralization than those without added ascorbic acid. With those on the 25.2 per cent , 40.2 per cent, and 78.2 per cent levels of dietary protein, the rats with added ascorbic acid had higher average skeletal mineralization values than did those without this supplement. The highest degree of average skeletal mineralization occurred in the animals on the 25.2 per cent protein level.

It is interesting to note that the mineralization values of all groups, except the lowest protein group, exhibited values in the region of 1.0 gm. of ivory per cubic centimeter of bone, which is considered to be in the normal range in the case of human bone, as determined by Mack (14). The 3.2 per cent rats showed values only of 0.7 gm. of ivory per cubic centimeter of bone, which is equivalent to the poorest classification of bone density in the case of humans, as established by Mack and associates (15).

In summary, the highest degree of skeletal mineralization was found in rats on the 25.2 per cent dietary protein level, while the poorest was found in those with 3.2 per cent protein. In addition, in all cases except the 3.2 per cent protein level, the rats with added ascorbic acid had a higher degree of skeletal mineralization than did those without supplements of this vitamin.

There were no sex trends as to the degree of skeletal mineralization.

RATIO OF TOTAL BODY WEIGHTS TO SKELETAL WEIGHTS OF EXPERIMENTAL ANIMALS

Table XVII has given the skeletal weights of the experimental animals, as well as the total body weights and the ratio of total body weight to skeletal weight.

The experimental animals on 3.2 per cent protein dietaries had the lowest skeletal weights, as well as the lowest total body weights; and the lowest ratio of total body weight to skeletal weight of any of the rats.

The only marked difference between the males and the females on this lowest protein occurred in the ratio of total body weight to skeletal weight with the ratio for the males being somewhat higher than that for the females (14.6 and 12.6, respectively, for the group without added ascorbic acid, and 14.1 and 13.2, respectively, for the group with supplementary vitamin C). The average male and female ratio was the same for both groups.

The 25.2 per cent protein groups were third highest in rank order for those animals receiving no added ascorbic acid and second highest for the ascorbic acid supplemented group on the basis of the ratio of total body weight to skeletal weight. The males in this dietary level, both with and without added ascorbic acid, had greater skeletal and total body weight, as well as higher ratios than did the females.

The ascorbic acid supplemented group showed greater values in weights, but lower ratios (23.4 and 25.2, respectively) than did the group without added ascorbic acid.

For the group of animals receiving 40.2 per cent dietary protein, the males and females followed a similar pattern to that of the 25.2

per cent protein group. No ascorbic acid differences were noted for any of the values; the males and females on both groups averaged a ratio of 26.2. This group on 40.2 per cent protein was fourth in rank order among the ratios of the other dietary groups. The high protein group, without added ascorbic acid, was second in rank order of ratios of total body weight to skeletal weight, and, for the group with added ascorbic acid, third. Both groups shared an average male and female ratio of 24.8. The males of this 78.2 per cent protein group, both with and without added ascorbic acid, had higher skeletal and total weights than did the females, but there were no definite patterns of sex or ascorbic acid differences for the ratio of total body weight to skeletal weight. No pattern of ascorbic acid differences was noted for either of the calculated weights.

CRANIAL MEASUREMENTS

Table XVIII has given the average cranial measurements of the experimental animals, as follows: squamosal distance, zygomatic width, and the ratio of the zygomatic to the cranial measurements. Figure 2 has given a diagram of these measurements.

The rats receiving the lowest protein dietary (3.2 per cent), both with and without added ascorbic acid, showed the highest ratios of zygomatic to cranial measurements, but the lowest measurements of the bones themselves of any dietary protein group. These values generally rose to the animals on the 40. 2 per cent protein level, and then dropped slightly at the highest protein level. The groups on 40. 2 per cent and 25. 2 per cent protein, both with and without ascorbic acid supplements, showed very closely parallel data.

The 3. 2 per cent protein group had practically the same squamosal and cranial measurements for males and females, with no differences, whether ascorbic acid was added to the diet or not. The same was true of the various calculated ratios.

As noted, the values for the animals on the 25. 2 and 40. 2 per cent protein diets were closely parallel. For the former groups, the male measurements somewhat exceeded the female, whether the animals had the additional ascorbic acid or not. The ratios went slightly in the opposite direction.

For the animals on 40. 2 per cent protein, there were no distinct sex differences.

The males on the 78. 2 per cent protein dietary, both with and without added ascorbic acid, had greater measurements than did the females for all three measurements (squamosal, zygomatic, and total cranial). For the ratio of the zygomatic to cranial measurements, there was no sex pattern observed. For the ascorbic acid supplemented group, as compared to the group without added ascorbic acid, the average male and female measurements were very similar except for the cranial measurements. These were 4.38 cm. and 4.21 cm., respectively, for those with and without added ascorbic acid. The ratio of zygomatic to cranial measurements averaged 1.92 for the ascorbic acid supplemented group and 1.86 for the group without added vitamin C in the diet.

To summarize the results of the cranial measurements of the experimental animals, it is noted that the lowest protein animals have smaller squamosal, zygomatic, and total cranial measurements and higher ratio of zygomatic to cranial measurements, than did any of the other protein groups.

The 25.2 per cent and the 40.2 per cent protein groups had almost the same values recorded for all of these measurements; and they were somewhat higher than the highest protein group.

The ascorbic acid supplemented groups on all levels of dietary protein (except 3.2 per cent animals), for males and females combined, exceeded the group without added ascorbic acid in the ratio of the zygomatic to the cranial measurements. This same pattern was followed by the squamosal, zygomatic, and total cranial measurements with the one exception of the zygomatic width of the 40.2 per cent males and females.

GENERAL DISCUSSION

The pathway for the synthesis of ascorbic acid in the rat has been of much interest in scientific investigations. Recent research reports by C. G. King and co-workers (2) have elucidated the major steps in the synthesis of vitamin C in the rat. This animal, like the majority of mammals, does not require an exogenous source of this dietary essential. King and his co-workers state that the central compound in this biosynthesis is glucuronic acid, a constituent of many polysaccharides in plants, bacteria, and animals (often considered essentially a protective agent important in many detoxification reactions). Glucuronic acid, which has a chemical structure resembling that of ascorbic acid, has been shown by Schmid (2) to arise from glycogen and hence glucose, as well as glycogenic amino acids.

In continuation of earlier work, Horowitz and King (11) showed that glucose-6-^{14}C, when injected intraperitoneally into chloretone-stimulated rats, was converted into and excreted in the urine as ascorbic acid-1-^{14}C.

Completing the scheme of biosynthesis of ascorbic acid from glucose to glucuronic acid to ascorbic acid, Horowitz and King (10) experimented with chloretone-treated male albino rats. The conversion of C^{14} labeled glucuronolactone to ascorbic acid was from four to eight times greater than that of similarly labelled glucose to ascorbic acid, indicating that the synthesis of ascorbic acid from glucose in the rats proceeds by way of glucuronolactone. On the basis of these experiments, King and Horowitz have suggested the following reaction:

D-glucuronic acid-gamma-lactone ⟶ 2 keto-L-gluono-gamma-lactone ⟶ L-ascorbic acid.

Yanovskaya and Kraiko (32) experimented with male white rats fed on a synthetic diet of casein, starch, salt mixture, yeast, sunflower oil, and fish fat, which provided normal growth. Different groups were given 20 mg. chloretone, veronal or medinal in water; and the amount of ascorbic acid excreted in the urine was estimated. The results which they obtained are considered to confirm the hypothesis that the biosynthesis of ascorbic acid in rats is increased to compensate for increased dissipation thereof; and that ascorbic acid is mobilized from those organs whose functional activity is increased in response to an external stimulus or influence.

The synthesis of vitamin C was studied by Yanovskaya (31) in white rats on two diets differing considerably in protein content (18 and 1.5 per cent, respectively), but adequate in all other components. At the end of the test period, the rats were killed and the vitamin C content of the liver, intestine, spleen, kidneys, and adrenal glands was estimated.

The results showed that a decrease in the protein content of the diet, all other conditions being equal, led to a significant decrease in the amount of ascorbic acid synthesized and to a lower ascorbic acid content of the tissues. In further experiments, rats on the low protein diet were given chloretone; and these showed an increased synthesis of vitamin C to a level almost equal to that of rats on the higher protein diet. Yanovskaya did not attribute the low ascorbic acid content of the tissues of rats suffering from protein deficiency to increased use of the vitamin or to the destruction of the activity of the enzyme system which acts in the biological synthesis of ascorbic acid.

The extent of the interaction of vitamin C and protein cannot be fully realized without a consideration of ascorbic acid deficiency. Rats in a state of inanition and guinea pigs on a vitamin C deficient diet, both exhibit various symptoms of this deficiency.

As reported in Nutrition Reviews, Gersh and Catchpole (21) have suggested that the depolymerization of the glycoprotein constituents of the ground substance of healing wounds of scorbutic animals would lead to an increase of their water solubility and to an increase of their concentration in blood serum. Pirani and Catchpole (21) have studied the level of serum glycoproteins in experimental scurvy and have found their concentrations higher in guinea pigs with acute or chronic scurvy than in normal animals, with a close correspondence between the serum glycoprotein levels and the estimated severity of the disease. In a group of scorbutic animals treated with ascorbic acid, there was a clinical improvement in some, but not all, while the serum glycoprotein concentrations fell somewhat (though still significantly higher than the levels of normals). These workers point to earlier work by Catchpole, and interpret his findings of increased serum glycoprotein concentrations in scorbutic animals as lending support to the concept of a depolymerized ground substance as a feature of scurvy. They suggest that the elevated serum glycoproteins could result either from the failure of glycoproteins to be polymerized in the absence of ascorbic acid, or from the depolymerization of glycoproteins. However, depolymerization and the elevation of serum glycoproteins may occur in a variety of conditions and sometimes in the presence of presumably adequate amounts of ascorbic acid intake and synthesis.

Whether or not the guinea pig in a scorbutic condition could be attempting an adjustment of the ascorbic acid deficiency by an effort toward the synthesis of vitamin C by the accumulation of glycoproteins, even though this animal does not normally produce this vitamin, is of interest to this author, considering the very recent elucidation of ascorbic acid synthesis in the rat by C. G. King and his co-workers (2).

Brown and Sturtevant (4) agree with other investigators that the rat is not dependent upon a dietary source of vitamin C; but the site of the synthesis of this factor has not been determined (either in the intestinal flora or in the tissues).

Ascorbic acid treatment of rats in certain types of sterility high-lighted the synthesis of ascorbic acid in the animal body, in a series of experiments by Sutton, Kaesar, and Hancord (24). Their results indicated that many of the animals showing abnormal reproductive performanced had lower ascorbic acid concentrations in certain body fluids than is normally found. These workers also found a decreased concentration of blood plasma ascorbic acid and a decrease in the urinary ascorbic acid for animals of vitamin A deficient diet. They found no particular gland or organ to be involved, but felt that ascorbic acid synthesis was a general metabolic function.

Morehouse and Guerrant (16) made a study to learn more about the effect of age and sex of the albino rat upon the hepatic ascorbic acid of normal healthy animals. They found the ascorbic acid content of the adult female to be considerably higher in pregnant animals. The concentration of hepatic amino acid in rats (over eight weeks of age) remained relatively constant.

According to Follis (6), a relationship may exist between ascorbic acid status and the production of adrenal cortical hormones, which is of interest in view of the extremely large concentrations of ascorbic acid in the adrenal gland. When pituitary corticotropic hormone is injected into rats, there is a decrease in ascorbic acid concentration of the adrenals. This anterior pituitary growth hormone reflects protein anabolic properties as well (12). The increase in plasma alpha-amino nitrogen following the administration of adrenocorticotropic hormone is a reflection of its growth-inhibiting properties.

H. Selye and D. J. Ingle (1) have reported that, if stimuli are continued over a sufficient period, the adrenal gland enlarges and ascorbic acid concentration increases rather than decreases. Some evidence suggests that ascorbic acid may be synthesized in the adrenal gland (1).

Mulinas, Pomerantz, and Jojkin (17), in studying the effects of pseudo-hypophysectomy in albino rats, found that a substantial increase in the ascorbic acid content of the adrenal glands occurred, this increase being greater in males than in females.

The fact that the blood is an indication of nutritional status has been elucidated by several investigators, both for vitamin C and for protein. Todhunter and McMillan (22) determined the ascorbic acid content of whole blood plasma as an indication of normal values for experimental animals. The ascorbic acid content of the plasma of male rats ranged from 0.87 to 0.28 mg., and of females from 0.33 to 0.019 mg. per 100 ml.

Wortis, et al. (30) established the fact that blood is an adequate and accurate index of the state of vitamin C nutrition. Also, the plasma amino acids apparently are in dynamic equilibrium with tissue amino acids and protein, according to Engel, Winton, and Long (12).

The importance both of protein and of ascorbic acid on wound healing has been emphasized by Wohl (28), who states that a high-protein diet stimulates fibroblastic activity and decreases the quality of wound secretion; and the benevolent influence of vitamin C on collagen formation has been demonstrated amply.

The nucleic acids and proteins in the liver of guinea pigs dying from experimental ascorbic acid deficiency was compared with controls and fasting rats by M. Fukuda and A. Sibatani (7). Values for total deoxypentose nucleic acid, as well as the amounts per nucleus were similar in scorbutic guinea pigs and fasting rats, showing that there was no necrotic degeneration before death. Loss of body weight of these two groups was comparable, but the amount of total pentose nucleic acid remained normal in scorbutic guinea pigs and decreased in the rats to one-third that of normal. Liver weight remained higher in the guinea pigs than in the rats. Protein N of scorbutic animals showed a loss comparable in order with that of body weight; but the decrease was less than in fasted animals. In prolonged starvation, loss of pentose nucleic acid was greater than that of protein.

Womack, Marshall, and Parks (29) found that the negative nitrogen balances of adult protein-depleted or undepleted rats fed rations containing low levels of amino acids (14.4 to 14.7 mg. essential amino acid nitrogen per day) with sucrose as the carbohydrate, were significantly improved when the essential amino acid nitrogen intake was increased to 24.7 and 25.1 mg. per day. Nitrogen losses of protein-depleted animals were less than those of undepleted animals at the same levels of total and essential amino acid nitrogen intake.

Beznak (3), studying the effects of prolonged administration of l-ascorbic acid and d-gluconic acid on the body weight, food, water, and salt solution consumption of albino rats, found that the effect of consuming ascorbic acid did not become apparent until after 70 days, when there was a marked increase in body weight and growth. Also, the animals receiving ascorbic acid supplements consumed more food and water.

Jolly (13), who conducted a preliminary study on feeding ascorbic acid to rats in the same laboratory as those in which the author worked, fed 0.25 per cent of ascorbic acid to one group of animals in comparison with no supplementary vitamin C to a comparable group. All other dietary factors were the same for the two groups of animals. Although the amount of ascorbic acid fed was only one-fourth that used in the study presently reported, with the experiment continued only 70 days, there was an indication toward the close of this study that growth was being favored by the ascorbic acid supplement. She also found indications of improved skeletal status and blood plasma ascorbic acid levels in the rats receiving an exogenous source of this vitamin.

The administration of thyroxine, a protein-like substance, leads to a rise of ascorbic acid content of the suprarenals and liver of rats, according to Paal and Brecht (18).

Schwartz and Williams (20) studied the effect of vitamins and natural products of liver ascorbic acid on sulfasuxidine-fed rats, and found that the effect of a natural stock ration in maintaining a high level of ascorbic acid in the liver, even in the presence of sulphasuxidine, was not due to the presence of increased amounts of the known vitamins in the ration. Graded increasing amounts of vitamins added to a purified diet did not have the same effect. Change in the protein intake also was not responsible, though the possibility that specific amino acids might contribute to the increased liver ascorbic acid could not be ruled out.

In studies undertaken by Patterson and Bourquin (19), and also reported by Sherman (22), it appeared that the ascorbic acid requirement was increased when the diet contained more than the average amount of protein. This confirms and extends the findings that vitamin C is concerned in the metabolism of certain amino acids, especially the aromatic amino acids, phenylalanine and tyrosine.

SUMMARY

Eight groups of albino rats were maintained on experimental diets varying in protein and ascorbic acid. The protein (casein) content of these diets was at four levels: 3.2, 25.2, 40.2, and 78.2 per cent of the total ration. One group of diets contained these four levels of protein without added ascorbic acid, while a second group contained these same levels of protein with one per cent ascorbic acid supplemented. These diets (made isocaloric as the protein content in the diet rose, by substituting cornstarch for casein) are considered adequate, according to present standards, for optimum growth, except for the 3.2 per cent protein diets, which were deficient in methionine for the amounts consumed by the experimental animals.

These animals were kept on the experiment for 140 days, unless death occurred earlier.

FOOD CONSUMPTION. On all dietary levels the males ate more than did the females, except on the lowest protein level. With the exception of the females receiving 40.2 per cent protein, the groups receiving added ascorbic acid consumed more food than did those without the ascorbic acid supplement. The most food was consumed by the

males on 40. 2 per cent protein with added ascorbic acid, while in the group with no added ascorbic acid the most food was eaten by the males at the 25. 2 per cent dietary protein level. The animals on the 3. 2 per cent protein consumed the least amount of food. Those on 40. 2 per cent protein level, regardless of sex or of ascorbic acid supplement, averaged the highest amount of total food of any of the protein level groups.

GROWTH CHARACTERISTICS. The rats on 3. 2 per cent protein showed no growth during the experimental period, and, with the exception of one female (without added ascorbic acid), died before the end of the test period.

Males tended to exceed females in growth progress. The rats with supplementary ascorbic acid exceeded the corresponding sex-protein group without added vitamin C (except for the 3. 2 per cent group). The rats on the 40. 2 per cent protein produced the best growth, with the exception of the males receiving added ascorbic acid, which were very slightly below the 25. 2 per cent protein consuming group. This was followed closely by the 25. 2 per cent group, with the highest protein level ranking next to the poorest. In general, the growth pattern tended to follow the food consumption pattern.

FOOD CONSUMPTION PER GRAM OF BODY WEIGHT. The rats on the 3. 2 per cent protein diet consumed the most food per gram of body weight, with no sex-ascorbic acid differences noted.

The 78.2 per cent protein group ate the least amount of total food per gram of body weight. The two intermediary groups (25.2 per cent and 40.2 per cent) showed closely paralleling values for food consumption per gram of body weight.

PHYSICAL APPEARANCE. The 25.2 per cent and the 40.2 per cent protein groups exhibited the best physical appearance. The 78.2 per cent protein groups were slightly less optimum in appearance, while the 3.2 per cent protein groups had the poorest appearance and many manifestations of dietary deficiencies.

BLOOD VALUES. a) When the values for the animals on both ascorbic acid groups were compared, those animals having massive dosages of ascorbic acid added to their diet averaged a higher blood plasma ascorbic acid content than did those animals not receiving additional vitamin C. The values averaged lowest for the low protein groups. There was no other pattern noted.

b) The 3.2 per cent protein groups had the lowest serum protein value of any of the dietary groups. At the final test (140 days) the ascorbic acid supplemented groups were consistently lower in total serum protein than the corresponding protein groups without additional ascorbic acid.

c) The blood serum albumin values for all dietary groups had averages which fell within a narrow range, with a trend toward lower values at the final test period for the groups with added ascorbic acid.

VITAL ORGANS. a) The lowest protein group of rats showed the lowest organ weights, with no outstanding sex-ascorbic acid differences. In the three highest levels of dietary protein, the vital organs of the males generally weighed more than did those of the females. There also was a trend toward an increase in organ weights with the rise in body weight.

b) The experimental animals receiving the 3.2 per cent protein dietary generally showed the lowest vital organ weight ratio to total body weight in comparison with all of the other protein groups for the spleen, liver, and testicles, but the highest ratio for the lungs, kidneys, heart, and brain. There was no distinct order noted for the suprarenals.

Relatively the same ratios were shown for the ratio of weights of vital organs to total body weight of the three higher protein levels (with the exception of an increased ratio for the kidneys and the liver with an increase of protein in the diet).

No sex or ascorbic acid differences were notable.

c) The percentage moisture of the brains averaged 78.2 per cent. There were no notable differences between any of the groups in this factor in relation to sex, or to ascorbic acid or protein content of the diets.

SKELETAL MATURATION. An increase of dietary protein tends to cause a slower rate of bone closure (one exception was the females on the 40.2 per cent protein level which had a faster rate of closure than did the 25.2 per cent group, regardless of ascorbic acid supplementation).

The phalanges closed sooner than the metacarpals on all dietary levels, except for the lowest protein groups; and the phalanges of the ascorbic acid supplemented groups closed sooner than did the phalanges of the groups without added ascorbic acid (except on the lowest protein dietaries). There was no notable difference between the males and the females in this respect.

SKELETAL MINERALIZATION. The highest degree of skeletal mineralization was found in the rats on the 25.2 per cent dietary protein level, while the poorest was noted for those animals consuming a 3.2 per cent protein dietary. In all except the lowest protein group, additional ascorbic acid gave a higher degree of mineralization. No sex trends, however, were noted.

RATIO OF TOTAL BODY WEIGHTS TO SKELETAL WEIGHTS. The lowest protein groups had the lowest skeletal weights and body weights, and the lowest ratio of total body weight to skeletal weight of any of the other dietary protein groups.

The males on the 25.2 per cent level, both with and without added ascorbic acid, had higher ratios than did the females. The ascorbic acid supplemented group showed greater values in weights but lower ratios than did the group without added ascorbic acid.

The two intermediary protein groups shared the distinction of having the highest skeletal weight. The 40.2 per cent protein groups had the highest ratio of skeletal weight to total body weight of any of the protein groups. The males and females followed the same general pattern as did the 25.2 per cent protein groups.

In the highest protein group, the males had higher skeletal and total body weights than did the females. No other pattern for sex or ascorbic acid differences was observed.

CRANIAL MEASUREMENTS. The lowest protein animals had smaller squamosal, zygomatic, and total cranial measurements and higher ratio of zygomatic to cranial measurements than did any of the other dietary protein groups.

The two groups on intermediate levels of protein shared almost identical values (being somewhat higher than the 78.2 per cent group).

Ascorbic acid supplemented groups on all levels of dietary protein (except the lowest protein), both males and females, exceeded those animals on corresponding dietary protein levels without added ascorbic acid in the ratio of zygomatic to cranial measurements. This same pattern was found for the other measurements, with the exception of the males and females on 40.2 per cent protein (squamosal distance).

CONCLUSIONS. On the basis of these experimental findings, the following conclusions have been drawn:

(1) Animals on an optimum diet consume more food than do those animals on a deficient diet. Males average higher food consumption than do females. Ascorbic acid tends to increase the food consumption of the experimental animals.

(2) The 25.2 per cent and the 40.2 per cent protein dietaries gave optimum growth for animals on this experiment. Ascorbic acid supplements to the diets of the animals tend to produce better growth than in those not receiving added ascorbic acid. Males average greater growth than do females.

(3) The intake of food per gram of experimental animal weight cannot be related directly to growth.

(4) Ascorbic acid supplements tend to increase blood plasma ascorbic acid values for the experimental animals.

(5) Total protein and albumin values are not markedly altered in any dietary protein level above the lowest protein group (3. 2 per cent). Ascorbic acid supplementation seemed to cause a decrease in blood serum total protein and albumin for animals on experiment for 20 weeks.

(6) Animals on 3. 2 per cent protein have vital organs weighing less than do the animals on higher protein intakes. There tends to be an increase in organ weight with the increase in body weight. The ratio of organ weights to total body weight shows some changes, as described, with the various diet groups.

(7) 3. 2 per cent protein promotes more rapid fusion of the epiphyseal centers to the adjoining bones. An increase in dietary protein tends to cause a slower rate of bone closure.

(8) The phalanges of the males and females receiving an adequate diet close earlier than do the metacarpals; also, ascorbic acid influences an earlier closure of the phalanges.

(9) Animals on the lowest protein level have the lowest skeletal weights, and the lowest ratio of total body weight to skeletal weight.

(10) Males receiving an adequate protein dietary have higher skeletal weights than do females.

(11) Neither dietary protein nor ascorbic acid supplementation influences the percentage of brain moisture.

(12) Animals on 3.2 per cent protein diets have smaller cranial measurements than do those on higher dietary levels. They also have a higher ratio of zygomatic to cranial measurements.

(13) Ascorbic acid seems to cause an increase in the ratio of the zygomatic to cranial measurements for those animals receiving optimum dietaries.

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