STUDIES ON GROWTH INHIBITION AND RECOVERY IN YOUNG MALE ALBINO RATS

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INTRODUCTION

RECOVERY THERAPY

Caloric starvation is a common condition in both nature and society. It has for centuries characterized many of the large nations. Two great world wars have added measureably to the clinical and biological significance of mass undernutrition; yet chronic inanition has had only scant experimental attention. Physiological studies on recovery and therapy in this type of starvation have been almost entirely neglected. It is the purpose therefore of this investigation to study semi-starvation itself and to determine the effects of therapeutic supplements, including adrenal cortex extract, growth hormone, vitamin B_1, vitamin B-complex, testosterone, liver extract, and insulin on the rate and degree of recovery. The studies made are approached from the standpoint of changes in the blood, organ and body weights, skeletal growth, food consumption, and metabolic rate.

Reduced caloric intake has been shown to result in atrophic and functional disturbances of the hypophysis, the resulting reduction in pituitary activity being followed by secondary effects in other endocrine glands and organs. In a review of the literature, Stephens (1) points out the histological and physiological evidence showing chronic inanition to be accompanied by structural and functional alterations of the pituitary. Both clinical and experimental data indicate atrophic and degenerative changes in the thyroid, adrenal cortex, gonads, and sex accessories associated with suppression of the anterior pituitary. It is in this association that Mulinos and Pomerantz (2) regard the state of chronic inanition as one

of "pseudohypophysectomy."

Vollmer (3) emphasizes that the atrophy of some of the endocrine organs in undernutrition is due less to the direct starvation of their tissues than to the failure of hypophyseal support. He states that even though the male rat continues to be underfed its genitalia are repaired by gonadotropin or testosterone. Trentin and Turner (4) found that the mammary gland responded to estrogen at low feed levels, but that increased amounts of estrogen were required at such levels because of the physiological suppression of the pituitary during underfeeding. Mulinos and Pomerants (5) were able to modify the effects of complete and chronic inanition in rats by means of pituitary implants. Thus a relationship between starvation syndromes and the endocrine glands seems well established.

Although histological changes in the endocrine glands occuring in experimental undernutrition appear to be corrected by refeeding, as shown by Stephens (6) and Jackson (7), there are hormone-influenced processes which recover slowly or incompletely. Jackson (8) points out that in rats, which have strong recuperation from malnutrition, a diet deficient only in calories may result in permanent dwarfing.

It may be that the changes which occur in undernutrition are expressions of a protective conservation mechanism to compensate for the reduced caloric intake, mediated through a limitation of pituitary activity; so that the therapeutic use of endocrine preparations would be contraindicated during actual caloric restriction. On the other hand, with the re-established alimentation of a recovery diet, it would seem reasonable to hypothesize the possibility of supporting the depressed glands with supplements until the apparently reversible changes resulting from underfeeding have been restored. There is no objective evidence to support either of these

points of view; and it is the purpose of this investigation to resolve the question.

Pituitary supplements have been tried on animals while being maintained in a semi-starvation state. Maline and Poserants (5) employed pituitary implants in adult rats in a state of chronic inanition during continued underfeeding with the result that the thymus was decreased, the testes were increased in size, the adrenal glands returned to normal weight, and the thyroid and spleen were unaffected.

Smith (9) made daily homeotransplants into hypophysectomized rats with the result that the decreased kidneys, adrenal cortex, thyroid, liver, spleen, and testes were restored or enlarged. The experimental animals which had failed to increase in body weight and which had ceased skeletal growth as a result of hypophysectomy were restored to normal or nearly normal condition as a result of the replacement therapy.

Some of the effects of pituitary extracts and growth preparations on normal animals have been determined by Kleiber and Cole (10), Le Roy et al (11), Bell and Cuthbertson (12), Schaffer and Lee (13), Freidgood (14), Bryon and Gaiser (15), Teel and Cushing (16), Hall and Selye (17), Handelsman and Gordon (18), Teel and Watkins (19), Korenchevsky (20), Duel at al (21), Riddle and Folhemus (22), and Lee and Freeman (23). The results obtained by most of these workers revealed a stimulation of appetite and growth, an enlargement of the adrenal glands, kidneys, and spleen, a decrease in the blood N.P.N. and metabolic rate, and an increase in the size and strength of the bones. Disagreement exists as to the effect on the thyroid, liver, and heart. The details of these results on normal animals as they apply to the results obtained on the animals under the conditions of this investigation will be elaborated upon in the discussion.

The foregoing constitutes the theoretical basis for the therapeutic use of hormones in general and of pituitary extracts in particular, as an aid to recovery from inanition. A commercial growth hormone extracted from the pituitary was employed in this experiment during recovery because: (1) it was the only available pituitary preparation for which physiological action was claimed by the manufacturers, (2) it was known to contain some thyreotropic and adrenotropic principles in addition to the growth hormone, (3) it was purified for clinical use and therefore was free from side effects, and (4) it has been found to exhibit protein conserving action according to reports by Draier (24), Gaebler and Bartlett (25), and Schaffer and Lee (13).

That there are other endocrine related effects of inanition for which other hormone supplements might prove beneficial is brought out in several investigations. An adrenal cortex relationship to the depressed carbohydrate absorption and metabolism typical of underfeeding has been shown by Chambers (26), Althausen and Stockholm (27), Dann and Chambers (28), Marassi (29), and Long, Katzin, and Fry (30). Hoskins and Freeman (31), Hartman (32), and Simpson, Dennison, and Korenchevsky (33) have reported appetite and growth stimulating properties for the adrenal cortical hormones. These effects, together with the atrophy of the adrenal glands in chronic inanition as already shown, constitute ample justification for the empirical use of a cortical extract in this experiment on recovery.

That there is a reduction in gonadotropin in chronic inanition and a consequent decrease in sex hormone secretion has already been shown. The atrophy of the testes and sex accessories in this connection suggests that injections of testosterone might make a substantial contri-

bution to the recovery of these organs. Further reasons for the use of testosterone in recovery from inanition is found in the work of Shay et al (34), Korenchevsky, Dennison, and Speyer (35), and Rubinstein and Solomon (36) who report growth and appetite stimulating effects for this hormone.

Insulin injections were employed during refeeding following chronic starvation for the express purpose of determining the effect of increased feed consumption on recovery. There was reason to expect the desired effect. E. R. Squibb & Sons (37) publishes a rather complete bibliography on insulin and non-diabetic malnutrition. Barnes and MacKay (38) and MacKay, et al (39) were able to produce a marked hyperalimentation in normal rats by administration of protamine zinc insulin. And Bodgatjan and Ostrowidoff (40) found that insulin stimulates appetite and increases the acidity, capacity, and emptying time of the stomach.

The clinical use of liver extract as a tonic in practically all types of convalescence and rehabilitation points to the possibility of its therapeutic use in recovery from inanition. The leucopenia frequently reported in both chronic and acute starvation and the leucocytosis reported by Powers and Murphy (41) from injections of liver extract, suggest benefits of liver to the blood in recovery. And Johnsen and Palmer (42) found a favorable effect of liver upon growth and appetite.

That there is a vitamin deficiency in most forms of undernutrition and that vitamin supplements constitute a rational therapy in recovery from inanition seems almost a priori; yet the use of vitamins in this way has been limited to the clinical field where observations were unsystematized, scanty, or lacking entirely. Keys, et al (43) administered vitamins orally in the form of Hexavitamin Tablets to 16 adult men recovering from experimental semi-starvation of 24 weeks' duration, but the

vitamin B_1 supplement here amounted to only 1 mg. per day. The animals of this experiment were brought to a state of chronic inanition by a diet which was inadequate in all respects, consequently the condition included and must have been in part caused by a vitamin deficiency. Vitamin B_1 and B complex were selected for recovery therapy in this experiment primarily because of the work of Sure, Kik, and Smith (44), who concluded that vitamin B has a growth stimulating effect unrelated to food intake, and that it also produces growth through stimulation of appetite and increased food intake.

THE BLOOD

It would be expected that the blood composition would in some way reflect the altered metabolism in starvation and recovery, yet there is only meager experimental data available on the effects of chronic inanition on the blood. Most of the investigations which have been made are old, unsystematic, fragmentary, difficult to classify with certainty under the chronic starvation category, and above all contradictory.

Regarding anemia, Minot (45) reports a slight secondary anemia with a reduced hemoglobin, but Jackson (8) states that the anemia of chronic starvation is deceptive due to blood redistribution and a diminution in hemoglobin, while Lazarus (47) reports that the results on anemia are uncertain.

Concerning the red cell count there is equal confusion. Kisseritzky (48) and Benedict (46) state that there is an increase in the number of red cells in chronic inanition which decrease upon refeeding, while Minot (45) and Jackson (8) found a reduction in the red cell count.

The density of the blood in chronic inanition marks another point of variance. Lust (49) states that the blood changes but slightly in

in water content, and Benedict (46) supports this theme by clearly stating that there is no material variation in the density of the blood. Jackson (8) reports that there is a decreased plasma protein in inanition causing a blood hydremia. The confusion reaches its climax with Kisser-etsky (48), who noted a concentration of the blood.

The disagreement appears also in studies made on the differential white count. Minot (45) reports a slight increase in lymphocytes and eosinophils in underfed humans, while Knollmark (50) noted a general but transient decrease in lymphocytes. Benedict (46) admits that the literature shows no agreement on the relative percentages of leucocyte types in chronic starvation, and Mergulis (51) simply states that the differential is variable.

There is the typical contradiction even on the number of white cells in chronic starvation. Minot (45) found the white count unaltered in humans after prolonged underfeeding, while Cabot (52) and others observed a leucocyte decrease to as low as 3,000.

Two recent studies agree with Cabot above on the effect of reduced calorie intake on the reduction in total leucocytes. There is also agreement on the differential, both reporting a reduction in granulocytes; but disagreement appears as to the dietary cause. Ershoff and Adams (53) attribute the leucopenia and agranulocytosis as due to a vitamin deficiency in the B group, and Wright and Skeggs (54) explain the cause in terms of protein deficiency.

The only study made on the effect of chronic starvation on rats was made by Miller, Friedman, and Benoi (55), who fed restricted diets of varying fat content to young rats for a period of 12 weeks and found that the plasma protein, hemoglobin, and hematocrit values corresponded

with those of normal rats of the same size rather than the same age.

The only systematic study which has been done concerning the blood in chronic inanition and recovery is the unpublished work of Keys et al (43) on semi-starved adult human males. It was found that a moderate anemia developed slowly during semi-starvation. In the first 12 weeks of rehabilitation the recovery in hemoglobin was very small, but in the subsequent period of 12 to 21 weeks it was more marked, with complete recovery being achieved by the 32nd week. During semi-starvation, the plasma protein decreased only a small amount and was nearly fully restored after 12 weeks of refeeding, but continued unrestricted feeding tended to diminish it again so that there was still a slight relative hyperproteinemia after 8 months of rehabilitation. In semi-starvation there was a progressive fall in blood sugar so that at the end of semi-starvation the sugar concentration was 63.39 mg. per 100 cc. or about 10 to 15 mg. below the normal average with the methods employed. During rehabilitation the blood sugar rose in close relation to the caloric intake. Other values determined by these authors are as follows:

	Hematocrit	R.B.C.	W.B.C.	Hemoglobin
Normal control	45.6			15.12
Starved 24 weeks	36.6	3.76	3,875	11.79
12 weeks recovery	39.5	4.17	5,398	12.76

The blood investigations made in this experiment on white rats is more comprehensive than that employed in the study above or in any previous studies. It includes observations not only on the red and white cell count, hematocrit, hemoglobin, plasma protein, and blood sugar, but also the specific gravity of the blood, the leucocyte differential, specific gravity of the plasma, and the blood N. P. N.

No study has been made on the blood in young growing animals during

recovery from chronic starvation.

ORGAN WEIGHTS

Since many of the significant physiological effects of inanition are reflected in the organ weights and since the weight of some of the endocrine glands constitute an index of the tropic action of hormones, it follows that such weight data would constitute a criterion of change for recovery and endocrine therapy.

The difference with which the organs respond to chronic inanition in young rats has been shown by other workers. Jackson (56) found that there was no significant change in the average weight of the brain, a slight increase in the weight of the liver and spleen, a 34% increase in the weight of the testes, and a weight loss of about 90% in the thymus. In another work, Jackson (8) states that the results on the spleen are variable, and that the pituitary may continue to increase in weight up to 33%. Stewart (57) found only a 5% increase in adrenal weight in an underfeeding period of 16 days, but continued underfeeding of 3 and 10 weeks resulted in weights 60% and 114% above normal respectively.

The weight response of organs to recovery from chronic inanition in young rats has also been investigated. Stewart (58) found the brain returned to normal, the kidneys were normal in proportion to body weight, the testes were at first subnormal but attained a normal weight before the adult body weight was reached, the liver was normal or above after 4 weeks, the thymus, though subnormal at 2 weeks, was 50%-70% above normal at 4 weeks, and after one month the adrenals showed a relative weight loss but continued feeding resulted in a normal weight relationship. Regarding the spleen, Jackson (8) states that refeeding after inanition restores the normal weight with a transient over-compensatory

regeneration being present.

In a more recent study on prolonged refeeding of underfed young male rats, Jackson (59) found that absolute weights were restored in only the brain, pituitary, kidneys, testes, and epididymides, while weight recovery in the skeleton, thymus, adrenals, heart, liver, and spleen were only relative. Refeeding to 60 weeks of age failed to overcome the dwarfing effect of the chronic underfeeding on the body weight.

THE METABOLIC RATE

Basal metabolism has had little study in chronic inanition. There is the question of whether an organism on restricted diet will adjust itself by reducing its basal metabolism as it does in acute starvation. Kleinperer (60), Svenson (61), and Magnus-Levy (62) in clinical observations hypothesized that there is such a metabolic adjustment to low caloric diets. Castaldi (63), as a war prisoner, speaks of "adaptation" to reduced diets. The experimental observations of Pashutin (64) on animals kept on reduced diets show a reduction in respiratory oxygen and carbon dioxide. Morgulis (51) concluded that under the condition of chronic inanition the basal metabolism passes through the same stages recognized in acute inanition.

No study has been made of the effect of chronic inanition and recovery on the metabolic rate in young animals. The only investigations on the metabolic rate in recovery from chronic inanition in adult animals are those of Morgulis (51) and Keys et al (43).

Morgulis performed his experiment using only one dog, the dog being its own control. The findings showed the metabolic rate increasing 100% over that of chronic inanition and 30% above normal after two weeks

of re-alimentation, and dropping to normal after five weeks. Keys et al (43) found that men underfed for 24 weeks used 36.5 cc. 0_2 per minute per square meter of body surface as compared with 122.2 cc. of the pre-starved control. After 12 weeks recovery the basal oxygen consumption was still considerably below the control; after 20 weeks it was slightly higher than the control; and after 32 weeks it was nearly the same as the control. The decline in B.M.R. as a result of starvation was 31%. The recovery was closely determined by the caloric intake. These workers did not find an increased metabolic rate in the initial stage of recovery as did Morgulis.

Benedict (46) points out that carbon dioxide is the end product of oxidation of all carbonaceous material, aside from those fragments of the protein molecule that are excreted in the urine. A measure of the carbon dioxide production furnishes, therefore, an estimate of total catabolism of considerable value. Inaccuracies in the determination of other respiratory and nitrogen factors are in part compensated for by the more accurate determinations of carbon dioxide. It was with these reasons in mind that the metabolic rates were calculated from carbon dioxide determinations in this experiment.

It is the purpose of this investigation to determine the effect of chronic inanition on the respiratory quotient and basal metabolic rate; and to determine the effect of realimentation upon the recovery of the normal metabolic rate. The only therapeutic supplement which was evaluated in this respect during recovery in Experiment I was the growth hormone.

SKELETAL GROWTH

An index of fundamental growth was considered important to the effect

of inanition and recovery, as well as a reliable end point for the growth action of the therapeutic supplements. Although the body weight provides some idea of growth, it may, as shown by Keys et al (43), to a great extent represent fat deposition rather than true growth.

The growth impulse in the skeleton of young animals is an irresistable force. Smith (65) points out that there is a retarded but continued growth of the skeleton during underfeeding. Winters, Smith, and Mendel (66) found that bones in animals stunted on low salt diets continue to increase in length regardless of a 30% to 40% lower weight than bones of animals stunted in other ways, shewing that the skeleton continues to grow even while being depleted of minerals. Morgulis (51) speaks of the work of Waters and Aron, who observed independently that though chronic underfeeding could keep animals at a constant body weight, it could not cause the cessation of skeletal growth.

In this experiment, the length of the femur was selected as a criterion of growth.

FOOD AND WATER ECONOMY

Perhaps the most obvious criterion for recovery from inanition is the body weight, although the establishment of normal weight does not indicate unmistakably that the organism is normal in every respect. Keys et al (43) found that weight recovery was greatest in high caloric diets, and that in all cases an undue proportion of regained weight was fat.

Thompson and Mendel (67) have shown that the weight gained by refeeding undernourished and stunted animals was greater in proportion to the food eaten than that gained by the controls. The weight gain of the starved mice was actually accomplished on a smaller intake of food.

Morgulis (51) states that the gross gain in weight may exceed the quantity of food consumed due to the fact that during re-alimentation the organism retains much water.

Other observations concerning water metabolism have been confined to the starvation period itself. According to Benedict (46), large amounts of water are lost during inanition, and starving subjects drink enormous volumes of water. He states that in fasting men there is considerable retention of this water by the body. He concludes that since there are no material variations in the density of the blood, other tissues must have the power of absorbing and retaining water. Keys, et al (43) also found the tissues of underfed men to become increasingly hydrated. They report that water intake more than doubled in semi-starvation. This was explained as due to the attempt to increase the bulk in the stomach or to the excessive diuresis which is typical of starvation.

The studies made in this experiment to determine the influence of food on inanition and recovery from inanition were approached in the following ways: (1) by determining the effect of starvation and refeeding on the metabolic rate, (2) by determining the effect of temperature on the daily weight ration during starvation, (3) by studying the course of recovery of body weight both as to the amount of food consumed and the time required, and (4) by determining the degree of absorption of food materials in underfed and normal rats by means of food-feces ratios.

There was reason to believe that food-feces ratios would throw light on the problem of food economy. It is conceivable, for instance, that a change in the ability of the digestive system to remove usable material from ingested food could contribute to body weight gain. Such

a change is at least suggested by Ficker (68), who speaks of the increased permeability of the epithelial lining of the digestive tract to microorganisms. Brooks, Marine, and Lambert (69) employed food-feces ratios to determine the efficiency of digestive functions in experimental obesity. Methods similar to those used by these workers were employed in this experiment.

METHODS

The studies made in this work were arranged in two separate experiments. In both experiments young male albino rats 30 days of age were employed in groups of ten, each group constituting an experimental unit. The rats were placed in individual cages and were underfed by restriction to a constant daily weight ration of a qualitatively adequate diet. At the end of the selected periods of underfeeding the animals were put upon full and adequate rations for various periods of time. During recovery the supplements were administered daily by intraperitoneal injection. At the close of the selected refeeding periods the animals were anesthetized by nembutal, exanguinated, and posted. In Experiment I each rat was underfed for 30 days and then refed for 35 days, receiving during the recovery a schedule of therapeutic injections as follows:

Group I	20 rat units	- "Antuitrin Growth" (Parke, Davis)
Group II	20 dog units	- "Adrenal Cortex Extract" (Upjohn)
Group III	5 mg.	- "Thiamine Hydrochloride" (Abbott)
Group IV	2 USP units	- "Solution Liver Extract" (Lederle)
Group V	½ ml.	- "Physiological Salt Solution" (Lilly)

In Experiment II each rat was underfed for 90 days and then refed 60 days, receiving during the recovery a schedule of therapeutic injections as follows:

Group VI	5 rat units	- "Antuitrin Growth" (Parke, Davis)
Group VII	1/10-5/10 units	- "Iletin Insulin" (Lilly)
Group VIII	.5 mg.	- "Methyl Testosterone" (Schering)
Group IX	1/5 ml.	- "Betalin Complex" (Lilly)

.5 mg. thiamin
.2 mg. riboflavin
.25 mg. pantothenic acid
.5 mg. pyridoxine

Group X ½ ml. - "Physiological Salt Solution (Lilly)

In addition to these groups of refed rats, two groups of fully fed controls were observed during recovery; one was the same weight as the underfed rats, and the other was the same age as the underfed rats.

In order to obtain data relevant to chronic inanition itself data covering all phases of this investigation were determined on a group of rats sacrificed at the end of the starvation period.

In order to determine the effect of temperature on the constant weight ration, a group of ten rats was removed during the final stage of inanition to a colder temperature which varied with the weather, while the remainder were left in the animal room maintained at a constant temperature of 75°F. plus or minus 2°. Food and weight loss data was determined on this group for a period of 16 days.

At the close of the 60-day refeeding period the animals of all the recovery groups were sacrificed. Blood was drawn from the heart with a "heparinized" syringe under nembutal anesthesia and determinations were made on the numbers of red and white cells, the specific gravity of the whole blood and plasma, the hemoglobin, hematocrit, and leucocyte differential.

After removing the blood as above, the organs and glands were dissected out and their fresh weights determined on an analytical balance. The larger organs were weighed in regular weighing vials, but the adrenal glands, pituitary, and the thymus and seminal vesicles of the small normal rats and the underfed rats were weighed in vials of fixative the tare weight of which had been predetermined. The femur was removed and measured for length by a vernier caliper.

In addition to the terminal data which was obtained from the blood at the close of the recovery period, there were also some progressive

data obtained in a separate series of animals without regard to recovery therapy. The gradual progressive changes in the total red and white cell count and in the specific gravity of the whole blood which occurred during underfeeding and recovery were determined by drawing blood from the tail of the animal at 7-day intervals. Progressive changes in the hematocrit, N. P. N. plasma protein, and blood sugar were determined by sacrificing 10 animals at the end of the underfeeding period and at refeeding intervals of 10, 20, 30, and 60 days, and drawing the blood for study from the heart with a "heparinized" syringe.

Daily food consumption was determined during the refeeding period by placing a given quantity in the cages and weighing the dry residue 24 hours later.

Food-feces ratios were obtained by determining food intake over a period of 5 days by weighing the dry residue in the cages of 10 control rats and of 10 experimental rats which had been underfed for 60 days. The dry weight of the feces was determined by collecting and desiccating the feces from both the control and experimental groups over this same period.

Water consumption was determined by recording the number of times cage bottles were refilled. Lines were marked on the bottles so that refilling represented a certain number of milliliters.

The animals were weighed daily in Experiment I and weekly in experiment II. All weights were taken at the same time each day. Periodic weighing at regular intervals was necessary during the underfeeding period to determine the constant weight ration.

The hematocrits were determined by centrifuging for one hour at 2500 R. P. M. with the blood in a Wintrope tube. Total white cell and red cell counts were determined by employing regular clinical dilution

pipettes, diluting fluids and hemocytometers. Hemoglobin was determined by the Sahli method. Differentials were made by Wright's method, with the direct microscopic count of 100 cells. The specific gravity of the whole blood and the plasma was determined by the copper sulfate method of Phillips et al (70). Plasma protein values were calculated by use of the nomogram of these authors. Blood N. P. N. was determined by nesslerization after digestion with an acid digestion mixture. Blood sugar was determined by the Folin-Wu method. All colorimetric determinations in connection with the above were made with a Klett colorimeter.

Sample metabolic rates were taken of the rats in a state of chronic inanition of 30 days duration, and after 10, 20, and 30 days refeeding. Similar determinations were made on other animals after a more prolonged underfeeding of 90 days, and at refeeding intervals of 2, 5, and 8 weeks. Determinations were also made on fully fed normal rats. A modification of the Haldane open-circuit was employed in making these metabolic studies. Air was drawn through the animal chamber at a rate of 2 liters a minute, after being made carbon dioxide and water free by passing it through moist soda lime, anhydrous calcium sulfate and activated alumina. Carbon dioxide was collected from the air leaving the animal chamber for a period of 2 to 4 hours by use of Ascarite. Water in the air was collected by the use of a Swartz tube immersed in a bath of dry ice and alcohol. The collecting tubes were weighed on an analytical balance to the nearest milligram, and the cage with animal (designed to include the urine and feces) was weighed on a torsion balance to the nearest approximate hundredth gram. Since Horst et al (71) have shown that oxygen uptake in the rat is abnormally high in the morning and in the late afternoon, these determinations were made between 10:00 A.M. and 4:00 P.M. And since Sherwood (72) reports that rats exhibit a marked diminution in metabolism during the summer

months, the experiments in metabolism were conducted during the winter. Food consumption data was being gathered on the rats while metabolism was being studied; so it is clear that the metabolic data collected during recovery are non-fasting. The metabolic rates are expressed as the number of calories liberated per square meter of body surface in 24 hours, the latter being determined by the following formula:

$$\text{square meters surface} = 9.1 \sqrt[3]{\text{weight of animal in grams}^2}$$

The calories were calculated from an average respiratory quotient based upon preliminary experiments, and from the amount of carbon dioxide released.

RESULTS

The numbers in the tables and points on the graph increments represent averages of a group of ten determinations on ten rats. The validity of the averages was determined by plotting the range and mean deviation. Only results that were found significant by this method are considered in the discussion.

The results of all determinations made on the blood are summarized in Tables 1 and 2.

Progressive changes in the red and white cell count and the specific gravity of the blood during chronic starvation and recovery are shown in Graphs 1 and 2.

The absolute and relative organ weights in chronic starvation, recovery, and recovery therapy are summarized in Tables 3 and 4, and the statistically valid effects are sketched in Tables 5 and 6.

Daily food rations necessary to maintain constant body weight during starvation are shown in Graph 3.

The body weight during the recovery of rats underfed for 30 and 90 days is compared in Graph 4.

Average body weights of the animal groups receiving experimental therapeutic treatment during recovery from inanition are shown in Graphs 5 and 6.

Food consumption and body weight of untreated rats recovering from chronic starvation are compared to normal rats of the same size in Graphs 7 and 8.

Alterations in the metabolic rate after periods of chronic starvation and recovery therapy are summarized in Table 7.

Changes in bone growth as indicated by femur length after periods of chronic starvation and recovery therapy are shown in Table 8.

The effect of variations in environmental temperature on the weight of rats in a state of chronic inanition is shown in Graph 9.

The results of recovery treatment on appetite, food utilization, weight gain, and water consumption are summarized in Tables 9 and 10.

The average total water consumption for underfed rats during a 30-day starvation period, including that indirectly consumed with the food, was 373 cc. The total amount of dry food allowed over this same period was about 150 grams. The ratio of food to water in the chronically starved rats accordingly is 1.0 : 2.5. The average total water consumption of normal fully fed rats including that consumed indirectly with the food over this same 30-day period was 984 cc. The total amount of dry food ingested by the normal rats during this time averaged 520 grams. The ratio of food to water in normal rats therefore is 1.0 : 1.9. Comparison of these two ratios make it clear that chronically starved rats consume more water in proportion to their food intake than normal fully fed rats.

Observation of 50 rats at the end of a 90-day period of chronic underfeeding revealed a marked erythema of the penis in 32 of the animals. This sign was taken as a symptom of a mild efferent urinary inflammation. At the end of the first week of recovery the symptoms had disappeared from 14 rats, and after two weeks refeeding the inflammation was absent in all but two rats. Obstruction of the urethra, cystitis, and death developed from one of these infections before recovery feeding was instituted.

Mortalities during underfeeding occurred in rats which showed a

very rapid weight loss. Although all rats in the early experiments were allowed an identical daily ration, there were some with an inherently greater energy demand and a vital calorie minimum which did not survive on the given amount of food. Rats that survived the first few days of restricted food intake usually lived throughout the selected starvation period. Other mortalities were probably due to pneumonia, since they usually followed in the wake of a cold night.

A wide range of individual differences in the differential leucocyte count appeared as a result of chronic inanition. This fact may make the averages below without significance:

Group	Eosinophils	Monocytes	Lymphocytes	Neutrophils
Starved	1	2	73	24
Refed 30 days	0	0	81	19
Normal control	1	0	81	18

The effects of the therapeutic injections employed in Experiment I on the blood sugar and N.P.N. were too slight to be significant. It was for this reason that similar determinations were not made in Experiment II. However, the values obtained are as follows:

Treatment	Blood Sugar	N.P.N.
Liver extract	140 mg./100 cc.	
Vitamin B_1	125 " "	
Saline control	134 " "	44 mg./100 cc.
Growth hormone	141 " "	49 " "
Cortex extract	127 "	
Fully fed control	149 "	52 "

The relationship between the weight of the dry food consumed and the weight of the dried feces is expressed in the data arranged below:

Experimental group	Average weight of dry feces per day	Average weight of food per day	Food-feces ratio
Starved	1.14 gm.	4.5 gm.	1.0 : 0.25
Normal	5.20 gm.	17.1 gm.	1.0 : 0.34

It is clear from these figures that the chronically starved rats removed more material from the ingested food than did the normal rats.

TABLE 1

Effects of Chronic Starvation and Recovery Therapy on the Total Erythrocytes, Total Leucocytes, Hemoglobin, Hematocrit, Specific Gravity of the Blood, and Specific Gravity of the Plasma of the Rat

Experimental group	Red cell number (no./cu./mm.)	White cell number (no./cu./mm.)	Hemo-globin (%)	Hemato-crit (%)	Specific gravity of blood	Specific gravity of plasma
Normal control (60 grams)	5,875,000	9,855	54.0	53-67	1.0471	1.0180
Underfed 90 days	8,557,000	5,405	68.3	42-58	1.0607	1.0213
B-Complex	8,796,000	17,010	82.0	45-55	1.0600	1.0225
Testosterone	8,788,000	11,600	84.0	46-54	1.0610	1.0226
Growth hormone	8,528,000	15,450	82.0	46-54	1.0580	1.0231
Insulin	8,202,000	12,370	75.0	44-56	1.0590	1.0226
Saline control	8,734,000	13,580	76.0	46-54	1.0600	1.0231
Mean	8,609,600	14,002	79.8	45-55	1.0594	1.0228
Normal control (6 months old)	8,920,000	20,129	82.0	47-53	1.0590	1.0230

TABLE 2

Effects of Chronic Starvation and Recovery on the Hematocrit, Specific Gravity, Non-protein Nitrogen, Plasma Protein, and Sugar of the Blood of the Rat

Experimental group	Hematocrit (%)	Specific gravity of blood	Non-protein nitrogen (mg./100 cc.)	Plasma protein (g./100 cc.)	Blood sugar (mg./100 cc.) Fasting	Non-fasting
Normal control (60 grams)	53-67	1.0471		3.77		
Underfed 90 days	41-59	1.0607	75.7	4.85	103.5	
Refed 10 days				5.12		
Refed 20 days	42-58		56.9	5.23	98.0	130.0
Refed 30 days	43-57			5.23	105.0	135.0
Refed 60 days	45-55	1.0594	38.4	5.40		
Normal control (6 months old)	47-53	1.0590	43.2	5.48	119.5	149.0

TABLE 5

Average Absolute Weights of the Organs of Rats which were Chronically Starved and then Refed and Injected with Therapeutic Supplements (Grams)

Experimental group	Pituitary	Adrenal	Brain	Spleen	Kidney	Testis	Epidydimides	Seminal Vesicles	Thymus	Heart	Liver
Underfed 30 days	.0028	.0100	1.351	.212	.530	.390	.164	.160	.041	.217	2.40
Refed 35 days											
Saline	.0116	.0163	2.295	.705	1.032	1.257	1.010	.473	.417	.777	8.70
Cortex	.0116	.0158	2.266	.734	1.148	1.256	.994	.405	.419	.908	10.22
Growth	.0116	.0246	2.219	.844	.966	1.103	.726	.334	.827	.782	9.87
Vitamin B_1	.0114	.0160	2.385	.727	1.163	1.212	.884	.457	.525	.861	11.28
Liver	.0112	.0165	2.386	.808	1.087	1.165	.846	.459	.450	.846	10.19
Normal control	.0139	.0205	2.403	1.355	1.164	1.354	1.200	.481	.370	1.025	11.65

Average Absolute Weights of the Organs of Rats which were Chronically Starved and then Refed and Injected with Therapeutic Supplements (Percentage weight on body weight)

Underfed 30 days	.0048	.0172	2.620	.362	.564	.669	.275	.266	.070	.370	4.10
Refed 35 days											
Saline	.0049	.0067	.960	.296	.434	.520	.425	.199	.175	.327	3.66
Cortex	.0047	.0063	.940	.297	.465	.510	.405	.164	.169	.368	4.14
Growth	.0049	.0103	.933	.352	.405	.460	.303	.140	.262	.329	4.15
Vitamin B_1	.0044	.0062	.930	.281	.450	.470	.341	.178	.220	.333	4.36
Liver	.0045	.0074	.965	.326	.438	.470	.341	.185	.182	.340	4.10
Normal control	.0047	.0070	.815	.460	.398	.460	.410	.164	.147	.350	3.96

TABLE 4

Average Absolute Weights of the Organs of Rats which were Chronically Starved and then Refed and Injected with Therapeutic Supplements (Grams)

Experimental group	Kidney	Adrenal	Thymus	Brain	Testis	Seminal vesicle	Spleen	Liver	Pituitary	Heart
Underfed 90 days	.334	.0094	.026	1.41	1.325	.015	.203	2.07	.0030	.20
Refed 60 days										
Growth	1.026	.0225	.381	1.72	1.054	.472	.679	10.00	.0101	.83
B-Complex	1.282	.0173	.475	1.82	1.369	.568	.775	13.70	.0108	1.11
Testosterone	1.282	.0139	.172	1.69	.821	1.122	.609	11.10	.0085	.95
Insulin	1.264	.0168	.435	1.85	1.342	.572	.702	12.60	.0108	.98
Saline	1.308	.0165	.411	1.80	1.287	.548	.726	12.50	.0100	.93
Normal control (same weight)	.347	.0080	.179	1.34	.156	.052	.899	2.65	.0021	.28
Normal control (same age)	1.075	.0201	.309	1.80	1.304	.529	.962	12.60	.0119	1.06

Average Absolute Weights of the Organs of Rats which were Chronically Starved and then Refed and Injected with Therapeutic Supplements (Percentage weight on body weight)

Underfed 90 days	.605	.0170	.0472	2.560	.590	.027	.363	3.76	.0054	.362
Refed 60 days										
Growth	.451	.0096	.1506	1.724	.428	.192	.284	4.18	.0043	.349
B-Complex	.411	.0056	.1535	.593	.446	.183	.247	4.42	.0035	.354
Testosterone	.532	.0059	.0725	.715	.350	.581	.256	4.56	.0032	.381
Insulin	.448	.0057	.1539	.658	.474	.203	.249	4.48	.0034	.346
Saline	.482	.0062	.1519	.673	.480	.201	.273	4.62	.0037	.343
Normal control (same weight)	.650	.0150	.3360	2.522	.291	.059	.562	4.95	.0039	.523
Normal control (same age)	.454	.0067	.1187	.605	.439	.177	.328	4.22	.0041	.355

TABLE 5

The Effect of Injections During Recovery on Organ Weights of Rats Chronically Underfed for 30 Days and Refed for 35 Days

Organ	Cortex Extract	Growth Hormone	Vitamin B_1	Liver Extract
Brain	no effect	no effect	no effect	no effect
Spleen	no effect	significantly hypergonic	no effect	no effect
Kidney	no effect	significantly hypogonic	no effect	no effect
Testes	no effect	slightly hypogonic	no effect	no effect
Epididymis	no effect	significantly hypogonic	no effect	no effect
Thymus	no effect	slightly hypergonic	no effect	no effect
Heart	no effect	no effect	no effect	no effect
Liver	no effect	no effect	no effect	no effect
Seminal vesicles	no effect	significantly hypogonic	no effect	no effect
Pituitary	no effect	no effect	no effect	no effect
Adrenal	no effect	significantly hypergonic	no effect	slightly hypergonic

TABLE 6

The Effect of Injections During Recovery on Organ Weights of Rats Chronically Starved for 90 Days and Refed for 60 Days

Organ	Growth	B-Complex	Testosterone	Insulin
Brain	no effect	no effect	no effect	no effect
Spleen	no effect	no effect	no effect	no effect
Kidney	no effect	no effect	significantly hypergonic	no effect
Testes	no effect	no effect	significantly hypogonic	no effect
Thymus	no effect	no effect	significantly hypogonic	no effect
Heart	no effect	no effect	no effect	no effect
Liver	no effect	no effect	no effect	no effect
Seminal vesicles	no effect	no effect	significantly hypergonic	no effect
Pituitary	no effect	no effect	no effect	no effect
Adrenal	significantly hypergonic	no effect	no effect	no effect

TABLE 7

Effect of Various Periods of Refeeding and Recovery Therapy on the Metabolic Rate of the Rat

(Calories per 24 hours per square meter body surface)

[1]Experimental group	Refed 10 days	Refed 20 days	Refed 30 days
Saline control	1710	1375	1330
Growth hormone	1395	1260	1150

[1] Before refeeding, these rats had an average metabolic rate of 814 as a result of a 30-day chronic starvation period.

[2]Experimental group	Refed 2 weeks	Refed 5 weeks	Refed 8 weeks
Saline control	1765	1532	1280
Growth hormone	1577	1417	1275
B-Complex	1720	1512	1301
Insulin	1635	1450	1370
Testosterone	1402	1475	1355

[2] Before refeeding, these rats had an average metabolic rate of 720 as a result of a 90-day chronic starvation period.

The average metabolic rate of fully-fed normal rats was 1162.

TABLE 8

Effect of Recovery Supplements on the Length of the Femur of the Rat after Chronic Starvation and Recovery

(Underfed for 30 days and refed for 35 days)

Experimental group	Length of femur, cm.
Normal at 60 grams	1.79
Underfed	2.12
Vitamin B_1	3.07
Growth hormone	3.04
Cortex extract	3.07
Liver extract	3.07
Saline control	2.98
Normal control	3.26

(Underfed for 90 days and refed for 60 days)

Experimental group	Length of femur, cm.
Normal at 60 grams	1.79
Underfed	2.68
B-Complex	3.32
Growth hormone	3.20
Testosterone	3.18
Insulin	3.29
Saline control	3.25
Normal control	3.47

TABLE 9

Effect of Therapeutic Injections on the Weight Gain, Appetite, Food Utilisation, and Water Consumption of Rats Chronically Starved for 30 Days and Refed for 35 Days

Experimental group	Initial weight (grams)	Final weight (grams)	Weight gain (grams)	Per cent gain	Per cent recovery (of normal)	Food consumption (grams)	*Per cent of food utilization	Water consumption (ml.)
Cortex extract	58.5	246.8	188.3	321.8	84.0	627.7	30.0	780.3
Saline control	58.5	238.3	179.8	307.0	81.0	628.8	28.6	817.8
Vitamin B_1	57.9	258.5	200.6	346.0	88.0	699.8	29.5	888.5
Growth hormone	58.6	238.2	179.6	306.8	81.0	587.4	30.6	841.3
Liver extract	58.2	248.6	190.4	327.2	84.5	621.7	30.6	936.3
Mean recovery	58.3	246.1	187.7	321.8	83.7	633.1	29.9	852.8
Fully-fed control (same age)	191.8	294.5	102.7	53.6		773.1	13.1	1,191.6
Fully-fed control (same weight)	60.5	195.2	134.7	222.2	66.3	576.0	23.4	

* $\frac{\text{Grams increase in weight}}{\text{Grams food consumed}}$

TABLE 10

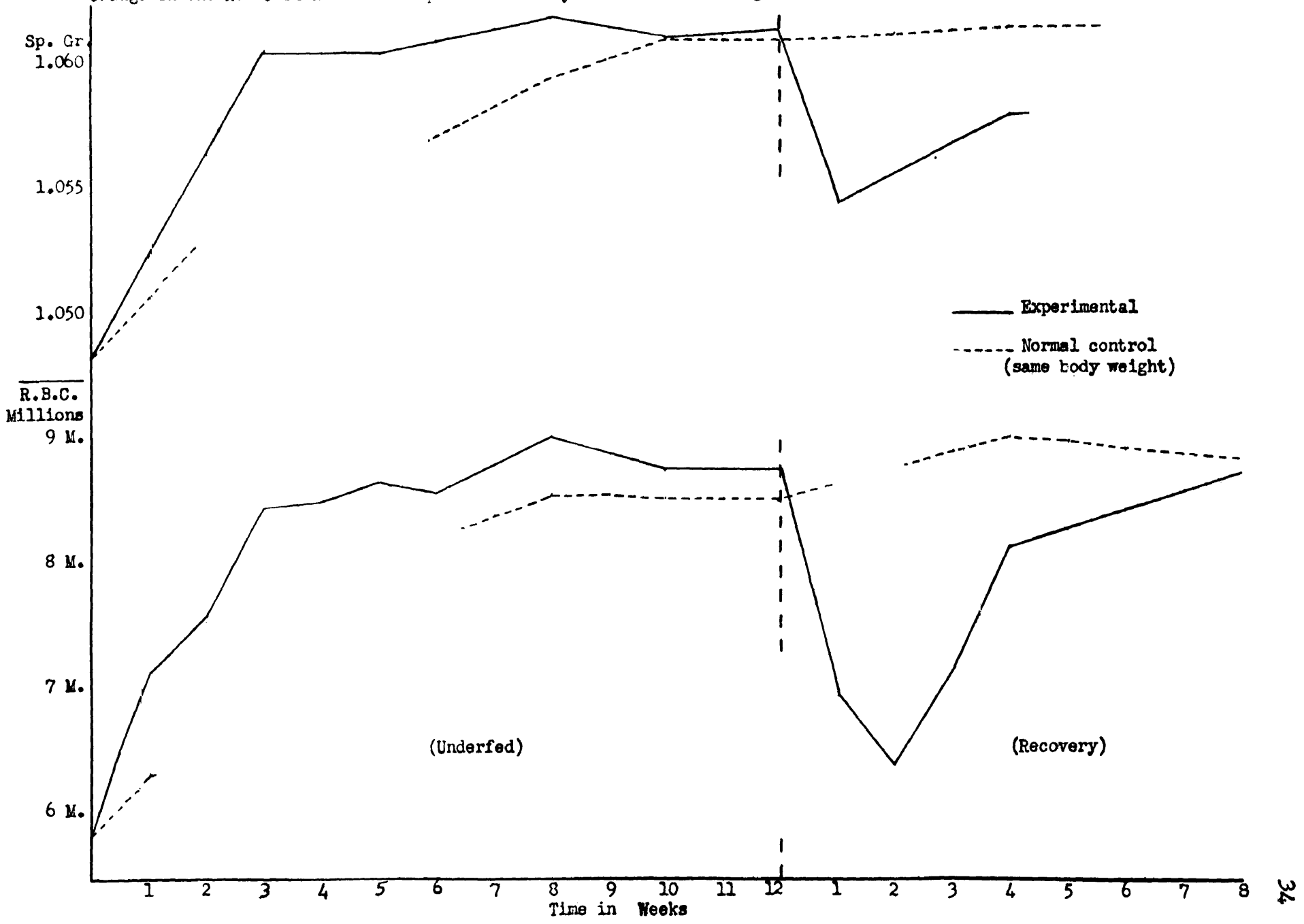
Effect of Therapeutic Injections on the Weight Gain, Appetite, Food Utilization, and Water Consumption of Rats Chronically Starved for 90 Days and Refed for 60 Days

Experimental group	Initial weight (grams)	Final weight (grams)	Weight gain (grams)	Per cent gain	Per cent recovery (of normal)	Food consumption (grams)	*Per cent of food utilization	Water consumption (ml.)
Growth hormone	59.5	237.8	178.3	300.0	66.9	858.9	20.4	1,665.0
B-Complex	59.9	309.8	249.9	411.0	87.5	1,148.7	21.8	2,244.0
Testosterone	59.0	237.6	178.6	301.0	66.9	930.8	19.1	1,654.0
Insulin	60.9	281.8	220.9	362.0	80.0	960.4	22.9	1,901.0
Saline control	62.4	268.4	206.0	330.0	75.5	929.6	22.2	1,705.0
Mean recovery	60.3	267.1	206.7	340.8	76.4	965.7	21.3	1,854.0
Fully-fed control	191.8	355.6	163.7	84.2		1,360.6	16.0	2,366.5

* $\frac{\text{Grams increase in weight}}{\text{Grams food consumed}}$

Graph 1

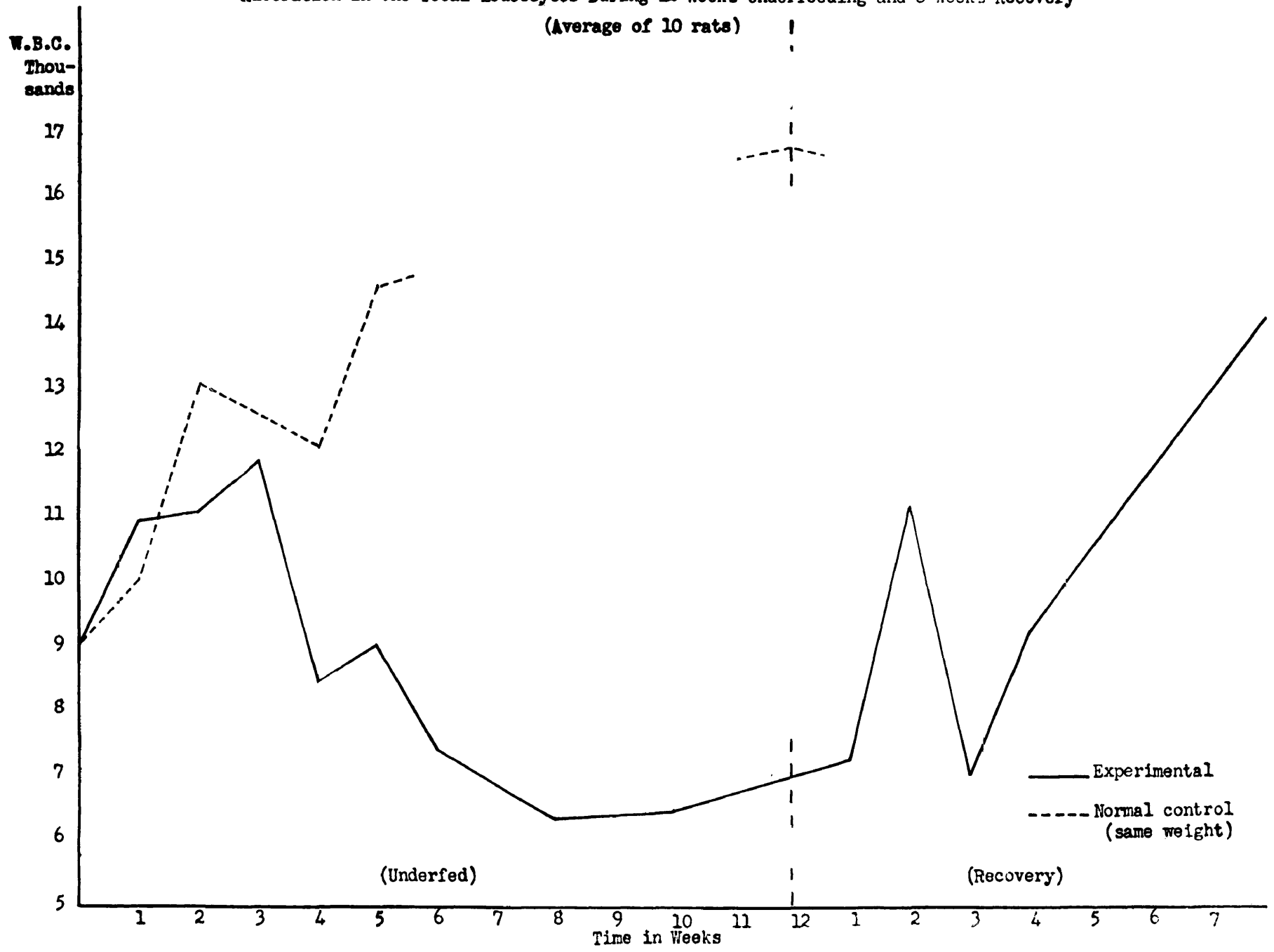
Change in the Red Cell Number and Specific Gravity of the Blood During 12 Weeks Underfeeding and 8 Weeks Recovery



Graph 2

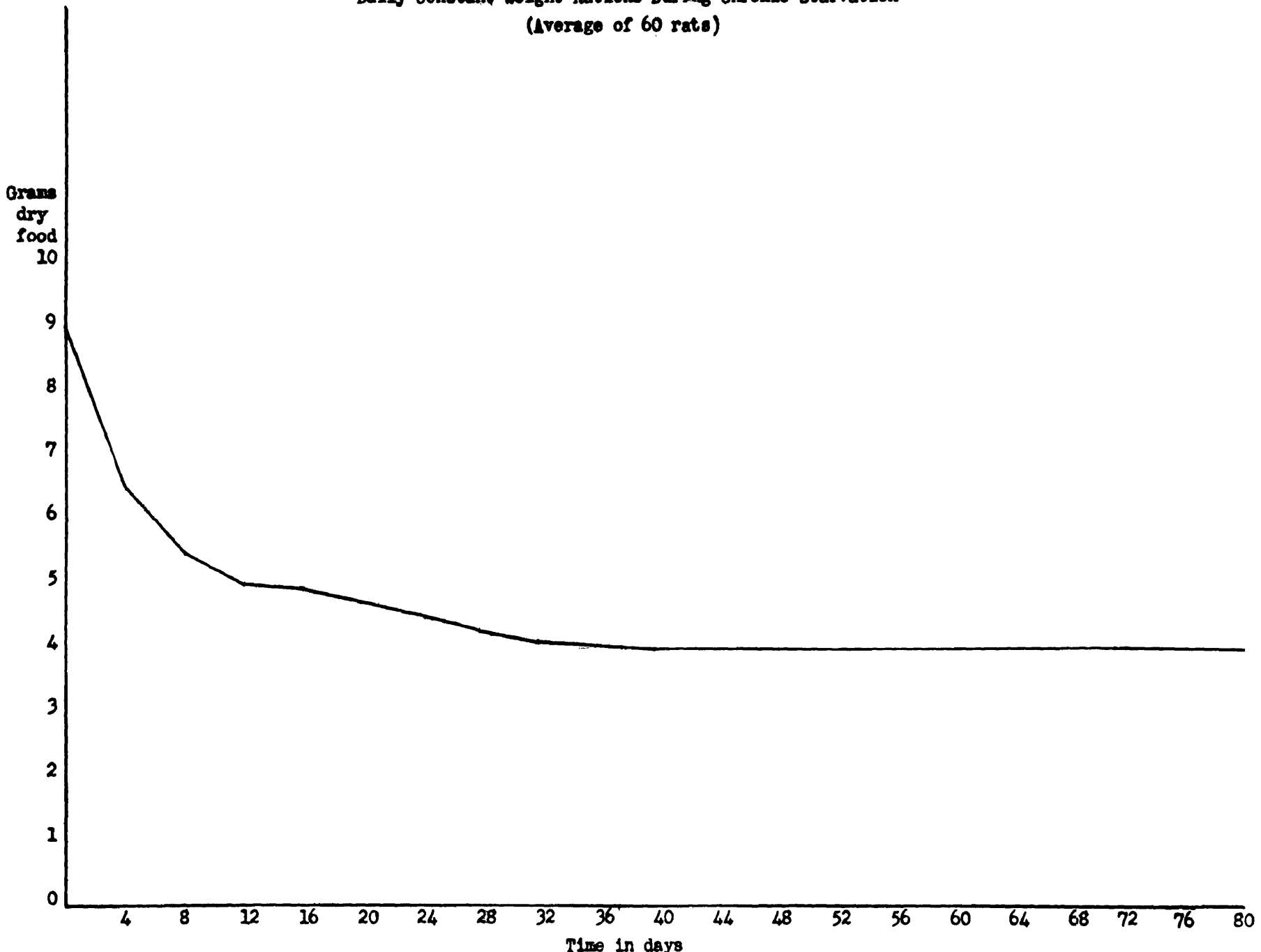
Alteration in the Total Leucocytes During 12 Weeks Underfeeding and 8 Weeks Recovery

(Average of 10 rats)



Graph 3

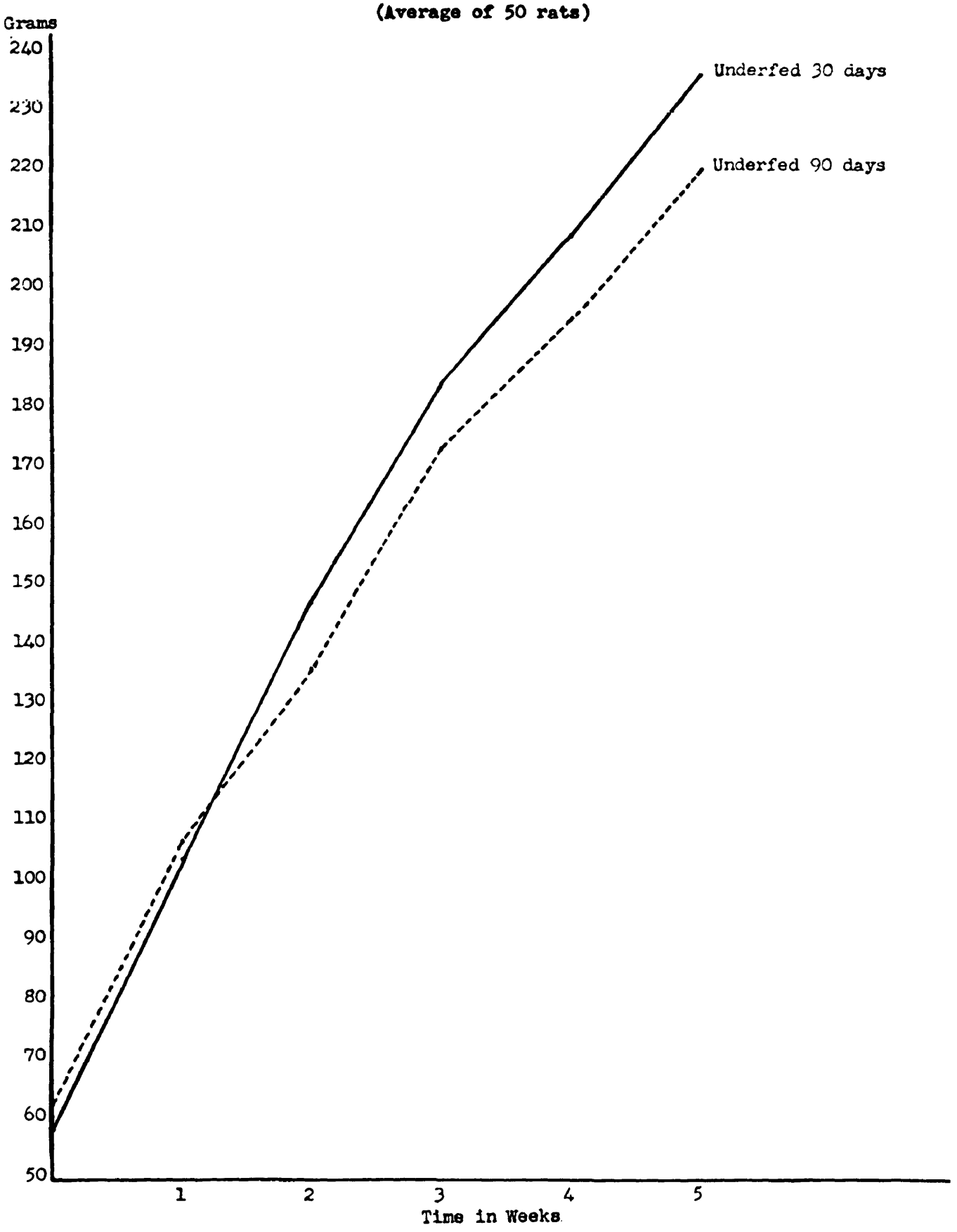
Daily Constant Weight Rations During Chronic Starvation
(Average of 60 rats)



Graph 4

Comparison of Body Weight During Recovery of Rats Underfed for 30 and 90 Days

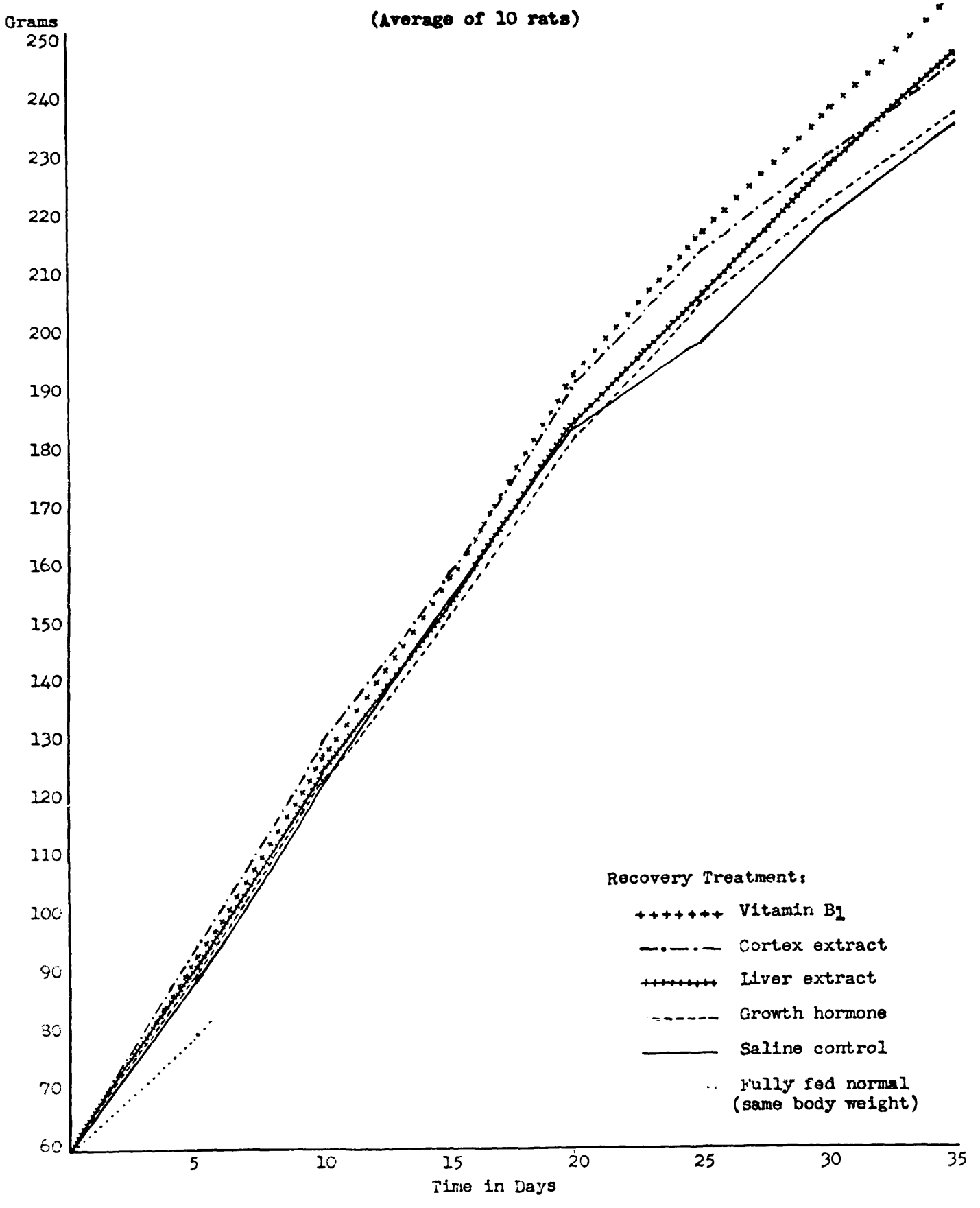
(Average of 50 rats)



Graph 5

Daily Body Weight During Recovery of Rat Groups Underfed for 30 Days

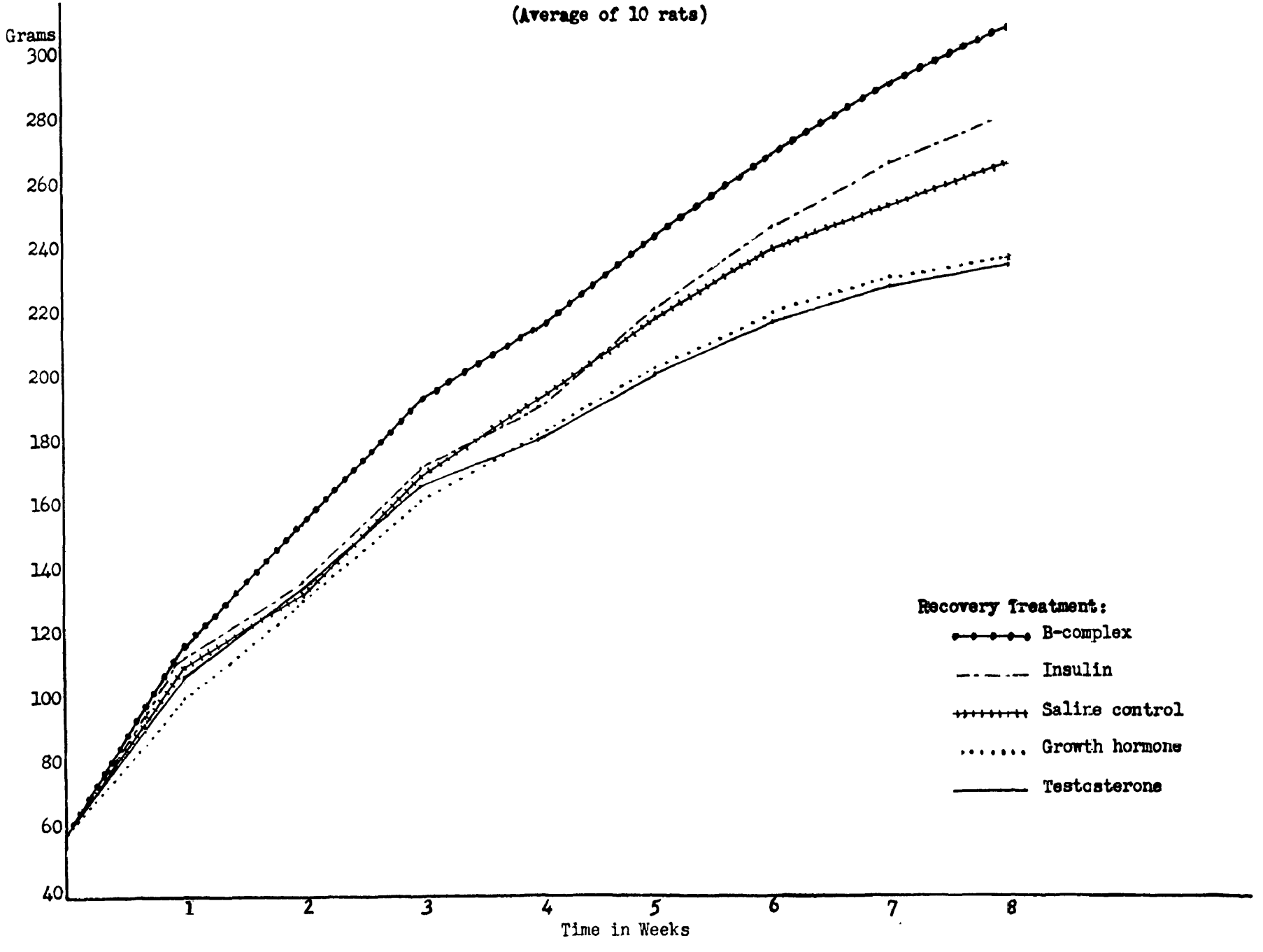
(Average of 10 rats)



Graph 6

Daily Body Weight During Recovery of Rat Groups Underfed for 90 Days

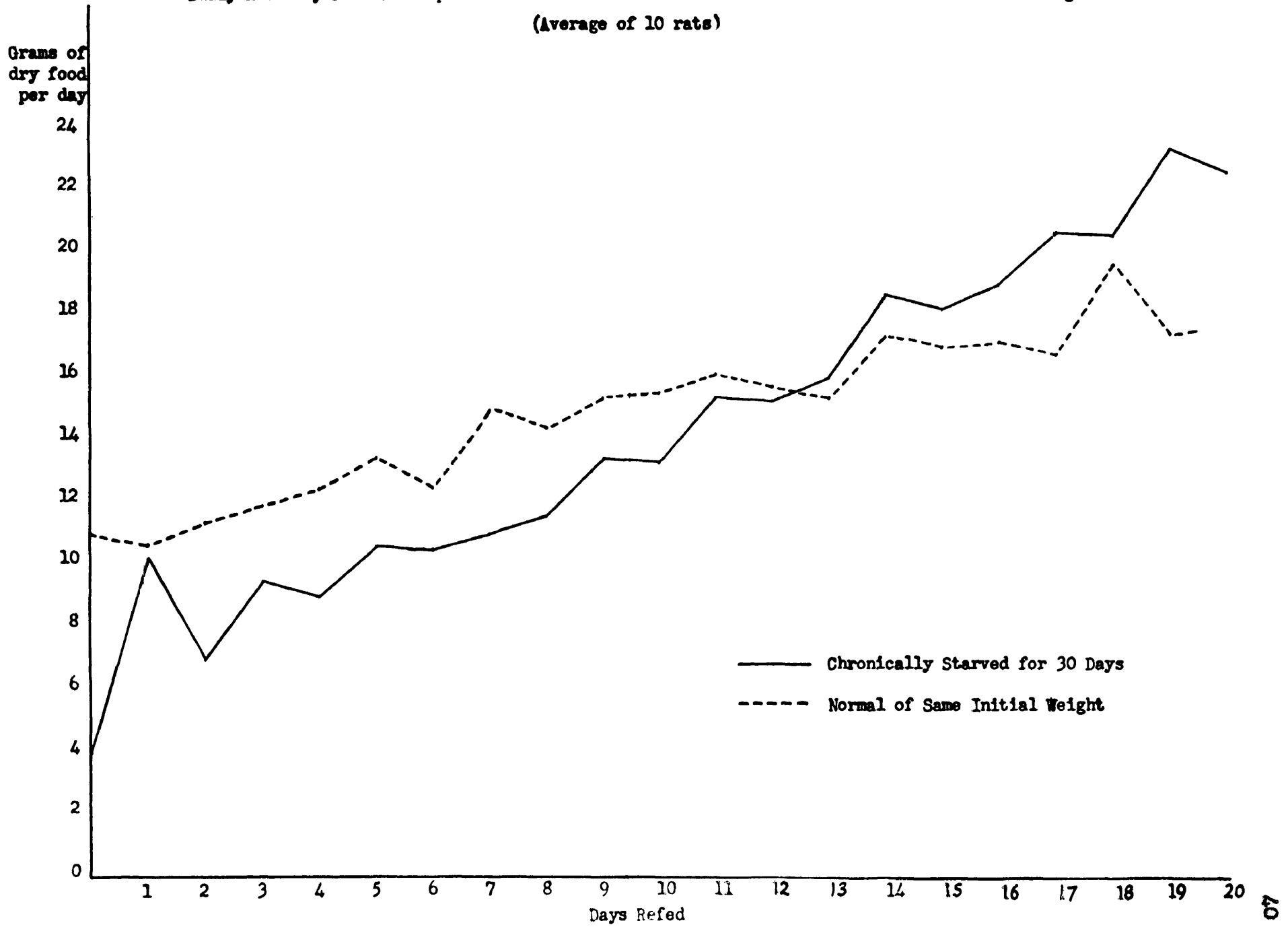
(Average of 10 rats)



Graph 7

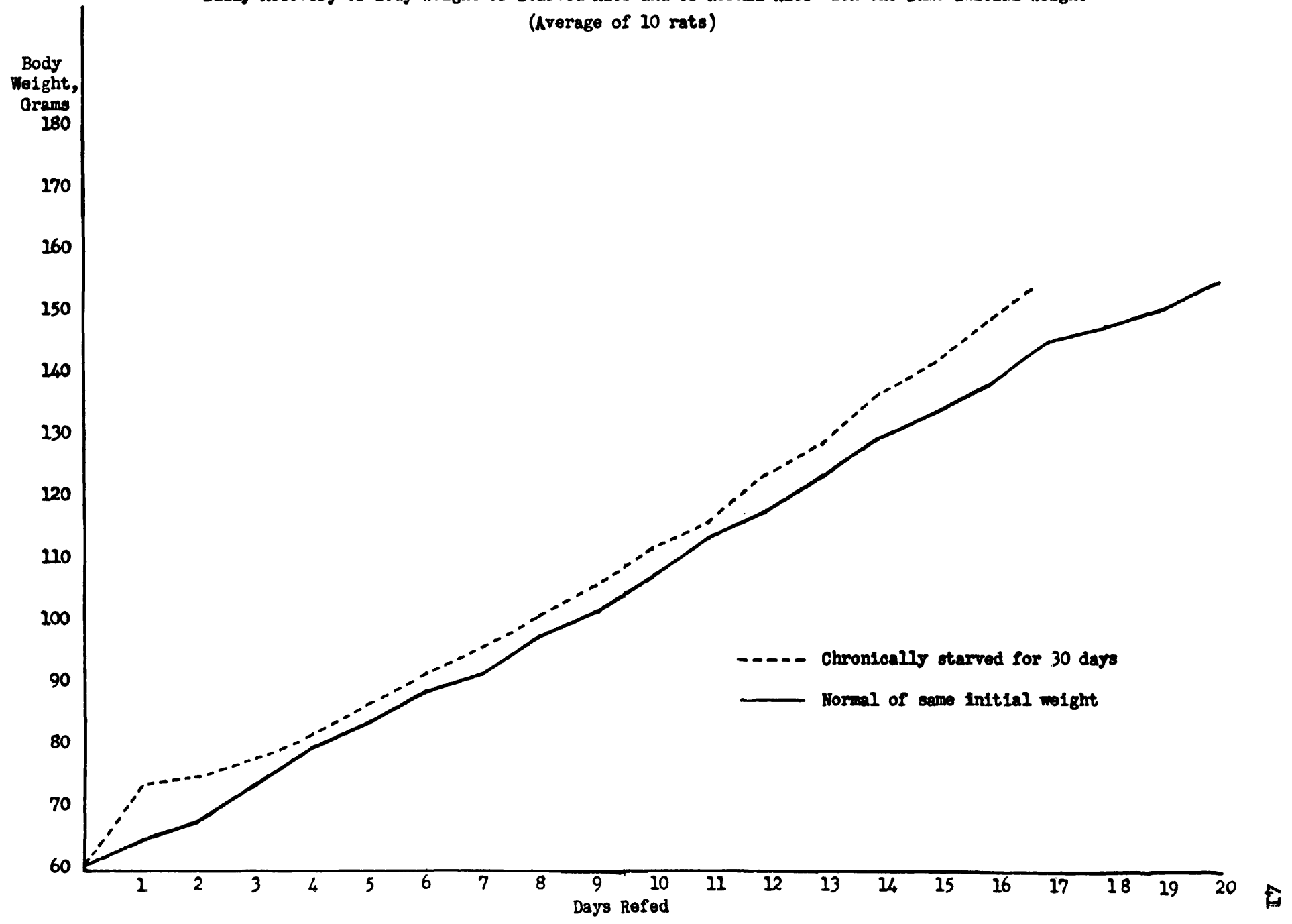
Daily Recovery Food Consumption of Starved Rats and of Normal Rats with the Same Initial Weight

(Average of 10 rats)



Graph 8

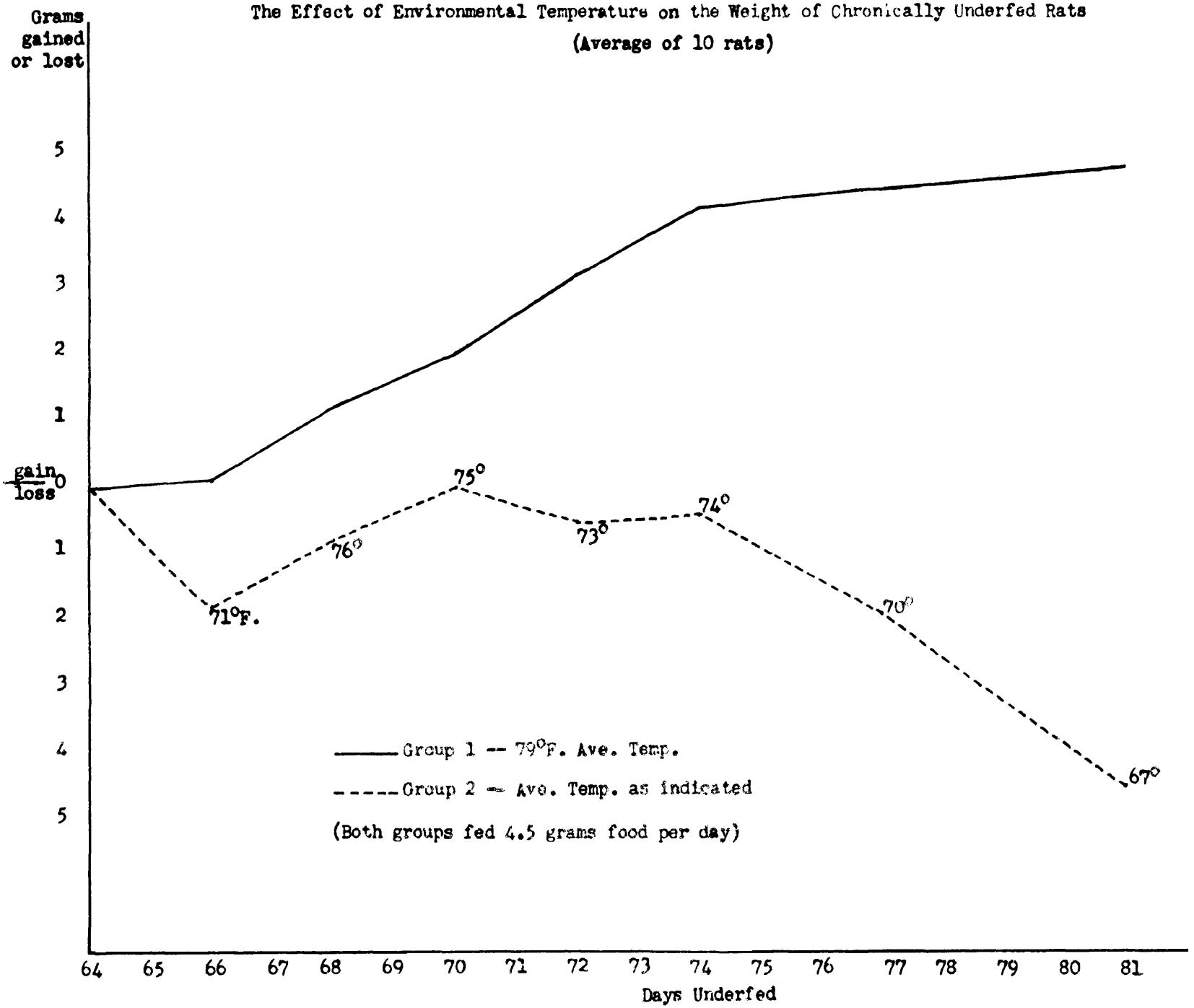
Daily Recovery of Body Weight of Starved Rats and of Normal Rats with the Same Initial Weight
(Average of 10 rats)



Graph 9

The Effect of Environmental Temperature on the Weight of Chronically Underfed Rats

(Average of 10 rats)



DISCUSSION

BLOOD

The Effects of Chronic Starvation on the Blood. The plasma protein, hemoglobin, and hematocrit values of the young underfed rats were higher than in normal rats of the same weight and lower than in normal rats of the same age; indicating that these features of the blood continue to increase with age during caloric restriction, although at a much reduced rate. These results are at variance with those of Miller, Friedman, and Desai (55) who considered the blood of underfed rats similar to that of rats of the same weight, and interpreted the blood values as a function of size.

The increase in the red cell count and the specific gravity of the blood during underfeeding is evidence of an adaptive function, the mechanism of which is either a direct stimulation of erythrocyte proliferation or a withdrawal of water from the blood producing a hemo-concentration. Examination of all the blood data points to a modification of the latter as the logical interpretation. The fact that the erythrocyte count and the specific gravity of the blood obtained from the tail was increased during underfeeding, while the hematocrit, plasma protein, and hemoglobin of the blood drawn from the heart were decreased; indicates a redistribution of the blood constituents to the effect of a peripheral hemo-concentration. It is difficult in view of these results to understand the "diminished peripheral circulation" and "deceptive anemia" postulated by Jackson (8). The difference between the erythrocyte count and specific gravity of the blood of the experimental rats and the normal rats becomes less marked as underfeeding is continued, showing that the adaptive

capacity wanes if inanition is prolonged.

The initial increase in total red cells is in agreement with Benedict (46) and Kisseritzky (48), but is in apparent disagreement with Minot (45), Jackson (8), and Keys, et al (43), all of whom found a reduction in red cells in chronic inanition. This difference probably arises from the fact that the investigators with whom we are at variance made their measurements after very prolonged periods of underfeeding. Since the curve of red cell increase in the underfed rats of this experiment had flattened by the end of the starvation period of 12 weeks, it is conceivable that continued inanition would have caused the curve to recede to points representing a definite red cell decrease in harmony with the above reports. The increased erythrocyte count reported by Minot (45) and Jackson (8) is probably the same transient adaptive increase as found in the underfed animals of this experiment.

Since the blood upon which hemoglobin determinations were made was taken from the heart it may be that the anemia found in the underfed rats was a deceptive one due to the peripheral hemo-concentration. This appears unlikely in view of the anemia reported by Minot (45), Jackson (8), Benedict (46), and Keys, et al (43) on blood derived from peripheral sources.

The specific gravity of the whole blood of the underfed rats was considerably increased initially but at the end of the starvation period it was only slightly above normal. Since these changes were accompanied by parallel changes in the red cell counts it is concluded that it is the variation in the numbers of red cells which caused the variations in the specific gravity. These results are in agreement with Benedict (46) who states that there is no material variation in the density of the blood

as a result of starvation, and with Lust (49) who found that the blood in chronic malnutrition changes but slightly in water content; but in sharp contrast with observations of a blood hydremia reported by Jackson (8) and Keys, et al (43).

One might have expected a blood hydremia on the basis of the increased water intake of the underfed rats. Benedict (46) noted that starving subjects drink enormous volumes of water and Keys, et al (43) state that water intake more than doubles in semi-starvation. However, since the density of the blood did not change it must be that there was a retention of water by tissues other than the blood or an increased loss of water for metabolic and excretory purposes.

A gradual reduction in total leucocytes appeared during underfeeding in this experiment. This result is in agreement with similar investigations by Cabot (52), Ershoff and Adams (53), Wright and Skeggs (54), and Keys, et al (43). Only Minot (45) found the white count unaltered after prolonged underfeeding. No measurements or observations were made in this investigation which explains the mechanism of this leucocyte reduction. Morgulis (51) states that the quickness of the onset of leucopenia shows that it cannot be due to a failure of new cells entering the blood stream but to a migration of leucocytes into the intestinal wall and lumen. However, in this experiment on young semi-starved rats no such sudden leucopenia occurred. Consequently, if the suddenness of leucocyte decrease in inanition constitutes the proof of leucocyte migration, then this experiment does not corroborate such an interpretation.

A review of the raw data of this investigation leads to the general conclusion of Morgulis (51) who states that the differential leucocyte count is variable during inanition. The average values tend to support

Knollmark's (50) finding of a transient decrease in lymphocytes. The slight increase in lymphocytes and eosinophils reported by Minot (45) and the decrease in granulocytes reported by Ershoff and Adams (53) and Wright and Skeggs (54) were not observed in the young underfed rats of this investigation.

It would be expected that acute starvation would increase the blood N.P.N. because of the ultimate catabolism of body proteins as a source of energy. There remains the question, however, of the effect of a semi-starvation in which enough food was provided to meet all energy demands except growth. The excessive protein metabolism indicated by the high N.P.N. in these underfed rats was not due exclusively to the metabolism of body proteins since enough carbohydrates and fats were permitted in a daily ration to prevent progressive weight loss by utilization of body tissues for energy.

Schaffer and Lee (13) conclude that the growth hormone decreases N.P.N. and is therefore a stimulant of protein anabolism. Teel and Cushing (16) and Teel and Watkins (19) found a similar reduction in N.P.N. as a result of injection of an extract of the anterior pituitary. Brailer (24) reported a decrease in nitrogen excretion after hypophysectomy. It is clear from the work of these men that the pituitary gland and more specifically, the growth hormone, has a profound effect on nitrogen metabolism. Since, as Mulinos and Pomerantz (2) have shown, chronic malnutrition creates a condition of pseudo-hypophysectomy it may be that the high N.P.N. in the underfed rats of this experiment is directly explicable as a result of a functional alteration of the pituitary. It is not made clear in this work or others whether the pituitary secretions, which are depressed in inanition, actually stimulate protein anabolism or merely inhibit

protein catabolism. The only other possible cause for such a high N.P.N. would be that mentioned by Gray (73), a relatively small available fluid in comparison to the nitrogen to be excreted. It is unlikely that the latter explanation is valid, however, since underfed animals use copious amounts of water, and according to Keys, et al (43) have an excessive diuresis.

It would have been surprising to have found a low blood sugar in animals as vivacious as were the underfed rats of this experiment, and no such reduction in blood sugar appeared. This result is in disagreement with a similar type of starvation conducted on adult human males by Keys, et al (43) in which a progressive fall in blood sugar was observed. The age and maturity of these human subjects as contrasted with the young immature rats used in this experiment may account for the difference in results.

The Effects of Refeeding on the Blood. Recovery of the plasma protein, hemoglobin, and hematocrit values was nearly complete after 60 days refeeding. However, comparable periods of underfeeding and recovery employed by Keys, et al (43) on adult men did not result in complete recovery of the corpuscular volume and hemoglobin, but the normal plasma protein value was nearly restored. It may be that recovery of the blood occurs more readily in young than in adult animals.

A marked hydremia similar to that observed by both Rosenstern (74) and Jacksen (8) occurred during early refeeding. This recovery hydremia was transient and is interpreted as due to the relatively rapid regeneration of the plasma. At the end of the 60-day refeeding period the specific gravity of the blood was nearly normal.

After 60 days' refeeding the blood N.P.N. was restored to the normal

range, indicating that the phase of pituitary function associated with protein metabolism had fully recovered.

The total erythrocytes, total leucocytes, and leucocyte differential were normal at the end of the 60-day recovery period.

The Effect of Therapeutic Supplements on the Blood During Recovery. No consistently significant benefits were seen in any feature of the blood as a result of the injection supplements employed during recovery.

ORGAN WEIGHTS

The Effect of Chronic Starvation on Organ Weights. The effects of chronic inanition on the relative organ weights as determined by this experiment were for the most part the same as those found by Jackson (56). There was no change in the average weight of the brain, an increase in the weight of the testes and pituitary, and a decrease in the weight of the thymus and spleen. The effects on the spleen and thymus as shown by these studies on rats are in accord with those observed on children by Stefko (75). Disagreement appears regarding only the kidneys and liver, Jackson (56) recording a slight increase in the weights of these organs, and this experiment showing no significant change in the kidneys and a decrease in the liver. While the difference in results on kidney weights is likely due to the age of compared controls, that on the liver is more difficult to explain. The reduced liver weights of this experiment do agree with those found by Stefko (75) in underfed children. The weight of the adrenal glands in the underfed rats was in nearly the same ratio to the body weight as that of normal rats of the same size, but is increased and consequently in accord with Stewart (57) when compared with controls of the same age. Since the relative weight of the adrenals decreases with age in normal animals, this latter comparison is logical. The relative weight of the heart is also reduced with body growth so that when compared with controls of the same age there was no significant change as a result of starvation. On the other hand, the weight of the heart was relatively less as a result of underfeeding when compared with normal controls of the same size. Stefko (75) observed a similar reduction in heart size in children suffering from chronic malnutrition.

Consideration to the age and size of the control rats does not otherwise alter the general effects of underfeeding as already stated, except in the case of the brain. The brain of an animal starved in any way at any age always constitutes a greater percent of the body weight than it does in a normal animal of the same age.

Since the weights of the kidneys, brain, pituitary, adrenal glands, and testes in chronic starvation were in normal or greater than normal proportion to the body weight, it is concluded that these organs are "preserved" or "protected" during underfeeding in young rats. Probably the growth impulse of these organs overbalances the effects of caloric restriction.

The thymus, seminal vesicles, spleen, and heart, on the other hand, sacrifice their growth and contribute their substance in a proportion greater than other organs and body tissues. The reduction of the thymus is probably a case of vitamin involution, since Stoerk and Zucker (76) found that in otherwise adequate diets, partial deficiency of vitamins of the B complex depressed the thymus weight. Sure (77) also concludes that in B_1 deficiency there is atrophy of the thymus. Reduction of the seminal vesicles in the chronic starvation of these rats is due to the diminished male sex hormone in connection with the inhibited testes. Mulinos and Pomerantz (78) employed this interpretation to similar results in adult rats.

The relatively greater weight of the testes and pituitary at the end of the underfeeding period shows that these organs continued to grow slowly during starvation in spite of the suspended growth of the body and other organs. In view of the depressing effects of chronic inanition on the pituitary gland of adult rats, as shown by Mulinos and Pomerantz (2), and more specifically in view of the disturbed protein

metabolism and reduced metabolic rates which occurred in the underfed rats of this investigation, one might have expected the pituitary to have had relatively less weight than the body. This is particularly true if consideration is given to the atrophic histological picture of the pituitary in chronic inanition in young rats described by Jacksen (7). However, an enlarged gland is not necessarily an active or over-active gland, as seen in the familiar response of the thyroid to endemic iodine deficiency; and the relative hypertrophy of the pituitary in the starved rats of this study may be just as indicative of a disturbed and inhibited function as if the gland had been reduced in size and weight.

The fact that the testes exhibited a marked weight gain in the presence of caloric restriction strongly suggests that the growth impulse in these organs was not independent of pituitary support. It may be, therefore, that the pituitary of young rats in chronic inanition continues to secrete a physiologically effective though reduced amount of gonadotropic hormone. A further evidence of gonadotropic secretion may be found in the fact that in over 200 young male rats, chronically starved from 30 days' age, there were only two instances of an undescended testis.

Another possible instance of continued pituitary support is in the case of the adrenotropic hormone. While chronic inanition in adult rats is accompanied by an adrenal atrophy, as shown by Mulinos and Pomerants (5), a marked adrenal hypertrophy occurred in the young rats of this investigation when compared with normal animals of the same age. It cannot be argued that the growth impulse prevents the adrenal atrophy in young rats, because the relative weight of the adrenal glands decreases with normal body growth.

The increases in the relative weights of the pituitary and adrenal

glands during chronic underfeeding may be attributed to the lack of vitamin B_1 in the reduced daily ration. This explanation is proposed in view of the work of Sure (77) who found that B_1 deficiency caused a marked hypertrophy of both of these organs.

The Effects of Refeeding on Organ Weights. The results of refeeding on the weights of organs corroborate those of Stewart (58), with normal recovery of the brain, kidneys, and testes. There is also agreement on the relative recovery hypertrophy of the thymus and liver. The relative hypertrophy of the spleen in recovery as observed by Jackson (8), however, is not confirmed by this experiment. Disagreement on this point is probably due to the tremendous individual variation in the weight of the spleen, and resolution would require repeating the condition with great numbers of individuals in both the experimental and control groups.

The recovery of the relative weights of the pituitary and adrenal glands as reported by Jackson (59) in prolonged refeeding was observed in this experiment in rats underfed for 30 days, but was not observed in those underfed for 90 days. It appears therefore that the extent to which these two organs are restored depends upon the length of the undernutrition period.

While most of the effects of chronic inanition on young male rats are reversible, it is clear that these animals are not free from the stigma of the underfed condition. The effect on the weights of the kidneys, brain, testes, seminal vesicles, liver, and heart appear to be completely reversible, since these organs regain their absolute weight. The effects on the body weight, body growth, the weight of the adrenal glands, thymus, spleen, and pituitary are, on the other hand, only

partially reversible since the recovery of these values was either lacking entirely or only relative to the partially recovered body weight.

The Effect of Growth Hormone on Organ Weights. An increase in the weight of the adrenal glands was effected by both small and large injections of growth hormone during recovery. This was probably due to traces of adrenotropic hormone which were known to be present in the preparation employed. Friedgood (14) and Hall and Selye (17) obtained similar results with growth extracts on normal animals. The hypogonic effect on the adrenals obtained by Kliober and Cole (19) with Evans' growth preparation may have been due to the very long period over which injections were made.

Large injections of growth hormone during recovery produced enlargement of the spleen. This result is interpreted as indicating a "splenotropic" action on the part of the growth hormone used. Friedgood (14) also noted an increase in the weight of the spleen in normal guinea pigs treated with an alkaline pituitary extract. However, using a similar extract, Riddle and Pelhemus (22) found no significant change in the spleen of young pigeons.

The growth hormone in large doses decreased the weight of the testes and sex accessories. This effect is interpreted as due to the inhibiting action on the pituitary gland of the growth hormone in general, and of the adrenocorticotropic hormone in particular. It is axiomatic that hormone supplements inhibit the gland which normally produced the hormone; and Koneff (83) has recently shown that the adrenocorticotropic hormone inhibits the anterior pituitary. Korenchevsky (20) obtained a similar reduction in testes and sex accessories in normal animals with an alkaline pituitary extract.

A decrease in the weight of the kidneys resulted from the large injections of growth hormone. No measurements taken in this experiment and no published studies of a related nature made it possible to interpret this effect. In view of the results it can only be stated that the growth preparation used has an "anti-renotropic" action. This effect of the growth hormone on young rats recovering from inanition is in contrast with the kidney enlargement obtained with a crude pituitary extract on normal animals by Hall and Selye (17).

No significant alteration in either the heart or liver appeared as a result of growth hormone administration. Other pituitary preparations have produced effects on normal animals which vary on either side of those obtained in this experiment. For example, extracts used by Lee and Freeman (23) and by Riddle and Folkhemus (22) produced a liver hypertrophy, while those employed by Klieber and Cole (10) and by LeRoy et al (11) resulted in liver reduction. Similarly, Hall and Selye (17) report an enlargement of the heart with their extract, while Klieber and Cole (10) found a reduction in heart size using Evan's extract. These disagreements undoubtedly arise from differences in the extracts, methods, and conditions employed.

While the growth hormone in heavy doses tended to increase and restore the normal weight of the spleen, it at the same time decreased the weights of the kidneys, testes, and sex accessories, and produced an abnormal hypertrophy of the adrenal glands. These results make it clear that large amounts of growth preparations are not indicated in recovery from chronic inanition. Since light injections of growth hormone restored the normal weight of the adrenal glands without producing undesirable alterations in other organs, it may be that small amounts of preparations containing the adrenotropic hormone would be useful in effecting an earlier cortical recovery.

The Effect of Testosterone on Organ Weights. The testes were decreased in weight as a result of injections of testosterone during recovery. This effect was probably mediated through the inhibiting action of testosterone on the gonadotropic function of the pituitary gland. The same interpretation has been proposed by Selye and Friedman (79) and Shay et al (34) for a similar effect of testosterone on normal animals. There is also the possibility that the depressing action of testosterone injections on the testes may have been due to the oil vehicle rather than to the hormone itself. Cameron, Guthrie, and Carmichael (80) found that daily injections of peanut oil for 17 or 18 days caused testicular atrophy and a decrease in rate of growth in the rat.

Testosterone administration produced a marked enlargement of the seminal vesicles; and since as stated above the testes themselves were inhibited by testosterone, it must be that the effect was due to the stimulating action of the hormone directly on the seminal vesicles.

A marked atrophy of the thymus occurred in rats treated with testosterone during refeeding. This effect was to be expected on the basis of the thymic involution which occurs normally in animals after puberty. Korenchevsky et al (35) obtained a similar reduction of the thymus in normal animals injected with testosterone.

Rats receiving supplements of testosterone during recovery exhibited a significant increase in the weight of the kidneys. This action has also been observed on normal animals by Korenchevsky et al (35), MacKay (81), and Luden et al (82). The mechanism and significance of this effect remains obscure.

The use of testosterone during recovery was attended with no measureable advantages, while presumably undesirable effects were abnormal hypertrophy

of the sex accessories, excessive thymic involution, and depression of the testes. It may be concluded, therefore, that the administration of the male sex hormone in the amounts employed in this experiment is without benefit if not actually contraindicated.

The Effects of Other Treatments on Organ Weights. Since the effects of the adrenal cortex extract on the thymus, seminal vesicles, and adrenal glands were very slight, the results are interpreted to be without significance. There may be in these small changes, however, a further evidence of the proposed adrenal-gonad relationship.

The cortex extract, vitamin B_1, liver extract, B complex, and insulin produced no alterations in the weights of the organs which would justify either their use or disuse in recovery from inanition.

FOOD AND BODY WEIGHT

The Effect of Chronic Starvation on Food Consumption and Body Weight. The fact that the animals being brought to a state of chronic inanition required less food to maintain body weight as the underfeeding was continued must be explained in terms of an adaptation or acclimitization. Two measurements were taken in this experiment which explain the probable mechanism here. For instance, it was found that chronic starvation resulted in an increased absorption efficiency on the part of the digestive tract. It was also found that starvation gradually effected a reduction in basal metabolic rate. Both of these effects would improve the food economy of the animal, the former actually providing more energy for the tissues, the latter reducing the basal energy demand.

The relatively increased water consumption of the underfed rats is in agreement with Benedict (46) who found that starving subjects drink enormous volumes of water, and with Keys et al (43) who found that water intake more

than doubled in semi-starved men. According to Benedict (46) there is a considerable retention of water by the body, and since there is no material variation in the density of the blood other tissues must have the power of absorbing and retaining water. Keys et al (43) interpret the increased water intake as due to the animals' attempt to increase the bulk in their stomach or to the excessive diuresis typical of starvation. The various treatments used during refeeding did not significantly alter the amount of water consumed.

Anorexia did not develop in any of the starved animals; food, upon realimentation, was accepted with vigor. No deleterious effects of ingestion of large amounts of food on the first day of recovery were noted in these rats, such as were observed by Benedict (46) and Keys et al (43) on humans. The decreased food intake on the second day may indicate some reaction or illness of the animals or may have been simply an appetite reduction due to the indulgence of the first day. The latter is the more likely explanation since it was observed that in both normal and refed rats there was a tendency for food consumption to alternate between high and low on successive days.

The Effect of Refeeding on Food Consumption and Body Weight. Upon refeeding, the rats which had been starved exhibited a remarkably rapid rate of growth. This rapid growth of animals whose bodies had been stunted by suspended growth may be emphasized by comparison with the growth rate found by Bryan and Gaiser (84) in normal animals fed a special growth ration. These workers found that normal rats fed on Wendel's special growth diet required 38 days to grow from 60 to 200 grams. The chronically starved rats of this experiment required only 22 and 30 days to grow through this identical range of body weight after being starved 30 and 90 days respectively.

Kopec and Latyszeuski (85) interpreted this increased growth rate of underfed animals as indicating a stimulating effect of starvation on growth. Jackson (8) states that the rapid growth of the body following periods of inanition may be due to the embryonic condition to which the cells are reduced. The extremely high metabolic rates found in this experiment during recovery may well support this concept of embryonic cell growth.

When refed, the rats which had been chronically starved showed a greater efficiency in food utilization than normal animals. Results similar to this have been obtained on mice by Thompson and Mendel (67) who concluded that the weight gained by stunted mice was greater in proportion to the food eaten than that gained by the controls. This food efficiency effect may be due to a lag effect of the reduced metabolism and increased intestinal absorption which were incurred during starvation. The reduced energy demand of a metabolic lag must have been very brief, however, because the data obtained in this experiment indicates that as soon as growth was resumed the metabolic rate was increased to values far above normal. The increased absorption of food in the starved rats as shown by the food-feces ratio suggests either a decrease in peristalsis or an increase in the permeability of the epithelial lining of the digestive tract, yet there remains the question of just how long this reduced peristalsis or enhanced permeability persists once realimentation is begun.

The recovery of the body weight in this experiment was, of course, somewhat proportional to the duration of refeeding. Prolonged inanition increased the time required for weight recovery, however. Animals underfed for 90 days required three times as long a period to reach a weight 75% of that of normal rats of the same age as did animals underfed for only 30 days. The mean weight of rats starved for 30 days and refed for 35 days was 236 grams, while that of rats starved for 90 days and refed for 35 days

was only 220 grams. In other words, after equal periods of refeeding the rats which had been held in a state of chronic inanition for a prolonged period recovered considerably less in body weight than rats which had been underfed for only a short time. It may be concluded, therefore, that while short periods of semi-starvation stimulate growth, prolonged starvation tends to nullify this effect. Whether the difference in growth rate following short and long periods of underfeeding is a reflection of the difference in the age of the animals, or whether it is a reflection of fundamental tissue changes associated with the malnutrition has not been determined.

The Effect of Liver Extract and Adrenal Cortex Extract on Food Consumption and Body Weight During Refeeding. Since the food consumption of recovery rats injected with adrenal cortex extract and with liver extract was the same or less than that of saline control rats, it must be concluded that the slight weight increase of these two groups over the controls was independent of appetite and therefore due to a growth factor in the case of each.

The growth action which was elicited by the liver extract was probably due to the presence of vitamin B and G both of which according to Solomon and Guerrant (86) and Graham and Griffith (87) play an important role in the physiological mechanism controlling the utilization of food. The appetite stimulating effect of liver extract as postulated by Johnson and Palmer (41) was not observed in these animals.

Rats treated with adrenal cortex extract during refeeding gained weight more rapidly than untreated controls. This action was marked until the 25th day after which the curve of effect dropped sharply, indicating that the animals became refractory to the extract after about 25 injections.

Treatment with liver extract during recovery produced a weight gain which was greater than the controls. The effect, though slight, was manifested throughout the entire refeeding period.

The results of the cortex extract in recovery is in harmony with the work of Hoskins and Freeman (31) who were able to effect a weight gain in human subjects following the use of a commercial preparation of adrenal cortex. The effect of the cortex extract in promoting growth is probably due to the "anabolic" function of the cortical hormones postulated by Simpson, Dennison, and Kornchevsky (33). Lockwood and Hartman (38) suggest the cortical extract aids in the utilization of vitamin C and B_1. The "anabolic" function proposed by Simpson et al (33) and supported by the results of this experiment may be in part due to this effect on vitamin utilization. The appetite stimulating action which Hartman (32) claimed for the cortex extract was not observed in these animals recovering from chronic inanition.

It is concluded that both adrenal cortex and liver extracts facilitated growth and food utilization during refeeding and hence promoted the recovery from chronic inanition.

The Effect of Vitamin Supplements on Food Consumption and Body Weight During Refeeding. The food consumption of recovery rats receiving supplements of vitamin B_1 and B complex was greater than that of control animals; and since the increased weight gain in these groups was nearly proportional to the amount of food consumed, it must be concluded that the increased recovery in weight was mediated through the appetite. This conclusion does not in itself eliminate the possibility of a specific growth property as reported by Sure, Kik, and Smith (44); it simply means that the only effect which could be measured in the course of maximum appetite and maximum growth was that of food consumption. The appetite and growth stimulating effect of vitamin B administration was consistant and marked throughout the recovery periods. The increased body weight brought about by vitamin B therapy was not restricted to fat deposition because, first, an unusual amount of fat was

not observed in the animals of this group during posting and, secondly, some of the organs (kidneys, thymus, liver, and heart) of the rats receiving vitamins in both experiments were increased in absolute weight over those of the saline injected controls.

In using small daily supplements of all of the principal vitamins, Keys et al (43) found no improvement in appetite, metabolic rate, or weight in human male adults recovering from semi-starvation. There was actually a relative retardation in overcoming anemia and a slight but consistant decrease in weight gain. The absence of positive results in this case may be due to the fact that prophylactic dosages were employed in an obviously therapeutic situation. In this connection Morris (89) points out that weight recovery may be the function of the quantity of vitamin B, since it was produced by increasing the dosage of B_1 sources by amounts considerably above those necessary to cure or protect against polyneuritis. The positive results obtained with B vitamins in this experiment with rats recovering from chronic inanition is undoubtedly due to the large amounts which were employed.

The Effect of Growth Hormone on Food Consumption and Body Weight During Refeeding. The growth hormone in large amounts caused the recovery rats to use considerably less food than the controls. Yet the animals receiving the hormone maintained a body weight gain equal to the controls. It is clear, therefore, that the growth hormone in the large quantities inhibits appetite but at the same time conserves weight. In terms of the work of Teel and Cushing (16), and Teel and Watkins (19), and Schaffer and Lee (13) this weight conserving action of the growth preparation is due to its protein sparing action.

The decrease in appetite which accompanied both small and large doses of growth hormone is difficult to explain in view of the opposite results

obtained by Teel and Cushing (16) and by LeRoy et al (11) with pituitary extracts. The latter investigators, however, did find that the initial appetite stimulation was followed later by appetite inhibition. Nevertheless it is clear that the refed, growth hormone injection rats of this experiment were able to maintain a weight gain and growth equal to the controls while consuming significantly less food. The fact that the growth hormone did not cause a greater weight gain than the controls is understandable in terms of the decreased food consumption, a possible inhibitory effect on the pituitary, or the animal's having become refractory to the growth principle during the early periods of recovery. On this last point, Handelsman and Gordon (18) observed that while weight increase was typical in 175-275 gram rats injected with a pituitary extract, such increase was less marked in lighter rats and completely missing in those under 100 grams. For this reason it may be that the growth injections given during early recovery only served to render the animals refractory to any possible later effects. It may also be that the increased growth following the use of various growth preparations by Duel et al (21), Schaffer (13), Kleiber and Cole (10), and Bryon and Gaiser (15) was not observed in the refed rats of this experiment due to the fact that so far as the pituitary and the growth hormone were concerned the growth rate was already at a maximum. The negative results obtained with the growth hormone in this experiment is not without parallel in other investigations. LeRoy et al (11) found that the last half of a 12-14 week injection period employing a pituitary growth extract resulted in a depression of growth, and Korenchevsky (20) was unable to obtain any decided influence on growth in rats injected with a pituitary extract.

When small amounts of growth hormone were injected, weight gain was not only less than in the saline controls but was less than would have been

expected on the basis of the reduced appetite. This result is interpreted as due to pituitary inhibition. Such an interpretation is supported by the fact that the metabolic rate was depressed by the growth hormone injections.

The Effect of Insulin on Food Consumption and Body Weight During Refeeding. The action of insulin in increasing the weight gain in the rats recovering from inanition is to be associated with the increased food consumption. Barns and MacKay (38) and MacKay et al (39) report a hyperalimentation in normal rats upon the administration of insulin, and Bogdantjan and Ostrowidoff (40) state that insulin stimulates the appetite in non-diabetics and that its action on the vagus increases stomach acidity and stomach capacity. The appetite stimulating effect did not appear in this experiment until the number of units injected had been increased over that initially employed.

The Effect of Testosterone on Food Consumption and Body Weight During Refeeding. While the testosterone injections had little or no effect on food consumption, there was a decrease in body weight gain. This result is in disagreement with the greater than normal weight curve obtained by Shay et al (34) on young male rats treated with one mg. testosterone three times weekly, and with the appetite stimulating action obtained by Korenchevsky et al (35) from testicular extracts from human urine. The work of Cameron et al (80) opens the question of whether the decrease in growth rate in this experiment was due to the action of the hormone itself or to the oil vehicle in which the hormone was prepared and marketed. The effect was probably due to pituitary inhibition. Such an interpretation is not only supported by the other evidences of pituitary inhibition in this experiment wherever testosterone was employed but also by work on normal and castrate rats by Rubenstein and Solomon (90) and Selye and Friedman (79). However,

in dealing with very small amounts of testosterone (50 gammas) in castrated rats, Rubenstein and Solomon (36) speak of a "probably significant" growth effect attributed to an inherent growth stimulating quality which strikes specifically at the accessory sex organs and more generally at other somatic tissue. These workers also mention a possible stimulating effect of testosterone on the growth function of the pituitary. There remains thus the possibility that smaller doses of testosterone than those employed in this experiment might facilitate recovery from inanition.

SKELETAL GROWTH

The bones in the rats continued to grow throughout the period of chronic starvation, although at a reduced rate. This continued growth of the skeleton during the suppression of body weight gain by caloric restriction is in agreement with the well known studies of Smith (65), Winters, Smith, and Mendel (66) and with the classical review of Morgulis (51).

Upon refeeding, the bones of the chronically starved rats did not regain normal size. This result confirms a similar observation made by Jackson and Stewart (91). It appears that chronic starvation produces a fundamental irreversable change in the mechanism of bone growth resulting in a degree of permanent skeletal dwarfing.

None of the therapeutic supplements employed during recovery in this investigation produced any consistent increase in bone growth. The bone growth promoting properties found by Bell and Cuthbertson (12) in a crude alkaline extract of the anterior pituitary was not observed in the commercial growth hormone employed in this experiment.

METABOLIC RATE

The Effect of Chronic Starvation on Metabolic Rate. The explanation for the decreased metabolism in chronic inanition must lie, in part, in

thyroid depression. Rabinovitch (92) noted an absence of proliferation, small acini, and a solid colloid in young guinea pigs underfed for only 10 days. The thyroid is probably inhibited because of the retarded pituitary thyreotropic secretion. There is also the probability of a direct effect of pituitary depression in causing reduced metabolism. According to Riddle et al (93) the anterior pituitary contains two hormones which affect the rate of oxygen consumption, one of which, prolactin, unlike the thyreotropic hormone does not act through the thyroid.

The ultimate cause of reduced metabolism in inanition may be traced to the specific deficiency of vitamin B. Vilma Sa (94) noted that in the course of B_1 avitaminosis the O_2 consumption drops below the normal value, and that in the terminal stages of the deficiency the oxygen value falls to less than half normal. Abderhalden and Wertheimer (95) associate this drop in basal metabolic rate which characterizes B avitaminosis in pigeons with a disturbance of oxidation-reduction relations in the animal. That the phenomenon is not general undernourishment was shown by the fact that the metabolic rate was restored to normal by yeast administration. A B_1-pituitary-thyroid relationship is found in the work of Sundhausen and Schulze (96 and 97). These workers found that in normal rats held on a diet free of vitamin B_1 the thyroid was quiescent and that the content of thyreotropic hormone in the anterior pituitary decreased, which was the cause of the quiescence of the thyroid. It was noted that a simple reduction of caloric intake in normal control rats did not elicit these effects. This same B_1-pituitary-thyroid relationship has been established by the use of vitamin B supplements instead of vitamin B deficiency. Giedosz (98) found that vitamin B_1 administered to guinea pigs stimulated the pituitary and thereby augmented the function of the thyroid. There is also the possibility of a synergistic action between the thyroid hormone and vitamin B.

Williams and Kendall (99) observed that the thyroid hormone appears to be less effective in promoting metabolic activity when there is a thiamine deficiency.

The caloric restriction employed in this experiment in bringing young rats to a state of chronic inanition was accompanied by a proportional restriction of minerals and vitamins. The amount of thiamine (vitamin B_1) contained in the daily ration during the underfeeding period was .02 milligrams. This constituted, according to Griffith and Harris (100), only about one fourth of the minimum daily requirements necessary for normal growth. Since other investigators have obtained a reduction in metabolic rate with vitamin B_1 deficiency without caloric restriction, the depressed metabolism in the underfed rats of this experiment is attributed to the specific deficiency of this vitamin, the effect being mediated through the pituitary and thyroid glands.

One might have expected little alteration in energy transformations in chronic inanition since this type of food restriction is purely quantitative. A marked or persistent decrease in the respiratory quotient was not anticipated; because although fats, carbohydrates, and proteins were not fed in their normal amounts, they were available in balanced proportions. Prolonged underfeeding at constant weight rations had presumably exhausted all food stores, since withholding the ration a few hours beyond the fixed feeding time invariably resulted in death. It would seem therefore than an animal without food stores and on small daily rations, would be metabolizing all three foodstuffs, thus resulting in a normal or nearly normal R.Q. Such, however, was not the case in the preliminary results obtained in this investigation. The respiratory quotients of these semi-fed rats were low, typically fasting R. Q.'s.

The explanation, however, arises from an entirely different quarter than does the low R. Q. of fasting and acute starvation. The principal cause is not in the oxidation of body proteins and stored fats, because, of these, the animals had little or none, but rather in the inability to oxidize carbohydrates. Dann and Chambers (28) found an almost complete suppression of the ability to oxidize ingested glucose in dogs after a three-week fast. The associated hyperglycemia and glycosuria were only partially reduced by insulin so that the effect must be in the sugar-oxidizing mechanism of the tissues themselves. Chambers (26) noted that as the amount of carbohydrate in the diet is decreased there is a diminished utilization. Marrazzi (29) observed that restriction of food intake decreased absorption of glucose.

The Effect of Environmental Temperature on the Metabolic Rate of the Unfed Rats. The weight loss of underfed rats exposed to cold environmental temperatures as compared to the weight gain of underfed rats held at constant optimum temperature is explained in terms of the effect on metabolic rate. The basal metabolism of the rat is increased according to Terroine and Trautman (101) and Benedict and MacLeod (102) with a fall in environmental temperature. It would be expected then that within the limits of the biokinetic temperature range, the body and food resources of the rats would be more quickly exhausted by a fast the lower the temperature. Nash (103) refers to this interpretation in the work of Horst, Mendel and Benedict, who starved rats at temperature of 26°C. and 16°C. and found that the rats at the former temperature lived on the average for 16½ days and lost 49 percent of their initial weight, while rats at the lower temperature lived only 11 days and lost 44 percent of their initial weight.

This effect of temperature on the metabolic rate is believed to be due to the thyroid as mediated through the pituitary gland. Prolonged exposure to cold, according to Turner (104), results in hyperplasia of the thyroid and a diminution of colloid, a response similar to that following stimulation by the thyrotropic hormone. This reaction to cold is due to nervous stimulation of the anterior pituitary which releases thyrotropic hormone and activates the thyroid. If this rationale is to be accepted, then it may be concluded from this experiment that chronic inanition does not inhibit the action of the pituitary to the extent that metabolic response to environmental temperature is lost.

The Effect of Refeeding on Metabolic Rate. The stimulation of metabolism which occurred in early recovery must be associated with the intense growth and regeneration of the active protoplasm. The influx of nitrogenous materials which naturally accompanied realimentation might offer an explanation, but this does not appear reasonable in view of the fact that the supply of nourishment continued to be great even as the metabolic rate gradually returned to normal. The possible explanation of increased body mass is equally invalid since weight gain continued at a rapid rate while the metabolic rate returned to normal. It is clear therefore that the enhanced metabolism of recovery must be interpreted as due to the active, growing, regenerating tissues. There is no evidence in the literature and no measurements were taken in this experiment which would indicate that the pituitary or thyroid are in any way responsible for this phenomenon.

The metabolic rate was nearly normal at the end of the refeeding periods, although the greater starvation period prolonged the time required for recovery.

The Effect of Growth Hormone and Testosterone on the Metabolic Rate During Refeeding. The reduction of the metabolic rate as a result of the growth hormone injections during recovery is in agreement with the results obtained on normal animals with similar growth extracts used by Kleiber and Cole (10) and by Teel and Cushing (16). The conclusion of Riddle, et al (92) that most "growth" preparations have a high calorigenic action due to the presence of thyreotropic hormone and prolactin; that the growth promoting action of growth extracts is due to the presence of these hormones; and that such throws doubt upon the existence of a separate "growth" hormone in the anterior pituitary is not confirmed by this experiment in which the growth preparation used reduced metabolic rate. The conclusion is also questioned by the work of Kleiber and Cole (10) and by Teel and Cushing (16) whose extracts reduced metabolism while promoting growth.

Injections of testosterone resulted in a reduction of metabolic rate to about the same extent as did the growth hormone. The action in the case of both hormones is pituitary inhibition. Experimental and clinical experience have established the principle that a hormone supplement will inhibit the gland which produces the hormone. While this explanation does not suffice for the testosterone, it is nevertheless true that the sex hormone had an inhibitory action on the pituitary. That such pituitary depression occurred with testosterone therapy in this experiment is supported by the reduced weight of the testes and the decreased body weight gain.

The fact that the testosterone and growth hormone did not maintain their depressing effect on the metabolic rate throughout recovery is probably because the animals became refractory to their action after

prolonged injection. This explanation appears more valid for the growth hormone, an extract, than for testosterone, a synthetic. It may be that the pituitary gland itself recovered in its secretory capacity so as to counteract the effects of the growth hormone and testosterone so far as metabolism was concerned.

MORTALITIES

The majority of mortalities occurred in the early experiments before it was learned that it was necessary to adapt the animals to semi-starvation by adjusting the amount of food to the daily weight change. It was soon observed that the animals varied in their ability to withstand undernutrition, and that rats of the same sex, strain, weight, and age would not all survive identical caloric restriction. This difference tended to disappear as the period of underfeeding was prolonged, so that later all rats were maintained on about the same amount of food. Mortalities which nearly always occurred at the beginning of starvation in the early experiments were eliminated entirely in the final complete studies by daily food-body weight adjustments.

There were also many mortalities before it was learned that rats in a state of chronic inanition were very sensitive to cold. Deaths which invariably followed a cold night were eliminated in the final experiments by keeping the animal room at a thermostatically controlled optimum temperature.

Caloric restriction beyond 30 days' duration resulted in reduced white blood cell numbers. This leucopenia probably made the rats sensitive to bacteria, and may account for the predisposition to pneumonia. The reduction in total leucocytes, together with the increased permeability of the intestinal wall to coliform bacteria offers an explanation for the

mild efferent urinary inflammations which were observed in the most of the rats after prolonged underfeeding.

The one mortality which occurred during recovery was due to complications which reflected symptoms in respiration and the blood. Autopsy revealed normal organs except that the stomach and intestines were empty, transparent, and filled with gas. Before dying, the rat struggled with breathing, and bronchial sounds were evident. The extreme hemo-concentration found in the blood may indicate a spontaneous atrophy of the adrenal cortex as the cause of death.

The sickness of one rat during refeeding appears to have been due to a transitory recession in the blood regeneration. The behavior and progress of the rat was typical until the 21st day of recovery, at which time food was refused and the eyes became very pale. Examination of the blood revealed an extreme hemo-dilution, numerous abnormalities of the erythrocytes, and many normoblasts. This blood picture is typical of that which is classified clinically as Mediterranean anemia. After five days of almost complete anorexia, the animal regained appetite and subsequently recovered a nearly normal weight and blood picture.

SUMMARY

Two hundred male rats, 30 days old and 50 grams weight, were brought to a state of chronic inanition by a qualitatively adequate constant weight ration. They were then refed and groups of ten were injected, each with a different supplement of hormone, vitamin, etc. Two different periods of starvation and recovery were studied. Observations were made on the blood, food consumption, weight gain, organ weights, metabolic rate, and bone growth on both starved and refed animals, as well as on fully fed controls. Food-feces ratios were determined on normal and underfed rats. The effect during chronic starvation of variations in environmental temperature on the body weight was also observed. All results were expressed as an average of ten animals.

The plasma protein, hemoglobin, and hematocrit continued to increase during caloric restriction, so that at the end of a 90-day starvation period these values were greater than in normal rats of the same size and less than in normal rats of the same age.

Chronic starvation resulted in a transient redistribution of the red cells, producing a peripheral concentration of the blood.

There was no variation in the density of the blood during starvation, except that which followed in the path of changes in the red cell numbers. A blood hydremia occurred almost immediately upon realimentation, however, as indicated by the decrease in the red cell count and specific gravity. This effect was temporary and in early recovery the density of the blood was rapidly returning to normal.

A gradual reduction in total leucocytes appeared during starvation, with the percentage of leucocyte types showing considerable variation and

average values tending to a decrease in lymphocytes.

Chronic starvation in these young rats did not significantly alter the blood sugar, but there was a marked increase in the non-protein nitrogen.

After 60 days' refeeding, the blood had recovered normal or nearly normal values for the red and white cell count, leucocyte differential, hematocrit, hemoglobin, plasma protein, specific gravity, and N.P.N.

No changes appeared and no measurable benefits were seen in any feature of the blood as a result of the treatments administered during recovery.

Chronic starvation decreased the relative weights of the liver, thymus, heart, seminal vesicles, and spleen; increased the relative weights of the adrenal glands, testes, and pituitary; and produced no change in the average weight of the brain and kidneys.

Refeeding resulted in the restoration of the normal weights of the brain, heart, kidneys, seminal vesicles, and testes, and a relative hypertrophy of the thymus and liver. The spleen, pituitary, and adrenals failed to recover a weight in normal proportion to the body. However, in rats underfed for only 30 days, the adrenal glands and pituitary were fully recovered after 35 days' refeeding.

Large injections of the growth hormone during recovery produced an enlargement of the spleen and adrenal glands, and a decrease in the weight of the testes, seminal vesicles, and kidneys. Small injections of growth hormone were without effect, except for an increase in the weight of the adrenals.

The administration of testosterone during refeeding decreased the weight of the testes and thymus, and increased the weight of the kidneys

and seminal vesicles.

No significant alterations appeared in the organ weights as a result of the use of vitamin B_1, B complex, insulin, cortex extract, or liver extract.

Except for the hypertrophy of the thymus and the reduced size of the spleen, the organs recovered in weight to an extent that was proportional or nearly proportional to the body weight. This rapid weight recovery of most of the organs upon nutritional improvement may have masked the effects of the treatments so that their benefits were not measurable.

The daily food ration necessary to maintain a constant weight in the starved rats became less as inanition was continued. Chronically underfed rats removed more material from the food consumed than normal rats. Anorexia did not develop as a result of chronic inanition; food was accepted with vigor, and ingestion of large amounts upon refeeding appeared to have no ill effects.

The amount of water consumed by the underfed rats was greater in proportion to the food intake than in normal rats.

The refed rats grew more rapidly and with greater food utilization efficiency than normal rats of the same size. This growth stimulating effect of starvation was reduced after prolonged underfeeding.

Rats treated with adrenal cortex extract and liver extract during recovery gained weight more rapidly than the untreated controls. The action of these two extracts in increasing weight gain was independent of appetite.

Supplements of vitamin B_1, B complex and insulin administered during recovery resulted in an increase in food consumption and body weight.

Rats injected with large doses of growth hormone exhibited a reduced

food consumption but at the same time maintained a weight gain equal to that of the untreated controls. Smaller doses of growth hormone also inhibited the appetite but failed to show the weight conserving effect.

Treatment with testosterone resulted in a weight gain considerably less than the controls, but food consumption was not reduced.

Reduced but continued bone growth occurred during chronic starvation. Although the growth rate was increased by refeeding, the bones did not regain normal size. None of the treatments employed during recovery produced any growth effect on the skeleton.

Chronic starvation lowered the metabolic rate to a marked degree, but an abnormally high metabolism appeared during the first few days of refeeding. This enhanced metabolism of early recovery was decreased in rats treated with testosterone and growth hormone. The metabolic rates in all the experimental groups was nearly normal at the end of the refeeding period.

A lowered respiratory quotient appeared as a result of semi-starvation, which was restored to the normal range upon realimentation.

A loss in body weight typical of normal animals during a lowering of environmental temperature appeared also in rats in a state of chronic inanition.

The experimental animals varied in their ability to withstand caloric restriction. Disregard of adaptive differences, using an identical daily food ration on all rats from the beginning of underfeeding, resulted in many mortalities. Careful food ration-weight loss adjustments during early starvation made it possible to later maintain all animals on about the same amount of food.

Prolonged inanition resulted in a mild efferent urinary inflammation

in about 60 percent of the rats. This became severe in only one case, producing a cystitis with urethral obstruction. Death followed from uremic poisoning. At the end of the first week of recovery, over half of the inflammations had cleared up, and after two weeks the symptoms remained in only two animals.

Only one rat became ill during refeeding. Anorexia in this animal appeared on the 21st day of recovery. Blood examination revealed an acute anemia of the clinical Mediterranean type. The rat showed improvement without treatment 5 days later, and subsequently recovered completely with a final body weight as great as the other experimental animals.

There was one mortality during refeeding. Symptoms before death were largely respiratory with labored breathing and bronchial sounds. Blood examination revealed an extreme concentration.

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