THE VITAMINS A AND C STATUS OF THE RAT AND GUINEA PIG, AS INFLUENCED BY THE LEVEL OF VITAMINS A AND C INTAKE, DIET COMPOSITION, CALORIE INTAKE AND EXPOSURE TO COLD.*

by

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Submitted in partial fulfillment for the
degree of Doctor of Philosophy in Biology
University of Ottawa
June 30, 1960

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* This research was aided by a grant from the National Research Council of Canada (MT 698)

To my Wife,

Thérèse

ACKNOWLEDGEMENTS

Words of thanks are not sufficient to express my gratitude for the quality of the immeasureable understanding and scientific leadership given to me by the 'patron' of this thesis, Dr. André DesMarais.

A sincere thank you is extended to Dr. Louis-Paul Dugal, Chairman of the Department of Biology, for his personal interest and assistance.

Many thanks to Drs. Q.N. LaHam, C. Godin, E.O. Dodson, Mr. J. Vaillancourt and other members of the departmental staff for their suggestions and encouragement.

Special acknowledgement is due to Miss Louise Sheppard whose technical assistance, executed with unselfish devotion and complete thoroughness, allowed me to visualize the completion of this thesis in a minimum of time.

A vote of gratitude is due to Mr. Jacques Hélie for his expert preparation of the histological material, Mr. G. Ben-Tchavtchavadze for the excellent photographs, and to the members of the technical and secretarial staff for their cooperation.

ABSTRACT

An enigma has existed since 1948 concerning the possible interrelationship of vitamin A and vitamin C. We first examined this possible relationship by taking advantage of the known effects of cold on tissue ascorbic acid. We found that vitamin A deficient rats had a decreased resistance to cold and that prior to becoming moribund, their vitamin C status was unaffected by the deficiency. The administration of vitamin C during the terminal stages of vitamin A deficiency did not alleviate the signs of vitamin A deficiency nor prolong survival in the cold.

The data of the above experiments called our attention to the importance and necessity of utilizing the pair-feeding technique and a vitamin A deficient test diet of a different composition. We found that the onset of vitamin A deficiency at room temperature occurred ten days earlier on a yeast-free vitamin A deficient test diet. In addition, the control animals on the new diet had a distinct tendency for a better weight gain, a smaller liver and an increased liver storage of vitamin A. Exogenous vitamin C administered from the onset of the experiment did not retard nor ameliorate the course of the deficiency or the survival of rats fed either the U.S.P. or a yeast-free vitamin A deficient test diet.

Administered vitamin C was readily taken up by the liver of rats on the yeast-free test diet but not by the liver of rats on the U.S.P. yeast-containing vitamin A deficient test diet. We believe this difference in composition of the test diets explains why a number of investigators were unable to find modifications in the ascorbic acid status of their animals after treatment with vitamin C.

Pair-feeding adequately accounted for the decrease in liver reduced ascorbic acid which occurred in terminal vitamin A deficiency. Evidently, no relationship exists between the rat's resources of vitamin A and its capacity to synthesize ascorbic acid.

Reduced glutathione does not seem to be the yeast factor which prevents the liver uptake of exogenous vitamin C. But, the administered reduced glutathione enhanced liver storage of vitamin A in normal rats and hastened the onset of the body weight gain plateau of vitamin A deficient rats by approximately five days.

Guinea pig experiments demonstrated that treatment with moderate amounts of vitamin A did not hasten nor retard the development of scurvy. In addition, it was found that the absence of liver vitamin A in the guinea pig had no effect on the uptake and storage of supplemented vitamin C.

Investigation into the beneficial effect of a high fat diet in the cold and the role of vitamins A and C revealed that increased fat in the diet exerts distinct beneficial effects in the cold, which are not enhanced when the diet is eaten in excess. We believe the mechanisms by which fat becomes a superior dietary constituent, are probably brought about by a conservation of energy resulting from a decrease in active lipogenesis and a decrease in specific dynamic action of non-fat foodstuffs. Fat promotes these decreases directly and also promotes an increased efficiency of the intestinal tract. The resultant energy conserved becomes available for heat production.

In the cold, indications were found supporting the hypothesis that ascorbic acid may be intimately associated with some phase of fat metabolism. The requirement of vitamin A in the cold was noticeably increased on the high fat diet.

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II. The effect of (a) vitamin A deficiency and (b) exogenous vitamin A administration (in doses which may or may not induce hypervitaminosis A), on the tissue content of Ascorbic Acid.

This problem has been reviewed by Ratsimamanga (159), Reid (161) and Terroine (212). All reached the conclusion that there exists data for and against a possible interrelationship and that it remained an unanswered hypothesis which only further experimentation would decide.

Ia. The effect of a vitamin C. deficiency on tissue vitamin A.:

The literature pertaining to this approach to the problem, is to our knowledge limited to that of Mitolo (131), who reported that the vitamin A. and B-carotene content of the livers of scorbutic guinea pigs averaged a little lower than in normal animals. Food intake was not controlled.

Ib. The effect of Ascorbic Acid administration on tissue vitamin A.:

Two clinical reports which probably prompted the research which followed are those of Stewart (202) and Kimble and Gordon (109). The former reported that ascorbic acid in daily doses of 150 mg. produced as great an improvement in dark adaptation of human subjects as did a daily dose of 24,000 I.U. of vitamin A. The latter (109) observed that the administration of ascorbic acid (dose not specified) caused an improvement in night vision which had not occurred with daily doses of vitamin A. (200,000 I.U.) alone.

Bassett et al. (9) found that the incorporation into the diet of 4 mg. ascorbic acid per 100 gm. of diet increased the plasma and tissue levels of vitamin A in foxes and mink, and concluded that the synthesis of vitamin C is dependent upon the supply of vitamin A. Mayer and Krehl (127) claimed that the survival time of vitamin A deficient rats was prolonged by the administration of ascorbic acid (100 mg./100 gm. diet).

On the contrary, Helmboldt et al. (95) administered 2.0 gm. of ascorbic acid subcutaneously for 14 days to six vitamin A. deficient calves without observing evidence of an amelioration of the deficiency signs. A lack of effect on the survival and physical condition of rats made deficient on the U.S.P. vitamin A. test diet supplemented with 5 gm. of ascorbic acid per Kg. of diet was reported by Guerrant (87).

IIa. The effect of vitamin A deficiency on the tissue content of Ascorbic acid:

Several investigators have shown a reduction in the ascorbic acid content of the organs of vitamin A deficient rats. Sure, Theis and Harrelson (206) using the pair-feeding technique found the vitamin C tissue level (heart and kidney) moderately (20%) decreased in vitamin A deficiency. Significant decreases (40%) in other organs including the liver, were found only after the rats were repeatedly depleted (2 or 3 times) over a period of 127 to 238 days. They also noted that 4 to 16 mg. of ascorbic acid given orally every day did not result in an increase in the tissue concentration. Sangiorgi (176) and Ghosh et al. (84) observed a decreased excretion of ascorbic acid in rats made vitamin A deficient. Sutton et al. (208) also observed that rats deficient in vitamin A excrete less ascorbic acid than non-deficient animals. Limiting the vitamin A intake (10,20,30 I.U. vitamin A/day/Kg.b.wt.) resulted in corresponding decreases in the blood plasma ascorbic acid. They also noticed that ascorbic acid excretion was invariably higher on the stock diet (not specified) than on the vitamin A supplemented U.S.P. test diet. Sjoberg (194) and Jonsson et al. (107) found decreases in rat serum ascorbic acid which they correlated with the clinical progress of vitamin A deficiency; and moreover,

correlated this with histological changes in the teeth and muscle which they believed were due to scurvy. They also noted that exogenous ascorbic acid did not benefit their vitamin A deficient rats. In cattle suffering from vitamin A deficiency Boyer et al. (19) (154) reported that there occurred a progressive decrease in plasma and cerebrospinal fluid ascorbic acid and that this could be raised by the administration of vitamin A. Eaton (59) recently reported that in such cattle, though they have a decreased plasma ascorbic acid level, that vitamin C failed to relieve the deficiency symptoms although the response to vitamin A is immediate. Other investigators experimenting with vitamin A deficient chickens (17) and horses (91) reported that plasma ascorbic acid levels were unaffected by the deficiency.

Irrespective of the previously mentioned works, this aspect of the problem became decisively split into two distinct "camps" with the publication in 1948 of the experiments of Mayer and Krehl (127) (128) at Yale, and that of Mapson and Walker (121) at Cambridge.

Mayer and Krehl:

They observed signs resembling scurvy in rats maintained on a high protein (60% casein) vitamin A deficient test diet; and also noted that a high protein intake made more acute the decrease in the efficiency of food utilization of the

deficient animals. Administration of ascorbic acid (50 mg. I.P.) to these animals alleviated the "scorbutic symptoms" but not the vitamin A deficiency symptoms. Rats fed 'ad libitum' for 37 days on a vitamin A deficient test diet containing 25% casein had decreased liver, adrenal and blood ascorbic acid levels. Administration of ascorbic acid (100mg./100 gm.diet) at this time returned the liver and adrenal vitamin C to values approaching that of the controls. (No mention of survival studies are made in this experiment). The fact that the exogenous ascorbic acid was taken up is of considerable interest because as we have previously noted, others (205) (107) had failed to observe this. Reid (161) suggested that this failure might be due to the use of yeast as a source of the B vitamins. Mayer and Krehl supplemented the B vitamins in crystalline form. (For a comparison of the diets of various investigators see table 23.)

The controversial experiment which provided the most emphatic data in favor of vitamin C, is the one in which rats of two different strains (Yale and repeated with Sprague-Dawley) fed their A_2 diet (25% casein), supplemented with ascorbic acid (100mg./100gms) from the onset of the deficiency, had a 100% survival at the 80th day of the deficiency, whereas the unsupplemented vitamin A deficient animals had all died within 40 days.

Mapson and Walker:

They determined the level of ascorbic acid in the liver, adrenal and whole blood of vitamin A deficient rats and compared these values to those of 'ad libitum' fed controls and 'pair-fed' controls. Moreover, they studied the effect of administering chloretone to determine whether the increased synthesis of ascorbic acid, which it is known to induce (115) (196), occurred in rats deprived of vitamin A. They confirmed the findings of previous investigators, that the ascorbic acid content of the liver and blood was diminished in vitamin A deficient male rats; and also that this diminution was not due to increased urinary excretion of ascorbic acid. However, they demonstrated that the reduced ascorbic acid values could be accounted for by the lowered food intake of the deficient animals. Moreover, the rise in urinary excretion of ascorbic acid in response to chloretone was less in vitamin A deficient rats than in non-deprived ones, but this difference was eliminated if the food intake was restricted to that of the deficient animals. In addition, they noted that vitamin A deficient female rats did not have lowered tissue ascorbic acid concentrations. They concluded that in their experiments no specific relationship was found between the rat's resources of vitamin A and its capacity to synthesize ascorbic acid.

IIb. The effect of Vitamin A administration on tissue ascorbic acid:

It is our contention that practically all the research which has been done in this field is not directly applicable to the problem, because it is primarily the study of excess vitamin A (hypervitaminosis A) which in itself is a pathological condition. However mention of some of the more significant contributions is made for the indirect insight which they do convey to the problem.

In 1922, Mouriquand and Michel (140) reported that an excess of cod liver oil interferes with the cure of scurvy in guinea pigs. Collet and Erickson (25) confirmed this finding but also observed that a more moderate (2,400 I.U./day) excess of vitamin A did not interfere with the ascorbic acid reqmt. Vedder and Rosenberg (224) reported that rats given large doses of vitamin A (100,000 I.U. daily) as jewfish oil, developed scurvy-like symptoms with a significantly decreased excretion of ascorbic acid. Supplementation with 5 mg. of ascorbic acid per day gave almost complete protection. Rodahl (169) confirmed this, and in further studies (168) found that large doses of ascorbic acid (50 mg./day subcutaneously) administered to either rats or guinea pigs did not afford protection against massive doses (50,000 I.U.) of vitamin A. But this quantity of vitamin C was beneficial

in counteracting a moderate excess (25,000 I.U.) of vitamin A. Morehouse (138) found that the concentration of ascorbic acid became progressively lower as the daily dosage of vitamin A was increased from 20,000 I.U. to 80,000 I.U. But Eeg-Larsen and Pihl (61) reported that the fall in serum ascorbic acid in rats treated daily with 20,000 I.U. to 40,000 I.U. of vitamin A was the same as that in rats showing the same weight loss after pair-feeding.

Neiman and Obbink (142) have written an excellent review on the biochemistry and pathology of hypervitaminosis A.

Concluding remarks:

Since 1955, when this subject was last reviewed (212), no data directly related to the solving of this vitamin A-vitamin C relationship debate have been published. We believe our experiments confirm the work of Mapson and Walker that decreased food consumption can account for the decreased level of liver ascorbic acid in vitamin A deficient male albino rats.

VITAMIN A and the THYROID

Introduction and history:

There are numerous reports concerning an antagonism between vitamin A and the thyroid (13) (135) (198). It was first suggested in 1932 by von Euler and Klussman (225), who found that injected thyroxine induced vitamin A deficiency, and that the loss of weight sustained by rats receiving thyroxine was largely compensated for by the simultaneous feeding of carotene. The fact that thyroxine hastened the onset (xerophthalmia and vaginal keratinization) of vitamin A deficiency in rats was confirmed by Greaves and Schmidt (85) and Sure and Buchanan (204). Weise et al. (226) found that surgical or chemical (thiouracil) thyroidectomy prolonged the lives of rats deprived of vitamin A. Moore (136) points out that the presumed interaction can be explained as effects on the metabolism of vitamin A which were due to an increase or decrease of B.M.R. resulting from an increase or decrease in circulating thyroid hormones. However he admits that the evidence that vitamin A can oppose the action of thyroxine in its effect on the metabolic rate, advances a stronger case for a specific antagonism. Logoras and Drummond (114) reported that vitamin A (1,000 I.U. per day orally) tended to protect rats against the loss of weight which injected thyroxine (100 ugm./2 days) caused; but vitamin A

did not protect against a similar increase in metabolic rate induced by 2,4, dinitrophenol. Similarly, the side effects of injected thyroxine (11mg./day) given to cows to increase lactation are reported to be ameliorated when vitamin A (100,000 I.U./day) is also supplemented (147). Belasco and Murlin (11) measured the B.M.R. of rats which had received thyroxin (0.2mg./100gm.b.wt. for 6 days) with or without vitamin A (2,000 I.U./day for 8 days). The administration of vitamin A decreased the metabolic rate by 30%. Sheets and Struck (192) observed a non-significant tendency for a decrease in metabolic rats when 20,000 I.U.vit. A/day was administered to rats.

I. Thyroid histology and function in vitamin A deficiency:

Assuming that an antagonism between vitamin A and thyroxine does exist, one might expect that in the lack of vitamin A, the thyroid activity would increase.

Histology:

McCarrison (141) and Spence (199) noted hypertrophy of the thyroid gland and abnormal distention of the follicles in vitamin A deficient rats. Others (132) (220) reported that not only where the follicles filled with colloid but that the epithelial cells were flattened. However, DeRuytter (38) stated that in severe avitaminosis A, the thyroid atrophied and showed epithelial degeneration. Coplan and Sampson (29)

noted that vitamin A deficiency produced definite hypertrophy in the thyroid of the female rat but consistant atrophy in the male. Several authors (50) (174) including Sure (203) and Remington et al. (163) stated that no significant changes in thyroid weight were seen during vitamin A deficiency. Drennan et al. (50) and Schulze et al. (182) reported "a tendency to activation".

The apparent lack of aggreement in many cases might be attributed to the variable composition of the vitamin A deficient test diet utilized. Some of the diets were probably low in iodine content, and/or may have contained foods which were capable of either inhibiting or stimulating the thyroid. Moreover the lack of true controls and pair-fed controls could have resulted in misinterpretation of the data. In addition, there is no doubt that the sex of the animal alters the response to vitamin A deficiency (134).

Thyroid function studies: I^{131}:

Lipsett and Winzler (113) are the sole investigators who have studied the effects of vitamin A deficiency on the function of the thyroid with radioactive iodine. On a U.S.P. Vit. A deficient test diet which contained only 16-17 ugm./Kg. of iodine, they observed that vitamin A deficient rats (on the diet 2 days from date of weaning) had thyroid weights which were almost twice that of the equi-weight but not simultaneous

controls. The histology of these glands showed some area of distended follicles with flattened epithelium, while other areas showed degenerative changes. The control thyroids had slightly enlarged follicles due to the low iodine in the diet. The total I^{131} taken up by the thyroids was not different but because the thyroids from the vitamin A deficient animals were larger, the uptake per mg. of thyroid was lower. Though the same percentage of injected I^{131} was picked up by the thyroids of both groups, the quantity of inorganic iodine found in the thyroids of the vitamin A deficient animals was higher, and the amount of bound iodine was lower. They believed their results indicated a decrease rate of conversion of inorganic iodine to organic iodine in the vitamin A deficient rat.

Thyroid function studies: B.M.R.:

In 1934, Chevallier and Baert (24) reported that the energy requirement of vitamin A deficient rats was increased by 13%. However, other workers (110) were unable to find a noticeable effect. According to Blaizot and Benac (16) they were able to confirm Chevallier and Baert's observation. The former authors (16) state that vitamin A deficiency provoques an increase of 10 to 25% in the oxygen consumption which develops before weight gain stops and ocular signs become noticeable. We question the reliability of these data

because 1. the measurements were made on non-fasting animals, 2. the controls were not pair-fed, 3. the results were expressed as a function of age rather than weight or surface area, 4. thyroid weight and/or histology was not attempted. They claim that fasting has little effect on the QO_2 reading because the rat eats often and little at a time. Our observations have been that the rat is a night animal and that activity, food consumption and excrement are increased during the night. Their non-fasting results would probably have been more reliable had their controls been pair-fed. They showed that with normal rats, the oxygen consumption expressed as a function of age was identical to that expressed as function of weight. We feel that this correlation would probably not exist in a deficiency state.

Also in 1955, in France, Olivereau and Serfaty (146) reported that vitamin A deficient male rats had thyroids which showed epithelial involution, but that the B.M.R. remained normal; but, there was an increase in the 'in vitro' oxygen consumption of certain tissues.

II. Thyroid histology and function in hypervitaminosis A:

According to Drill (58) and Simkins (193), the consensus of many workers, who reported on the weights and histologies of the thyroid gland when excess vitamin A was administered, is that a high intake of vitamin A causes a decrease in the

weight of the gland, diminution of colloid and epithelial hyperplasia. The mechanism by which this occurs has been postulated by Sadhu et al.. Ray and Sadhu (160) have shown that there is a decreased liver activity in hypervitaminosis A and that therefore there is a decreased destruction of thyroxine which leads to a hyperthyroxinemia (173) which causes a decreased secretion of T.S.H., and small sized thyroids. Direct opposition of large doses of vitamin A to the action of T.S.H. was reported by Schneider (178) and Fellinger and Hockstaid (71). Danowski et al. (31) reported that the supplementation of 30,000 I.U. of Vitamin A per day to rats for 12 weeks induced a slight but definite decrease in P.B.I.. No conclusion can be drawn from this latter work because the free thyroxine blood level and thyroid histology were not considered.

Though no mention has since been made, Belasco and Murlin (1940) (11) and Sadhu and Brody (1947) (172) suggested that the double bond of vitamin A might be capable of taking up one or both forms of circulating iodine and thus when vitamin A was present in excess, somehow reduce the effectiveness of thyroxine.

III. Thyroxine therapy vs. hypervitaminosis A and vitamin A therapy vs. thyrotoxicosis:

The use of thyroxine in the therapy of hypervitaminosis

A, and the use of excess vitamin A in the therapy of experimental and clinical thyrotoxicosis was first suggested by Fasold and Peters (70). They claimed that in the rat, thyroxine (0.5mg. s.c. 3Xweek) was effective in counteracting the effects of excess vitamin A (approx. 30,000 I.U./day). Baumann and Moore (10) repeated the experiment and found that rats suffered more from the combined excess of vitamin A and thyroxine than from either alone. Fasold and Peters (70) also asserted that vitamin A could prevent the toxic effects of excess thyroxine, however, they admitted that arachis oil, used as the solvent for their vitamin A, could itself protect against excess thyroxine. Nevertheless, confirmatory evidence for the hypothesis was reported by several workers (182) (1) (155), and in 1947 by Sadhu and Brody (172). These latter investigators showed that 30,000 I.U./day/rat itself depressed the rate of oxygen consumption by 10%. The same dose of vitamin A depressed the increased metabolism of thyroxine (1mg./kg./week) injected rats by 20%. These animals (thyroxine plus vit.A) had an oxygen consumption 20% greater than that of the untreated controls. Completely oxidized vitamin A had no effect.

In addition to the experiments mentioned, many confusing and contradictory clinical reports have been published. No thorough re-investigation of this hypothesis has been made

IV. Thyroxine and the conversion of carotene to vitamin A:

We have avoided the use of carotene as a dietary source of vitamin A because one would then be obliged to consider the influence of thyroxine on the conversion of carotene to vitamin A. This subject has been reviewed by Moore (136) and Lowe and Morton (116).

V. Thyroxine and the storage and utilization of vitamin A:

Consideration of the consensus of recent investigators concerning the influence of thyroxine on the storage and utilization of vitamin A is necessary if a proper interpretation of the possible effect of cold exposure on vitamin A expenditure is to be discussed.

Storage:

There are indications that a short term increase in metabolic rate may influence the storage of vitamin A. Logoras and Drummond (114) found that in rats given daily doses of 3,000 I.U. of vitamin A, treatment with either thyroxine or dinitrophenol increased the average liver stores by 20% However, when the metabolic rate is increased or decreased for a longer duration due to a hyper- or hypo- thyroid state, the liver storage of small daily doses (100-150 I.U.) of vitamin A has been reported to be equal to that of control animals (106) (139), although the liver size in the experimental groups is either enlarged or reduced.

Expenditure:

In 1948, Johnson and Baumann (106) found that the expenditure of vitamin A is significantly affected by the rate of growth. Weise (226), Sobel et al. (94), Morgan and White (139) and Arnrich (3) all agreed that the changes in growth rate have a greater influence upon vitamin A utilization than changes in metabolic rate. Recently, Frape et al. (78) studied the relationship of vitamin A to the thyroid function of young pigs. They state that dietary vitamin A (0 to 64,000 I.U./lb. diet) had considerable influence on the rate of thyroxine secretion. Insufficient and excessive intakes of vitamin A lowered the rate of secretion. They believe that vitamin A exerts a direct effect on thyroid function because they found a negligible relationship between thyroid secretion rate and growth rate in the pig.

VITAMIN A DEFICIENCY and the TESTIS

Introduction:

In our investigation into the effect of vitamin A deficiency on the vitamin C status of the rat, we have taken the wet weight of the testis, and in some experiments, the histology of the gland and the wet weight of the seminal vesicles, as a reliable relative index of the progress of the deficiency.

Vitamin A deficiency:

Atrophy of the testis is a constant occurence in vitamin A deficiency. In 1924, Gross (86) and in 1925, Wolbach and Howe (228) found a reduction to about half the usual size in rats. Many investigators confirmed this finding in rats (122) (69), mice (230) and guinea pigs (229). Mason (123) reported that the germinal epithelium of the testis is injured earlier and more severely than other epithelial structures. He noted, however, that the injuries caused to the testis by deficiency of vitamin A, unlike those caused by vitamin B deficiency can be cured by prolonged treatment with the vitamin.

According to Moore (135) "the extent to which vitamin A is concerned in the development and maintenance of the epididymes, seminal vesicles and prostate glands, has not been completely elucidated". Mayer and Goddard (126) believe the

seminal vesicles fail to develop because of a deficiency in the function of the testis which is a consequence of a pituitary abnormality. On the other hand, Mason (123) and Sutton and Brief (207) both concluded that the lesion in the testicular epithelium is a primary effect of vitamin A deficiency, and not a secondary effect of a pituitary lesion.

RESPONSE TO COLD AS INFLUENCED BY DIETARY COMPOSITION.

Introduction:

Since 1950, there has appeared an increasing number of publications concerning the response of animals maintained at low environmental temperature to diet composition. A number of observations and their underlying reason have become established fact. With cold exposure there is an increase in the caloric requirement (217) due, at least partially, to an increase in heat production. That is, the resting metabolism (12) and the overall metabolic rate (189) (92) are elevated during cold exposure. Associated with this increased energy requirement is an increased requirement for both specific and basic organic constituents.

I. Specific constituents:

The known specific constituents include vitamin A (67) (158),, Riboflavin and Pyridoxine (65), Pantothenic Acid (66) and Ascorbic Acid.

Ascorbic acid:

Dugal et al. (56) (54), have shown that there exists not only an increased requirement for vitamin C in the cold, but also that: a. Barly during the exposure to cold, abdominal organ levels of ascorbic acid increase and become re-established at a higher level (56); b. Exogenous ascorbic acid confers a

definite protection to animals exposed to cold (54) (41). This latter observation has been confirmed by several investigators (125) (79) (17). DesMarais (40) has since shown, that the beneficial effects of ascorbate administration are probably mediated though the thyroid hormones. It increases the efficiency of low doses of thyroxine administered to cold exposed rats, preventing, at the same time, some of the typical signs of an alarm reaction. He has since demonstrated that this ascorbate effect is not mediated through a direct effect on the thyroid-T.S.H. relationship (42) and has thus postulated that it exerts its effect at the peripheral site of action of the thyroid hormones.

II. Basic Organic constituents:

The basic organic constituents are protein, carbohydrate and fat. Mitchell and Edman (129) reviewed the literature on this subject in 1950, and concluded that: a. A high protein intake is not beneficial in cold tolerance; b. A high carbohydrate diet is superior in this respect to one in protein; and c. Dietary fat under some conditions improves still further the beneficial effects of carbohydrate foods.

Fat:

Recent investigations have been focused on whether, in the cold, fat is preferentially utilized and metabolized for heat production and/or exerts its beneficial effect by

supplying a larger number of calories per unit weight and volume of diet.

In 1937, Kayser (108) stated that fats were utilized preferentially for heat production. Later, Dugal et al. (55) reported on the beneficial effects of high fat diets on the resistance to cold in rats; and Forbes et al (73), comparing diets in resting animals at room temperature, reported that high fat diets were more efficiently utilized. The latter authors postulated that there was a higher energy expense of food utilization with high carbohydrate diets which was probably related to the cost of the synthesis of fat from carbohydrates. Templeton and Ershoff (210) studying survival on single food diets, suggested that adaptation to cold was necessary for the efficient utilization of fat (margarine). If rats were exposed to cold (2°C) for 12 days prior to the start of margarine feeding, the time of survival in the cold, on this food was increased from 10 days for non-adapted rats to 26 days for the pre-adapted animals. The survival time of rats fed a sucrose diet under the same conditions was increased from 17 days to 25 days. Moreover, sucrose was the better diet for survival when animals were at room temperature or exposed to cold without prior adaptation.

A thorough investigation on the effects of diet and cold on body composition has been published by Pagé and

Babineau (150). Irrespective of environmental temperature, rats on a high fat diet had greater body weight gains than animals on a low fat-high carbohydrate diet. At room temperature, increased fat deposition accounted for the excess weight of the high fat group, whereas in the cold (3°C) no significant difference in fat content was found. Thus, in the cold, the high fat diet had a beneficial effect because growth was actually enhanced as opposed to a mere increase in energy reserves. Contrary to Mitchell's hypothesis (130) that the beneficial effects of high fat diets in the cold might be due to increased insulation (increased laying down of subcutaneous fat), Pagé and Babineau (150) (148) demonstrated that the distribution of fat (subcutaneous vs. adipose vs. skeletal) in rats exposed to cold was not affected by the amount of fat in the diet. R.Q. studies by Chénier (23) (151) support the idea that rats exposed to cold oxidize fat preferentially, and that adaptation to cold is characterized by an increased lipogenesis from carbohydrate to fat. Because this latter phenomena occurs only during and after adaptation to cold, Chénier states that it probably explains why increased dietary fat cannot pre-adapt an animal to cold.

On the other hand, Sellers et al. (188) (183) reported observations which indicated that moderate variations in the

fat content of the diet did not have any effect on the growth rate or survival in the cold. They also observed that the caloric intake was remarkably constant regardless of the amount of lipid in the diet. The oxygen consumption ($M.R._{30C}$) of their groups of rats fed high fat diets and low fat diets respectively and in the cold for 3 months did not differ significantly. Baker and Sellers (6) found that the glycogen content of the heart, liver, diaphragm and the perirenal fat of acclimatized rats was lower than that of room temperature controls. Blood sugar levels and the glycogen of skeletal muscle did not differ significantly. After insulin, acclimatized rats in the cold showed a greater fall in blood sugar than did normal controls at room temperature. They (Sellers et al.) believe these results are evidence of an increased utilization of carbohydrate in conditioned animals.

Evidence that glucose is not used preferentially by cold acclimated rats has been advanced by Pagé et al. (152) and Depocas et al. (37). Both observed that, in cold acclimated rats, more of a given dose of glucose is deposited as liver glycogen and Depocas et al. (37) found that over a two and one-half hour period at -5°C, less of the C^{14} glucose given intra peritoneally appeared as $C^{14}O_2$ in cold acclimated rats than in controls.

Effect of cold on the fat content of the liver:

Cold has been reported to have a curative effect on neutral fat fatty liver (16) (185) but not on a cholesterol fatty liver (21). Recently, Treadwell et al. (215) confirmed this and stated that the rat at 1°C has an increased capacity for metabolizing fat, even in the absence of an adequate dietary supply of lipotropic factors.

Concluding remarks:

Further research is necessary. The recent reports: 1. of Hausberger and Milstein (93) on the superiority of lipogenesis in adipose tissue over that found in hepatic tissue, and the observation that fasting or feeding a high fat diet abolished lipogenesis in adipose tissue; 2. of Hill et al. (104) that hepatic lipogenesis is also depressed by an increasing fat intake even when the carbohydrate intake is adequate; 3. of Smith and Fairhurst (195) of a highly significant depression of the P/O ratio in liver mitochondria from cold adapted rats, which is not due to an increased degradation of A.T.P.; and 4. of Felts and Masoro (72) that cold acclimation modifies the hepatic response to fasting as evidenced by a maintenance of liver glycogen, a lack of fatty acid infiltration and the observation that liver slices were better able to oxidize labelled acetate and palmitate to labelled CO_2 as compared to fasted non-adapted

animals; are indicative of a trend toward an understanding of the process of acclimation to cold at the cellular level and a realization of the interdependence of the liver and the striated muscle mass for thermogenesis in the cold, which in turn may be enhanced by specific dietary modifications.

THE PROBABLE MECHANISM OF ADAPTATION TO COLD

Introduction:

Because we have utilized cold as a tool in attempting to discover the site of action of Vitamin C and its possible interrelationship with Vitamin A, and with diets of various compositions; we submit a brief review of the literature concerning the most probable mechanism by which homeotherms (especially the rat) adapt to cold.

Adaptation, acclimation, acclimatization, or conditioning to cold are terms which are often used synonymously to describe these phenomena which can be defined as: the physical and chemical responses of homeotherms when subjected to temperatures below that which the animal is normally accustomed to. The term acclimation has been popularized by the Canadian National Research Council group with the understanding that it would apply to the changes, metabolic and others, which are observed in animals exposed to cold under artificial conditions. On the other hand, the term acclimatization has been applied to the seasonal changes which occur in animals exposed to cold in nature, while adaptation to cold was to be used only to mean the genotypic changes which are produced in species subjected to variations in climatic conditions. Since workers in the field

often use acclimation and acclimatization interchangeably, it has been suggested that "conditioning to cold" be used instead of "acclimation". It seems imperative that a uniform terminology be adopted. For practical purposes we will use the term of the author being quoted.

I. Adaptation:

The process of adaptation begins when a homeotherm is placed in a moderate cold (usually 2 to 6°C.). In general, the milder the temperature, the lower the mortality and the longer the time necessary for adaptation to set in. At 2°C a rat is considered adapted after approximately 4 weeks exposure. At this time the animal usually has regained his initial body weight and is gaining. This animal is now able to withstand short periods of exposure to acute cold (-5°C to -20°C) which are usually lethal to room temperature animals.

The development of the adaptation phenomenon has been postulated as being mediated primarily through the agency of the pituitary (219), adrenal (190), and the thyroid (39) (112b) glands.

Adrenal Medulla:

Depocas (33) has shown that within three minutes after rats are exposed to cold (6.0°C), the metabolic rate is increased 2,5 to 3 times. Moreover, pre-adapted animals are

not only better able to maintain this augmentation but can accentuate it if the need arises. He (35) has also shown that this increase is due primarily to an increased release (secretion) of nor-adrenaline. However, Thibault (214) showed that the calorigenic action of adrenaline is reduced by 90% in the absence of the thyroid. This has been confirmed by Swanson (209).

Pituitary-Thyroid:

This initial increase of the metabolic rate promotes a fall in the concentration of thyroid hormones in the peripheral blood. Harris (89) states that this decrease in the concentration of the peripheral and blood thyroid hormones serves as the stimulus to the thyroid gland. However, he believes this stimulus is mediated through the central nervous sytem by a nervous reflex rather than by the classical feedback mechanism. He has shown that the actual stimulation of the thyroid gland by T.S.H. is mediated through the hypothalamic control of the anterior pituitary secretion of T.S.H. (90). Moreover, he has relegated the feedback mechanism of target organ hormones "to maintaining an optimal level of activity in the pituitary organ system under basal conditions". In doing so he has explained how an increased blood concentration of thyroid hormones (such as during some stages of cold exposure) can be maintained without eliciting

a vicious circle of events. Incidentally, Harris and co-workers (89) have also shown that cold is the only environmental stimulus which causes an excitation of the thyroid. All other stresses, including those involving physical trauma, cause an inhibition of the thyroid. The results of other investigators (42) (189) (112) (200) has made it increasingly evident that the pituitary-thyroid relationship is necessary for the establishment and the maintenance of adaptation to cold.

Adrenal cortex:

The work of DesMarais (39) and Héroux and Hart (103) demonstrates that adrenal hypertrophy is not necessary for acclimation and that adaptation can be acquired with a minimum of cortical hormones. Other workers (5) (143) (53) have shown that adrenal function can be maintained in the cold without a hypersecretion of ACTH. However, the thermoregulatory efficiency of the thyroid hormones is considerably decreased in the absence of adrenal cortical hormones (200) (40). Thus the normal functioning of the thyroid in the cold, is assured by a minimum quantity of cortical hormones and adrenal hypertrophy is probably an index of non-adaptation (41). Consequently, the adrenal can be considered a second line of defence, which is brought into play when the thyroid fails to fully meet the challenge (41).

II. The maintenance of body temperature in the cold:

The site or sites, in the rat, of heat production and/or heat conservation in the cold has been postulated as being a function of all or some of the following:

1. Insulation: a. thickness of fur and/or skin.
   b. thickness of subcutaneous fat.
2. Changes in peripheral circulation.
3. Mechanical thermogenesis (shivering) of muscle.
4. Chemical thermogenesis of the viscera.
5. Chemical thermogenesis of muscle (striated).

Insulation:

It is difficult to ascribe increased resistance to cold to the development of an increased amount of insulation. Sellers (190) has shown that hair is not the only factor of great importance. Moreover, Héroux (102) reported that a reduction and not an increase in pelt weight occurs in the cold (6.0°C.) Pagé and Babineau (150) have shown that subdermal insulation (subcutaenous fat) is relatively unchanged in the cold, and that feeding a high fat diet results in the deposition of excess fat in adipose tissue rather than subcutaneously.

Circulatory changes:

The following is a modification of a recent evaluation written by DesMarais (45). It is a matter of common experience to observe circulatory changes in the skin of the rat exposed

to cold. Ears and tail become pale and later extremely red and then eventually succumb to cold injury. This indicates vasoconstriction followed by vasodilatation and finally an inability to surmount injury induced by cold: first, conservation of heat, second rewarming of tissues. The latter probably occurs too late to reverse the existing cold injury in these appendages which are wholly dependent on the circulation for heat. (ie. have no underlying muscle with which there is "direct vertical vascular convection (27)". Recent observations by Héroux et al. (98) show that rats conditioned to cold have a larger number of "opened" skin capillaries with which is associated an increased skin temperature. Thus the skin reaches a state of permanent hyperemia. This phenomenon protects the skin from frostbite (if it has not already occured) but it is far from being economical as far as heat conservation is concerned. Héroux has found that rats acclimatized to cold as opposed to conditioned to cold (ie. rats kept outdoors in winter and permitted to nest and huddle as opposed to artificial cold room conditions) have a similar increase in vascularization, but the skin temperature is lowered rather than increased. Maintaining a lower skin temperature decreases the thermal gradient between the body and the environment, thus decreasing the rate of heat loss. Indications are that important differences must exist in the

way homeotherms build up their resistance to cold in nature, as compared with those exposed to cold under laboratory conditions, and these await further research.

Chemical thermogenesis vs. Mechanical thermogenesis (shivering):

Sellers and coworkers (184) demonstrated that cold acclimated rats could increase heat production independently of muscular tone and shivering. This evidence in conjunction with that of Cottle and Carlson (30), Dohoffer et al (49) and Davis and Mayer (32) implies that a well developed chemical thermogenesis exists in cold adapted animals.

Chemical thermogenesis of muscle vs. that of viscera:

Depocas (34) has demonstrated that the presence of the abdominal viscera is not essential for the manifestation of chemical thermogenesis in the cold acclimated albino rat. He states that "shivering is the process by which warm acclimated rats increase their heat production in the cold," however once rats have become adapted, "muscular chemical thermogenesis is the main process". One cannot deny that the abdominal viscera contribute to cold adaptation. The viscera are responsible for the higher metabolic rate ($M.R._{30^{o}C.}$) of cold acclimated rats. We believe these organs (especially the liver) play a role which is not directly associated with heat production, and that these organs undergo the observed morphologic and metabolic modifications so as to support the increased heat production (chemical thermogenesis) of the striated muscle mass.

III. Adaptation to artificial vs. natural cold:

Héroux et al. (97-101) have shown that "while the metabolic adjustments such as increased peak metabolism and decreased shivering were similar in outdoor rats (natural acclimatization to average -10°C. in period from October to January) and indoor rats (thermal acclimation to +6°C. cold room), the physiological adjustments to attain this end were different". Under the two types of cold exposure, the rats were observed to have a reduced muscle growth. However, the enlargement of the thyroids, pituitary, heart, and digestive tract; and the reduction of the mesenteric and subcutaneous fat; and of the pelt weight, which have repeatedly been found in the "indoor" cold-acclimated rats, did NOT take place in the "outdoor winter" rats. Adrenal hypertrophy did not occur in the "outdoor winter" rats. As compared to their respective (outdoor summer and room temperature) controls, "in vitro" adrenal steroid production was increased in the "outdoor winter" rats but decreased in the "cold room" rats. Winter exposed rats did not have a higher thermoneutral metabolic rate. Though their thyroids showed evidence of increase activity, there were no signs of cold injury such as occurs in "cold room" rats. Details concerning these differences and others which were observed will not be considered further in this review.

It is clear that increased resistance to cold can be brought about without some of the anatomical and physiological changes characteristically associated with acclimation under rigorous conditions to continous artificial cold exposure. The importance and the implications of Héroux's work are self-evident. It does not negate the postulated mechanism of adaptation to cold which has been elucidated. However, these experiments probably do approach more closely the process of adaptation as it possibly occurs in man (180) (105).

It is our opinion that the biochemical mechanism of adaptation to cold will become more difficult to determine and evaluate if care is not taken to relate the findings to the conditions under which adaptation was obtained. Moreover, such practices as the excision of the tail before exposure to cold (22), the coating of metal cages with a polyethylene film or other non-conducting material (215) might conceivably introduce results which could not be compared to the existing data (derived from experiments performed under rigorous conditions) without some hesitation. The effects of allowing animals to nest and huddle in wooden or plastic cages in continuous artificial cold has yet to be examined.

EXPERIMENTAL: DESCRIPTION OF METHODS:

Introduction:

Even though the primary purpose of each experiment in this thesis may be different, the methods utilized are the same.

Vitamin C.:

The determination of reduced ascorbic acid was made by the 2,6 dichlorophenolindophenol titration method of Bessey and King (14). Tissues analyzed: 1. Liver (in all experiments): approximately 500 mg. from the median lobe of the liver. 2. Neutral Fat (experiment X only): approximately 1000 mgs. of perirenal fat. 3. Muscle (experiment XI only): approximately 5 grams of abdominal muscle.

The liver and neutral fat were homogenized on a glass tissue grinder (13mm.O.D. by 100mm. in length). Muscle was homogenized in a semi-micro monel metal Waring blendor.

Vitamin A.:

The determination of liver vitamin A was made by the method of Gade and Kadlec (80) (81). The vitamin is solubilized in the test material (in this case, liver) with a surfactant and extracted with a mixed solvent (ethyl and petroleum ether).

Weighed liver samples (approximately 5 gm.) may be frozen (81) for the determination of vitamin A at a later date,

however, this requires a rapid and thorough freezing of the tissue and a reliable freezer. We preferred extracting the vitamin A from fresh liver samples and storing it as described below.

The fresh or frozen liver sample is blended with surfactant in a semi-micro Waring blendor. This mixture is gently agitated with mixed solvent in a 125 ml. glass stoppered erlenmeyer which has been placed on a Magne-stirrer. Duplicate aliquots (3 to 5 ml.) of the solvent layer are removed into clean test tubes, and then evaporated to dryness under vacumn while the tube is swirled in a +45°C. water bath. If the aliquot is to be stored, the test tube with contents is flushed with dry nitrogen, stoppered and placed in the refrigerator. These aliquots, unless accidentally unstoppered, preserved the vitamin A for at least six weeks without a noticeable decay. Later, (usually within two weeks) the residue is dissolved in a known quantity of chloroform. The vitamin A content of a 2ml. aliquot of the chloroform solution is determined by the Carr-Price method (22), using a Coleman Jr. Model 6 A Spetrophotometer.

Organ wet weight:

The following tissues were weighed on a Cent-O-Gram triple beam scale to 0.01gm.

1. Total liver and sample for Vit. A determination.

2. Kidneys.

3. Testes.

4. Perirenal fat of left side of rat (150).

The following were weighed on a Roller-Smith torsion balance to 0.1 mg.

1. Thyroids.

2. Adrenals.

3. Seminal vesicles.

Liver; dry weight, defatted dry weight:

A sliver of liver (approximately 100 mg.) is placed in a pre-weighed 2 ml. fine porosity, büchner micro-funnel, and immediately re-weighed to determine the exact wet weight. The sample is then dried for 24 hours in a 90°C. oven and re-weighed. From the difference between the wet weight and the dry weight, we calculate percent water or percent dry weight. The dry tissue is matted and washed three times with boiling absolute ethanol and three times with boiling acetone and again dried for 24 hours before re-weighing. From the dry weight data and the defatted dry weight data, we calculate percent fat. From the defatted dry weight and fresh weight data we calculate percent defatted dry wt.

Thyroid histology:

The thyroids were fixed in Bouin's fixative and processed by the method of LaHam and DesMarais (45). They have

shown that with their technique it is "histologically possible to differentiate the follicles containing iodinated thyroglobulin (stained blue) from those which do not (stained yellow): and in the case of mixed follicles (blue and yellow) to estimate with reasonable accuracy the proportion of active colloidal substance".

Oxygen Consumption:

Oxygen consumption measurements were made in rats at thermal neutrality (± 30°C.) in experiments No. 5, 10 and 11. The method utilized is that of Depocas and Hart (36) and involves the use of the Pauling oxygen analyzer in an open circuit system. The Q_{O_2} is calculated from measurements of the partial pressure changes in a metered portion of the CO_2 free air leaving the animal cage. The Q_{O_2} values obtained are unaffected by the R.Q. (respiratory quotient) of the animal (36).

Statistical treatment:

The data have been tabulated and subjected to the analysis of variance (197); and when according to the tables, the F was significant, the Duncan test (57) was used to locate the differences between the means which were significant. Where unequal numbers of replications were encountered, the extension of the multiple range tests by Kramer (111) was used to group the means. Linear correlations were tested by the least square method (197).

Meaning of Group Symbols:

OA: on a vitamin A deficient Test diet.

+A: on test diet supplemented with 500-1,000 I.U. vitamin A per week.

nA: (Exp. I. only) on test diet supplemented with 56 I.U. vit. A/week.

N: (Exp. I only) normal Purina Laboratory Chow Meal.

L: Exps. X and XI.) Purina Laboratory Chow Cubes.

F: (Exps. X and XI.) High Fat Diet (see table 54).

H: (Exps. X and XI.) High Carbohydrate Diet (see table 54).

K: This symbol prior to any of the above group symbols (eg. KF) means that the group was exposed to cold (2.0°C.).

C: This symbol placed after any of the above group symbols including groups exposed to cold, (eg. ±AC or KFC) signifies that the group received exogenous vitamin C. ('Redoxon' liquid, Hoffmann-LaRoche).

EXPERIMENTAL: EXPERIMENTAL RESULTS:

EXPERIMENT 1.

Introduction:

The main conclusion of our review of the literature concerning the possible interrelationship between vitamin A and ascorbic acid was that, due to the contradictory results obtained by different workers, this problem remained unsolved. In our attempt to resolve this enigma, our first objective was to determine whether a relationship could be found between the two vitamins. It was decided to place rats on a vitamin A deficient diet, wait for the deficiency signs to appear, and then measure the level of ascorbic acid in the liver. This organ is the preferential site of ascorbic acid synthesis in the rat (21). In addition and consequent to Dr. DesMarais's previous experience, it was decided to expose some of the animals to cold in order to bring out differences which might not be observable at room temperature, since it is a recognized fact that the needs for ascorbic acid are increased in cold exposed animals (41).

EXPERIMENTAL PROTOCOL:

Sixty male albino rats of the Wistar strain, ranging in weight between 55 and 68 grams, were divided into four groups of 15 animals each and caged individually in metal cages with

wire-screen bottoms. Group N was placed on Purina Fox chow meal. The remaining three groups were placed on a vitamin A deficient test diet (*) without supplement (group 0A) or with either 280 (group +A) or 28 (group nA) I.U. of vitamin A (**) twice weekly 'per os'. Both the weights of the animals and the amount of diet consumed were recorded every other day. Animals of group 0A (vitamin A deficient) showed a levelling off of weight gain and early clinical signs of vitamin A deficiency 85 days after the beginning of the experiment. At this time, five animals of each group were killed and the following determinations were made: Liver ascorbic acid and vitamin A; Wet weight of liver, testes, adrenals and thyroids; and thyroid histology. The remaining animals were placed in a cold room, the temperature of which varied from +1.00 to +4.0°C., until half of any one group were dead; this occured in the 0A group after 55 days of exposure to cold (total 140 deficiency days). From this day on the remaining animals were killed at a rate of 3 to 6 per day. The determinations previously mentioned were made. In this experiment, weighed samples of the liver of each animal were placed in aluminum dishes and frozen. These frozen samples were later analyzed for vitamin A. This method of freezing the liver samples was not utilized in subsequent experiements (see Description of Methods).

* U.S.P. XIV specifications - G.B.I. Inc. (see table 23)
** Vitamin A acetate (synthetic) - G.B.I., diluted in corn oil.

RESULTS and DISCUSSION:

Table 1 depicts which of the determinations have or have not, according to the analysis of variance, revealed differences between the groups at the 1% level of significance.

1. Body Weight Gain and Survival (Fig. 1 and Table 2 and 3).

The weight gain in all groups prior to exposure to cold (at 85th day) was not significantly different (table 2); however, at this time one deficient animal (OA) had died and several showed signs of the deficiency (anorexia, decreased weight gain, decreased activity, poor posture and the pathognomonic xerophthalmia). Moreover, body weight gain in the deficient group was beginning to level off (Fig. 1).

After 55 days of exposure to cold, only 50% of the deficient animals (Group KOA) had survived; whereas survival was 100% in the other groups. The KOA animals also had a significantly greater weight loss as compared to the other groups in the cold (table 3).

The time necessary for the deficiency to develop in this experiment was rather long when compared to that reported in the literature; however, these rats were not ordered specifically for Vitamin A experimentation and the supplier had probably supplemented the weanlings with cold liver oil and therefore, a longer time was necessary to deplete the

animals. In our subsequent experiments the time necessary to render the animals deficient was shorter.

The decreased survival and extensive weight loss of the deficient animals in the cold (KOA) might well be an indication that vitamin A deficient animals have a decreased resistance to cold (133) (64); however, because there were no simultaneous room temperature deficient and control animals, one cannot ascribe the effect to cold exposure 'per se' or simply to the increasing severity of the deficiency.

2. Liver vitamin A.

No extensive data on this subject are presented in this experiment because the number of reliable determinations were limited. Many liver samples were inadequately frozen and/or were partially thawed due to mechanical failure of the refrigerator. In subsequent experiments the vitamin A was immediately extracted and stored as described previously.

We are only able to state that in no case was vitamin A present in the liver of deficient animals (groups OA and KOA); whereas the liver of all other groups contained vitamin A.

3. Liver ascorbic acid concentration (table 4).

There are no significant differences between the means of all groups on the 85th day (room temp.). At this time, the vitamin A deficient animals (OA) had no detectable liver

vitamin A and several showed clinical signs of the deficiency, but no significant weight loss. Apparently ascorbic acid synthesis was not impaired.

After exposure to cold, and as the deficiency progressed, there was a tendency for a decrease in the ascorbic acid concentration in the liver of the deficient (KOA) and purina chow control (KN) animals. The supplemented group (K+A) had a tendency for an increased liver ascorbic acid concentration from the value on the 85th day. Specifically, the KOA and KN groups had a significantly lower liver ascorbic acid concentration as compared to K+A. We hesitate to attribute this a given change to cold exposure ~~and/~~or to the progressing deficiency state, or both.

4. Liver weight (table 5).

Because we later wish to introduce 'total liver ascorbic acid', we believe total liver weight changes should be considered now.

There are no significant differences between the weights of the livers in those groups killed on the 85th day (prior to the exposure of the remaining animals to cold). With exposure to cold, a slight increase in liver weight was observed in groups KN, KnA and K+A. Group KOA liver weight was significantly decreased when compared to the other cold exposed groups. This latter observation may in some degree

be attributed to the slight increase in liver weight in the control groups. During the cold exposure, body weight did not change considerably in the control groups and thus the tendency to gross hypertrophy of the liver is probably due to cold (102). The deficient animals in the cold were not able to maintain their body weight, nor augment their liver weight. Statistical analysis for a linear correlation between body weight and liver weight gave an r = 0.760 which we interpret as a lack of a correlation, in part due to the decreased body weight gain and increased liver size in the cold.

5. Total Liver Ascorbic Acid (Table 6).

These values were obtained from ascorbic acid concentration multiplied by the weight of the liver. As mentioned previously, the liver is the preferential site of ascorbic acid synthesis in the rat. We believe that 'total liver ascorbic acid' is an important index of the ascorbic acid status of the rat because it represents the total synthesizing mass or potential of the animal. The synthesizing mass may or may not have access to a limited capacity for storage.

Sure et al. (206) noted that the ascorbic acid concentration did not change in short term starvation and in vitamin A deficiency. We starved six rats (approx. 250 gm. body weight) for seven days (at this time, perirenal fat was not exhausted) and also found that as compared to

control animals, the liver ascorbic acid concentration values were not significantly different ($t = 1.97$; $p = 0.1$). However, liver weight was reduced by half ($t = 11.98$; $p = 0.001$) and, therefore 'total liver ascorbic acid' was also reduced by approximately one half, ($t = 12.74$; $p = 0.001$). We believe this latter expression of the data gives an authentic assesment of the actual ascorbic acid status of the animal. If we assume that the total liver cell number has not drastically changed during this period of starvation, then the decrease by one half of the total liver weight actually means that there are twice as many cells per unit mass. Although the concentration of ascorbic acid per gram of tissue has not changed, it (the concentration) is more than likely decreased by nearly half per unit cell. This same pattern was also found to apply in the case of liver vitamin A; I.U. of vitamin A per gram ($t = 1.29$; $p = 0.3$) as compared to total liver vitamin A in I.U. ($t = 4.71$; $p = 0.001$).

The expression of the results in this manner does not, in this experiment, lead to observations which are substantially different from those given for the liver ascorbic acid concentration data. There are no significant differences between groups killed at room temperature on the 85th day. After cold exposure, the deficient group (KOA) had a significantly lower total ascorbic acid status as compared to

Groups KnA and K+A, but not as compared to Group KN. A clear conclusion is not possible and one wonders whether the vitamin C status of the animal would be significantly lowered in vitamin A deficiency as compared to controls on the same type of diet if cold had not been utilized.

6. Testes Weight (Table 7).

The results show that the cold exposed groups (KnA, K+A and KOA) have smaller testes than did the same groups killed on the 85th day at room temperature. Only the vitamin A deficient animals (KOA) have testes significantly smaller than those in all other groups. The weight of the testes, in this experiment, was correlated to the body weight with a coefficient r = 0.952. Thus, the testicular weight changes may simply be secondary to body weight changes. Héroux and Gridgeman (102) concluded that the genitals were not directly affected by exposure (4 weeks at 6°C.) to cold. On the other hand, Dugal (51) and Perrault (153) have observed, in different situations of cold exposure, a definite decrease in the weight of the testes, which appears to affect both the spermatogenic and endocrine functions of the gland (51). These changes are probably secondary to body weight changes during the initial exposure to cold, however they become marked and unrelated to body weight as the duration and degree of exposure is increased.

It has been shown that vitamin A deficiency in the rat is accompanied by a regression of testicular weight (228) (123). In our experiment, it might be that either exposure to cold exaggerated the effects of the deficiency, or that conversely, the effects of cold were enhanced by the deficiency; it might also be an interplay of the two separate processes. A more enlightening interpretation awaits a more thorough knowledge of the effects of cold and of vitamin A deficiency on testicular morphology and function.

7. Thyroid and Adrenal Weight (Table 8 and 9).

The thyroid weights of all groups killed on the 85th day are not significantly different. The thyroid weight in the groups exposed to cold is not significantly different but tends to be greater than that of the room temperature groups. The supplemented groups KnA and K+A have significantly heavier thyroids as compared to the room temp. groups N, +A, and OA. The thyroid weight of groups KnA, KN and KOA are not significantly different from their weight on the 85th day (nA, N, and OA), but are on the heavier side. Increased thyroid weight and/or function is expected in cold exposure (39) and the vitamin A deficient animals did not appear to lose this tendency. Nonsignificant changes in thyroid weight do not mean that the gland has not undergone a substantial increase in follicular activity when exposed to cold. We

simply state that the thyroids of the deficient animals appeared capable of increasing their activity in the cold; a discussion of thyroid histology in vitamin A deficiency is undertaken in experiment V.

Adrenal weight: The only groups in the cold which showed an increase in adrenal weight over their initial controls killed before exposure to cold are groups KN and KOA, respectively significant at the 1% and 5% levels. Curiously enough these two groups had the greatest dietary difficulties in the cold. The deficient group's diet lack is self explanatory. The laboratory chow (meal) diet fed the control group in the cold contains roughage and therefore a larger amount of diet needs to be eaten to extract the necessary calories. The semi-synthetic diet is apparently a more adequate diet in the cold. Little significance can be attached to adrenal weight changes in the absence of a thorough morphologic and metabolic study. We have bothered to record the data in hopes that a definite trend may evolve which could probably be utilized as a guide in further specific experimentation.

CONCLUSIONS:

Vitamin A deficiency is accompanied by metabolic changes reflected in body and organ weight changes. This deficiency

state impairs resistance and adaptation to cold. Before anorexia and resulting weight loss occur#, but after the liver vitamin A stores of the rat are depleted, the vitamin C status is not affected. As the deficiency state progresses further and is hastened by exposure to cold to the point where the animals are moribund and in a state of protein deteriation, the liver ascorbic acid status tends to be adversely affected. Questions arise as to whether 1. the ascorbic acid status would be as drastically reduced in the terminal stages of the deficiency when not in the cold, where the requirement for ascorbic acid is greater. 2. Would the exogenous administration of ascorbate ameliorate the deficiency and/or the response of deficient animals to cold exposure? These questions are not only prompted by the evidence for the increased protection afforded by vitamin C in the cold (56) (41); but also Mayer and Krehl (127) have reported that vitamin A deficient rats develop scurvy-like symptoms which were cured with the administration of ascorbic acid and which, in one of their experiments, prolonged the survival of such vitamin A deficient rats.

EXPERIMENTS II, III, and IV.

Introduction:

The purpose of these experiments was primarily to determine whether ascorbate administration could ameliorate vitamin A deficiency in the rat.

EXPERIMENTAL PROTOCOL:

In all three experiments, Vitamin C was administered to both vitamin A deficient and control animals for 10 days prior to sacrifice. This was done only at room temperature ~~only~~ in Experiment II, and simultaneously at room temperature and in the cold in Experiments III and IV. Both, the treatment and exposure to cold were commenced on the same day. This day is the 48th deficiency day in Experiment II; the 38th deficiency day in Experiment III; and the 32nd deficiency day in Experiment IV.

Grouping: In each of the experiments, male albino rats of the Wistar strain, ranging in weight between 37 and 57 grams (mean 45 grams), were caged individually and divided into four room temperature groups in Experiment II, and into four room temperature and four cold room groups in both Experiments III and IV. Experiment II: Each control group (N and NC) had 10 animals. Each experimental group (0A and 0AC) had 15 animals. Five rats of each of these latter experimental groups (total 10 rats) were sacrificed

as initial controls (OAic) on the day of onset of the 10 day treatment period (day 48). Experiments III and IV: Each room temp. control group (+A and +AC) had 5 rats and each cold room control group (K+A and K+AC) had 7 animals. Each room temp. experimental group (OA and OAC) had 8 animals, and in the cold consisted of 12 rats (KOA and KOAC).

Diet: All groups were fed 'ad libitum'. All experimental groups were on the U.S.P. vitamin A deficient test diet. The control animals in Experiment II were on Purina Fox Chow meal. The control animals in Experiments III and IV were on the U.S.P. test diet supplemented with 250 I.U. of vitamin A twice weekly 'per os'.

Cold room and treatment: The cold room temperature fluctuated between + 1.0°C. and + 4.0°C. (mean ± 2.0°C.). In all three experiments, Ascorbic acid**, 150 mg. in two divided doses was injected intraperitonealy morning and evening during the last 10 days prior to sacrifice. When the vitamin A deficient animals of Exp. III were placed in the cold (on the 38th day of the deficiency), survival was poor and therefore all the remaining animals were sacrificed after only 4 days of exposure and treatment. Experiment IV is a repetition of the Exp. III protocol. In Exp. IV, the treatment and exposure to cold was instituted 6 days earlier

** Redoxon (Ascorbic Acid U.S.P.) Hoffmann-LaRoche.

(on the 32nd day of the deficiency), and the shock of cold exposure ameliorated by gradually lowering the cold room temperature from + 10.0°C. to + 2.0°C. during the first five days of the ten day exposure period. The onset and duration of the treatment and exposure to cold for a given experiment is recorded on the body weight graphs (Figs. 2, 3, and 4).

At autopsy: At autopsy, the following determinations were carried out: Liver ascorbic acid and vitamin A; wet weight of liver, testes, adrenals and thyroids. In Exp. III the wet weight of the seminal vesicles was also recorded as an index of testicular endocrine function (218). The thyroids were processed for histological examination.

RESULTS AND DISCUSSION:

1. Survival and Body Weight Gain (table 10).

EXP. II (Fig. 2): Survival in the experimental (OA) groups was as follows; Group OA: 13/14(93%) and in Group OAC: 10/14(74%).

On the 48th day (on which administration of vitamin C began), the body weight gain of both vitamin A deficient groups (OA and OAC) had already become significantly inferior to that of the control groups (N and NC) ($t = 12.01$; $p = 0.001$). Treatment of the deficient animals with exogenous vitamin C did not ameliorate their weight losing status and actually tended to promote decreased survival. Treatment with ascorbate had no significant effect on the controls.

EXP. III (Fig. 3 and Table 11): This experiment was terminated after four days of exposure to cold and treatment with vitamin C because survival in the cold of the vitamin A deficient animals was extremely poor (6/11(55%) in group KOA and 4/10(45%) in group KOAC). At this time, survival at room temperature in the vitamin A deficient groups was 5/6(84%) in group OA and 6/6(100%) in group OAC.

These data indicate that rats deficient in vitamin A are less resistant to a cold stress and are a confirmation of our observations in EXP. I. It is evident that if the protocol of this experiment is to be successfully completed,

the vitamin A deficient animals must be entered into the cold at an earlier stage in the deficiency.

The weight gain data reflect the survival results in that the weight change was most acute in those groups with the lowest survival (KOA and KOAC). Weight gain but not survival was better in group KOAC. It is possible that the short term administration of vitamin C exerted the observed beneficial effect on weight gain. The administration of vitamin C to normal animals (+A's) at room temperature tends to produce a lag in weight gain which usually does not occur in the cold (K+AC vs. K+A). This phenomenon is not statistically significant but is often observed and has not been critically examined.

EXP. IV (Fig. 4 and Table 12): Because the ten day exposure to cold and treatment with vitamin C was inaugurated earlier (on the 32nd day), the survival in this experiment was only slightly affected in one group (KOA, 10/11(91%). In general, weight gain was retarded in the cold.

Group KOAC had a significantly lower weight gain as compared to the room temperature deficient and +A groups but not as compared to the cold room groups.

2. Vitamin A:

A) I.U. vitamin A per gram of liver (Table 13): In all three experiments, irrespective of whether the deficient

animals received vitamin C or were exposed to cold, vitamin A was never detectable in the liver. It is obvious that, as compared to all the control animals, which always had unquestionable quantities of liver vitamin A, the deficient animals were significantly depleted of all liver stores. Treatment with vitamin C could not exert a beneficial effect by sparing vitamin A stores because these deficient animals were completely depleted prior to the onset of the treatment. However, the vitamin C had an opportunity to possibly 'replace' for the vitamin A and favorably modify the deficiency state and/or survival, but this did not seem to occur.

Short term exposure to cold and/or treatment with vitamin C had no significant effect on the liver vitamin A concentration of the control animals. If no effect occurs in normal animals, one hesitates to believe that it would have an effect in deficient animals, however this does not nullify the possibility.

B) Total liver [redacted] vitamin A (table 14): We have already mentioned the absence of liver vitamin A in all the deficient animals. The data of Experiments II and III are not significant.

EXP. IV (Table 15): These data are significant at $p = 0.05$, We attribute the observed difference between group

K+AC amd +AC to the considerably lower total liver vitamin A of group +AC. These animals received their supplement of vitamin A at the same time as the other groups and would be expected to have a comparable status. The mean liver weight in this group (EXP. IV Table 18) is significantly less than that of the comparable room temperature +A group; and it is probably related to the decrease in body weight which we have associated with a lag in weight gain during the initial vitamin C treatment days.

3. Liver ascorbic acid concentration (Table 16).

No statistically significant differences were found in experiments II and III. However, in experiment II we do observe that the deficient animals (OA) potentially tend to have lower concentration values when compared to the control (N) and deficient animals which received vitamin C (OAC). Apparently the administration of exogenous vitamin C to control animals (NC) on a fox chow diet did not cause, as compared to group N, a noticeable change in the liver concentration. In experiment III, both the room temperature and cold room deficient animals which received vitamin C (OAC and KOAC) potentially tend to have a greater concentration value than their deficient controls (OA and KOA). Both control groups which received vitamin C (+AC and K+AC) potentially tend to have lower concentration values than their controls

(+A and K+A). These potential tendencies are not significant and are mentioned only as indexes of the probable direction the results might take under other experimental conditions.

EXP. IV: Although the administration of vitamin C and exposure to cold were initiated before weight gain plateaued, the treatment with ascorbate did not benefit the deficient animals.

As in the previous experiments, the liver ascorbic acid concentration of the deficient groups (OA and KOA) was not significantly different from that of the normal controls (+A and K+A).

The administration of vitamin C to normal rats (+AC as compared to +A) significantly lowered the liver ascorbic acid concentration. A tendency to this response was noted in experiment III.

The mean liver ascorbic acid concentrations of the deficient groups (OAC and KOAC) which received vitamin C is significantly lower than that in group +A but not from that in groups +AC, OA and KOA.

Our observations in these three experiments point out the need for a study of the responses of animals on various diets and experimental conditions to doses of vitamin C. It is baffling that the administration of vitamin C did not

alter the liver concentration values of the animals on Fox Chow in experiment II but actually diminished, to the point of statistical significance, the liver concentration of ascorbic acid in the control animals in experiment III (compare +A with +AC). No studies, to our knowlege, have been made on the effect of exogenous vitamin C on the total body ascorbic acid status of the rat. Thus we have no indication as to whether the observed response is to be expected. Dugal (52) has suggested that, as in many endocrine glands, the rate of synthesis may be diminished when an exogenous source of the particular chemical substance is administered. This is plausible and must be considered. It is also possible to expect that some part of the administered ascorbic acid might be capable of being stored, as in the case with vitamin A. Of course vitamin C is synthesized by the rat and is not a true vitamin. We wonder whether our data and the conflicting results of other investigators could somehow be linked to the differences in the composition of the diets utilized to produce vitamin A deficiency in the rat. Both Sure et al. (206) and Jonsson et al. (107), utilizing yeast containing vitamin A deficient test diets, reported that administered vitamin C was not apparently available to their animals. Whereas Mayer and Krehl (127), utilizing a vitamin

A deficient test diet in which the crystalline B vitamins were incorporated in the place of yeast, observed that ascorbic acid administered to deficient animals restored the tissue ascorbic acid levels to normal. We designed our next experiment (V) so as to test this possibility.

4. Total liver ascorbic acid (Table 17):

EXP. II: The total liver ascorbic acid of the deficient group (OA) is significantly less than that of the control group (N). This difference is primarily a consequence of the significantly different liver weights between these two groups. This is discussed further below. The administration of exogenous vitamin C had no significant effect on either the control (N vs NC) or deficient (OA vs OAC) animals.

EXP. III: Group +A (room temp. control) has a significantly greater mean total liver ascorbic acid than all other groups except group +AC (r.t. control + vit. C.) and group OAC (r.t. deficient + vit. C.). As in the previous experiment the higher total liver ascorbic acid value of the control groups (+A and +AC) is most likely due to heavier liver weights. The total liver ascorbic acid data of the room temperature deficient group which received vit. C. (OAC) are not statistically different from either group +A nor the

untreated room temp. deficient group (OA). Within the control groups (all groups with +A in symbol), those which received vitamin C (+AC and K+AC) potentially tend to have lower total values, when compared to their respective untreated control (+A and K+A). Within all the deficient groups, those which received vitamin C (OAC and KOAC) potentially tend to have higher total values when compared to the respective untreated deficient groups (OA and KOA).

EXP. IV: Group +A has a significantly greater mean total liver ascorbic acid than all other groups including group +AC. The significantly greater value in group +A is probably the resultant of a high liver ascorbic acid concentration and a heavy liver; whereas in comparison, group +AC had a significantly lower liver ascorbic acid concentration and a significantly lower liver weight. This effect of exogenous vitamin C on control animals does not visibly affect the well being of the animals. It may be that when a normal rat is not under physical stress or in a given deficiency state, or both, it does not need the administered vitamin C and therefore cannot tolerate the excess. This is questionable because one would expect excess water soluble vitamin to be excreted without a noticeable effect on body weight; however, it is our experience, that normal animals tend to show a lag in body weight gain during the initial week or so of vitamin C

administration. Matsko et al. (124) has shown that when ascorbic acid, in excess of six times the M.D.R., is administered to guinea pigs, their weight gain is retarded. To our knowledge research concerning this problem has not been done. It is a basic problem and should not be avoided simply because the rat synthesizes its own ascorbic acid.

Though the liver ascorbic acid concentration data of deficient animals, as compared to controls, have often been nonsignificant, we note that expression of the results as total liver vitamin C often gives significant results which are for the most part attributable to liver weight changes. One cannot deny that to some extent liver weight is related to body weight; and we question whether the differences observed could have been a function of the increased caloric consumption and therefore increased body and organ weights in the control animals. Mapson and Walker (121) attributed the decrease in tissue ascorbic acid during vitamin A deficiency to the reduction in food intake which accompanies deficiency. Our next experiment was designed so as to control this factor by utilizing the pair-feeding technique.

5. Liver weight (table 18):

EXP. II: There exists a linear correlation between the liver weight and body weight ($r = 0.9620$). The control animals (N and NC) have significantly larger liver weights as

compared to the deficient animals (OA and OAC). The decreased body weight and liver weight of the deficient animals is probably a consequence of the anorexia which accompanies terminal vitamin A deficiency. The administration of exogenous vitamin C has no significant effect.

EXP. III: A processing of these data for linear correlation was not undertaken. The liver weight of group +A (r.t. control) is significantly greater than all other groups except its' room temperature counterpart which received vitamin C (+AC). These heavier liver weights are probably related to the increased body weight in these groups.

The deficient cold room groups (KOA and KOAC) have significantly smaller liver weights when compared to the room temperature controls (+A and +AC), but not when compared to the cold room controls K+A and K+AC.

No definite effects are attributable to vitamin A deficiency or the short term treatment with vitamin C.

EXP. IV: A linear correlation study was negative ($r = 0.6904$). It is evident that though the liver weight was linearly correlated to body weight in experiment II, this correlation is apparently lost when some of the animals are exposed to cold. In the cold there occurs a liver weight gain retardation, especially during the initial days of cold

exposure; and with 'conditioning', liver hypertrophy develops. This phenomenon coupled with the rapidity with which morbidity in vitamin A deficiency accelerates when animals are exposed to cold, perhaps partially accounts for the lost of linear correlation. Actually, no data are available on what type of correlation, if any, exists between body and organ weights during various lengths of exposure to nonfreezing cold.

As in experiment III, the room temperature control group (+A) has a significantly heavier liver weight than most other groups. Though the mean body weight gain of the +A group was approximately 20 grams greater (N.S.*) than that of the room temp. deficient groups OA and OAC, the mean liver weight of group OA, but not group OAC, is significantly less than that of group +A. This apparent beneficial effect of vitamin C administration on the liver weight of deficient animals was not observed in the cold. The added stress of cold may be too great for vitamin C to exert such an effect. The mechanism whereby vitamin C becomes beneficial to deficient animals at room temperature might conceivably be a transient stimulating effect of the vitamin on food consumption or utilization, or a direct metabolic effect consequent to the lack, due to anorexia, of a dietary substance(s) which previously hindered the absorption or utilization of the vitamin. This latter hypothesis would be in agreement with

* see page 79

the observed higher ascorbic acid concentration values in group OAC in experiment II and III. It also would be in agreement with the observed effect on the +AC groups in these experiments. This emphasizes the importance of an experiment utilizing pair-feeding and test diets of different compositions.

6. Testes weight (Table 19):

EXP. II: As in experiment I, there exists a linear correlation between the wet weight of the testes and body weight (r = 0.930). The control groups N and NC have significantly greater testes weights as compared to all the deficient animals. As the deficiency progressed (OAic. vs OA and OAC), testicular weight decreased as did body weight and other organ weights. Treatment with vitamin C had no effect on the testicular weight of either the normal or deficient animals.

EXP. III: No significant changes occurred.

EXP. IV: The linear correlation between the testes weight and body weight is very poor (r = 0.4278). The body weight changes due to the short exposure to cold were greater than the changes in testicular weight during this period. Testicular weight changes occur slowly and apparently when body weight is slowly changing, testicular weight also changes accordingly (Exp. I and II). However, when body weight changes occur rapidly, testicular weight is not as labile (Exp. IV).

The testicular weight of the deficient group in the cold which received vitamin C (KOAC) is not significantly different from any other group in the cold. This group is significantly different from the room temp. groups OA and +A. Both exposure to cold and the administration of vitamin C seem to have contributed to the intensification of the deficiency state and/or the effects of the lack of vitamin A at this particular site. The data on the wet weights of the seminal vesicles (Table 20) closely mimic the testicular weight changes. Vitamin C has no significant effect.

Testicular weight and function are apparently not adversely affected until very late in vitamin A deficiency. Perhaps the observed changes in testes weight and function are merely the result of the lack of dietary nutrients other than vitamin A and especially the lack of proteins. The effect on the testes is reversed without visible lesions remaining when vitamin A is administered to deficient rats, even very late in the deficiency (134). This does not necessarily mean that vitamin A directly cures the testicular condition. It may simply be consequent to the return to normal feeding. Experimentation on the effect of vitamin A deficiency on the gonads should be undertaken in animals after they have reached sexual maturity. Weanling rats on a test diet have no detectable liver vitamin A at the time they reach sexual maturity, approximately three weeks after being placed on the diet.

7. Thyroid weight (Table 21):

EXP. II: No significant differences exist between the thyroid weights of the deficient animals (on the 58th deficiency day) and those of the fox chow controls.

EXP. III: The room temperature deficient groups (OA and OAC) have significantly heavier thyroids as compared to all animals exposed to cold and as compared to the room temperature group +AC, but not group +A. This response (increased thyroid weight) is probably a transient one, because it is not seen in the presumably later stage of the deficiency in which the deficient animals in the cold (KOA and KOAC) are assumed to be. Of course, the exposure to cold may be the modifying factor. Moreover, this particular response was not seen in the other experiments. It does indicate the importance of a serial study in thyroid histology and function during vitamin A deficiency.

The administration of vitamin C had no effect on thyroid weight.

EXP. IV: No significant difference were found. A ten day exposure to cold and/or treatment with vitamin C does not cause a significant change in the thyroid weight of either deficient or control animals.

A consideration of the effect of vitamin A deficiency on thyroid weight and histology is given with the thyroid data of experiment V.

8. Adrenal weight (Table 22):

As pointed out in experiment I, the significance of the wet weight of the adrenal without a consideration of the histology and hormonal physiology has a very limited importance. All data in these experiments are expressed at $p = 0.05$.

EXP. II: Deficient groups (OAic. OA and OAC) have significantly smaller adrenal weights as compared to the controls (N and NC). The adrenal weight of the deficient animals at the term of the experiment (OA and OAC) potentially tend to be increased ~~adrenal weights~~ as compared to the initial control group (OAic) sacrificed ten days earlier.

EXP. III: Even though the period of cold exposure in this experiment was short, the adrenal weights of the controls exposed to cold tend to be the heaviest. The deficient animals tend to have smaller adrenals.

EXP. IV: The effect of cold on adrenal weight is reflected in the significant increase of the adrenal weights in the control groups exposed to cold (K+AC and K+A) as compared to their room temperature counterparts (+AC and +A). The deficient animals were unable to respond to the stress. Treatment with vitamin C caused a significant increase in the adrenal weight of the control exposed to cold (K+AC as compared to K+A). Treatment with vitamin C had no remarkable effect on the other groups. Thérien (213) reported that the

administration of vitamin C to normal animals exposed to cold prevented an increase in adrenal weight. Her treatments lasted over a considerably longer time and the animals were not on a semi-synthetic diet as in this experiment. Our data are much too limited to allow for any definite conclusions.

CONCLUSIONS:

1. No consistent significant changes in the ascorbic acid status of the rat could directly be associated with vitamin A deficiency.

2. Vitamin A deficient animals have a decreased resistance to cold.

3. The administration of vitamin C during the terminal stages of vitamin A deficiency (after the liver is known to be depleted of vitamin A), does not alleviate the deficiency nor does it tend to prolong the survival. Vitamin C is not able, under these conditions, to 'replace' vitamin A. This does not exclude the possibility that, if vitamin C was administered daily from the onset of the deficiency, it might be able to conserve the vitamin A stores from as rapid a depletion.

4. The data has called to our attention the importance and necessity of utilizing the pair-feeding technique and a vitamin A deficient test diet which does not contain yeast as the source of the B vitamins.

EXPERIMENT V:

Introduction:

We designed this experiment to determine if the effect of vitamin A deficiency on the liver ascorbic acid status would be significantly different in male rats, simultaneously fed two different vitamin A deficient test diets. The diets are practically identical in all respects except that one (U.S.P. XIV) contains 8.0% yeast and the other diet contains the B vitamins in crystalline form (L.D. DIET). One half of the animals in each group were pair-fed and the remainder fed ad libitum. In addition we studied the effect of the administration of ascorbic acid to animals on both diets.

EXPERIMENTAL PROTOCOL:

This experiment has two identical 'groupings' of rats; both of which were assembled on the same day from the same shipment of animals. Each 'grouping' was fed a different vitamin A deficient test diet. One diet was the usual U.S.P. XIV test diet and the other was a diet prepared to our specifications (G.B.I.) and referred to in this text as the L.D. (Lachance-DesMarais) test diet. Table 23 gives the composition of these diets and compares them to the diets utilized by three other research teams. For further comparisons with other vitamin A deficient test diets the reader is referred to György (88).

Each 'grouping' consists of four groups of individually caged, male albino rats of the Wistar strain, ranging in weight between 32 and 50 grams (mean of each individual group was 40.3 ± 0.5 grams). The two deficient groups (OA and OAC) had 15 animals in each group; whereas, the two control groups (+A and +AC) each had 12 animals.

The groups labelled OAC and +AC received 75mg. of ascorbic acid (Redoxon), administered orally every two days. All control animals ('+A' in their group symbol) received an oral supplement of 500 I.U. of vitamin A in corn oil twice weekly.

Pair-feeding (205): The food consumption of seven individual animals in each deficient group (OA and OAC) was measured every two days. The exact quantity of diet eaten by a given deficient animal was allowed for the next two days to a correspondingly mated control animal in group +Apf and +ACpf. All other animals were fed ad libitum.

When the deficient (OA and/or OAC) group of a particular 'grouping' showed clinical signs of the deficiency and their weight gain plateaued, the experiment for this 'grouping' was terminated. This first occurred on the L.D. vitamin A deficient test diet (terminated on the 44th deficiency day). The same event occurred 10 days later for the animals on the U.S.P. XIV vitamin A deficient test diet. When sacrificed

the following determinations were made: Liver ascorbic acid and vitamin A; wet weight of liver, testes, seminal vesicles, adrenals and thyroids. The thyroids were processed for histological examination.

For three weeks during the experiment, that is from the 14th deficiency day to the 38th deficiency day, the oxygen consumption (36) of five animals from each of the four pair-fed and the four deficient groups was measured twice. A total of 124 separate measurements were made at the rate of six per working day.

RESULTS* AND DISCUSSION:

1. Survival:

Survival in the deficient groups on the U.S.P. diet was as follows: Group OA 13/15(87%) and Group OAC 14/15(93%). Survival in the deficient groups on the L.D. diet was as follows: Group OA 14/15(93%) and Group OAC 15/15(100%). These data establish the fact that the animals were probably suffering from the deficiency state but had not reached the terminal stage of rapid mortality.

2. Body Weight Gain (Figs. 5, 6 and 7; Table 26);

From the overall growth response (Fig. 5), we note that the time necessary for the development of vitamin A deficiency on the L.D. diet, is shorter by approximately 8-10 days. The slope of the growth curve is more acute for the control animals on the L.D. diet. This observation is confirmed by the fact that the total weight gain (Table 26) reached by the control (+A) animals on the U.S.P. diet, was attained 10 days earlier on the L.D. diet. Though not significantly different, the body weight gain of the control (+A) rats on the L.D. diet is 5.8 grams/day whereas, it is 5.3 grams/day on the U.S.P. diet. Over a longer duration it is highly probable that the difference would become significant. Except for the source from which the vitamins

* A tabulation of the significance of the results is given in Tables 24 and 25.

are derived, the diets are very similar in composition. Thus the observed improvement in the body weight gain of the controls on the L.D. diet could possibly be attributed to a greater availability of the vitamins when added in crystalline form to the diet, as compared to their availability from dried yeast; or to the supplementation of a ratio of crystalline 'B' vitamins which is superior to that naturally occuring in dried yeast. The added cystine in the L.D. diet may possibly be responsible for the earlier onset of the deficiency signs. This latter problem is further discussed in experiment VI.

Body weight gain on the U.S.P. diet (Table 26): The weight gain of both pair-fed groups (+Apf and +ACpf) was not significantly different from that of the deficient animals (OA and OAC). This means that the pair-feeding was adequate. The 'ad libitum' fed controls (+A and +AC) had significantly greater weight gains than did both the pair-fed and deficient animals. Treatment with ascorbate had no apparent effect.

Weight gain on the L.D. diet (Table 26): The weight gain of a given pair-fed group (+Apf or +ACpf) was not significantly different from its respective vitamin A deficient reference group (OA and OAC respectively). This is apparently due to the fact that the deficient group which received vitamin C (OAC) had a better, but not significantly better, weight gain than the untreated deficient animals (OA).

The ad libitum fed controls (+A and +AC) had a significantly greater weight gain than both deficient groups. The administration of vitamin C tended to retard the growth of the ad libitum fed controls (+AC) and for this reason the weight gain of this group is not significantly greater than that of the pair-fed animals.

3. Liver vitamin A:

At autopsy, none of the deficient animals on either diet had detectable liver vitamin A. The administration of vitamin C from the onset of the experiment (prior to the depletion of liver stores) did not 'conserve' the liver vitamin A. No significant differences in liver vitamin A concentration or in total liver vit. A were found between the control groups on a particular diet. Exogenous ascorbate had no apparent effect.

The control animals on the L.D. diet received a total of 7,500 I.U. of vitamin A from the onset of the experiment to its termination (44-48 days); whereas, the control animals on the U.S.P. diet received a total of 10,000 I.U. of vitamin A from the onset of the experiment to its termination (55-58 days). The mean concentration and total liver vitamin A in the controls on the U.S.P. diet was 28.7 I.U./gm. of liver and 269.0 I.U./whole liver respectively; whereas, the mean concentration and total liver vitamin A in the control animals

on the L.D. diet (which received less vitamin A due to the shorter experimental time) was 52.5 I.U./gm. of liver and 424.7 I.U./whole liver respectively. It is probable that the L.D. diet promoted a better absorption and storage of vitamin A. However, even though the liver is usually considered as containing 95% of the body reserve of vitamin A (63) it remains possible that the rate of utilization on the L.D. diet was decreased because of some other dietary factor being present or absent; or the kidneys containing a greater percentage of the vitamin A administered to the animals on the U.S.P. diet. Of interest, in connection with our observation, is the report of Braude et al. (20) describing increased liver weights and decreased vitamin A storage in large white pigs placed on a diet containing 14% dried yeast and 2% minerals in lieu of 10% fish meal and 6% barley meal. We also observed an effect on liver weight due to diet. We have pointed this out in the section on liver weight.

4. Liver ascorbic acid status:

U.S.P. diet: Ascorbic acid concentration (Table 28): No significant differences between the groups was found. As in the previous experiments, the control groups (+AC and +ACpf) which received vitamin C potentially tend to have lower mean concentration values.

U.S.P. diet: Total liver ascorbic acid (Table 29):

We observe that there is a significant decrease in the total ascorbic acid in groups OA and OAC as compared to the ad libitum fed controls but not as compared to the pair-fed controls. The higher value in the ad libitum fed animals is due to the heavier liver weight of these animals. Pair-feeding as compared to ad libitum feeding was able to account for a 50% reduction in the total ascorbic acid status. The value found in the deficient group (OA) is 38% (N.S.*) lower than that of the pair-fed controls (+Apf). This latter difference is substantial but leaves some doubt. The results on the L.D. diet are less dubious.

L.D. diet: Ascorbic acid concentration (Table 28): The administration of vitamin C to animals on this diet resulted in increased liver ascorbic acid concentrations. The composition of the diet is therefore an important factor as to whether exogenous ascorbic acid will be taken up by the liver. This is discussed further below. The mean liver ascorbic acid concentration of the deficient animals (OA) is significantly lower than that of all the treated groups, irrespective of whether they were vitamin A deficient (OAC), pair-fed (+ACpf) or ad libitum fed (+AC) controls. The group OA value is not significantly different from that of the control groups +A and +Apf. Comparison of the group +Apf mean to that of the ad libitum fed +A group demonstrates

*N.S.: not a significant difference

that pair-feeding (a form of fasting) does not alter the liver concentration of ascorbic acid. As pointed out in Experiment I, this does not mean that the actual concentration per cell is not decreased.

The uptake of exogenous vitamin C by vitamin A deficient animals fulfills the prerequisite of it being present if it is to be able to ameliorate vitamin A deficiency; moreover, it signifies that vitamin A is not necessary for the uptake of vitamin C.

L.D. diet: Total liver ascorbic acid: (Table 29) The total liver ascorbic acid status of the deficient animals (OA) is not significantly lower than that of the pair-fed controls (+Apf). Pair-feeding reasonably accounts for the decreased ascorbic acid status of vitamin A deficient rats. This is also discussed further below. Group OAC tends ($p = 0.05$) to have a greater total liver ascorbic acid status as compared to group OA. The mean value in group OAC is not significantly different from that of the ad libitum fed control group (+A) and the remaining groups which received vitamin C. One would believe that these animals (OAC) have an unquestionably better ascorbic acid status than the deficient (OA) animals and are therefore in a position to benefit therefrom.

The presence or absence of some agent(s) in the U.S.P. diet, somehow prevents the absorption and/or utilization and

storage of ascorbic acid. Although no vitamin E has been added to the U.S.P. diet, the quantity present in cottonseed oil is probably sufficient (60). Vitamin K has also not been supplemented; however, the intestinal synthesis of vitamin K is considered an adequate source in the normal rat (47). Vitamin B_{12} is definitely lacking in the U.S.P. diet and is present in the L.D. diet; but, B_{12} was lacking in the Mayer and Krehl diet and they observed increased tissue levels with the administration of vitamin C. Choline and cystine have not been added to the U.S.P. diet; however, yeast contains substantial amounts of choline and although the cystine content of yeast is low, it is not the limiting amino acid in casein.

We believe that it is the presence of at least 8% dried yeast that is responsible for the observed phenomena. It has been reported that active yeast absorbs ascorbic acid from its medium (223); however, it is extremely unlikely that dried yeast is reactivated in the gut (201). The gamut of the possible agent or agents present in dried yeast which could be responsible for the observed phenomena is too extensive for discussion here.

In any event, the presence of yeast in the diets utilized by Jonsson et al. (107), Sure et al. (206, Sharman et al. (191) and Guerrant (87) probably accounts for their

finding that exogenous vitamin C was not apparently available to their animals and/or did not benefit vitamin A deficient rats.

Pair-feeding reasonably accounts for the decreased ascorbic acid status of vitamin A deficient rats. In 1948, the same year Mayer and Krehl published their report in favor of a vit. A - vit. C relationship, Mapson and Walker (121) utilizing a yeast containing diet demonstrated that "the lowered urinary excretion and tissue concentration of ascorbic acid found in vitamin A deficient male rats could be attributed to the reduction in food intake which accompanies this condition". Moreover, "the rise in urinary excretion of ascorbic acid in response to chloretone was less in vitamin A deficient rats than in ad libitum fed ones, but this was eliminated if the food intake of the controls was restricted to that of the deficient animals". In 1954, Robertson et al. (166), utilizing the Mayer and Krehl diet demonstrated that vitamin A deficient rats were able to synthesize collagen as well as the controls, and stated that the suggestion that A-vitaminotic rats might have scurvy (127) should be critically re-examined.

We firmly believe that our results confirm those of Mapson and Walker, and that no further doubt should exist concerning the complete lack of a specific relationship

between the rat's resources of vitamin A and its capacity to synthesize ascorbic acid. This does not nullify the possibility that exogenous vitamin C might prolong the survival of vitamin A deficient rats. The protocol of Experiment VI is designed to test this possibility.

5. Liver weight (Table 27):

U.S.P. Diet: A check for linear correlation with body weight gave an r = 0.9162. The liver weight of the pair-fed controls (+Apf and +ACpf) is not significantly different from that of the deficient animals (OA and OAC). The liver weight of the ad libitum fed controls (+A and +AC) is significantly greater than that of the deficient animals. Those groups which received vitamin C potentially tend to have smaller liver weights. This probably accounts for the lack of significance between the liver weight of group +AC as compared to group +Apf. These results support the contention that the pair-feeding was reasonably adequate.

L.D. diet: A check for linear correlation with body weight gave an r = 0.9044. These results pattern the weight gain results. The liver weight of the pair-fed groups is not significantly greater than that of the deficient animals. The ad libitum fed controls (+A and +AC) have significantly heavier livers as compared to both deficient groups (OA and OAC) and the +Apf group. The pair-fed control group which

received vitamin C (+ACpf) has a mean liver weight which is not significantly different from that of the ad libitum fed controls. An explanation for a similar situation with body weight gain has been given.

The mean body weight of all animals killed at term on the U.S.P. diet was 224.8 grams; whereas it was 235.9 grams on the L.D. diet. The mean liver weight of these animals of approximately the same mean body weight was higher on the U.S.P. diet (8.87 gm. or 4.75 gm./100 gm. body wt.) as compared to that on the L.D. diet (7.82 gm. or 3.98 gm./100 gm. body wt.). This observation is in line with the previously mentioned (Liver vitamin A discussion) report of Braude et al. (20).

6. Testes weight (Table 30):

U.S.P. Diet: The weight of the testes is significantly smaller in the deficient animals (OA and OAC) as compared to all the controls. Pair-feeding and treatment with vitamin C have absolutely no effect on the weight of the testes. No significant differences were found between the means of all the groups (control and deficient) in the wet weight of the seminal vesicles (Table 24). Though the weight of testes of the deficient animals is decreased, their endocrine function as measured by this index, has not yet become impaired. Spermatogenic function in vitamin A deficiency is discussed below.

L.D. Diet: Only the testis weight of group OA is significantly lower than in all other groups. The administration of vitamin C seems to have aided the deficient animals, as reflected in the better body weight gain, slightly higher liver weight and now a heavier testicular weight. The administration of vitamin C has no effect on the testicular weight in the control groups. The observed influence of vitamin C on testicular weight is probably not a direct effect but rather a result of the effect of vitamin C on the body weight, in general, of the vitamin A deficient group. The wet weight of the seminal vesicles (Table 31) was significantly decreased in group OA as compared to the ad libitum fed controls only.

Irrespective of the type of diet or treatment (Vit. C.), the spermatogenic funtion of the testes in vitamin A deficient animals is curtailed. Fig. 9 depicts the histological picture which is encountered as compared to the normal appearance of the gland (Fig. 8). Pair-feeding does not reduce the number of active seminiferous tubules and therefore cannot account for the drastic curtailment of the spermatogenic function evidenced in vitamin A deficiency.

7. Adrenal weight (Table 32): All data expressed at $p = 0.05$

U.S.P. diet: The weight of the adrenals of both deficient groups (OA and OAC) are significantly lower than

that of the ad libitum treated controls (+AC). Pair-feeding has no effect on adrenal weight. The observed results are apparently due to a slight decrease in the adrenal weight of the deficient animals which received vitamin C (OAC) plus a slight increase in the adrenal weight of the ad libitum fed treated controls (+AC).

L.D. diet: The adrenal weight changes on this diet further indicate that the type of diet exerts an important influence on the type of response. The adrenal weights of the untreated control animals (+Apf and +A) are significantly greater than that found in all other deficient (OA and OAC) and treated control (+ACpf and +AC). Either vitamin C administration promoted a decreased adrenal weight (213) or pair-feeding resulted in a stress response (increased adrenal wt. in grp. +Apf) which was ameliorated by the administration of vitamin C (+ACpf). The administration of vitamin C had no effect on the adrenal weights of the vitamin A deficient animals. If it is true that the adrenals respond to exogenous vitamin C, it might possibly be that the adrenals in vitamin A deficiency lose the ability to respond.

Extensive studies concerning the status of the adrenals in vitamin A deficiency are needed. We believe that serious consideration should be given to the type of diet utilized to produce the deficiency in such experiments.

8. Thyroid:

Thyroid weight (Table 33): weight data expressed at $p = 0.05$ U.S.P. diet: Thyroid weight is significantly greater in group OA than in all other groups. The weight of the thyroids, as compared to the ad libitum fed controls (+A), is significantly lower in groups OAC, +ACpf and +Apf. The response of the thyroid, as measured by its wet weight, has been very erratic in all the previous experiments. In experiment III, we noted an increased thyroid weight in group OA. This was not observed when the protocol was repeated (Exp. IV); however, the duration of the deficiency was not as long in this latter experiment. A more thorough discussion is given below.

L.D. diet: On this diet, no significant differences in thyroid weight were found.

Thyroid status in vitamin A deficiency:

We are reasonably certain that the wet weight and histology of the thyroid of both deficient and control animals is unaffected by the exogenous administration of vitamin C and/or pair-feeding. This lack of a direct effect of vitamin C on the thyroid confirms the results of DesMarais (39).

Vitamin A deficiency apparently does not exert an adverse effect on the thyroid function, during the early and

middle stages (prior to extensive weight loss) of the deficiency.

Oxygen consumption measurements made at thermal neutrality (B.M.R.) on 62 deficient and 62 control animals on either diet and/or treatment, between the 14th and 38th deficiency days, gave no significant results (Table 34). This confirms the recent observation of Olivereau and Serfaty (145).

After body weight ~~body weight~~ loss has occurred, evidence of alterations in thyroid histology are seen. However, thyroid weight is not apparently affected.

Thyroid histology and function (as measured by the histochemical reaction of the colloid (43) and follicular morphology) was found to be different in deficient animals from that in controls. Representative photomicrographs of a normal (+A) thyroid (Fig. 10) as compared to that of thyroids from vitamin A deficient animals (Figs. 11, 12) are submitted as evidence.

Out of a total of 143 deficient animals, excluding those placed in the cold, only 22 rats had tentatively heavier thyroids. Most of these latter animals were sacrificed during the terminal stages of the deficiency. The deficiency in all these cases of heavier thyroids had been induced on the U.S.P. vit. A deficient test diet.

As compared to the normal rat, the thyroid of the vitamin A deficient rat manifests i) an increase in the number of follicles containing yellow colloid (black on the black and white prints), ii) an infiltration of connective tissue and iii) most follicles have a low cuboidal or squamous epithelium. This histological picture resembles that of the thyroid gland response to insufficient thyrotrophic hormone (T.S.H.) (45).

How early in the development of vitamin A deficiency this 'involution' begins has not been evaluated. At the stage we have examined this gland (after the body weight gain plateau), the animal is most likely in a state of protein catabolism and therefore the rate of protein synthesis by the pituitary and other organs (including the thyroid) is probably curtailed. It is possible that vitamin A has a specific role in T.S.H. production, however we do not believe a specific relationship is likely. It is becoming increasingly evident that vitamin A plays a role in the metabolism of sulphur containg compounds (experiment VI.) and thus vitamin A is probably associated with the synthesis of most complex protein molecules including the hormones.

The frequently observed lesion in the thyroid of vitamin A deficient rats (Figs. 11-13) has been characterized by Van Dyke (222) as "a cystic metaplasia of the thyroid parenchyma

embryologically derived from the ultimobranchial body". To our knowledge, no previous worker in the field of the thyroid-vitamin A relationship had described such a lesion. Van Dyke's interest in this structure has been mainly concerned with its possible relationship to thyroid tumorigenesis.

We have observed distinct metaplastic changes as early as the 21st deficiency day. The lesions are not frequent before weight gain has plateaued. At this time the animal is entering the terminal stages of the deficiency and the clinical signs are evident. These lesions are not affected by treatment with vitamin C, although, Van Dyke has demonstrated that this pathological process can be reversed with the administration of vitamin A.

CONCLUSIONS:

1. The onset of vitamin A deficiency, as judged by body weight gain changes and clinical signs, is shorter by approximately ten days on a yeast free vitamin A deficient test diet.

2. Body weight gain potentially tends to be greater and liver weight smaller on the L.D. diet. The liver storage of vitamin A is apparently better on this yeast free diet.

3. Orally administered vitamin C is readily taken up by the liver of both vitamin A deficient and control rats on

the L.D. diet. This response probably explains why a number of investigators were unable to find modifications in the ascorbic acid status of their control and vitamin A deficient animals after treatment with vitamin C.

4. Pair-feeding adequately accounts for the decreased ascorbic acid status of vitamin A deficient rats. It is evident that no relationship exists between the rat's resources of vitamin A and its capacity to synthesize ascorbic acid.

5. Exogenous vitamin C did not retard nor ameliorate the course of vitamin A deficiency in rats on either diet.

6. The oxygen consumptions of vitamin A deficient rats prior to body weight loss was unaffected by the deficiency. As compared to the normal rat, the thyroid of the vitamin A deficient rat (after body weight gain plateau) presents a histological picture resembling the response of this gland to insufficient thyrotrophic hormone. A metaplasia of the thyroid parenchyma embryologically derived from the ultimobranchial body was frequently encountered. It was usually located towards the center of the gland at the level of the parathyroids. The presence of this lesion does not appear to alter thyroid function. Treatment with vitamin C has no effect on the metaplastic changes nor on the thyroid as a whole.

7. The spermatogenic function, but not the endocrine function (as measured by the weight of the seminal vesicles) of the testes, is drastically curtailed in vitamin A deficiency. Treatment with vitamin C has no effect.

8. The wet weight of endocrinological organs (testes, adrenals and thyroids) are not significantly affected by pair-feeding. This indicates that these organs are not rapidly affected by body weight changes and therefore the fractional expression of their weights should be avoided.

EXPERIMENT VI:

Introduction:

In the previous experiment, the administration of vitamin C to vitamin A deficient rats had a questionable tendency (N.S.) to promote apparently better body and organ weights. Mayer and Krehl (127) reported that treatment with vitamin C at least doubled the survival of vitamin A deficient rats. This experiment was designed to test this possibility and thus either confirm Mayer and Krehl's report or further demonstrate the lack of a specific relationship between these two vitamins.

We have shown that the presence of yeast apparently prevents the absorption and/or utilization and storage of administered vitamin C. There are many possible substances, present in yeast, which might be responsible for the observed phenomena. Glutathione is commercially extracted from yeast (157) and also has been intimately related to ascorbic acid (120) (18). We believe that these reasons warrant its being considered the 'culprit'. Both reduced and oxidized glutathione or a mixture of both should be tested because the actual state in which the tripeptide exists in dried yeast is unknown. In this experiment we have studied the effect of reduced glutathione on the liver ascorbic acid

status of rats receiving and not receiving exogenous vitamin C. The experiment also presents us with the opportunity to study the effect of reduced glutathione on vitamin A deficiency.

EXPERIMENTAL PROTOCOL:

Eighty-one male albino rats of the Wistar strain, ranging in weight between 28 and 41 grams (Mean 32.6 grams), were caged individually in metal cages with wire screen bottoms. They were divided into eight groups as follows:

Group	Symbol.	N/group	Vit.A.	Vit. C.	G.S.H.	N.sac at 36 day
1.	+A	9	+	0	0	5
2.	+AC	9	+	+	0	5
3.	+AG	5	+	0	+	5
4.	+ACG	10	+	+	+	5
5.	OA	16	0	0	0	6
6.	OAC	15	0	+	0	6
7.	OAG	7	0	0	+	7
8.	OACG	10	0	+	+	6

Diet: All animals were ad libitum fed the L.D. vitamin A deficient test diet for the first 16 days. Thereafter, all control groups (+A in group symbol) were restricted to 25.0 grams of diet every two days. This was done in an effort to restrict the body weight gain of these animals to that of the deficient ones. Pair-feeding would have been a superior method but technical difficulties prevented us from utilizing it.

Treatments: All control animals received a biweekly oral supplement of 500 I.U. of vitamin A dissolved in corn oil. From the 16th deficiency day each rat in the groups

having the letter 'C' in their group symbol received 75 mg. of vitamin C (Redoxon) every two days 'per os'. On the morning of the same day vitamin C was to be administered, 12.5 mg. of reduced glutathione* dissolved in distilled water was administered to each rat in those groups with the letter 'G' in the group symbol.

On the 36th deficiency day, a representative number of animals in each group (see grouping chart above) were sacrificed and the remaining animals were maintained for a survival study. At autopsy the following determinations were made: liver ascorbic acid and vitamin A; wet weight of the liver, testes, seminal vesicles, adrenals and thyroids; thyroid histology.

RESULTS** AND DISCUSSION:

1. Survival:

On the 36th deficiency day, a representative number of animals in each group were sacrificed and the remaining animals kept for a survival study. At this time (36th deficiency day), survival in the deficient groups was 100% in Groups OAG (7/7) and OACG (13/13); 13/14(93%) in Group OAC, and 11/14(79%) in Group OA. Sixteen days later (52nd deficiency day) survival was: 2/5(40%) in Group OACG: 2/10(20%)

* Glutathione (reduced) - Nutritional Biochemical Corp.
** A tabulation of the significance of the results is given in table 35.

in Group OA; and 3/9(33%) in Group OAC. These remaining animals were losing weight and showed severe signs of vitamin A deficiency. Irrespective of treatment, no vitamin A deficient animals survived beyond the 60th deficiency day.

Our results clearly demonstrate that exogenous vitamin C definitely does not prolong survival. As previously stated, the L.D. diet and the quantity of vitamin C administered is very similar to that utilized by Mayer and Krehl (127). In connection with our results we note that Robertson et al. (166) (167), in their work on collagen synthesis, utilized the Mayer and Krehl diet A_1; and administered 25mg. of vitamin C every two days to vitamin A deficient rats. None of these animals had increased survival.

Mayer and Krehl investigated the production of vitamin A deficiency on several different diets. One of these diets contained lard which they noticed prolonged survival and is now known to contain biologically active vitamin A. (96). We suggest that, due to some technical error, the test diet containing lard rather than the A_1 diet supplemented with vitamin C was utilized in their survival study. Contrary to interpretations of several investigators, Mayer and Krehl did not state that they observed 'scorbutic like' signs in vitamin A deficiency produced on all their test diets.

This occurred only when the test diet contained 60% casein. On that particular diet, the administration of vitamin C relieved the 'scorbutic' signs but did not alleviate the vitamin A deficiency.

2. Body Weight Gain (Fig. 14; Table 36):

Because all the control animals were restricted to a given amount of diet, the differences between the control groups are small and not significant. With the exception of Group +AG as compared to Group OAG, no significant differences in weight gain exists between a given experimental group and its control. The difference between a given control group and the respective deficient group tends to be wide and so the data are not as comparable as that resulting from the use of pair-feeding.

As compared to Group +A the control animals which received vitamin C (+AC), as in most previous experiments, potentially tend to have a lower weight gain; and the animals which received both glutathione and vitamin C (+AGG) potentially tend to have a better weight gain.

As compared to Group OA, the deficient animals which received glutathione only (OAG) potentially tend to have a poorer weight gain and the deficiency body weight gain plateau occurs 5 days earlier. In experiment V we noted that on the L.D. test diet, the vitamin A deficiency body weight gain plateau occurred earlier than on the U.S.P.

XIV test diet. In this experiment, supplementation of reduced glutathione to deficient animals on the L.D. diet further hastened the development of the deficiency plateau (5 days). We believe this incriminates the presence of additional sulphur containing compounds (additional cystine of L.D. diet and, in addition, glutathione in this experiment) as the factor(s) which hastens the development of vitamin A deficiency. This favors the hypothesis that vitamin A may be linked with the metabolism of sulphur containing compounds (117) (137). The importance of the sulphur linkage in the structure and maintenance of the protein of hair, keratin and other proteins is well established. Similarly, the importance of vitamin A for the maintenance of epithelial surfaces is also well established.

3. Liver weight (Table 37):

The differences in liver weight between the control groups are not significant. The liver weight of Group OACG (vitamin A deficient plus glutathione and vitamin C) is significantly greater than that of groups OAa nd OAG. This response is probably associated with the higher weight gain in this group (OAGG). The liver weights in group OA and OAG are significantly less than those in Groups +A and +AG. There is no significant difference between the mean liver weight of Group OAC as compared to +AC; and of Group OAGG as compared to +ACG.

From these data, one realizes that the restriction in food consumption imposed on the controls was not adequate enough because the organ weights are not as comparable as in the previous experiment. It also seems possible that liver weight is more labile in vitamin A deficiency than is the body weight.

No beneficial effects on liver weight can be attributed to treatment with vitamin C, reduced glutathione or both.

4. Liver vitamin A (Table 40):

In all the deficient animals, no liver vitamin A was detectable on the 36th day. In the control animals, at $p = 0.05$, the total liver vitamin A stores of Group +AG was significantly greater than in Groups +ACG and +AC; but not as compared to Group +A. All control rats received the same supplement of vitamin A and this latter phenomenon coupled with the observation that exogenous glutathione hastened vitamin A deficiency (Group OAG reached a body weight plateau 5 days before the other deficient groups) might possibly be a further indication that vitamin A has a role in the metabolism of sulphur containing compounds.

5. Liver ascorbic acid concentration (Table 38):

The mean liver ascorbic acid concentration of the vitamin A deficient group (OA) is significantly less than that found in all groups which received vitamin C. This Group OA value is not different from that of its proper control (+A), nor from that of those groups which received reduced glutathione (OAG and +AG).

As in the previous experiment, this diet (L.D.) allows the orally administered vitamin C to be taken up by the liver. The administration of reduced glutathione potentially tends to augment and/or preserve the liver ascorbic acid status. The dipeptide is apparently readily absorbed and as reported in the literature, has a physiological relationship with ascorbic acid (120) (18) (181). Reduced glutathione does not benefit vitamin A deficient rats and actually tends to promote an earlier onset of the body weight changes associated with vitamin A deficiency.

6. Total Liver ascorbic acid (Table 39):

The total liver ascorbic acid in the deficient group (OA) is significantly less than that in all other groups exept group OAG. Although the liver weights of the deficient animals were generally smaller, the total ascorbic acid status of the deficient animals which received vitamin C (OAC and OACG) is not significantly different from that of the control groups. The significantly greater liver ascorbic acid value of the control (+A) group as compared to the deficient OA group is primarily due to the larger liver in the +A group.

7. Testes weight (Table 41):

All four control groups have statistically heavier testes as compared to all four deficient groups. Not only has there probably occurred a decrease in testicular weight

due to the deficiency, but also the controls were not sufficiently restricted in calories and do not tend to be comparable.

No effects can be attributed to treatments.

8. Seminal vesicle weight (Table 42):

There is considerable overlapping in these data. Groups OA, OAC and OAG have significantly smaller seminal vesicles as compared to the treated control groups +AG and +ACG. None of the mean weights are significantly greater or smaller than that of the true control group, +A. It is interesting that the heaviest seminal vesicle weights (N.S.) in either the control or deficient series, is in the group which received both ascorbic acid and glutathione (OACG and +ACG).

The data to date indicate that the endocrine function of the testes in vitamin A deficiency is not rapidly affected. Treatment with vitamin C or reduced glutathione has no significant effect on this organ.

9. Thyroid weight and histology: Adrenal weight:

No significant weight differences were found. Treatment with reduced glutathione and/or vitamin C did not affect the thyroid picture nor prevent the keratinizing metaplasis of the ultimobranchial body in the thyroids of the deficient rats.

CONCLUSIONS:

1. Contrary to the report of Mayer and Krehl (127), the administration of vitamin C to rats rendered deficient on a comparable diet (Table 23) definitely did not prolong survival.

2. Reduced glutathione does not seem to be the yeast factor which prevents the liver uptake of exogenous vitamin C. The administration of reduced glutathione complements the liver ascorbic acid status of rats (N.S.) irrespective of whether the animal is vitamin A deficient or not. It does not reduce the severity of ~~ameliorate~~ the deficiency.

3. Reduced glutathione enhances the liver storage of vitamin A in normal rats and moreover hastens the body weight plateau in vitamin A deficient rats by approximately five days. This lends support to the hypothesis (117) that one of the functions of vitamin A may be linked with the metabolism of sulphur containing compounds.

EXPERIMENT VII.

Introduction:

In order to further confirm our observations concerning the nonrelationship of vitamin A to vitamin C, we decided that experiments utilizing the guinea pig were necessary. In this experiment, we have studied the effect of exogenous vitamin A on the development and duration of scurvy..

EXPERIMENTAL PROTOCOL:

Fifty-seven young male guinea pigs of a mixed strain, ranging in weight between 121 and 215 grams (mean 160.3 grams), were caged individually in metal cages with wire screen bottoms. The experiment had five groups. Four groups were fed, ad libitum, Purina Dog Chow Checkers (broken into small pieces in an ice crusher). This diet is scorbutigenic (44). 20 animals were placed on this diet with no supplement of vitamin A other than that present in the diet (group S+0); 13 animals with an oral supplement of 3,000 I.U. of vitamin A in corn oil twice a week (Group S+3); 13 animals with a bi-weekly oral supplement of 6,000 I.U. of vitamin A (Group S+6). A control group of 6 animals received the diet plus 10mg. of vitamin C daily (Group DC). Another control group of 5 animals was ad libitum fed Purina Guinea Pig Chow (pellets, which have vitamin C incorporated) (Group GP).

This experiment was to be terminated when half of the

animals in any one group on the scorbutigenic diet had died. This occurred in Group S+0 on the 18th deficiency day. All the remaining animals were sacrificed and the following determinations made: Liver ascorbic acid and vitamin A; wet weight of the liver, thyroids and adrenals; thyroid histology.

RESULTS* AND DISCUSSION:

1. Survival:

Survival at the time the experiment was terminated was as follows: Group S+0 11/20 (55%); Group S+3 10/13 (77%); Group S+6 8/13 (61%); Group DC 5/6 (83%); Group GP 5/5 (100%). Survival in the Dog Chow control group (DC) is not 100% because one animal, although gaining weight, suddenly died and showed no apparent external pathology. An autopsy was not performed. Scurvy was pronounced in the S+0, S+3 and S+6 groups. Vitamin A administration had no significant effect on survival.

2. Body Weight Gain (Fig.15 and Table 44):

Weight gain was obviously drastically curtailed by scurvy and probably ~~aggravated~~ further curtailed by treatment with vitamin A. All the scorbutic animals, including those which received vitamin A, had a poor general appearance, fragile bones and the same relative number of subcutaneous hemorrhages (i.e. number of subcutaneous hemorrhages found on the medial surface of both thighs).

* A tabulation of the significance of the results is given in Table 43.

3. Liver weight (Table 45):

The liver weight of the group fed commercial guinea pig chow (GP) is significantly greater than that of all other groups. The liver weight of the remaining groups are not significantly different from each other. Liver weight is not significantly affected in the scorbutic animals as compared to the control group DC. This may be attributable to the rapidity with which the deficiency develops in young guinea pigs.

4. Liver vitamin A (Table 47 and 48):

The scorbutic animals were able to absorb and store the orally administered vitamin A. The concentration of vitamin A in the GP group is not statistically different from that in the scorbutic group which received no additional vitamin A (S+0), and the control animals on dog chow plus vitamin C (DC). This is probably related to the fact that the liver in the GP group is significantly heavier and the concentration is therefore lower.

The total liver vitamin A data (Table 48) demonstrates that the vitamin A status of the animals on dog chow without additional supplements of vitamin A (Groups S+0 and DC) was similar. Both had absorbed approximately the same quantity of vitamin A from the diet (calc. probable 200 I.U. per day). The control animals on guinea pig chow had a significantly different total vitamin A status as compared to all other

groups. This group not only had a heavier mean liver weight but also had access to a calculated 1,200 I.U. of vitamin A per day in their diet. The scorbutic groups which received additional vitamin A (S+3 and S+6), each have a total liver vitamin A status significantly different from that found in any other group.

Our results demonstrate that the absorption and storage in the liver of supplemented doses of vitamin A is not impaired in guinea pigs rendered scorbutic. This is additional evidence that no relationship exists between an animal's resources of vitamin A and its ascorbic acid status.

5. Liver Ascorbic acid (Table 46):

The scorbutic animals, irrespective of treatment with vitamin A, had practically identical ascorbic acid concentration and total liver values. These values were significantly lower than those found in the control groups (DC and GP). The guinea pig chow control group had a significantly higher liver ascorbic acid status as compared to the dog chow group DC. Dog chow is not an ideal diet for guinea pigs. One of the principal reasons is purely mechanical. The cubes were crushed to pellet size in an ice crusher and were foreign to the animals as compared to the pellets. Other than the absence of vitamin C, the chemical composition (analysis available from company (156) of Purina guinea pig chow as compared to Purina Dog Chow Checkers is

remarkably similar. However the ingredients utilized in the manufacture of each diet may be in some respects different and also probably those ingredients common to both diets are probably present in different proportions.

6. Thyroid and Adrenal:

No significant weight differences were found (Table 43).

On the dog chow diet, no histological changes in the thyroid could be associated with scurvy, or supplement with vitamin A.

The reaction of the thyroid colloid to the staining technique utilized (45) seems different when the animal is fed dog chow as compared to when the animal is fed guinea pig chow, (Fig. 16 and 17 respectively). The increased amount (proportion) of yellow colloid in the gland when dog chow is fed, indicates a decrease in organicly bound iodine. Both diets contain 1.00 ppm of iodine. Although not dramatic, this demonstrates the important influence diet composition and food consumption can have on thyroid function. It emphasizes the need for a more thorough consideration of the nutritional status of animals utilized in thyroid research, other than the simple consideration of sufficient iodine content.

CONCLUSIONS:

1. The absorbtion of moderate oral doses of vitamin A supplemented to guinea pigs is apparently unaffected by a developing scorbutic state.

2. Treatment with vitamin A (3,000 and 6,000 I.U. biweekly) does not hasten nor retard the development of scurvy. The presence of substantial amounts (2 to 4 times that found in normal controls fed guinea pig chow) of vitamin A in the liver of scorbutic guinea pigs has positively no effect on the liver ascorbic acid status. The doses of vitamin A that we administered are not in the realm of those utilized to induce hypervitaminosis A. Our results confirm the observation of Collet and Erickson (25) that moderate doses of vitamin A do not interfere with tissue ascorbic acid levels.

EXPERIMENTS VIII

Introduction:

The number of publications concerning the vitamin A status of the guinea pig are few (119). In the following experiments (VIII and IX), we have produced vitamin A deficiency in the guinea pig. Our criteria have been that no liver vitamin A was detectable and that growth was retarded. We will not make extensive comments concerning the pathological physiology of vitamin A deficiency in the guinea pig. We feel this problem needs a thorough evaluation.

The purpose of our experiments was to determine if the liver ascorbic acid level of the guinea pig, administered a constant supplement of vitamin C, would be adversely affected by the absence of liver vitamin A.

The vitamin A deficient test diet utilized in our experiments has a composition which is a compromise between that recommended by Collins and Elvehjem (26) (28) and by Reid and Briggs (62). A comparison of the compositions is given in Table 50.

EXPERIMENT VIII

EXPERIMENTAL PROTOCOL:

Three groups of guinea pigs were assembled from a stock of animals which had been on the test diet of our composition for two weeks, and had been supplemented with 50 I.U. vitamin A biweekly 'per os' plus 10 mg. of

vitamin C given orally every day. The chosen mixed strain guinea pigs were individually caged and ranged in weight between 211 and 305 grams (mean 255 grams). One group of 6 animals was sacrificed on the second day of the experiment as initial controls (Group IC). The control group (+A) had 5 animals and were supplemented with 500 I.U. of vitamin A twice a week and 10 mg. of vitamin C per day. The deficient group (OA) had 8 animals and received 10 mg. of vitamin C/day. When weight gain had stopped and was dropping and moreover 3 animals had died in the experimental group, the experiment was terminated. The following determinations were made: Liver ascorbic acid and vitamin A; wet weight of the liver, thyroids and adrenals; thyroid histology. A tabulation of the significance of the results is given in Table 49.

RESULTS AND DISCUSSION:

1. Survival and Body Weight Gain (Fig.18):

When the experiment was terminated, 5/8 (62%) of the Group OA animals had survived. Body weight gain changes were significantly different ($t = 3.15\emptyset$, $p = 0.02$). Group +A had gained 51.8 + 26.9* grams whereas the OA group had gained -37.8 + 8.9 grams. The deficient animals had a poor general appearance but no distinct xerophthalmia.

2. Liver vitamin A:

The initial control group (IC) had only very slight traces of liver vitamin A, although these animals had been

* ± standard error

receiving 50 I.U. twice a week during the previous two weeks. For this reason the control +A animals were supplemented with 500 I.U. of vitamin A biweekly throughout the experiment. When the experiment was terminated, the +A group had a mean of 33 I.U. of vitamin A per gram of liver and a mean total of 393 I.U. of vitamin A per whole liver. The deficient animals had no detectable liver vitamin A.

3. Liver ascorbic acid status (Table 51):

The liver ascorbic acid concentration of Group OA is significantly lower (at $p = 0.05$ but not at $p = 0.01$) as compared to both the +A and IC groups. The deficient animals had anorexia and lost considerable weight. Even so, the mean concentration value is 50% greater than that found in scurvy (Exp. VII), and the animals did not show signs of scurvy (e.g. joint tenderness, fragile bones, loose teeth, subcutaneous and joint hemorrhages).

The total liver ascorbic acid of Group OA is significantly (at $p = 0.05$ but not at $p = 0.01$) lower than that in the I.C. group but is not significantly different from that found in scurvy. Body weight changes and the fact that the animals were supplemented with a minimal amount of vitamin C can reasonably account for the observed tendency toward a decreased ascorbic acid status in the vitamin A deficient liver.

4. Liver weight:

There are no significant differences in the wet weight of the liver between the three groups. One might have expected the deficient animals (OA), which lost considerable weight during the last 10 days of the experiment, to have had a decreased liver weight; however this did not occur. This is a further reminder that liver weight does not necessarily parallel body weight changes. The increased liver weight in cold and in choline deficiency are further examples.

5. Thyroid:

Wet weight: no significant differences. Histology: The histological picture is not visibly different between groups. Pathological lesions (metaplasia) as seen in the rat thyroid were not observed.

6. Adrenal weight (Table 52): Data at $p = 0.05$.

The mean adrenal weight of Group OA is significantly greater than that in both Group +A and the IC group. As a general stress response, it represents an affirmation that a detrimental condition existed in the group.

CONCLUSIONS:

1. The liver ascorbic acid concentration of guinea pigs, rendered deficient of liver vitamin A on a completely synthetic diet and orally supplemented with 10 mg. of vitamin C per day, had a tendency to be lower than that

found in the control (+A) and initial control (IC) groups.

2. The total liver ascorbic acid value of the deficient animals (OA) tended to be lower than that of the initial controls (IC) but was not significantly different from that of the control (+A) group.

Even though the deficient animals were rapidly losing weight and a few had died, the ascorbic acid values found were not as low as those found in scurvy; and these vitamin A deficient animals did not show signs of scurvy.

EXPERIMENT IX

Introduction:

This experiment has essentially the same protocol as that of experiment VIII. We decided that an attempt should be made to slow the rate at which vitamin A deficiency apparently develops and to sacrifice the animals before weight changes had become marked.

EXPERIMENTAL PROTOCOL:

Two groups of six male, mixed strain guinea pigs, ranging in weight between 386 and 495 grams (mean 455.5 grams), were placed in two separate group cages. Both groups received 75 mg. of vitamin C per day 'per os'. The control +A animals received 500 I.U. of vitamin A, administered orally once a week. Both groups were ad libitum fed a mixture of 50% ground Purina Guinea Pig Chow (passed through a Hobart coffee grinder at the 'coarse' setting) and 50% of the L.D.G.P. vit. A deficient test diet previously described (Table 50). The animals were on this diet mixture for 36 days and were then given the pure test diet during the last 16 days of the experiment. When the experiment was terminated (when the OA Group body weight gain began to plateau), the following determinations were made: Liver ascorbic acid and vitamin A; wet weight of the liver and thyroids. The data and their significance are tabulated in Table 53.

RESULTS AND DISCUSSION:

No significant differences were found in body weight gain (Fig. 19), liver weight and thyroid weight.

1. Liver vitamin A:

No detectable vitamin A was present in the livers of the deficient animals. The control (+A) guinea pigs had a mean liver vitamin A concentration of 44.6 I.U. and a total liver mean value of 914.0 I.U. of vitamin A.

The slope of the weight gain curve (prior to the 'plateau') of the deficient animals in the two experiments are similar. It is not difficult to believe that the animals were probably already deficient of liver vitamin A when they were placed on the pure test diet alone. A calculated 1,200 I.U. of vitamin A (1,000 I.U. from carotene) is available per day to a guinea pig who daily eats at least 20 grams (156) of Purina Guinea Pig Chow. If, when this dietary source of vitamin A is diluted by half, the animal becomes deficient, then the daily requirement of the adult guinea pig is very high or a substantial portion of the vitamin A and/or carotene in the chow is not readily available to the animal. We have seen in experiment VII that orally administered preformed vitamin A is taken up by the guinea pig liver. However, it has repeatedly been demonstrated that guinea pigs consuming a diet rich in carotene show little tendency to store vitamin A (13).

Probably very little of the carotene present in guinea pig chow is available to the animal. If this is the case, then a calculated 300 I.U. of vitamin A is available daily; and when the chow is diluted by half, only 150 I.U./day is available.

In experiment VII we noticed that 50 I.U. vitamin A biweekly did not allow liver storage in measurable quantities. In this experiment, 500 I.U. of preformed vitamin A per week gave liver values comparable to that found on guinea pig chow alone in experiment VII. The requirement of vitamin A in our guinea pigs was at least as calculated 50 I.U. of preformed, orally administered, vitamin A per week; and for moderate liver stores, at least 500 I.U. per week when orally administered or 1500 I.U. per week when contained in the diet.

In addition, we believe that the turnover of vitamin A in the guinea pig is considerably more rapid than in the rat. The obvious usefulness of such an experimental animal for research into the yet unknown (other than its role in vision) mechanism and site of action of vitamin A demands that the animal be given more consideration in vitamin research. A serious handicap in the past was the lack of an adequate synthetic diet. We feel that the synthetic diet we utilized is adequate but that the pelleting of such a diet is a definite prerequisite for long term experimentation.

2. Liver Ascorbic Acid:

There is no significant difference between the groups in either liver concentration or total liver ascorbic acid. We sincerely believe that the absence of vitamin A in the liver of the guinea pig has no bearing on the ascorbic acid status of the guinea pig.

Although the experiments are not strictly comparable, it is interesting to note that the liver ascorbic acid values in this experiment are considerably higher; probably because a higher exogenous dose of vitamin C was administered. This probably indicates that, to a certain extent, guinea pig liver cells are capable of storing ascorbic acid. No one, to our knowledge, has extensively studied the ascorbic acid status of the rat or guinea pig when and after receiving varying supplements of vitamin C. Experiments utilizing the combined techniques of radioactive tracers and metabolic balance studies are in order. Careful consideration should be given to the type and quantity of diet utilized in such studies.

CONCLUSIONS:

1. The absence of liver vitamin A in the guinea pig has no effect on the uptake and storage of supplemented vitamin C.

2. The requirement for vitamin A in our guinea pigs

has been calculated to be at least 50 I.U. of preformed, orally administered vitamin A per week; and for moderate liver storage, at least 500 I.U. per week when orally administered.

EXPERIMENTS X AND XI:

Introduction:

The purpose of the following two experiments was to study the effect of a high fat diet on acclimation (conditioning) to cold as compared to an isocalorically fed (Exp. X) and an ad libitum fed (Exp. XI) high carbohydrate diet. In both experiments, these diets were compared to an ad libitum fed commercial ration (Purina Laboratory Chow). In addition, in Experiment X, we attempted to determine if a relationship existed between the beneficial effects of exogenous ascorbic acid in the cold (reviewed on page 16) and diet composition.

EXPERIMENTAL PROTOCOL:

Experiment X:*

Eighty-four male, wistar strain, albino rats, ranging in body weight between 160 and 186 grams (mean 170.5 grams), were individually caged and divided into 12 groups. There were four groups on each of three different diets. On a given diet, two groups were exposed to cold ($+2.0^0 \pm 2.0^oC$) and one of these received 75mg. of vitamin C administered per os twice a day ($\pm$ 9:AM and $\pm$ 4:PM): the other two groups were the respective controls, with and without vitamin C, at room temperature.

* This experiment was performed with the cooperation of Dr. Claude Godin and the students of Bio. 517 (Laboratory of Nutrition). We express our sincere thanks to Dr. Godin and Messrs. John Burba, Jacques Dunnigan, Stanley Magwood and Earl Potvin.

Group	Diet	Vitamin C	R.T. Symbol	& N.	Cold Symbol	& N.
1-2	Lab.Chow	0	L	6	KL	8
3-4	Lab.Chow	+	LC	6	KLC	8
5-6	High Fat	0	F	6	KF	8
7-8	High Fat	+	FC	6	KFC	8
9-10	High CHO.	0	H	6	KH	8
11-12	High CHO.	+	HC	6	KHC	8

The composition of the diets utilized as compared to those utilized by Pagé and Babineau (150), and as compared to the chemical analysis of Purina Laboratory Chow is given in Table 54. All animals were allowed to adapt to the respective diet for a period of one week prior to the onset of the experiment. The laboratory chow animals were fed ad libitum. The animals on the semi-synthetic diets were fed equicaloric amounts sufficient to maintain growth. Every two days, the room temp. rats were allowed that weight of their particular diet equal to 53 calories per day. Similarly, the animals in the cold were allowed 96 calories per day.

During the 5th and 6th week of the experiment, the oxygen consumption (M.R.$^{30.0^{o}C}$) of a different fasted rat in each group (total 12 rats) was measured each day. A total of 111 measurements were made on the 12 groups.

The experiment was terminated after 44 days of exposure to cold had elapsed. The following determinations were made:

Liver ascorbic acid and vitamin A; wet weight of the liver, kidneys, testes, adrenals, thyroids and the perirenal fat of the left side of the animal; perirenal fat ascorbic acid; dry weight and fat content of the liver; thyroid histology.

Experiment XI.

Forty-eight male, wistar strain, albino rats, ranging in weight between 162 and 191 grams (mean 174.2 grams), were individually caged and divided into 6 groups. Three groups of 6 animals each were kept at room temp. and three groups of 10 animals each were placed in the cold (± 2.0° ± 2.0°C.). Each group at a given environmental temperature (symbol "K" for groups in cold) were ad libitum fed a different diet; either high fat (symbol 'F'), high CHO (symbol 'H') or Purina Laboratory Chow (symbol 'L'). All the animals were allowed one week to adapt to the diet prior to the onset of the experiment.

During the 5th and 6th week of the experiment, the oxygen consumption (M.R.$_{30.0^{\circ}C.}$) of a different fasted rat in each group (total 6 rats) was measured on each day. A total of 59 measurements were made on the 6 groups.

The experiment was terminated after 42 days of exposure to cold. The following determinations were made: Liver ascorbic acid; abdominal muscle ascorbic acid; wet

weight of the liver, kidneys, testes, adrenals, thyroids, and perirenal fat of the left side of the animal; dry weight and fat content of the liver; and thyroid histology.

RESULTS:

The significance of the results are tabulated in Table 55 for Experiment X and in Table 65 for Experiment XI.

In order to conserve space and to avoid lengthy descriptions of the results, (which are, at times, of mediocre individual importance); we have presented our interpretation of the data of each experiment in the following abbreviated form. The discussion is found later in the text and is based, with few exceptions, upon the data 'in toto' of both experiments.

EXPERIMENT X

Subject:	Response to Cold vs. R.T. control on; High Fat Diet; (Restricted calories)	High CHO.Diet;	Lab. Chow; (Ad libitum)	Other remarks
Body Weight Gain. 44 days. Table 56 Fig. 20	N.S.**	N.S.	d.73%***	As compared to other groups in the cold, fat groups KF and KFC have i.36%(0.05)

Liver:wet weight in gms. Table 57(A)	w/o* Vit.C.:	i.5%(N.S.)	: i.45%	: R.T.> Cold due to b.wt. HC>H 10%(N.S.)	
	Vit.C.:	i.24.5%	: i.27%		
wet wt. per 100 g. b.wt. Table 57(B)	w/o Vit.C.:	i.15%	: i.49%	: i.26%	
	w/ Vit.C.:	i.30%	: i.31%	: i.20%	
	Vit.C. tends to promote the hypertrophy on a hi. fat diet				
Liver: defat.dry wt. in gms. Table 57(C)	w/o Vit.C.:	i.4.5%(N.S.)	:i.47%	: R.T.> Cold	
	w/ Vit.C.:	i.22%(0.05)	: i.39%		
defat.d.wt./ 100g. b.wt. Table 57(D)	w/o Vit.C.:	i.13%(N.S.)	: i.45%	: i.19%	
	w/ Vit.C.:	i.29%	: i.53%	: i.12%(N.S.)	

These dry weight data are a direct reflection of the whole weight data. The increase in liver dry weight in the cold signifies either an increase in cellular protein or in the number of cells.

Liver: % defat. dry weight. N.S. This may be an indication that no. of cells has not changed. 'Hypertrophy' in the cold has not been critically examined and defined.

Total liver fat in gms. N.S.

* W/O: Without, W/: with
** N.S.: no significant differences were observed.
*** Percent differences given as i. (increase) or d. (decrease) are significant at p = 0.01 unless otherwise indicated.

EXPERIMENT X continued

Subject:	Response to Cold vs. R.T. control on; High Fat Diet; (restricted calories)	High CHO .Diet; Lab. Chow; (Ad libitum)	Other remarks
% liver fat.N.S.			
Liver: Vitamin A I.U./gm. Table 60(A)	w/o Vit.C.: d.42%(NS)	: d.25%(NS) : i.8%(NS)	
	w/ Vit.C.: d.42%(NS)	: d. 9%(NS) : i.2%(NS)	
Total I.U. Vitamin A Table 60(B)	w/o Vit.C.: d.36%(NS)	: 0%(NS) : d.8%(NS)	
	w/ Vit.C.: d.31%(NS)	: i.15%(NS) : 0% (NS)	
	High fat diet in the cold potentially i. reqmt, vit. A. Assuming that absorption of vitamin A is best on fat diet this increase reqmt. probably indicates a vitamin A role in fat metabolism.		
Liver: Ascorbic A. mg./gm. Table 58(A) N.S.			
Total liver Ascorbic A. Table 58(B)	w/o Vit.C.: i.20.5%(0.05)	: i.50% : nil(NS)	
	w/ Vit.C.: i.8.6%(N.S.)	: i.35% : nil(NS)	
	Vit. C. promotes hypertrophy on a high fat diet but not on a high CHO diet. If total liver asc. a. value is taken as an index of cellular activity, then exogenous vit. C. decreases this activity on a high fat diet and high CHO diet but probably has more significant effect on the high fat diet.		
Kidney: wet weight in gms. Table 59(A)	w/o Vit.C.: i.37%	: i.37% : i.4%(N.S.)	
	w/ Vit.C.: i.23%	: i.35% : i.13%	
wet wt. per 100g. b.wt. Table 59(B)	w/o Vit.C.: i.53%	: i.39% : i.57%	
	w/ Vit.C.: i.29%	: i.39% : i.38%	
	Kidney hypertrophy signif. less on high fat diet + vit. C. Beneficial effect of vit. C. observed on Lab. Chow is N.S.		

EXPERIMENT X continued

Subject:	Response to Cold vs. R.T. contron on: High Fat Diet; High CHO. Diet; Lab. Chow; (restricted calories) (ad libitum)			Other remarks:
Perirenal Fat: wet weight in grams Table 61(A)	w/o Vit.C.: d.59%	: d.37%(0.05)	: d.85%	:FC 22% < F(0,05) :KFC 38% > KF(0.05) :HC 13% > H(NS)
	w/ Vit.C:: d.27%(0.05)	d.50%(0.05):	d.71%	
wet wt. per 100g. b.wt. Table 61(B)	w/o Vit.C: d.58%	: d.35%(0.05):	d.78%	:KHC 13%<KH(NS) :LC 39%>L(0.01) :KLC 39%>KL(NS)
	w/ Vit.C: d.25%(0.05)	d.40%(0.05):	d.69%	
	Animals on high fat diet in the cold maintained more perirenal fat than other groups. Addition of vitamin C (On high fat diet) tended to augment or preserve the fat depot in the cold.			
Perirenal Fat: Ascorbic A. mg./gm. Table 62(a)	w/o Vit.C: i.50%(NS):	i.33%(NS)	: i.125%	
	w/ Vit.C: i.19%(NS):	i.120%(0.05):	i.100%(NS)	
Total P.Fat ascorbic acid. Table 62(B)	w/o Vit.C: d.35%(NS):	d.22%(NS)	: d.74%	
	w/ Vit.C: d.16%(NS):	d.23%(NS)	: d.54%	
	Our interest derived from work of Pagé and Babineau (149) on brown fat ascorbic acid content. Our results on neutral fat are equivocal. Defatted dry weight determinations of this tissue would probably have made the data more meaningful. The total ascorbic acid data parallel the total wt. of perirenal fat data.			
Testes: wet weight in grams.	N.S.			
Adrenals: wet weight in mgs. Table 63	w/o Vit.C: i.47%	: i.30%	: 0%(NS)	
	w/ Vit.C: i.46%	: i.20%(NS)	: i.35%	:LC 26%<L
	Adrenal hypertrophy was greatest on high fat diet. Vit. C. admin. tends to decrease the response on hi. CHO diet.			

EXPERIMENT X continued

Subjecté	Response to Cold vs. R.T. control on; High Fat Diet; (Restricted calories)	High CHO. Diet: Lab. Chow; (Ad libitum)	Other remarks

Thyroids:
wet weight N.S.
in mgs.

Histology: Activity of gland increased due to cold but no observable differences can be attributed to diet composition.

Oxygen Consumption:

M.R. 30.0°C. in ml./hr.					
	w/o Vit.C.:	i.31%	: i.16%(NS)	: i.4%(NS)	:KHC 12%<KH
	w/ Vit.C.:	i.20%(0.05)	: i.33%	:: i.1%(NS)	:KFC 4%>KF

Table 64

Admin. of Vit.C. tend to decr. Q_{O_2} on high fat diet :HC 2%?H

but exerts the reverse effect on the high CHO. diet :FC 5%<F
:LC 8%?L

EXPERIMENT XI:

Midway through this experiment, the room temperature, ad libitum fed, rats on the high carbohydrate diet began to lose weight. Although none died, their general appearance was poor but showed no signs of any known deficiency. We believe these animals could not tolerate the high carbohydrate content of the diet when ad libitum fed. In view of this, the comparison of the response of the animals on this diet in the cold as compared to those at room temperature is exagerated by the very marked decrease in the general status of the room temperature animals. The animals on this diet in the cold (KH) did not appear to be affected by this particular condition and they are thus capable of being compared to the other animals in the cold.

We have not given 'regressed weight' data. Although we admit the increased statistical accuracy conferred by this mode of expression, one must realize that it involves extensive statistical manipulations. We hope to undertake such studies in the future. We feel our results, as expressed, illustrate the beneficial effect of ~~an~~ a~~d libitum fed~~ high fat diet in the cold as compared to Purina Laboratory Chow.

Please note that a change has been made in the arrangement of the following text-chart. In experiment X,

the results on the restricted calorie intake of diet (high fat and high CHO.) were compared, in that order, to the ad libitum fed laboratory chow results. Because, in this experiment (XI), the results obtained at room temperature with the ad libitum feeding of the high CHO. diet are problematical, we have compared the ad libitum fed high fat diet results to the results obtained on the laboratory chow, in that order, and have recorded the high CHO diet results last.

Subject: EXPERIMENT XI:	Response to cold vs. R.T. control on: High Fat Diet; (all diets ad libitum fed)	Purina Lab. Chow;	High CHO. Diet;
Survival	: R.T. 6/6 (100%) : Cold 10/10(100%)	: R.T. 6/6 (100%) : Cold 7/10 (70%)	: R.T. 6/6 (100%) : Cold 4/10 (40%)
Body weight Gain 42 days Table 66 Fig. 21	: d.16%	: d.39%	: i.300%(N.S.) : best in cold!
	In the cold: gain is best on high fat: potentially better than lab. chow and signif. better than on high CHO.		
Liver: wet weight in gms. Table 67(A)	: i.1%(NS)	: d.20.5%	: i.59%
	No significant differences btwn. each other in the cold.		
wet wt. per 100g. b.wt. Table 67(B)	: i.17.5%(0.05)	: i. 8.0%(NS)	? i.57%
	In the cold: KH sign. greater than all other groups.		

Subject:	Response to cold vs. R.T. control on: High Fat Diet; Purina Lab. Chow; High CHO. Diet; (all diets ad libitum fed)		
Liver: Defat. dry wt. in gms. Table 67(C)	: N.S.	: d.18%	: 1.66%
	In the cold: parallel wet wt. changes, no differences between groups.		
Total liver fat in gms. Table 67(D)	: N.S.	: d.24.0%(NS)	: i.172%
	Between groups: total liver fat tends to be greater on high fat diet.		
% defatted dry weight. Table 67(E)	: N.S.	: N.S.	: N.S.
	In the cold: The percent is signif. less on high fat diet then on high CHO. diet, and tends to be less than that on Lab. Chow.		
% liver fat: Table 67(F)	: d.6.5%(NS)	: d.5.5%(NS)	: i.60%
	Between groups: percent liver fat signif. greater on fat diet then on Lab. Chow. KH tends (0.05) to have more liver fat then Lab. Chow groups.		
Liver: Ascorbic A. mg./gm. Table 68(A)	: N.S.		
Total liver Ascorbic A. Table 68(B)	: L sign. greater (0.05) than all other groups due to i. liver weight at room temp. H sign lower value (0.01) than in all other groups due to d. liver weight at room temp. No effect can be attributed to cold.		
Abdominal muscle: Ascorbic A. mg./gm. Table 69	: N.S.	: N.S.	: N.S.
	Although muscle mass reportedly decreases in the cold (102), the ascorbic acid concentration remains suprisingly constant on a given diet. However, if the no. of cells has not changed then the actual cellular concentration is decreased. We failed to measure some index of muscle mass but realize the importance of such a measure in that it could contribute toward a more thorough understanding of ascorbic acid changes at the level of chemical thermogenesis in the cold.		

Perirenal Fat:
wet weight in grams. Table 70(A) : d. 61% : d. 73% : i. 150%(N.S.)

wet wt. per 100g. b.wt. Table 70(B) : d. 55% : d. 71% : i. 152%(N.S.)

In the cold: the decrease in P. fat is less on the fat diet than on Lab. chow. Thus animals on a fat diet have more active reserve energy. The high CHO. diet group (KH) has twice as much perirenal fat as compared to the room temperature (H) group. The animals in the cold did not have the condition suffered by their room temp. counterparts and this may be related to the fact that cold stimulated depot fat synthesis; and therefore, the animals on this diet were better able to utilize CHO. However, this was done at the cost of decreased weight gain, decreased survival, marked liver hypertrophy etc.

Kidneys:
wet weight in gms.
Table 71(A) : : N.S. : N.S. : i.24%(NS)

wet wt. per 100g. b.wt.
Table 71(B) : : i.20% : i.32% : i.22%

In the cold: Hypertrophy least on high fat diet, as compared to Lab.chow animals. The kidneys of animals on high CHO diet are significantly heavier at room temp. as compared to other groups, thus increase in the cold seems small.

Testes:
wet weight in grams.
Table 72 : N.S. : N.S. : d. 32%

Animals on high CHO in the cold have sign. decrease as compared to all other groups in the cold and at room temp.

Thyroids:
wet weight in mgs. : i.12%(N.S.) : i.16%(N.S.) : i.13.5%(N.S.)
Table 73

In the cold: Thyroid weight tends (0.05) to be lowest on the High CHO diet.

Oxygen Consumption: N.S.

DISCUSSION AND CONCLUSIONS:

It is apparent from the data of these two experiments, that a high fat diet is beneficial in the cold. Rats on such a diet have a superior body weight gain, increased survival, decreased degree of liver hypertrophy and the weight of the perirenal fat (index of total body fat (150) was nearly equal to that of control animals fed Lab. chow at room temperature.

An important observation, however, is that such a high fat diet fed ad libitum (Exp. XI) gives results which are not superior (and probably slightly inferior) to those results obtained when the intake of such a high fat diet was restricted (Exp. X). Just as a high fat diet at room temperature is not as efficient as a low fat diet (such as lab. chow), a high fat diet when consumed in excess probably tends to exert a similar retarding effect in the cold. Assuming that all or some phase of fat metabolism is increased in the cold, the dietary quantity and the type of fat which can be optimally utilized can be advantageously increased proportionately, but an excess probably hampers the mechanism.

The beneficial effects which increased dietary fat is able to confer is not restricted to the field of cold physiology. Deuel et al. (48) and Samuels et al. (175) demonstrated that the maximum work capacity of rats

was considerably prolonged as the proportion of fat in the diet was increased. Using different experimental conditions, Ershoff (68) was unable to confirm this.

Fasting animals undoubtedly survive longer when the prefast diet has been composed of fat rather than carbohydrate or protein (164) (165). In connection with this, one must remember that animals in the cold have a decreased muscle mass (102). It seems likely that this active tissue mass (chemical thermogenesis) has little energy available for net protein growth, thus, in a sense, the animal in the cold is fasting. At room temperature, Scheer et al. (177) found that the loss of weight by young rats when subjected to severe caloric restriction was less in the groups receiving high fat diets than in those receiving the isocaloric low fat diets.

The metabolic pattern changes during the time animals are on a high fat diet. Lunbaek and Stevenson (118) advanced the hypothesis that this greater efficiency of fat during fasting was due to the fact that the animals receiving the fat did not experience a loss of energy entailed in the transformation of carbohydrate to fat. Hausberger and Milstein (93) have since shown that the 'in vitro' rate of lipogenesis in adipose tissue is proportional to the carbohydrate content of the diet, but in addition was in some way also regulated by the

fat of the food. They (93) as well as Masoro et al. (72) demonstrated that the protein content of the diet does not exert a major influence on subsequent 'in vitro' lipogenesis of hepatic and adipose tissue. These results also support the earlier reports of Forbes et al. (74) (75) (76). Utilizing pair feeding tests, they demonstrated that, in the rat, the greater efficiency of the diet having the highest fat content (30%) was manifested by significant gains in fat energy and by a decrease in total heat production. Moreover, the fat content had little effect upon nitrogen utilization.

Roberts and Samuel (164) reported that rats on a high fat diet had a lower susceptibility to insulin. This has been confirmed 'in vitro' by Hausberger and Milstein (93). To date, the role of insulin in the cold and its relation to thyroid function has not been examined. This field of research is 'wide open'.

Although the consensus of opinion concerning the importance of the reduction of heat loss in achieving adaptation has diminished in favor of increased energy exchange and heat production (195), the possibility, other than insulative changes, of a decrease in heat loss due to a saving in energy expenditure, should not be overlooked.

Forbes et al. (77) attributed the beneficial effect of high fat diets (at room temp.) to a decreasing

energy expenditure (which they called the "associative dynamic action") whereby a smaller amount of waste energy is lost as a result of Specific Dynamic Action (S.D.A.). Although Rubner (171) has shown that the extra heat produced due to S.D.A. may be used by the body in lieu of stimulation of metabolism by shivering (which is of very little importance in comparison to chemical thermogenesis in muscle of adapted animals), the extra heat produced by the metabolism of foodstuffs usually represents waste heat. Any condition which would reduce the output of waste heat will obviously result in a greater efficiency of food utilization.

We believe the following observations demonstrate the probable importance of such mechanism. In experiment XI, the question arose as to why a rat consuming 112 calories per day in the cold had a better general appearance, weight gain, survival and perirenal fat status as compared to a rat consuming 156.5 calories per day??? The former rat received his energy primarily from fat and the latter primarily from commercial (high CHO) laboratory chow. We believe that, not only is fat probably 'preferentially utilized', but also, the gross efficiency of the digestive tract is considerably better on the high fat diet.

Rats fed lab. chow, at room temp., evacuate 15.5 ± 0.74 * grams of feces every two days. Animals of a comparable age, who have been in the cold 40 days, evacuate

* Std error

52.6 ± 2.03 grams of feces every two days (i.240%). During this time the former (room temp. animals) consumed daily 33.4 ± 1.74 grams of laboratory chow (ca. 117 calories) or approximately 66 grams of food in two days. The cold room rats consumed daily 44.2 ± 2.32 grams of laboratory chow (calc. 156 calories), or approximately 88 grams of food in two days. This is a 'weight of food (6% fiber) efficiency' of 66/15.5 = 4.25 at room temperature, and 88/52.6 = 1.67 in the cold. Assuming the laboratory chow animals had eaten twice as much in the cold, with no greater 'loss', their 'food efficiency' would be 176/52.6 = 3.4 which is still less than that at room temperature.

The rats on a high fat diet, at room temp., evacuate 9.75 ± 0.69 grams of feces every two days. Animals of a comparable age, who have been in the cold 40 days, evacuate 15.6 ± 0.54 grams of feces every two days (i.58%). During this time the former (room temp. animals) consumed 19.3 ± 1.19 grams of high fat diet (ca. 93.2 calories) per day or approximately 38 grams of diet in two days. The cold room rats consumed 23.3 ± 0.90 grams of high fat diet (ca. 112 calories) per day or approximately 46 grams of food in two days. This is a 'weight of food (24% fiber) efficiency' of 38/9.75 = 3.90 at room temperature, and 46/15.6 = 2.95 in the cold.

The importance of the fiber content should by no means be overlooked. However, if the fiber content of the lab. chow was theoretically increased to 24% of the diet, then the caloric value of the chow would be decreased to a calculated 3.78 calories per gram. This would mean that the animal would have to eat at least 55 grams (i.22%) of chow in order to maintain the same number of calories as previously. Moreover, this weight of food would be nearly two times that normally eaten at room temp. and there necessarily must be a physical limit to how much an animal can consume. In addition, a further increase in roughage would probably further increase the frequency of evacuation.

The importance of fat and possibly the fat to roughage ratio is evident. Fat furnishes an increased amount of calories per unit weight and moreover slows gastric motility. Fat thus prolongs the absorption time and effectively decreases the S.D.A.

We realize that our results need confirmation, but we are positive that this occurred throughout our experiments in the cold; we simply failed to recognize its value and so made a limited number of measurements.

Given that increased dietary fat content is beneficial in the cold, the report of Sellers and You (187) concerning the development of coronary artherosclerosis in the cold on a high fat diet containing 2% cholesterol, and on a

commercial stock ration containing only 3.2% fat is worthy of serious consideration. Although many possible etiologic agents are proposed, a high level of dietary cholesterol when choline is present in the diet, was incriminated in the case of the synthetic diet. The authors could not explain the less drastic but similar results on the commercial ration and stated that it contained no cholesterol. We do not wish to debate the relationship between cholesterol and artherogenesis, however we wish to indicate that the commercial ration does contain choline and substantial quantities of yeast squalene. Deuel (46) states that the "evidence is so overwhelming for the participation of squalene (which is capable of being absorbed) in the acetate-cholesterol reaction, that one can accept is as a proven fact." In addition, Schoenheimer and Breush (179) were unable to demonstrate any increased cholesterol synthesis in mice which were fed considerable amounts of lard and other fats. Similar results were observed by Alfin-Slater et al. (2) on rats. Cold however has a definite effect. Vahouny et al (221) state that "while a cold environment has a consistent lipotropic influence on hepatic triglycerides, there is a simultaneous increase in blood and liver esterified cholesterol levels in rats fed a cholesterol-fat diet". A comparable deposition of cholesterol was not

found in animals on the low-sterol diet, and they (221) suggest that cold had an effect on cholesterol absorption from the intestinal tract or that the normal metabolism of cholesterol in the liver is impaired in the cold.

In a related vein, the relationship between an increased carotenoid content to cholesterol content in the adrenals and artheroma needs further investigation (4).

Returning to Experiment X, we note that adrenal hypertrophy was greatest on the high fat diet. This does not necessarily indicate that adrenal function is increased. Gieger, El Rawi and Catz (83), have shown that the adrenals and thyroids are not required for adaptation at room temperature to a high fat diet. Fat adapted animals have been found to survive longer following adrenalectomy than animals on a high CHO. regime. The increased adrenal weight is probably a reflection of non-adaptation not only to a cold stress but also to a high fat diet. This problem needs further study.

In our experiments we did not observe increased liver ascorbic acid concentrations when animals were exposed to cold. We were unable to confirm the earlier observations of Thérien (213). Although no data is given here, we have witnessed the dramatic beneficial effects of exogenous vitamin C on the survival of young rats in the cold. Moreover, from other experiments we have no doubt that vitamin C

substantially aids animals to acclimatize to cold. The mechanism of this action is unknown. We believe our experiment (X) sheds light on this problem. Our observations are not necessarily definitive but may be indicative.

1. The administration of ascorbic acid to lab. chow animals at room temp. tends (0.05) to decrease weight gain. This is not observed in the cold. 2. Whereas, vitamin C administration had no effect on the degree of liver hypertrophy on the lab. chow and high CHO fed animals in the cold, it significantly increased liver hypertrophy on the high fat diet. The administration of vitamin C had no effect on the liver fat content on any of the diets fed.3.The kidney is hypertrophied in the cold on all diets but potentially tends to be the least hypertrophied on the high fat diet plus vitamin C. 4. Perirenal fat is markedly decreased with exposure to cold, however this decrease is significantly less on the high fat diet plus vitamin C. 5. Basal oxygen consumption (M.R. $30.0^{\circ}C.$) is increased on the high fat diet by 31%; but this potentially tends to be decreased with the addition of vitamin C. Thus if basal needs are decreased, the amount of energy saved is potentially available for heat production. In this respect we should note that basal oxygen consumption was increased by the administration of vitamin C to the animals on the high

carbohydrate diet. We were technically unable to measure Q_{O_2} (M.R. 2.0°) in the cold and realize such data is needed.

We conclude that there is evidence for a vitamin C-High Fat diet relationship in the cold. Such a relationship may simply be an additive reaction of the beneficial effect conveyed by the two separate dietary constituents. However there does exists evidence for a direct relationship. Investigation of the reported intimate association between choline oxidase and ascorbic acid (227) (8) as affected by cold exposure is needed. The recent reports concerning cold and lipotropic factors (221) (186) add importance to the possible fruitfulness of such aforementioned investigations. The report that the utilization of phospholipids in brain and liver is catalyzed by iron compounds, especially if ascorbic acid is added (62), further incriminates ascorbic acid in phospholipid metabolism. Also of interest, is the report of increased fatty acids and phospholipids (but unchanged cholesterol) in the blood of scorbutic guinea pigs (144). Although not confirmed by Baldwin (7), Terbruggen (211) reported that the liver of scorbutic guinea pigs contained 10 to 19% fat (normal 3.4-3.8%).

In connection with their work on brown adipose

tissue and ascorbic acid, Pagé and Babineau (149) concluded "that ascorbic acid is linked with some phase of fat metabolism". We wish to reaffirm this belief and we sincerely hope that further interest will be taken in the elucidation of this particular role of vitamin C.

Summary: (Experiment X and XI)

1. Rats fed a high fat diet withstand a cold stress (2.0°C. for 42 days) better than rats fed high carbohydrate diets.

2. The observed beneficial effects of a high fat diet in the cold were not increased, (ie. not markedly enhanced), when the diet was fed ad libitum as compared to when it was fed in restricted amounts. The supplemental calories which a high fat diet can furnish is therefore not the only reason why such a diet promotes increased resistance to cold. The dietary quantity and type of which can be advantageously utilized is probably proportional to the increase in fat metabolism in the cold, and excess fat probably hampers metabolism in the same fashion in the cold as it obviously does at room temperature.

3. We suggest that increasing the fat content of the diet in proportion to the increase in metabolism in the cold decreases the need for increase lipogenesis and that the energy thus saved becomes available for heat production. In addition, the decreased energy expenditure due to a smaller amount of waste energy being lost as a result of specific dynamic action probably benefits the animal. We have shown that the gross efficiency of the digestive tract

in the cold is considerably better when a high fat diet is fed. We believe this allows for an optimum utilization of the mechanisms of energy conservation previously mentioned.

4. Indications are that the mechanism by which ascorbic acid exerts its beneficial effect in the cold may be intimately associated with some phase of fat metabolism. We believe particular emphasis should be placed on its role in phospholipid metabolism.

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EXPERIMENT I

Table 1

Source of Variance		Total	Groups	Indiv. in Grps.	F	p
	d.f.	49	7	42	-	-
Liver: Ascorbic Acid M.mg./gm.	S.sq.	0.270	0.102	0.168		
	M.sq.	--------	0.015	0.004	3.75	0.01
Liver: Total Ascorbic Acid.	S.sq.	59.969	34.783	25.186		
	M.sq.	---------	4.969	0.599	8.29	0.01
Liver: Wet weight in gms.	S.sq.	253.54	122.67	130.87		
	M.sq.	---------	17.52	3.12	5.61	0.01
Thyroid: Wet weight in mgs.	S.sq.	957.29	366.02	591.27		
	M.sq.	---------	43.71	14.08	3.10	0.01
Testes: Wet weight in gms.	S.sq.	20.40	11.05	9.35		
	M.sq.	---------	1.58	0.22	7.18	0.01
Adrenal: Wet weight in mgs.	S.sq.	3784.3	1448.3	2336.0		
	M.sq.	---------	206.9	55.6	3.72	0.01
	d.f.	54	3	51		
Weight Gain 85 days (room temp.)	S.sq.	75096.2	5078.2	70018.0		
	M.Sq.	---------	1692.73	1372.90	1.23	N.S.
	d.f.	29	3	26		
Weight Gain from 85th. to day 145 (cold room)	S.sq.	46247.2	28173.2	18074.0		
	M.sq.	---------	9391.06	695.15	13.51	0.01

EXPERIMENT I

Table 2 BODY WEIGHT GAIN AT ROOM TEMP.

Ranges. p:	(4)	(3)	(2)	
Rp:				
Results.				
Group :	nA(14)*	+A(14)	N(13)	OA(14)
Mean :	318.7	311.0	296.7	296.2

Table 3 BODY WEIGHT GAIN IN THE COLD.

Ranges. p:	(4)	(3)	(2)	
Rp:	64.0	62.5	59.8	
Results.				
Group:	K+A(9)	KnA(9)	KN(6)	KOA(6)
Mean:	-6.3	-16.0	-20.5	-89.0

Table 4 LIVER ASCORBIC ACID CONCENTRATION.

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)	
Rp:	0.093	0.092	0.091	0.089	0.088	0.085	0.082	
Results.								
Group:	K+A	nA	KnA	+A	N	OA	KN	KOA
Mean:	0.316	0.292	0.273	0.261	0.249	0.236	0.206	0.174

Table 5 WET WEIGHT OF THE LIVER.

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)	
Rp:	2.89	2.87	2.83	2.78	2.73	2.66	2.55	
Results.								
Group:	K+A	KN	KnA	N	nA	OA	+A	KOA
Mean:	13.62	12.52	11.92	11.43	10.41	10.28	9.76	8.70

* Number in parenthesis is N (ie. number of animals in group). These data is the same in each group for all tables in a given experiment and is therefore given only on the weight gain table and/or body weight gain graph of a particular experiment.

EXPERIMENT I

Table 6 TOTAL LIVER ASCORBIC ACID.

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	1.27	1.26	1.24	1.22	1.20	1.17	1.12

Results. Group:	K+A	KnA	nA	N	KN	+A	OA	KOA
Mean:	4.28	3.27	3.04	2.84	2.58	2.53	2.48	1.39

Table 7 WET WEIGHT OF THE TESTES.

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	0.74	0.73	0.72	0.71	0.70	0.68	0.65

Results. Group:	nA	+A	N	OA	KN	KnA	K+A	KOA
Mean:	3.29	3.27	3.18	3.12	2.82	2.55	2.51	1.86

Table 8 WET WEIGHT OF THE THYROIDS.

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	6.16	6.11	6.02	5.92	5.82	5.66	5.42

Results. Group:	K+A	KnA	KOA	nA	KN	OA	+A	N
Mean:	21.97	21.82	19.71	18.50	17.48	15.82	15.36	14.94

Table 9 WET WEIGHT OF THE ADRENALS.

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	12.23	12.13	11.96	11.76	11.56	11.25	10.67

Results. Group:	KN	K+A	KnA	KOA	+A	nA	OA	N
Mean:	52.70	45.06	43.86	43.70	40.40	40.26	34.40	33.96

Table 10 Body Weight Gain.

Source of Variance		Total	Groups	Indiv. in Groups	F	P
EXP. III	d.f.	46	7	39	-	-
	S.sq.	12973.5	6902.1	6071.4		
	M.sq.	-------	986.0	155.7	6.33	0.01
EXP. IV	d.f.	57	7	50		
	S.sq.	32308.3	12981.2	19327.1	-	-
	M.sq.	-------	1854.5	386.5	4.80	0.01

Table 11 .
EXP. III Weight gain during last 10 days.

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)	
Rp:	20.5	20.3	20.0	19.6	19.3	18.8	18.0	
Results.								
Group:	+A(5)	+AC(5)	K+AC(8)	K+A(8)	OA(5)	OAC(6)	KOAC(4)	KOA(6)
Mean:	24.8	15.2	12.5	6.9	6.8	0.0	-2.8	-19.8

Table 12 .
EXP. IV Weight gain from day 0 to day 42.

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)	
Rp:	32.0	31.7	31.3	30.8	30.0	29.4	28.2	
Results.								
Group:	+A(5)	OA(6)	OAC(7)	+AC(5)	KOA(10)	K+AC(7)	K+A(7)	KOAC(11)
Mean:	190.2	173.7	169.9	166.8	162.9	157.6	154.0	136.5

Table 13 I.U. Vitamin A per Gram of Liver

Source of Variance		Total	Groups	Indiv.in Groups	F	p
EXP. II	d.f.	18	1	17	-	-
	S.sq.	429049.4	37643.0	391406.0		
	M.sq.	--------	37643.0	23023.9	1.63	N.S.
EXP. III	d.f.	25	3	22		
	S.sq.	600114.5	75733.8	524380.7		
	M.sq.	--------	25244.6	23835.5	1.05	N.S.
EXP. IV	d.f.	23	3	20		
	S.sq.	7841.8	2283.9	761.3		
	M.sq.	--------	761.3	277.9	2.74	N.S.

Table 14 I.U. Vitamin A per Total Liver.

Source of Variance		Total	Groups	Indiv.in Groups	F	p
EXP. II	d.f.	18	1	17	-	-
	S.sq.	64937359.0	12395072.0	52542287.0		
	M.sq.	----------	12395072.0	3090722.7	4.01	N.S.
EXP. III	d.f.	25	3	22	-	-
	S.sq.	30347355.5	2390725.8	27956629.7		
	M.sq.	----------	796908.6	1270755.9	0.63	N.S.
EXP. IV	d.f.	23	3	20		
	S.sq.	561630.4	196162.9	365467.5		
	M.sq.	--------	65387.6	18273.4	3.58	0.05

Table 15 EXP. IV Total Liver Vitamin A in I.U.

Ranges. p:	(4)	(3)	(2)	
Rp:	248.2	241.9	230.2	
Results.				
Group:	K+AC(7)	K+A(7)	+A(5)	+AC(5)
Mean:	534.8	484.2	459.9	284.3

Table 16 Liver Ascorbic Acid in mgs. per gm.

Source of Variance		Total	Groups	Indiv. in Groups	F	p
EXP. II	d.f.	47	4	43	-	-
	S.sq.	0.2272	0.0367	0.1905		
	M.sq.	------	0.0092	0.0042	2.18	N.S.
EXP. III	d.f.	44	7	37	-	-
	S.sq.	0.2059	0.0457	0.1602		
	M.sq.	------	0.0065	0.0043	1.51	N.S.
EXP. IV	d.f.	57	7	50	-	-
	S.sq.	0.2042	0.0656	0.1385		
	M.sq.	-------	0.0937	0.0277	3.38	0.01

EXPERIMENT II

Ranges. p:	(5)	(4)	(3)	(2)
Rp:				
Results.				
Group:	OAC	N	NC	OA^{ic}	OA
Mean:	0.270	0.253	0.253	0.232	0.192

EXPERIMENT III

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:							
Results.							
Group:	KOAC	OAC	K+A	+A	+AC	OA	KOA	K+AC
Mean:	0.330	0.326	0.310	0.309	0.279	0.272	0.246	0.245

EXPERIMENT IV

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	0.85	0.84	0.83	0.82	0.80	0.78	0.75
Results.							
Group:	+A	K+A	OA	KOA	K+AC	OAC	KOAC	+AC
Mean:	0.391	0.352	0.350	0.318	0.314	0.302	0.287	0.269

Table 17 Total Liver Ascorbic Acid in mgs.

Source of Variance		Total	Groups	Indiv. in Groups	F	p
EXP. II	d.f.	47	4	43	-	-
	S.sq.	49.330	27.606	21.724		
	M.sq.	------	6.900	0.500	13.66	0.01
EXP. III	d.f.	44	7	37	-	-
	S.sq.	219.115	97.918	121.197		
	M.sq.	-------	13.99	3.28	3.80	0.01
EXP. IV	d.f.	57	7	50	-	-
	S.sq.	33.467	14.933	18.534		
	M.sq.	------	2.13	0.37	5.76	0.01

EXPERIMENT II

Ranges. p:	(5)	(4)	(3)	(2)
Rp:	1.48	1.45	1.42	1.36

Results.

Group:	N	NC	OAC	OA^{ic}	OA
Mean:	2.82	2.54	1.38	1.33	0.91

EXPERIMENT III

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	0.94	0.93	0.92	0.90	0.89	0.87	0.83

Results.

Group:	+A	+AC	OAC	K+A	KOAC	K+AC	OA	KOA
Mean:	3.39	2.58	2.56	2.39	2.31	2.04	2.01	1.70

EXPERIMENT IV

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	0.99	0.98	0.97	0.95	0.94	0.91	0.87

Results.

Group:	+A	K+A	K+AC	OAC	KOA	OA	+AC	KOAC
Mean:	4.18	3.08	2.94	2.77	2.74	2.72	2.34	2.24

Table 18 Wet Weight of the Liver in Gms.

Source of Variance		Total	Groups	Indiv.in Groups	F	p
EXP. II	d.f.	48	4	44	-	-
	S.sq.	425.163	358.388	66.775		
	M.sq.	-------	89.597	1.518	59.04	0.01
EXP. III	d.f.	46	7	39	-	-
	S.sq.	127.430	69.910	57.521		
	M.sq.	-------	9.990	1.470	6.80	0.01
EXP. IV	d.f.	57	7	50	-	-
	S.sq.	97.863	40.527	57.336		
	M.sq.	------	5.780	1.147	5.05	0.01

EXPERIMENT II

Ranges. p:	(5)	(4)	(3)	(2)
Rp:	2.57	2.53	2.46	2.35

Results.

Group:	N	NC	OA^{ic}	OAC	OA
Mean:	10.97	9.90	5.33	5.02	4.62

EXPERIMENT III

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	1.97	1.97	1.93	1.90	1.86	1.81	1.74

Results.

Group:	+A	+AC	K+AC	K+A	OAC	OA	KOAC	KOA
Mean:	11.25	9.32	8.31	7.83	7.69	7.44	7.16	7.09

EXPERIMENT IV

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	1.74	1.73	1.70	1.68	1.65	1.60	1.53

Results.

Group:	+A	K+AC	OAC	K+A	+AC	KOA	KOAC	OA
Mean:	10.62	9.38	9.25	8.75	8.65	8.60	7.73	7.70

Table 19 Wet Weight of the Testes in Gms.

Source of Variance		Total	Groups	Indiv.in Groups	F	p
	d.f.	46	4	44	-	-
EXP. II	S.sq.	42.237	28.549	13.688		
	M.sq.	------	7.137	0.311	22.95	0.01
	d.f.	46	7	39	-	-
EXP. III	S.sq.	3.840	0.880	2.960		
	M.sq.	-----	0.130	0.080	1.62	N.S.
	d.f.	57	7	50	-	-
EXP. IV	S .sq.	3.094	0.943	2.141		
	M.sq.	-----	0.136	0.043	3.18	0.01

EXPERIMENT II

Ranges. p:	(5)	(4)	(3)	(2)
Rp:	1.15	1.14	1.10	1.06

Results.

Group:	NC	N	OAic	OAC	OA
Mean:	3.34	3.12	1.90	1.50	1.49

EXPERIMENT IV

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	0.34	0.33	0.33	0.32	0.32	0.31	0.29

Results.

Group:	OA	+A	+AC	KOA	K+A	OAC	K+AC	KOAC
Mean:	2.91	2.91	2.80	2.76	2.73	2.73	2.69	2.53

Table 20 Wet Weight Seminal Vesicles in mgs.

Source of Variance		Total	Groups	Indiv.in Groups	F	p
	d.f.	57	7	50	-	-
EXP. IV	S .sq.	3058803.7	919815.7	2138988.0		
	M.sq.	---------	131402.2	42779.7	3.07	0.01

Table 20 continued. EXPERIMENT IV

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)	
Rp:	336.9	333.8	329.1	324.4	318.2	309.6	296.3	
Results.								
Group:	OAC	+A	OA	+AC	KOA	K+AC	K+A	KOAC
Mean:	836.7	756.5	701.6	688.9	674.2	563.5	536.3	443.9

Table 21 Wet Weight of the Thyroids in mgs.

Source of Variance		Total	Groups	Indiv.in Groups	F	p
	d.f.	48	4	44	-	-
EXP. II	S.sq.	520.67	83.73	436.94		
	M.sq.	------	20.94	9.93	2.10	N.S.
	d.f.	46	7	39	-	-
EXP. III	S.sq.	453.69	210.87	242.82		
	M.sq.	------	30.12	6.23	4.83	0.01
	d.f.	57	7	50	-	-
EXP. IV	S.sq.	406.90	58.34	348.56		
	M.sq.	------	8.33	6.95	1.19	N.S.

EXPERIMENT III

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)	
Rp:	4.09	4.05	4.00	3.93	3.87	3.76	3.60	
Results.								
Group:	OA	OAC	+A	+AC	KOAC	KOA	K+AC	K+A
Mean:	19.2	18.7	15.7	14.7	14.6	14.3	13.6	13.3

EXPERIMENT IV

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)	
Rp:								
Results.								
Group:	+A	KOA	K+A	OA	OAC	KOAC	K+AC	+AC
Mean:	16.1	16.1	15.2	15.1	14.9	13.8	13.6	13.4

Table 22 Wet Weight of the Adrenals in Mgs.

Source of Variance		Total	Groups	Indiv.in Groups	F	p
	d.f.	48	4	44	-	-
EXP. II	S.sq.	3277.10	2077.58	1199.52		
	M.sq.	-------	519.39	27.26	19.05	0.01
	d.f.	46	7	39	-	-
EXP. III	S.sq.	1665.07	999.40	665.67		
	M.sq.	-------	142.77	17.07	8.36	0.01
	d.f.	56	7	49	-	-
EXP. IV	S.sq.	1418.43	732.90	685.53		
	M.sq.	-------	104.70	13.71	7.63	0.01

EXPERIMENT II

Ranges. p:	(5)	(4)	(3)	(2)
Rp:	8.3	8.1	7.9	7.5

Results.

Group:	NC	N	OAC	OA	OA^{ic}
Mean:	42.4	36.2	29.6	27.2	24.1

EXPERIMENT III

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	5.2	5.1	5.0	4.9	4.8	4.7	4.5

Results.

Group:	K+AC	K+A	+A	+AC	KOAC	OA	OAC	KOA
Mean:	39.4	37.7	36.8	33.6	31.5	29.2	27.8	27.4

EXPERIMENT IV

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	4.6	4.6	4.5	4.4	4.3	4.2	4.0

Results.

Group:	K+AC	K+A	KOA	KOAC	OAC	+A	+AC	OA
Mean:	42.9	37.7	33.6	32.5	32.4	32.4	32.1	30.2

Table 23

Composition of some Vitamin A Deficient Test Diets.

Ingredients	U.S.P. XIV	L.D. Diet	Mayer & Krehl(127)	Sure et al.(206)	Jonsson et al.(107)
Vit. free Casein	18.0%	22.0%	25.0%	16.0%	20.0%
Starch	65.0%	68.0%		51.0%	60.0%
Sucrose			65.7%		
Cottonseed Oil	5.0%	5.4%			
Corn Oil			5.0%		
Crisco				15.0%	
Cocoa Fat					10.0%
Salt Mix					
U.S.P.XIV	4.0%	4.3%			
Philips			4.0%		
McCollum				10.0%	
Osborne					5.0%
Choline Chloride	none	0.1%	0.1%	none	none
L-Cystine	none	0.2%	0.2%	none	none
Vitamin Supplements in Grams per 100 Lbs.					
Alpha Tocopherol	none	4.540	4.540	none	none
Menadione(Vit. K)	none	4.540	4.540	none	none
Vit. D.(Viosterol)	0.5	0.500	0.005		
Thiamine Hcl		0.227	0.227		
Riboflavin		0.363	0.227		
Pyridoxine HCl	Yeast 8.0%	0.227	0.227	Irradiated Yeast 10.0%	Irradiated Yeast 5.0%
Niacin		1.816	2.270		
i-inositol		4.540	4.540		
Ca.Pantothenate		1.816	2.270		
p-Aminobenzoic Acid		4.540	4.540		
Folic acid		0.023	0.023		
Biotin		0.023	0.023		
Vitamin B_{12}	none	0.001	none	none	none

Table 24 U.S.P. XIV Vit. A. Deficient Test Diet.

Source of Variance		Total	Groups	Indiv.in Groups	F	p
	d.f.	43	5	38	-	-
Body Weight Gain.	S.sq.	37967.2	22433.7	15533.5		
	M.sq.	-------	4486.7	408.8	10.97	0.01
Liver: Ascorbic A. mg./gm.	S.sq.	0.25843	0.4600	0.21243		
	M.sq.	-------	0.0092	0.00559	1.64	N.S.
Liver: Total Ascorbic A.	S.sq.	47.642	24.572	23.070		
	M.sq.	-------	4.914	0.607	8.09	0.01
Liver: Wet Weight in mgs.	S.sq.	268.551	173.639	94.912		
	M.sq.	-------	34.728	2.498	13.90	0.01
Testes: Wet Weight in gms.	S.sq.	31.648	13.835	17.813		
	M.sq.	-------	2.767	0.469	5.90	0.01
Seminal Ves. Wet Weight in mgs.	S.sq.	3449309.1	536184.7	2913124.4		
	M.sq.	---------	107236.9	76661.2	1.40	N.S.
	d.f.	42	5	37	-	-
Thyroids: Wet Weight in mgs.	S.sq.	599.25	185.18	414.07		
	M.sq.	------	37.04	11.19	3.31	0.05
	d.f.	41	5	36	-	-
Adrenals: Wet Weight in mgs.	S.sq.	826.29	240.87	585.52		
	M.sq.	------	48.17	16.26	2.96	0.05
	d.f.	21	3	18	-	-
Liver: Total I.U. Vitamin A.	S.sq.	380298.5	33319.4	346979.1		
	M.sq.	--------	11106.5	19276.6	0.58	N.S.

Table 25 L.D. Vitamin A. Deficient Test Diet.

Source of Variance		Total	Groups	Indiv.in Groups	F	p
	d.f.	43	5	38	-	-
Body Weight Gain.	S.sq.	89650.5	58337.8	31312.7		
	M.sq.	-------	11667.5	824.0	14.16	0.01
Liver: Ascorbic A. mg./gm.	S.sq.	0.27378	0.12325	0.02465		
	M.sq.	-------	0.02465	0.00396	6.2	0.01
Liver: Total Ascorbic A.	S.sq.	31.735	21.285	10.450		
	M.sq.	-------	4.257	0.275	15.48	0.01
Liver: Wet Weight in gms.	S.sq.	229.151	123.091	106.060		
	M.sq.	-------	24.618	2.791	8.82	0.01
Testes: Wet Weight in gms.	S.sq.	12.341	6.333	6.008		
	M.sq.	-------	1.267	0.158	8.01	0.01
Seminal V.: Wet Weight in mgs.	S.sq.	2637593.2	877477.8	1760115.4		
	M.sq.	-------	175495.6	46318.8	3.79	0.01
Thyroids: Wet Weight in mgs.	S.sq.	458.33	56.06	402.27		
	M.sq.	-------	11.21	14.37	0.78	N.S.
Adrenals: Wet Weight in mgs.	S.sq.	1421.71	601.94	819.77		
	M.sq.	-------	120.39	21.57	5.58	0.01
	d.f.	21	3	18		
Liver: Total I.U. Vitamin A.	S.sq.	392783.4	1032.9	391750.5		
	M.sq.	--------	344.3	21763.9	0.02	N.S.

EXPERIMENT V — Table 26 — BODY WEIGHT GAIN in Grams.

U.S.P. XIV DIET	Ranges. p:	(6)	(5)	(4)	(3)	(2)	
	Rp:	38.4	37.8	37.2	36.2	34.6	
	Results.						
	Group:	+A(5)	+AC(5)	+ACpf(6)	+Apf(6)	OA(11)	OAC(11)
	Mean:	221.6	220.4	180.8	174.0	170.5	159.8
L.D. DIET	Ranges. p:	(6)	(5)	(4)	(3)	(2)	
	Rp:	54.5	53.6	52.7	51.3	49.2	
	Results.						
	Group:	+A(5)	+AC(5)	+ACpf(6)	+Apf(6)	OAC(11)	OA(11)
	Mean:	250.0	228.2	204.8	179.5	172.9	138.5

EXPERIMENT V — Table 27 — WET WEIGHT OF THE LIVER in Gms.

U.S.P. XIV DIET	Ranges. p:	(6)	(5)	(4)	(3)	(2)	
	Rp:	3.00	2.95	2.90	2.83	2.71	
	Results.						
	Group:	+A	+AC	+Apf	+ACpf	OA	OAC
	Mean:	11.97	11.06	8.63	8.22	7.32	6.02
L.D. DIET	Ranges. p:	(6)	(5)	(4)	(3)	(2)	
	Rp:	3.17	3.12	3.07	2.99	2.86	
	Results.						
	Group:	+A	+AC	+ACpf	+Apf	OAC	OA
	Mean:	10.70	9.31	8.41	6.31	6.22	5.99

EXPERIMENT V — Table 28 LIVER ASCORBIC ACID in mg./gm.

U.S.P. XIV DIET	Ranges. p:	(6)	(5)	(4)	(3)	(2)	
	Rp:						
	Results. Group:	+A	+Apf	+AC	+ACpf	OAC	OA
	Mean:	0.303	0.291	0.276	0.267	0.237	0.212
L.D. DIET	Ranges. p:	(6)	(5)	(4)	(3)	(2)	
	Rp:	0.120	0.118	0.116	0.113	0.108	
	Results. Group:	+ACpf	+AC	OAC	+A	+Apf	OA
	Mean:	0.336	0.322	0.316	0.279	0.277	0.194

EXPERIMENT V — Table 29 TOTAL LIVER ASCORBIC ACID

U.S.P. XIV DIET	Ranges. p:	(6)	(5)	(4)	(3)	(2)	
	Rp:	1.48	1.46	1.43	1.39	1.33	
	Results. Group:	+A	+AC	+Apf	+ACpf	OA	OAC
	Mean:	3.60	3.04	2.52	2.24	1.57	1.44
L.D. DIET	Ranges. p:	(6)	(5)	(4)	(3)	(2)	
	Rp:	1.00	0.98	0.96	0.94	0.90	
	Results. Group:	+A	+AC	+ACpf	OAC	+Apf	OA
	Mean:	3.00	2.97	2.78	2.06	1.69	1.16

EXPERIMENT V

Table 30 WET WEIGHT OF THE TESTES IN GRAMS

U.S.P. XIV	Ranges. p:	(6)	(5)	(4)	(3)	(2)	
	Rp:	0.96	0.95	0.93	0.91	0.87	
DIET	Results. Group:	+ACpf	+AC	+A	+Apf	OAC	OA
	Mean:	2.93	2.90	2.82	2.78	1.97	1.95
	Ranges. p:	(6)	(5)	(4)	(3)	(2)	
L.D.	Rp:	0.75	0.74	0.73	0.71	0.68	
DIET	Results. Group:	+A	+AC	+Apf	+ACpf	OAC	OA
	Mean:	2.81	2.81	2.57	2.56	2.23	1.78

Table 31 WET WEIGHT OF THE SEMINAL VESICLE

	Ranges. p:	(6)	(5)	(4)	(3)	(2)	
L.D.	Rp:	309.9	305.1	298.4	289.7	275.3	
DIET	Results. Group:	+AC	+A	+ACpf	+Apf	OAC	OA
	Mean:	824.3	818.1	578.9	545.5	542.2	422.5

Table 32 WET WEIGHT OF THE ADRENALS IN MGS.

U.S.P. XIV	Ranges. p:	(6)	(5)	(4)	(3)	(2)	
	Rp:	5.8	5.7	5.6	5.4	5.1	
DIET	Results. Group:	+AC	+ACpf	+A	+Apf	OA	OAC
p = 0.05	Mean:	34.2	31.9	30.9	30.5	28.4	26.9
	Ranges. p:	(6)	(5)	(4)	(3)	(2)	
L.D.	Rp:	6.7	6.6	6.4	6.2	5.9	
DIET	Results. Group:	+Apf	+A	+ACpf	+AC	OA	OAC
p = 0.05	Mean:	39.6	38.3	32.3	31.8	30.2	29.9

EXPERIMENT V — Table 33 — WET WEIGHT OF THE THYROIDS IN MGS.

U.S.P. XIV DIET	Ranges. p:	(6)	(5)	(4)	(3)	(2)	
	Rp:	1.52	1.49	1.46	1.42	1.35	
	Results. Group:	OA	+A	+AC	OAC	+ACpf	+Apf
p = 0.05	Mean:	17.9	14.7	13.9	13.3	12.7	12.5

L.D. DIET	Ranges. p:	(6)	(5)	(4)	(3)	(2)	
	Rp:						
	Results. Group:	+ACpf	+A	OAC	+AC	OA	+Apf
p = 0.05	Mean:	16.9	16.0	14.5	14.2	13.7	12.9

EXPERIMENT V — Table 34 — OXYGEN CONSUMPTION (M.R. 30°C.)

Source of Variance		Total	Groups	Indiv. in Groups	F	p
Oxygen Consumption ml./hour	d.f.	123	7	116	-	-
	S.sq.	2230.28	238.54	1991.74		
	M.sq.	-------	34.08	17.17	1.98	N.S.

Table 35

Source of Variance		Total	Groups	Indiv. in Groups	F	p
	d.f.	44	4	37	-	-
Body Weight Gain.	S.sq.	39483.91	17759.11	22224.80		
	M.sq.	--------	2465.59	600.67	4.10	0.01
Liver: Wet weight in gms.	S.sq.	72.923	48.153	24.770		
	M.sq.	-------	6.879	0.695	10.27	0.01
Liver: Ascorbic A. mg./gm.	S.sq.	0.34664	0.13421	0.21243		
	M.sq.	-------	0.01917	0.00574	3.34	0.01
Liver: Total Ascorbic A.	S.sq.	19.998	11.567	8.431		
	M.sq.	-------	1.652	0.228	7.25	0.01
Testes: Wet weight in gms.	S.sq.	19.571	13.371	6.200		
	M.sq.	-------	1.910	0.168	11.40	0.01
Seminal Ves. Wet weight in mgs.	S.sq.	1268664.3	585096.0	683568.3		
	M.sq.	---------	83585.1	18474.8	4.52	0.01
Thyroids: Wet weight in mgs.	S.sq.	531.53	147.37	384.16		
	M.sq.	----------	21.05	10.38	2.03	N.S.
Adrenals: Wet weight in mgs.	S.sq.	2354.65	452.99	1901.66		
	M.sq.	----------	64.70	51.40	1.25	N.S.
	d.f.	19	3	16	-	-
Liver: Total I.U. Vitamin A.	S.sq.	355402.01	153999.2	201402.8		
	M.sq.	---------	38499.8	10070.1	3.82	0.05

EXPERIMENT VI

Table 36 BODY WEIGHT GAIN IN GRAMS.

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	40.0	39.9	39.6	38.7	38.1	37.1	35.6

Results.

Group:	+ACG(5)	+AG(5)	+A(5)	OACG(6)	+AC(5)	OAC(6)	OA(6)	OAG(7)
Mean:	183.0	172.6	161.2	150.0	149.6	139.5	132.2	119.4

EXPERIMENT VI

Table 37 WET WEIGHT OF THE LIVER IN GMS.

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	1.34	1.33	1.32	1.29	1.27	1.24	1.19

Results.

Group:	+AG	+A	+ACG	+AC	OACG	OAC	OA	OAG
Mean:	7.51	7.10	6.34	6.26	6.07	5.06	4.65	4.55

EXPERIMENT VI

Table 38 LIVER ASCORBIC ACID IN MG./GM.

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	0.124	0.123	0.122	0.120	0.118	0.115	0.110

Results.

Group:	+ACG	OAC	OACG	+AC	+AG	+A	OAG	OA
Mean:	0.401	0.358	0.342	0.334	0.321	0.296	0.284	0.206

EXPERIMENT VI

Table 39 TOTAL LIVER ASCORBIC ACID IN MGS.

Ranges. p:	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	0.78	0.78	0.77	0.75	0.74	0.72	0.69

Results.

Group:	+ACG	+AG	+AC	+A	OACG	OAC	OAG	OA
Mean:	2.49	2.38	2.12	2.09	2.07	1.79	1.29	0.94

EXPERIMENT VI

Table 40 TOTAL LIVER VITAMIN A

Ranges. p:		(4)	(3)	(2)
Rp:		163.1	159.5	155.5
Results.				
Group:	+AG	+A	+AC	+ACG
Mean:	509.2	370.3	334.1	298.5

EXPERIMENT VI

Table 41 WET WEIGHT OF THE TESTES IN GMS.

Ranges. p:		(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:		0.67	0.67	0.66	0.65	0.64	0.62	0.59
Results.								
Group:	+ACG	+AG	+A	+AC	OACG	OAC	OAG	OA
Mean:	2.87	2.76	2.74	2.70	1.85	1.80	1.58	1.55

EXPERIMENT VI

Table 42 WET WEIGHT OF THE SEMINAL VESICLES.

Ranges. p:		(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:		221.9	221.4	219.3	214.7	211.1	206.0	197.3
Results.								
Group:	+ACG	+AG	+AC	OACG	+A	OAC	OA	OAG
Mean:	505.0	490.0	403.0	357.0	352.6	267.3	193.2	191.1

Table 43

Source of Variance		Total	Group	Indiv.in Groups	F	p
	d.f.	38	4	34	-	-
Body Weight Gain.	S.sq.	157973.7	112918.6	45055.1		
	M.sq.	--------	28229.6	1325.1	21.30	0.01
Liver: Wet weight in gms.	S.sq.	561.455	452.845	108.610		
	M.sq.	--------	113.211	3.190	35.90	0.01
Liver: Ascorbic A. mg./gm.	S.sq.	0.25466	0.23798	0.01668		
	M.sq.	--------	0.05949	0.00049	121.42	0.01
Liver: Total Ascorbic A.	S.sq.	96.776	93.233	3.543		
	M.sq.	--------	23.308	0.104	223.69	0.01
Adrenals: Wet weight in mgs.	S.sq.	36547.20	4628.03	31919.17		
	M.sq.	---------	1157.01	938.80	1.23	N.S.
	d.f.	37	4	33		
Thyroids: Wet weight in mgs.	S.sq.	4042.69	586.87	3445.82		
	M.sq.	-------	146.72	104.72	1.40	N.S.
	d.f.	34	4	30		
Liver: Vitamin A I.U./gm.	S.sq.	1171051.7	939644.1	231407.6		
	M.sq.	---------	234911.0	77135.9	3.04	0.05
Liver: Total I.U. Vitamin A.	S.sq.	49854206.0	39209950.9	10644255.1		
	M.sq.	----------	9802487.7	354808.5	27.60	0.01

EXPERIMENT VII

Table 44 **BODY WEIGHT GAIN**

Ranges. p:	(5)	(4)	(3)	(2)	
Rp:	76.8	75.7	73.9	70.8	
Results.					
Group:	GP(5)	DC(5)	SO(11)	S6(8)	S3(10)
Mean:	156.2	48.0	10.4	-8.5	-9.9

At p = 0.05 Grp. DC greater than S6 and S3.

Table 45 **WET WEIGHT OF THE LIVER**

Ranges. p:	(5)	(4)	(3)	(2)	
Rp:	3.76	3.71	3.62	3.47	
Results.					
Group:	GP	DC	SO	S3	S6
Mean:	17.52	9.39	8.06	7.32	6.46

Table 46 **LIVER ASCORBIC ACID STATUS**

As. A. mg./gm.

Ranges. p:	(5)	(4)	(3)	(2)	
Rp:	0.046	0.046	0.045	0.043	
Results.					
Group:	GC	DC	S+6	S+3	S+0
Mean:	0.294	0.126	0.063	0.060	0.057

Total Liver **ASCORBIC ACID**

Ranges. p:	(5)	(4)	(3)	(2)	
Rp:	0.68	0.67	0.65	0.63	
Results.					
Group:	GP	DC	S+0	S+3	S+6
Mean:	5.11	1.19	0.46	0.44	0.40

Table 47 **LIVER VITAMIN A in I.U./Gm.**

p = 0.05

Ranges. p:	(5)	(4)	(3)	(2)	
Kramer Rp:	888.7	866.5	844.3	802.6	
Results.					
Group:	S+6	S+3	GP	DC	SO
Mean:	446.2	147.7	40.6	17.7	13.8

EXPERIMENT VII Table 48 **TOTAL LIVER VITAMIN A**

Ranges. p:	(5)	(4)	(3)	(2)
Rp:	125.7	123.9	120.9	115.8

Results.

Group:	S+6	S+3	GP	DC	S+O
Mean:	2978.2	1033.2	699.6	165.4	112.2

EXPERIMENT VIII Table 49

Source of Variance		Total	Group	Indivs.in Groups	F	p
	d.f.	15	2	13	-	-
Liver: Wet weight in gms.	S.sq.	95.797	0.588	95.209		
	M.sq.	------	0.294	7.324	0.04	N.S.
Liver: Ascorbic A. mg./gm.	S.sq.	0.01234	0.00517	0.00716		
	M.sq.	-------	0.00259	0.00055	4.69	0.05
Liver: Total Ascorbic A.	S.sq.	2.634	0.985	1.649		
	M.sq.	-------	0.492	0.127	3.88	0.05
Thyroids: Wet weight in mgs.	S.sq.	1468.71	292.68	1176.03		
	M.sq.	-------	146.34	90.46	1.62	N.S.
Adrenals: Wet weight in mgs.	S.sq.	20319.44	15041.05	5278.39		
	M.sq.	--------	7520.23	406.03	18.52	0.01

Table 50

Composition of some Synthetic Guinea Pig Diets.

Ingredients	Lachance & DesMarais	Collins & Elvehjem (26)	Reid & Briggs (162)
Vit. free Casein	30.0%	30.0%	30.0%
Sucrose	12.0%	40.0%	10.3%
Starch	20.0%		20.0%
Cerelose	8.0%		7.8%
Corn Oil	7.4%	7.4%	7.3%
Salt Mix			
Hegested	4.0%	4.0%	
Briggs			6.0%
Roughage			
Solka Floc	15.0%	15.0%	
Cellophane spangles			15.0%
Potass. Acetate	2.5%	2.5%	2.5%
Mg. Oxide	0.5%	0.5%	0.5%
Choline Chloride	0.35%	0.35%	0.2%

Vitamin Supplements in Grams per Kilogram.

Ascorbic Acid	suppl. orally	suppl. orally	2.0
Vitamin A	none	sup 1060 IU/wk.	6.0
alpha tocopherol	0.020	sup 12mgs/wk.	0.020
Menadione (Vit.K)	0.002	sup 0.2 mg/wk.	0.002
Vitamin D_3	0.04mg.	sup 20 IU/wk.	0.04mg.
Thiamine HCl	0.02	0.02	0.016
Riboflavin	0.03	0.03	0.016
Pyridoxine HCl	0.02	0.02	0.016
Niacin	0.2	0.2	0.2
i-Inositol	2.0	2.0	2.0
Ca. pantothenate	0.08	0.08	0.04
p-Aminobenzoic Acid	0.1	0.1	none
Folic acid	0.01	0.01	0.01
Biotin	1.0mg.	1.0mg.	0.6mg.
Vitamin B_{12}	0.04mg.	0.04mg.	0.04mg.

EXPERIMENT VIII Table 51 **LIVER ASCORBIC ACID STATUS**

Ranges. p:	(3)	(2)	
Kramer Rp:	0.075	0.072	
Results.			
Group:	IC	+A	OA
concn. Mean:	0.118	0.109	0.075

Ranges. p:	(3)	(2)	
Kramer Rp:	1.14	1.09	
Results.			
Group:	IC	+A	OA
total Mean:	1.57	1.37	0.97

EXPERIMENT VIII Table 52 **WET WEIGHT OF THE ADRENALS.**

Ranges. p:	(3)	(2)	
Rp:	63.84	6.070	
Results.			
Group:	OA	+A	IC
Mean:	205.9	141.9	138.2

Table 53

Group	N	Body weight Gain	Liver weight	Liver As.A. mg/gm.	Total As. A.	Thyroid Wt.
OA	6	191.2 ± 1.76**	21.20 ± 2.08	0.154 ± 0.019	3.26 ± 0.39	92.4 ± 3.13
+A	6	192.6 ± 1.63	20.84 ± 0.54	0.168 ± 0.012	3.47 ± 0.35	87.6 ± 12.36
Difference		1.4 ± 23.99	0.36 ± 2.16	0.014 ± 0.023	0.21 ± 0.52	4.8 ± 0.38
't'		0.058	0.017	0.62	0.40	0.38
p		0.50	0.50	0.50	0.50	0.50

** ± Standard error.

EXPERIMENT IX

Table 54

Composition of High Fat and High Carbohydrate Diets.

Ingredients	L.D. High Fat	Page & Babineau	Purina Lab.Chow	L.D. High CHO	Page & Babineau
Protein			25.0%		
Vit. free Casein	19.2%	21.55%		15.0%	15.0%
Carbohydrate(N.F.E.)			47.5%		
Sucrose	13.3%	32.45%		77.7%	74.0%
Fat			6.0%		
Wheat Germ Oil	2.4%	5.0%		3.0%	2.5%
Crisco		25.0%			2.5%
Mazola Oil		10.0%			
Corn Oil (50% Hydrogenated)	36.8%				
Fiber			4.5%		
Cellulose	24.0%	1.45%			
Ash			7.0%		
Salt Mix					
U.S.P.XIV	4.0%			4.0%	
unknown		5.75%			4.0%
Cystine	0.2%			0.2%	

Vitamin Supplements in Grams per 100 Lbs.

Vitamin A	oral suppl 500 IU/wk	oral suppl 200 IU/day	22,000IU per Lb.	oral suppl 500 IU/wk	oral suppl 200 IU/day
Carotene			138,000 IU per Lb.		
alpha Tocherol	5.678	none	unknown	4.540	none
Menadione(Vit.K)	0.284	0.063	unknown	0.227	0.045
Vitamin D	0.625	oral suppl 35 IU/day	11,000IU per Lb.	0.500	oral suppl 35 IU/day
Thiamine HCl	0.284	0.262	0.450	0.227	0.182
Riboflavin	0.454	0.327	0.363	0.363	0.227
Pyridoxine HCl	0.284	0.327	0.200	0.227	0.227
Niacin	2.270	1.961	4.500	1.816	1.362
i-Inositol	5.678	6.538	unknown	4.540	4.540
Ca. pantothenate	2.270	1.961	0.680	1.816	1.362
p-Aminobenzoic A.	5.678	none	unknown	4.540	none
Choline Cl.	45.000	56.750	77.000	45.000	56.750
Biotin	0.028	0.130	unknown	0.023	0.090
Folic Acid	0.028	0.196	unknown	0.023	0.136
Vitamin B-12	0.001	none	unknown	0.001	none

EXPERIMENT X

Table 55

Source of Variance		Total	Groups	Indiv.in Groups	F	p
	d.f.	75	11	64	-	-
Body Weight Gain.	S.sq.	109262.0	73685.0	35577.0		
	M.sq.	--------	6698.6	555.9	12.05	0.01
	d.f.	76	11	64		
LIVER:						
Wet weight in gms.	S.sq.	153.24	97.49	55.75		
	M.sq.	--------	8.76	0.86	10.34	0.01
wet wt.per 100g.b.wt.	S.sq.	27.738	23.909	3.830		
	M.sq.	--------	2.174	0.059	36.90	0.01
defat.dry wt. in gms.	S.sq.	88.978	82.667	6.311		
	M.sq.	--------	7.543	0.097	77.80	0.01
defat.d.wt. 100g.b.wt.	S.sq.	2.673	2.120	0.553		
	M.sq.	---------	0.193	0.008	22.60	0.01
% defat. dry weight	S.sq.	867.03	109.09	757.94		
	M.sq.	---------	9.92	11.66	0.10	N.S.
Total liver fat in gms.	S.sq.	2.091	0.239	1.853		
	M.sq.	---------	0.022	0.028	0.76	N.S.
% liver fat	S.sq.	423.63	96.66	326.96		
	M.sq.	---------	8.79	5.03	1.75	N.S.
	d.f.	73	11	62		
LIVER:						
Ascorbic A. mg./gm.	S.sq.	0.2902	0.0415	0.2487		
	M.sq.	---------	0.0038	0.0040	1.00	N.S.
Total liver Ascorbic A.	S.sq.	27.786	8.971	8.815		
	M.sq.	---------	0.815	0.142	5.73	0.01
	d.f.	68	11	57		
KIDNEY:						
wet weight in gms.	S.sq.	10.04	7.39	2.65		
	M.sq.	---------	0.671	0.046	14.60	0.01
wet wt. per 100g. b.wt.	S.sq.	2.805	2.445	0.350		
	M.sq.	---------	0.222	0.0055	40.4	0.01

Table 55 continued

Source of Variance		Total	Groups	Indiv. in Groups	F	p
	d.f.	72	11	61		
LIVER:						
Vitamin A	S.sq.	7359829.0	6793214.0	556615.6		
I.U./gm.	M.sq.	----------	617564.9	9288.8	66.5	0.01
Total I.U.	S.sq.	474296913.0	437670191.0	36626722.0		
Vitamin A	M.sq.	----------	39788199.1	600438.0	66.2	0.01
	d.f.	76	11	65		
PERIRENAL FAT:						
wet weight	S.sq.	64.899	45.254	19.645		
in grams.	M.sq.	----------	4.114	0.302	13.7	0.01
wet wt.per	S.sq.	8.272	4.952	3.321		
100g.b.wt.	M.sq.	----------	0.450	0.051	8.8	0.01
	d.f.	72	11	61		
Ascorbic A.	S.sq.	0.1055	0.0609	0.0446		
mg./gm.	M.sq.	----------	0.0055	0.0007	7.57	0.01
Total P.F.	S.sq.	0.0764	0.0570	0.0194		
Ascorbic A.	M.sq.	----------	0.0052	0.0003	16.3	0.01
	d.f.	76	11	65		
TESTES:						
wet weight	S.sq.	23.29	2.39	20.90		
in grams.	M.sq.	----------	0.217	0.321	0.68	N.S.
	d.f.	68	11	57		
ADRENALS:						
wet weight	S.sq.	5745.1	3835.4	1909.7		
in mgs.	M.sq.	---------	348.7	29.4	11.87	0.01
	d.f.	75	11	64		
THYROIDS:						
wet weight	S.sq.	1827.6	360.6	1467.0		
in mgs.	M.sq.	---------	32.78	22.92	1.43	N.S.
	d.f.	110	11	99		
O_2 CONSUMPTION:						
M.R.30.0 °C	S.sq.	1813.90	588.54	1255.36		
in ml./hr.	M.sq.	---------	53.50	12.38	4.32	0.01

EXPERIMENT X

Table 56 BODY WEIGHT GAIN

Ranges. p:	(12)	(11)	(10)	(9)	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	31.2	31.1	30.8	30.6	30.3	30.1	29.6	29.3	28.6	27.9	26.7

Results.

Group:	L	LC	KFC	FC	F	KF	KH	H	KL	HC	KLC	KHC
Mean:	146.7	122.7	72.9	72.8	70.0	69.6	51.3	50.4	46.0	43.6	43.0	42.8

Table 57 LIVER: WET WEIGHT AND DRY WEIGHT

Ranges. p:	(12)	(11)	(10)	(9)	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	1.23	1.22	1.21	1.20	1.19	1.18	1.17	1.15	1.13	1.10	1.05

Results.

	Group:	L	KH	KHC	LC	KL	KLC	KFC	HC	KF	H	F	FC
(A)	Mean:	8.87	8.32	7.98	7.85	7.48	7.39	6.79	6.28	5.72	5.69	5.52	5.38

Rp:	0.32	0.32	0.32	0.31	0.31	0.31	0.30	0.30	0.29	0.29	0.27

Results.

	Group:	KH	KHC	KL	KLC	HC	KFC	H	KF	LC	L	F	FC
(B)	Mean:	4.15	4.03	3.30	3.14	3.05	2.88	2.78	2.65	2.62	2.61	2.31	2.22

Rp:	0.41	0.41	0.41	0.40	0.40	0.40	0.39	0.39	0.38	0.37	0.35

Results.

	Group:	L	KHC	KH	LC	KL	KLC	KFC	HC	H	KF	FC	F
(C)	Mean:	2.50	2.22	2.20	2.09	1.94	1.91	1.68	1.60	1.49	1.39	1.38	1.3

Rp:	0.12	0.12	0.12	0.12	0.12	0.12	0.11	0.11	0.11	0.11	0.10

Results.

	Group:	KHC	KH	KL	KLC	HC	KFC	L	H	LC	KF	FC	F
(D)	Mean:	1.12	1.10	0.85	0.80	0.77	0.73	0.73	0.72	0.70	0.64	0.57	0.56

Table 58

Ranges. p:	(12)	(11)	(10)	(9)	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	N.S.										
Results. Group:	FC	KLC	KHC	LC	KF	KFC	KL	HC	KH	H	L	F
(A) Mean:	0.362	0.333	0.333	0.327	0.323	0.316	0.316	0.314	0.299	0.283	0.276	0.275
Rp:	0.50	0.50	0.49	0.49	0.48	0.48	0.47	0.47	0.46	0.44	0.43	
Results. Group:	KHC	LC	L	KLC	KH	KL	KFC	HC	FC	KF	H	F
(B) Mean:	2.68	2.57	2.50	2.47	2.43	2.38	2.13	1.98	1.96	1.85	1.62	1.51

Table 59

Ranges. p:	(12)	(11)	(10)	(9)	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	0.28	0.28	0.28	0.28	0.28	0.27	0.27	0.27	0.26	0.25	0.24	
Results. Group:	KLC	KL	KHC	KH	L	LC	KF	KFC	HC	H	FC	F
(A) Mean:	2.65	2.55	2.49	2.47	2.45	2.33	2.29	2.18	1.84	1.80	1.77	1.67
Rp:	.098	.098	.097	.096	.095	.094	.093	.092	.090	.087	.084	
Results. Group:	KH	KHC	KL	KLC	KF	KFC	H	HC	LC	FC	L	F
(B) Mean:	1.27	1.25	1.14	1.08	1.07	0.94	0.91	0.90	0.78	0.73	0.72	0.70

Table 62 PERIRENAL FAT ASCORBIC ACID

Ranges. p:	(12)	(11)	(10)	(9)	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	0.036	0.036	0.035	0.035	0.035	0.035	0.034	0.034	0.033	0.032	0.031

Results. Group:	KL	KHC	KLC	KH	H	L	KF	KFC	HC	LC	FC	F
(A) Mean:	0.061	0.055	0.042	0.036	0.027	0.027	0.026	0.025	0.025	0.021	0.021	0.013

Rp:	0.024	0.024	0.023	0.023	0.023	0.023	0.022	0.022	0.022	0.021	0.020

Results. Group:	L	FC	LC	KFC	F	HC	H	KF	KHC	KH	KLC	KL
(B) Mean:	0.072	0.044	0.043	0.037	0.037	0.030	0.027	0.024	0.023	0.021	0.020	0.019

Table 63 WET WEIGHT OF THE ADRENALS

Ranges. p:	(12)	(11)	(10)	(9)	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	7.3	7.3	7.2	7.2	7.1	7.1	7.0	6.9	6.7	6.5	6.3

Results. Group:	KH	KLC	L	KL	KF	KFC	KHC	LC	H	HC	FC	F
Mean:	53.4	51.5	51.4	50.5	46.6	46.4	44.6	38.1	37.6	37.3	32.2	31.2

Table 64 OXYGEN CONSUMPTION (M.R. 30°C.)

Ranges. p:	(12)	(11)	(10)	(9)	(8)	(7)	(6)	(5)	(4)	(3)	(2)
Rp:	4.66	4.63	4.60	4.56	4.51	4.47	4.41	4.35	4.27	4.14	3.98

Results. Group:	KHC	KL	L	KF	KFC	KH	KLC	LC	H	FC	HC	F
Mean:	23.95	23.73	22.90	22.73	21.82	21.38	21.27	21.15	18.42	18.22	17.97	17.33

EXPERIMENT XI

Table 65

Source of Variance		Total	Groups	Indiv.in Groups	F	p
	d.f.	36	5	31		
Body Weight Gain.	S.sq.	110720.4	95475.9	15244.5		
	M.sq.	-------	19095.2	491.8	38.8	0.01
LIVER; wet weight in grams.	S.sq.	217.56	152.50	65.06		
	M.sq.	-------	50.50	2.10	14.5	0.01
wet wt. per 100g. b.wt.	S.sq.	20.09	15.05	5.04		
	M.sq.	-------	3.01	0.16	18.5	0.01
	d.f.	33	5	28		
defat. dry wt. in gms.	S.sq.	12.713	8.427	4.286		
	M.sq.	-------	1.685	0.153	11.0	0.01
Total liver fat in gms.	S.sq.	3.873	1.984	1.889		
	M.sq.	-------	0.397	0.067	5.9	0.01
% defatted dry weight	S.sq.	74.22	43.84	30.38		
	M.sq.	-------	8.77	1.05	8.35	0.01
% liver fat	S.sq.	185.47	118.62	66.85		
	M.sq.	-------	23.72	2.30	10.31	0.01
	d.f.	35	5	30		
LIVER: Ascorbic A. mg./gm.	S.sq.	0.0442	0.0032	0.0410		
	M.sq.	-------	0.0007	0.0137	0.05	N.S.
Total liver Ascorbic A.	S.sq.	24.005	12.735	11.270		
	M.sq.	-------	2.55	0.38	6.71	0.01
ABDOMINAL MUSCLE: Ascorbic A. mg./gm.	S.sq.	0.00119	0.00036	0.00083		
	M.sq.	-------	0.00007	0.00003	2.64	0.05
	d.f.	36	5	31		
PERIRENAL FAT: wet weight in grams.	S.sq.	146.83	119.82	27.01		
	M.sq.	-------	23.96	0.87	27.5	0.01
wet wt. per 100g. b.wt.	S.sq.	8.324	6.902	1.423		
	M.sq.	-------	1.380	0.046	30.0	0.01

EXPERIMENT XI — Table 65 continued

Source of Variance		Total	Groups	Indiv.in Groups	F	p
	d.f.	36	5	31		
KIDNEY:						
wet weight	S.sq.	5.274	2.248	3.026		
in gms.	M.sq.	-----	0.450	0.098	30.07	0.01
wet wt. per	S.sq.	1.368	1.156	0.212		
100g. b.wt.	M.sq.	-----	0.231	0.007	4.61	0.01
TESTES:						
wet weight	S.sq.	11.307	5.153	6.154		
in grams	M.sq.	-----	1.031	0.198	5.19	0.01
THYROIDS:						
wet weight	S.sq.	1323.3	502.6	820.7		
in mgs.	M.sq.	-----	100.5	26.5	3.80	0.01
	d.f.	58	5	53		
O_2 CONSUMPTION:						
M.R. 30.0°C	S.sq.	828.8	116.3	712.5		
in ml./hr.	M.sq.	-----	23.3	13.4	1.73	N.S.

EXPERIMENT XI — Table 66 — BODY WEIGHT GAIN

Ranges. p:	(6)	(5)	(4)	(3)	(2)
Rp:	42.8	41.8	41.2	40.3	38.6

Results.

Group:	L	F	KF	KL	KH	H
Mean:	164.0	142.7	119.5	99.4	26.0	6.5

EXPERIMENT XI

Table 67 LIVER: WET AND DRY WEIGHT, FAT CONTENT.

Ranges. p:	(6)	(5)	(4)	(3)	(2)
Rp:	2.80	2.73	2.70	2.63	2.52
Results. Group:	L	KF	F	KL	KH	H
(A) Mean:	15.60	12.61	12.46	12.40	12.22	7.68
Rp:	0.78	0.76	0.75	0.73	0.70	
Results. Group:	KH	KL	KF	L	H	F
(B) Mean:	5.59	3.95	3.80	3.65	3.56	3.23
Rp:	0.76	0.74	0.73	0.71	0.68	
Results. Group:	L	KH	KL	KF	F	H
(C) Mean:	3.98	3.31	3.26	3.13	2.99	1.99
Rp:	0.50	0.49	0.48	0.47	0.45	
Results. Group:	F	KF	KH	L	KL	H
(D) Mean:	1.15	1.07	0.95	0.83	0.63 *	0.35
Rp:	1.98	1.94	1.91	1.86	1.78	
Results. Group:	H	KH	KL	L	KF	F
(E) Mean:	27.3	27.1	26.5	25.5	24.9	24.1
Rp:	2.93	2.86	2.82	2.75	2.64	
Results. Group:	F	KF	KH	L	KL	H
(F) Mean:	9.1	8.5	7.4	5.4	5.1	4.6

* significant at p = 0.05 only

EXPERIMENT XI Table 68 **LIVER ASCORBIC ACID STATUS**

Ranges. p;	(6)	(5)	(4)	(3)	(2)
Rp:	N.S.				

Results.

	Group:	KH	L	KL	F	KF	H
(A)	Mean:	0.270	0.264	0.263	0.262	0.257	0.234

Rp:	1.19	1.16	1.15	1.12	1.07	

Results.

	Group:	L	F	KH	KL	KF	H
(B)	Mean:	4.13	3.31	3.29	3.24	3.21	1.83

EXPERIMENT XI Table 69 **ABDOMINAL MUSCLE ASCORBIC ACID**

Ranges. p:	(6)	(5)	(4)	(3)	(2)
Rp:	0.010	0.010	0.010	0.009	0.009

Results.

Group:	L	KL	F	KF	KH	H
Mean:	0.028	0.027	0.023	0.023	0.022	0.017

EXPERIMENT XI Table 70 **WET WEIGHT OF THE PERIRENAL FAT.**

Ranges. p:	(6)	(5)	(4)	(3)	(2)
Rp:	1.80	1.76	1.73	1.69	1.62

Results.

	Group:	F	L	KF	KL	KH	H
(A)	Mean:	5.68	3.39	2.19	0.91	0.52	0.21

Rp:	0.414	0.404	0.398	0.389	0.373	

Results.

	Group:	F	L	KF	KL	KH	H
(B)	Mean:	1.457	0.792	0.647	0.285	0.229	0.091

* - - - significant at p = 0.05 only

EXPERIMENT XI — Table 71 WET WEIGHT OF THE KIDNEYS.

Ranges. p:	(6)	(5)	(4)	(3)	(2)
Rp:	0.60	0.59	0.58	0.57	0.54
Results.					
Group:	L	KL	KF	KH	F	H
(A) Mean:	3.19	3.09	2.94	2.86	2.85	2.30

Rp:	0.160	0.156	0.154	0.150	0.144
Results.					
Group:	KH	H	KL	KF	L	F
(B) Mean:	1.317	1.081	0.987	0.883	0.747	0.738

EXPERIMENT XI — Table 72 WET WEIGHT OF THE TESTES.

Ranges. p:	(6)	(5)	(4)	(3)	(2)
Rp:	0.86	0.84	0.83	0.81	0.77
Results.					
Group:	F	L	KL	KF	H	KH
Mean:	3.37	3.18	3.18	3.00	2.97	2.01

EXPERIMENT XI — Table 73 WET WEIGHT OF THE THYMUS.

Ranges. p:	(6)	(5)	(4)	(3)	(2)
Rp:	8.9	9.7	9.6	9.3	8.9
Results.					
Group:	KL	KF	F	L	KH	H
Mean:	28.5	28.3	25.3	24.6	20.1 *	17.7

* - - - significant at p = 0.05 only

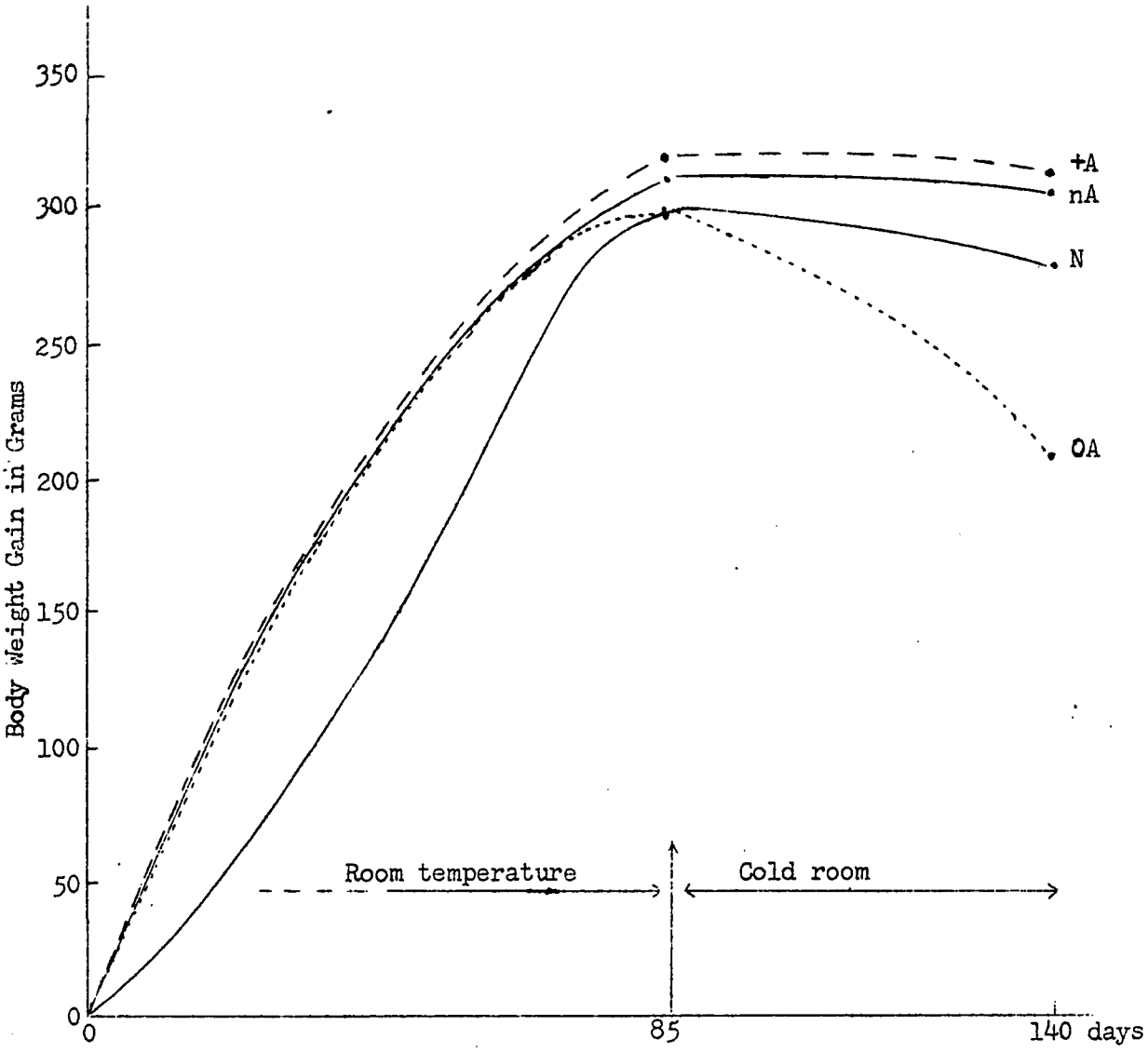

FIGURE 1 (Experiment I)

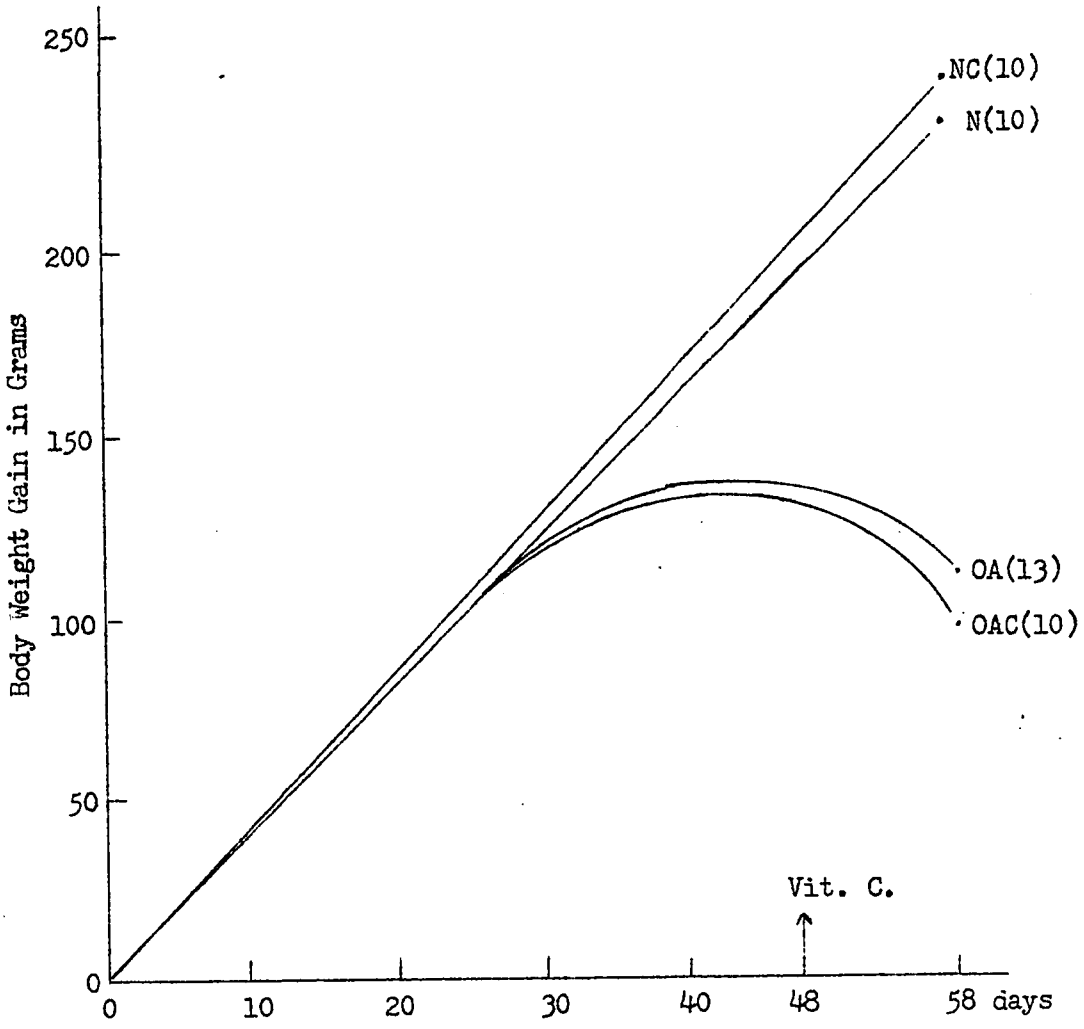

FIGURE 2 (Experiment II)

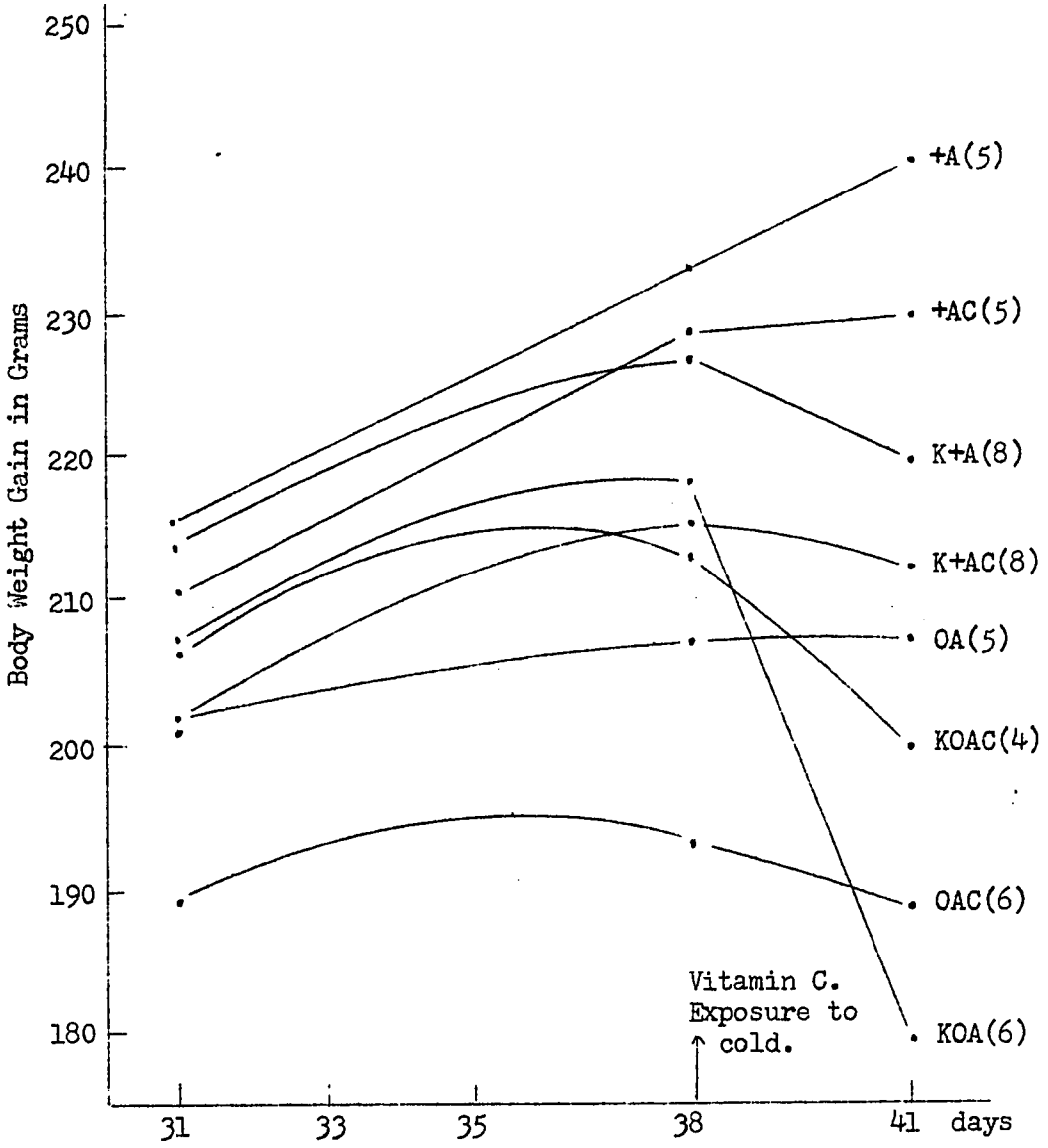

FIGURE 3 (Experiment III)

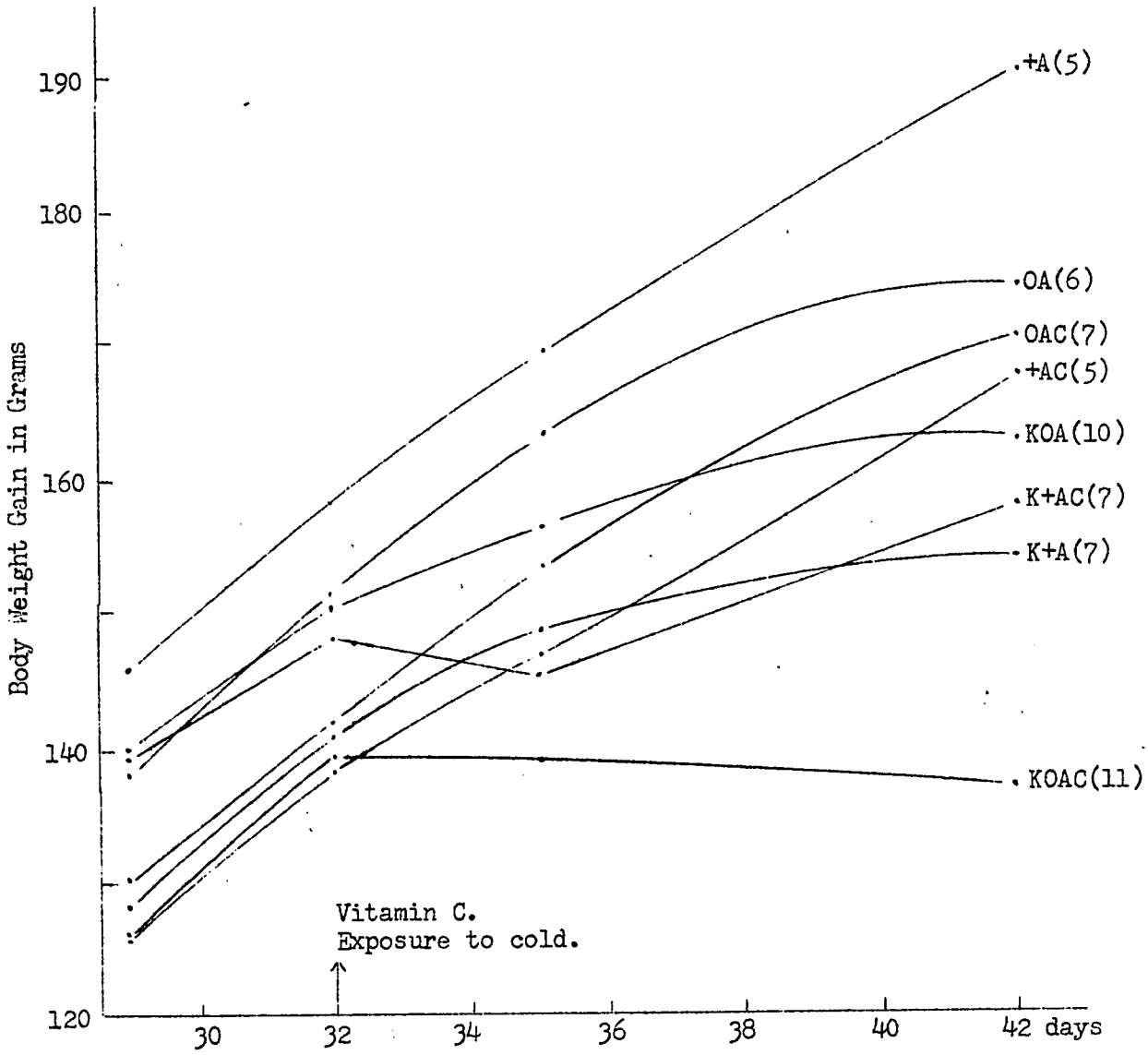

FIGURE 4 (Experiment IV)

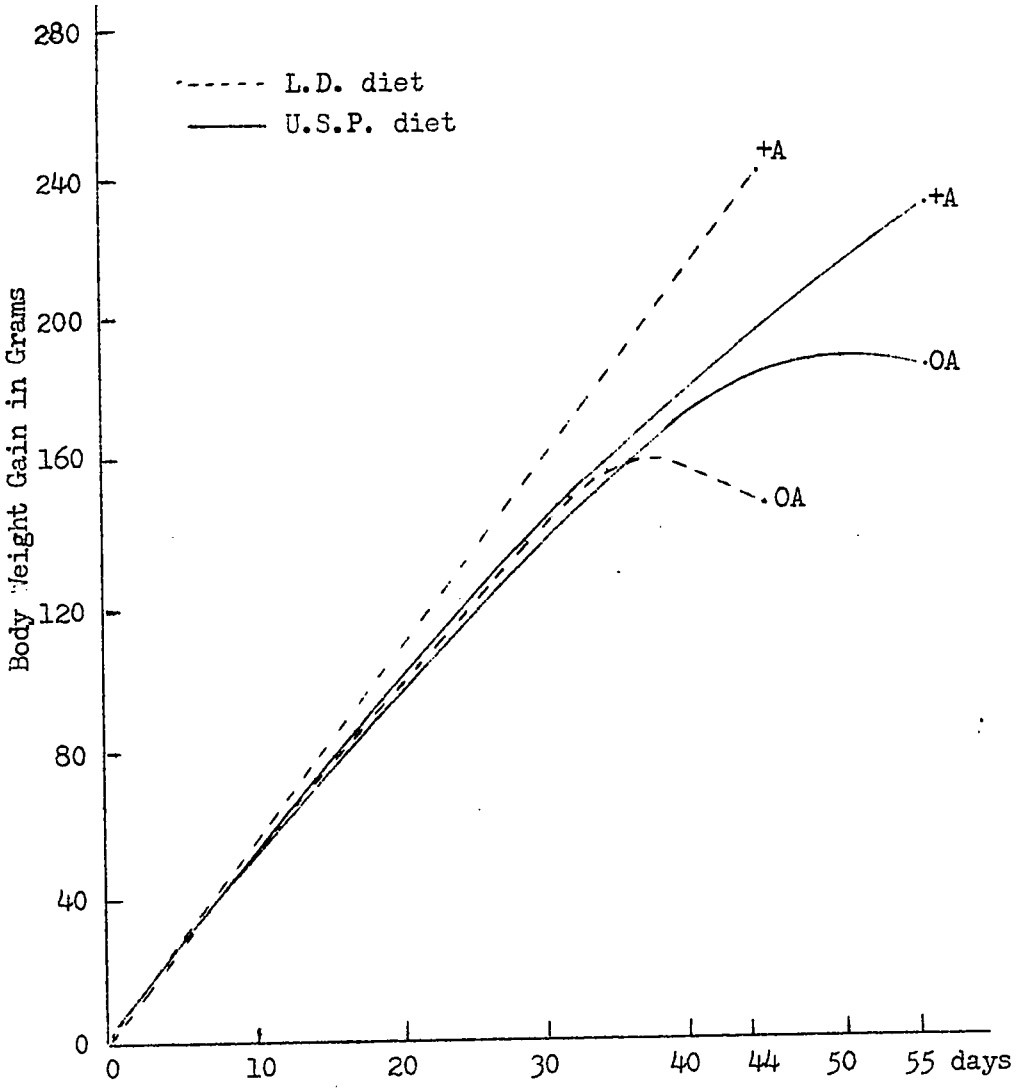

FIGURE 5 (Experiment V)

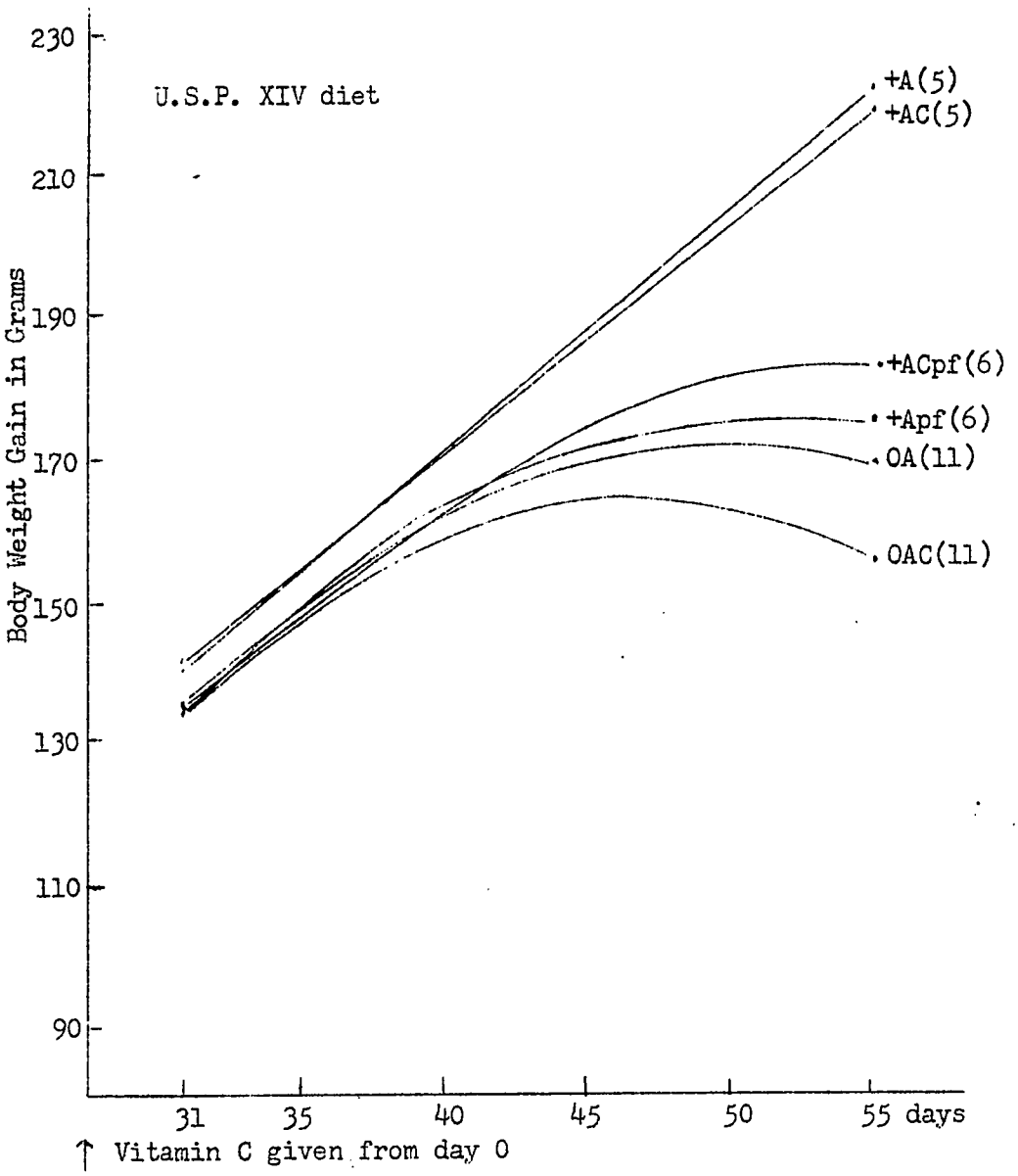

FIGURE 6 (Experiment V)

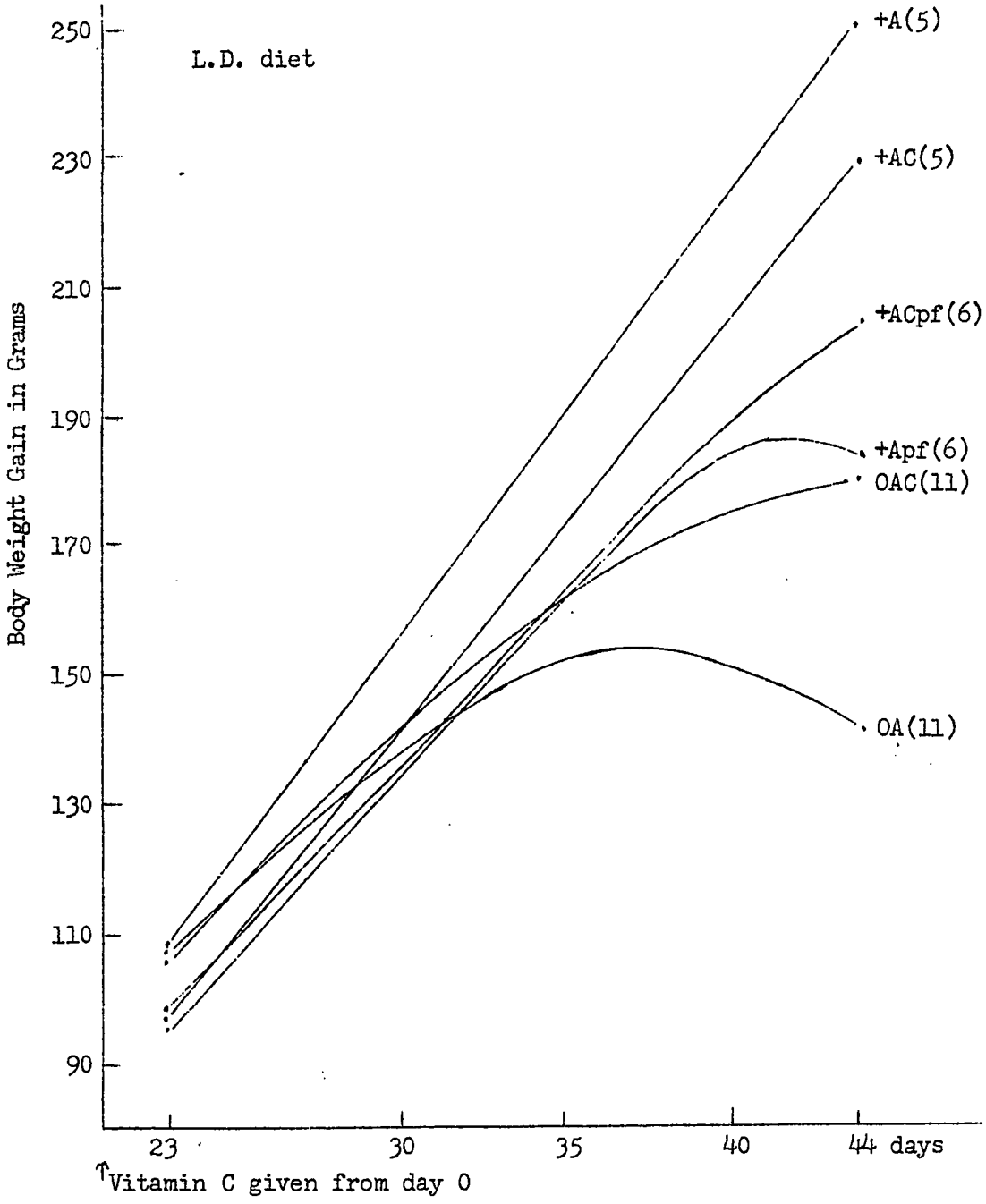

FIGURE 7 (Experiment V)

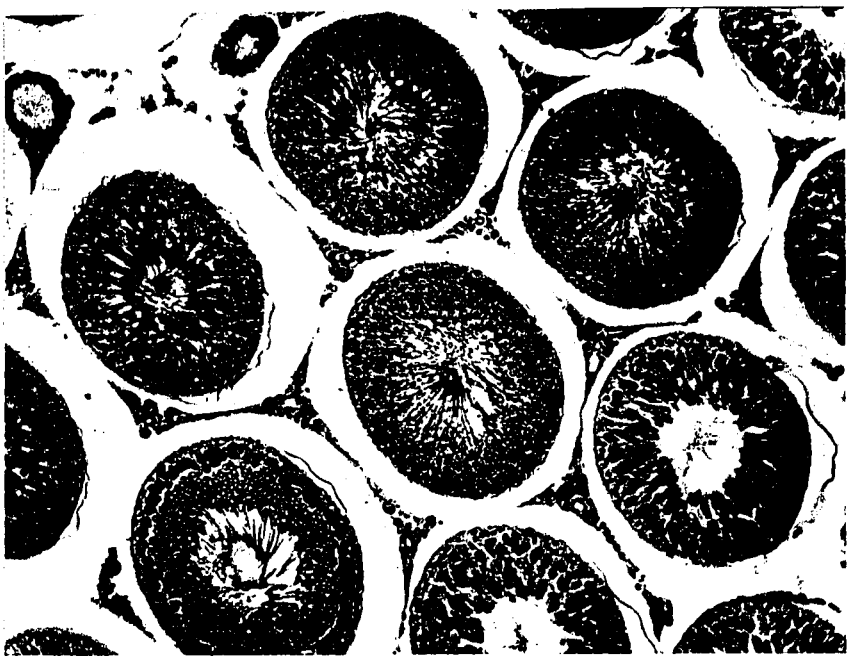

Figure 8. Testis of normal rat (125 X). Aniline Blue and Orange G.

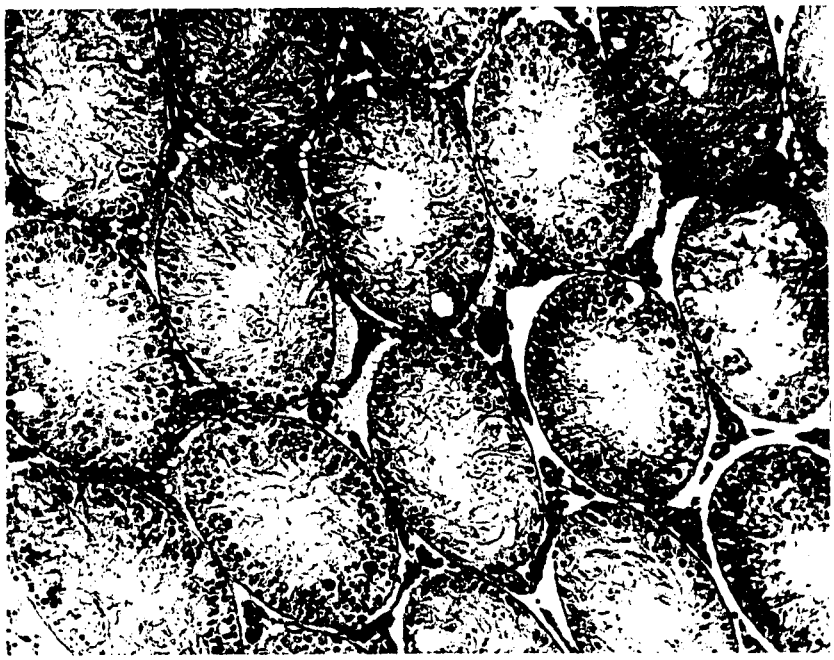

Figure 9 A. Testis of vitamin A deficient rat (125 X) at time of body weight gain plateau. Note loss of spermatogenic function.

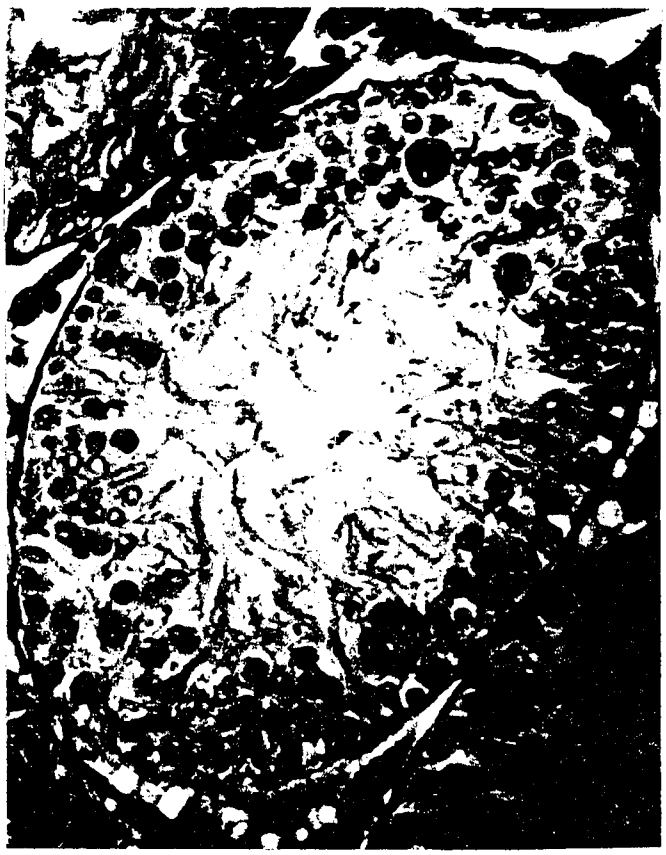

Figure 9 B. Seminiferous tubule of vitamin A deficient rat enlarged from figure 9 A. (450 X)

Primary spermatocytes are apparently unaffected. Remaining cells are practically all at stages VII through IX in their development (112a). As the number of these latter cells increases, they apparently aggregate into multinuclear-like structures.

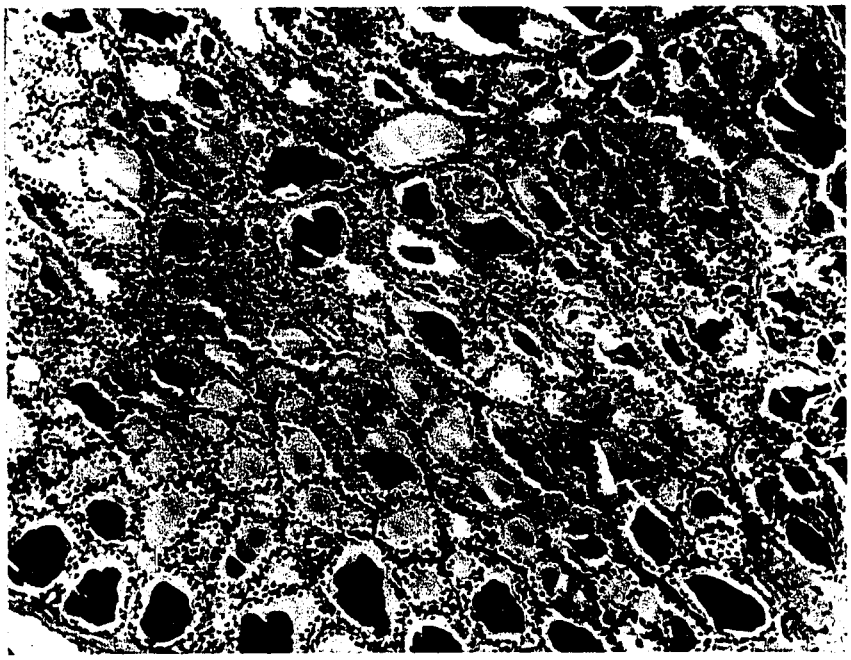

Figure 10. Thyroid gland of normal rat (125 X). The yellow staining areas of the colloid appear as black, the blue staining ones are light grey and the white areas denote the absence of colloid or tissue.

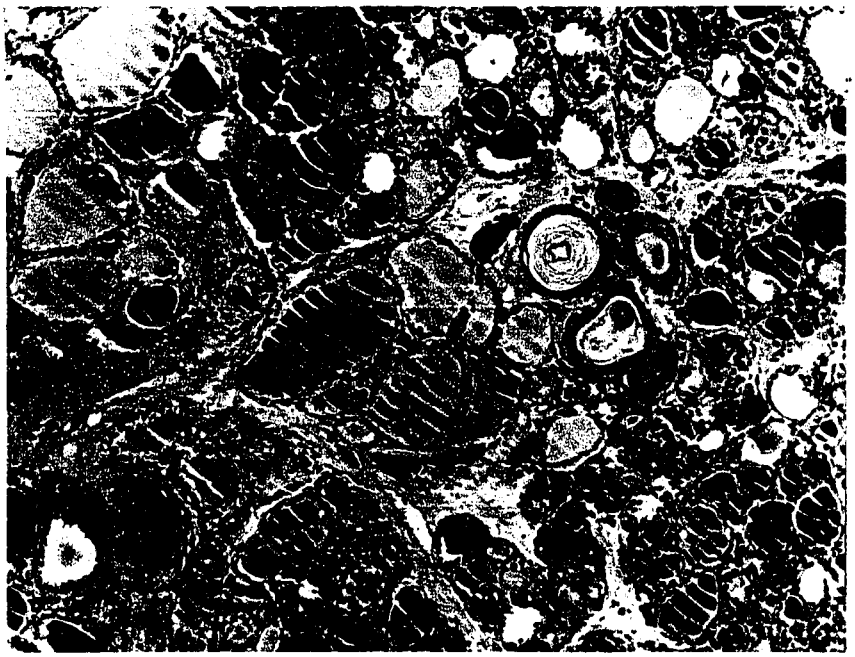

Figure 11. Thyroid gland of vitamin A deficient rat (125 X). Note the high incidence of yellow colloid, connective tissue infiltration and the onset of keratinizing metaplasia.

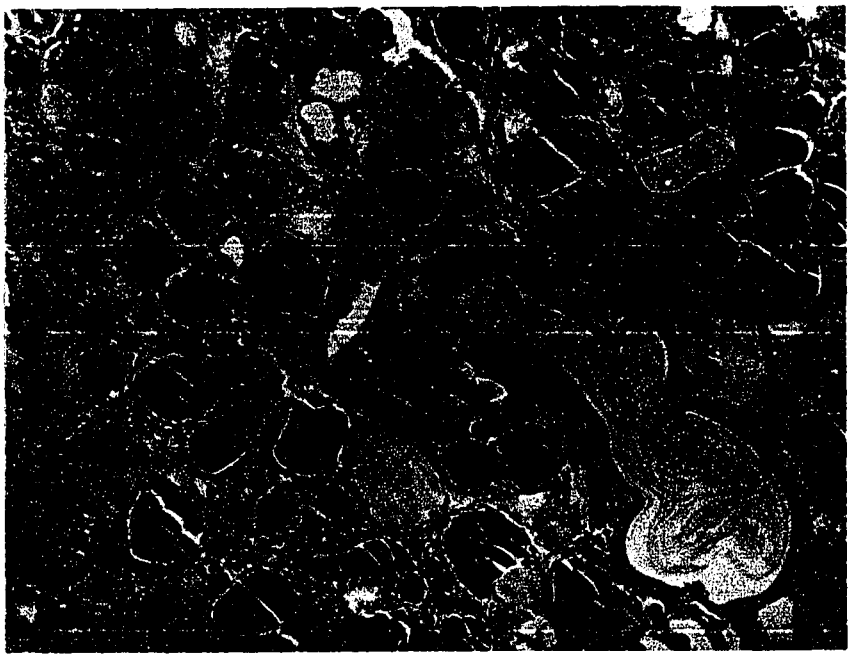

Figure 12. Thyroid gland of vitamin A deficient rat (125 X). Same remarks as for figure 11. Note the large metaplastic cyst.

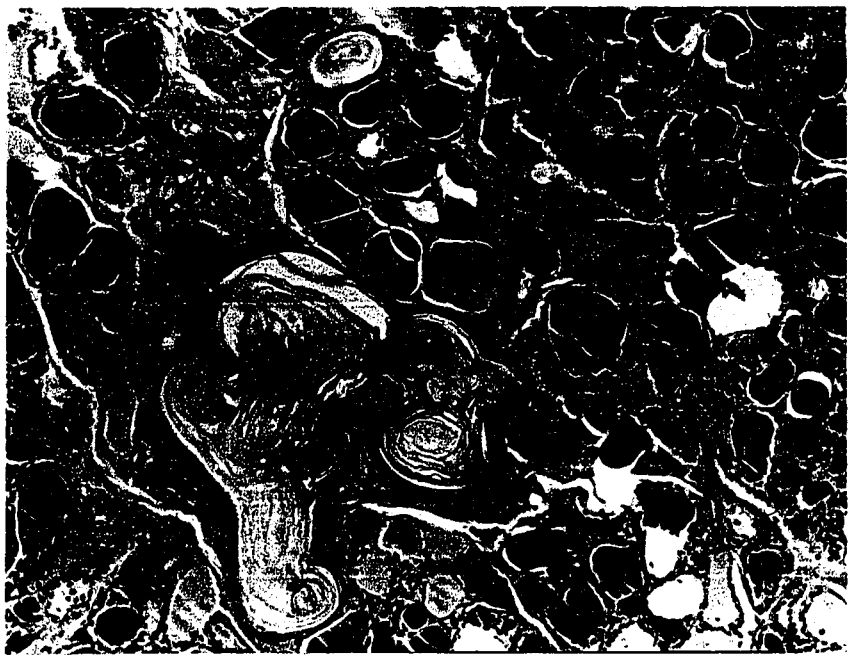

Figure 13 A. Thyroid gland of vitamin A deficient rat (125 X). Same remarks as for figure 12.

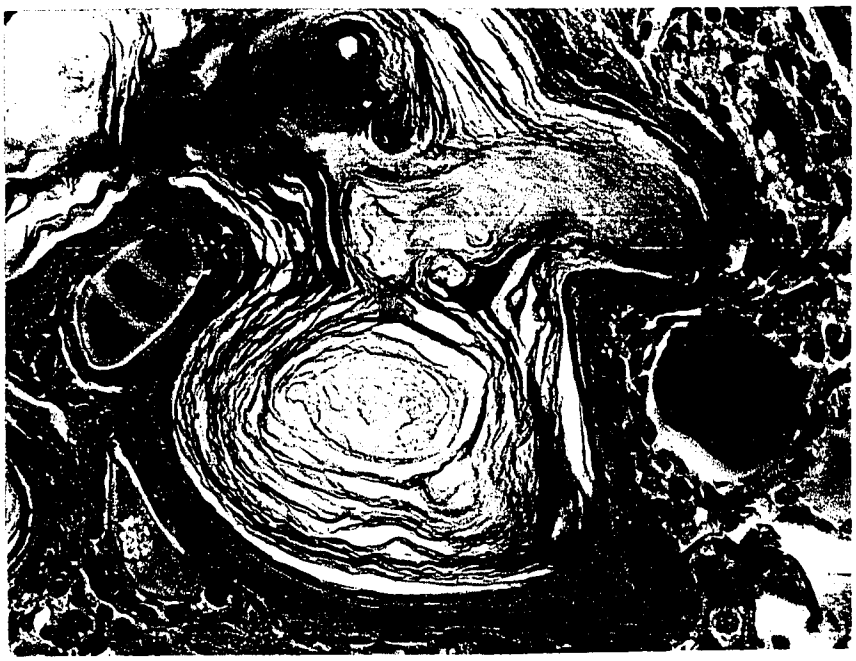

Figure 13 B. Higher magnification (500 X) of a portion of the cystic area shown in figure 13 A.

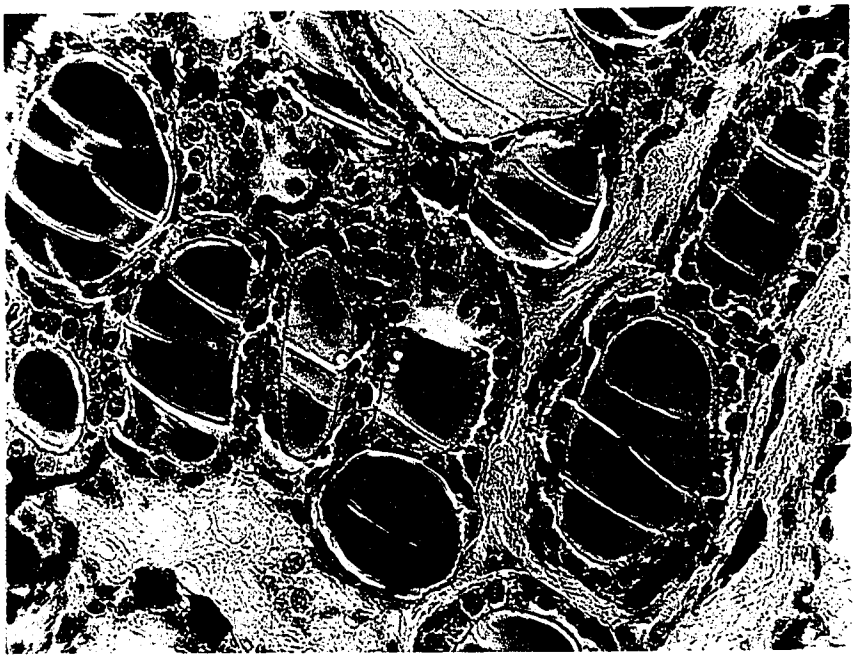

Figure 13 C. Higher magnification (500 X) of area of the gland shown in figure 13 A. Note the connective tissue infiltration, the low follicular epithelium and the incidence of yellow staining colloid.

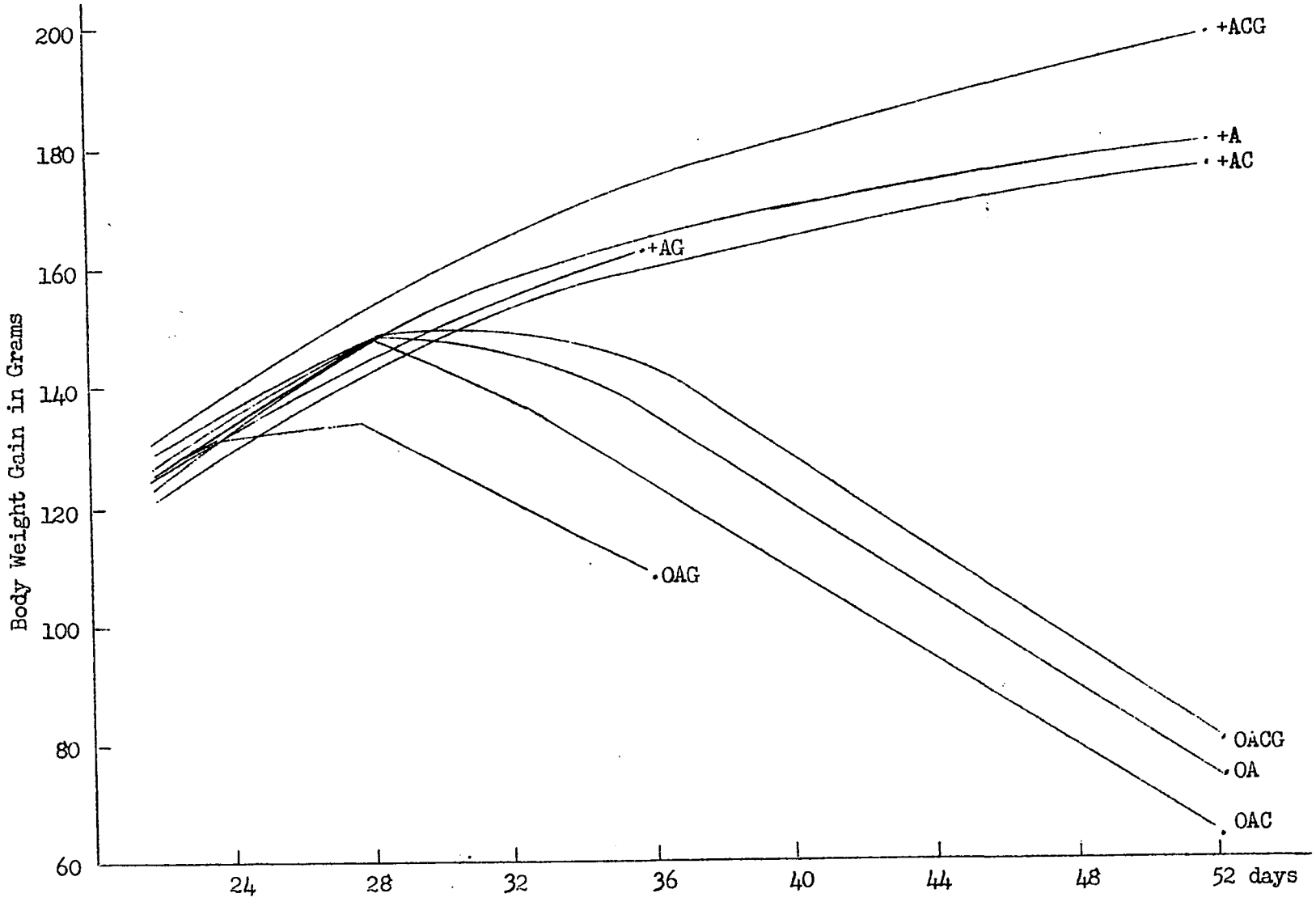

FIGURE 14 (Experiment VI)

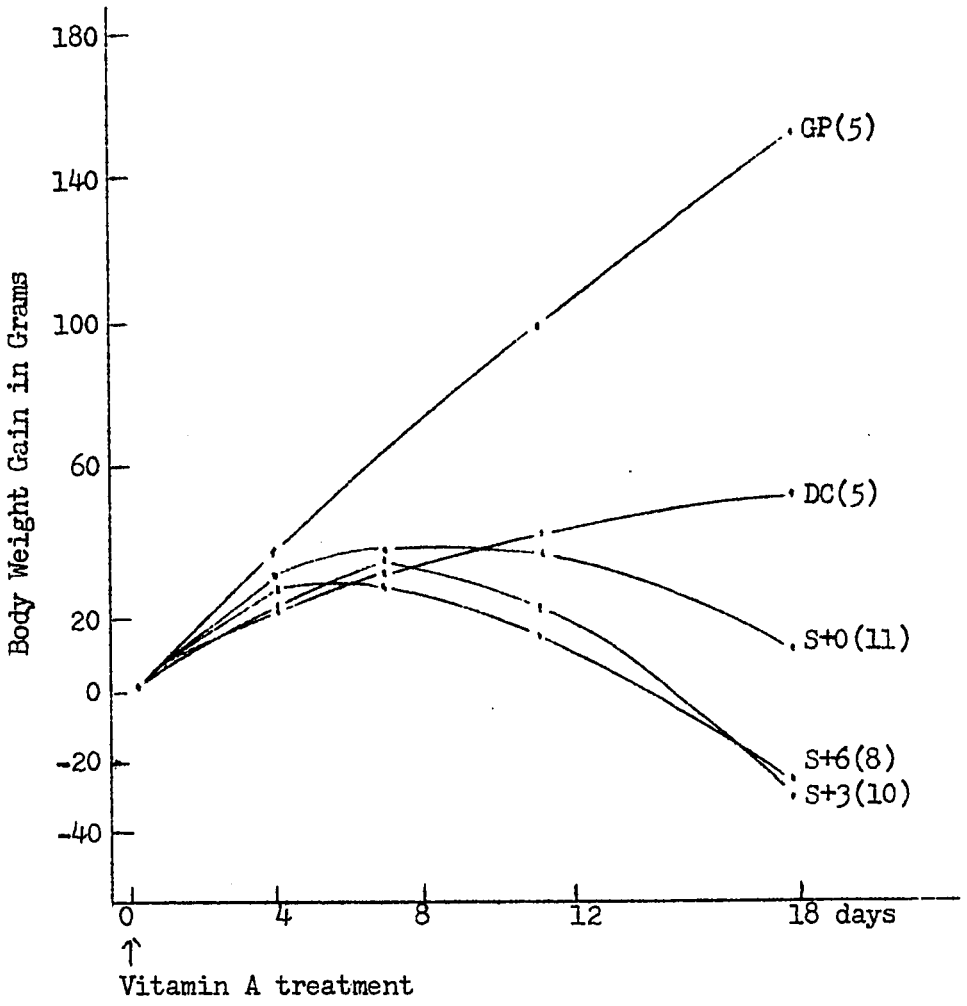

FIGURE 15 (Experiment VII)

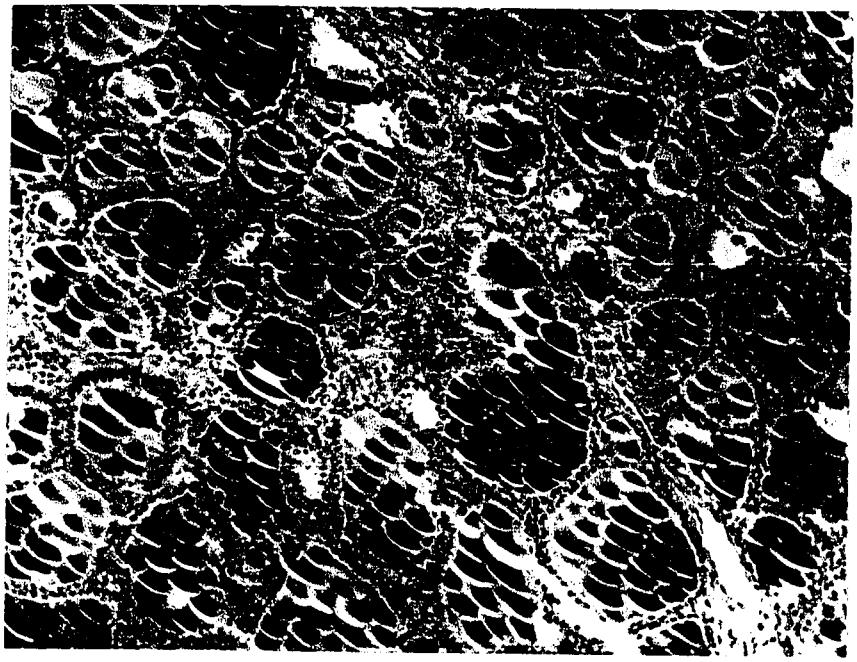

Figure 16. Thyroid gland of normal guinea-pig fed Purina Dog Chow supplemented with vitamin C. Microphotograph (125 X) of the center of the gland depicting an increase incidence of follicles containing yellow colloid and differences in the size of the follicles as compared to the gland shown in figure 17.

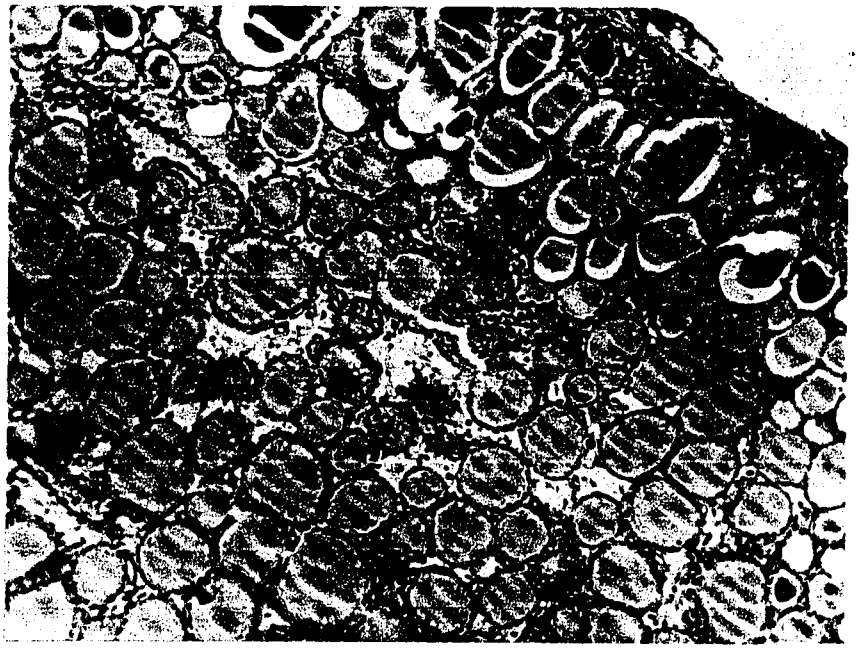

Figure 17. Thyroid gland of normal guinea-pig fed Purina Guinea Pig Chow (125 X). Note the smaller round follicles in the center and the occurence of yellow staining material in the colloid of only the peripheral follicles.

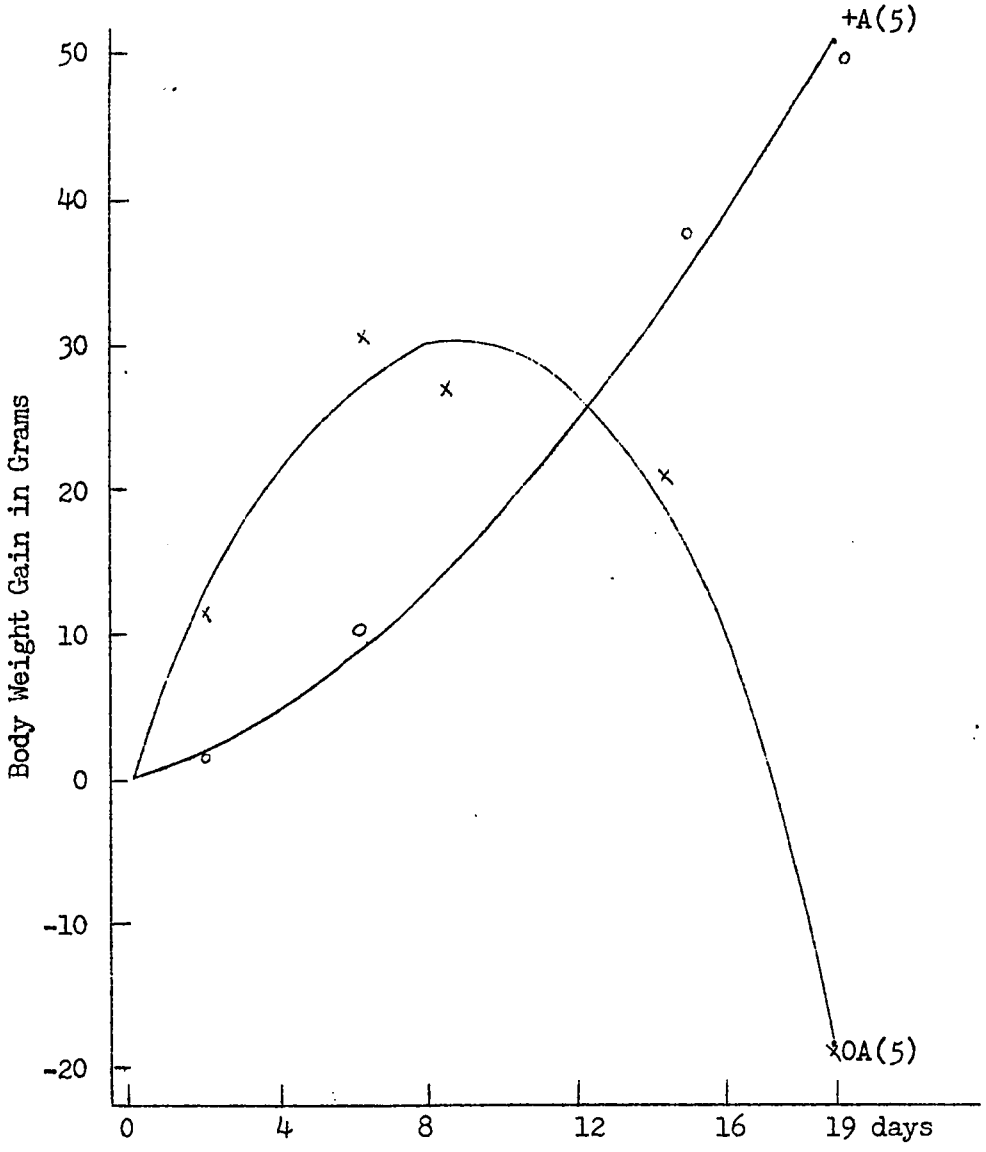

FIGURE 18 (Experiment VIII)

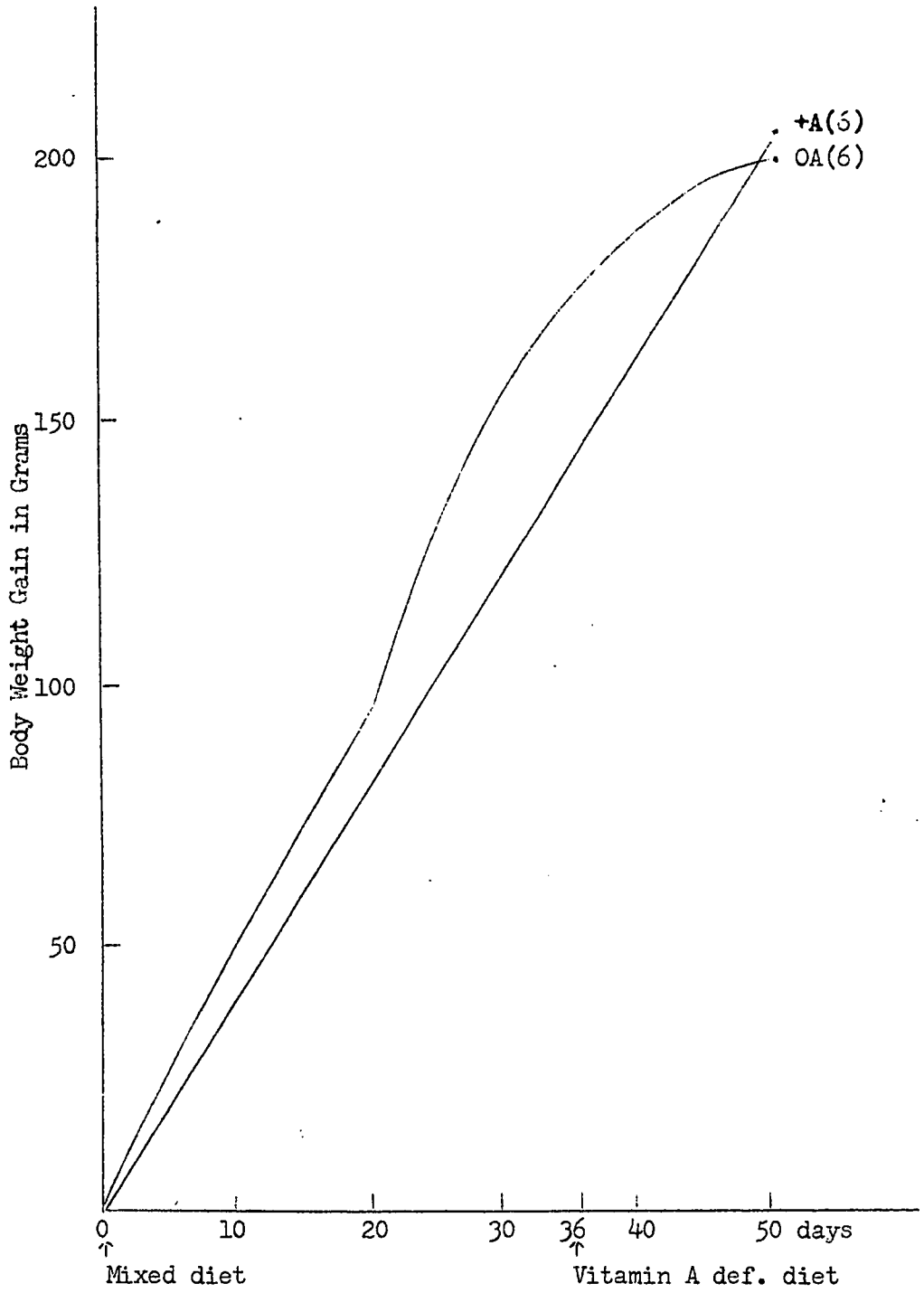

FIGURE 19 (Experiment IX)

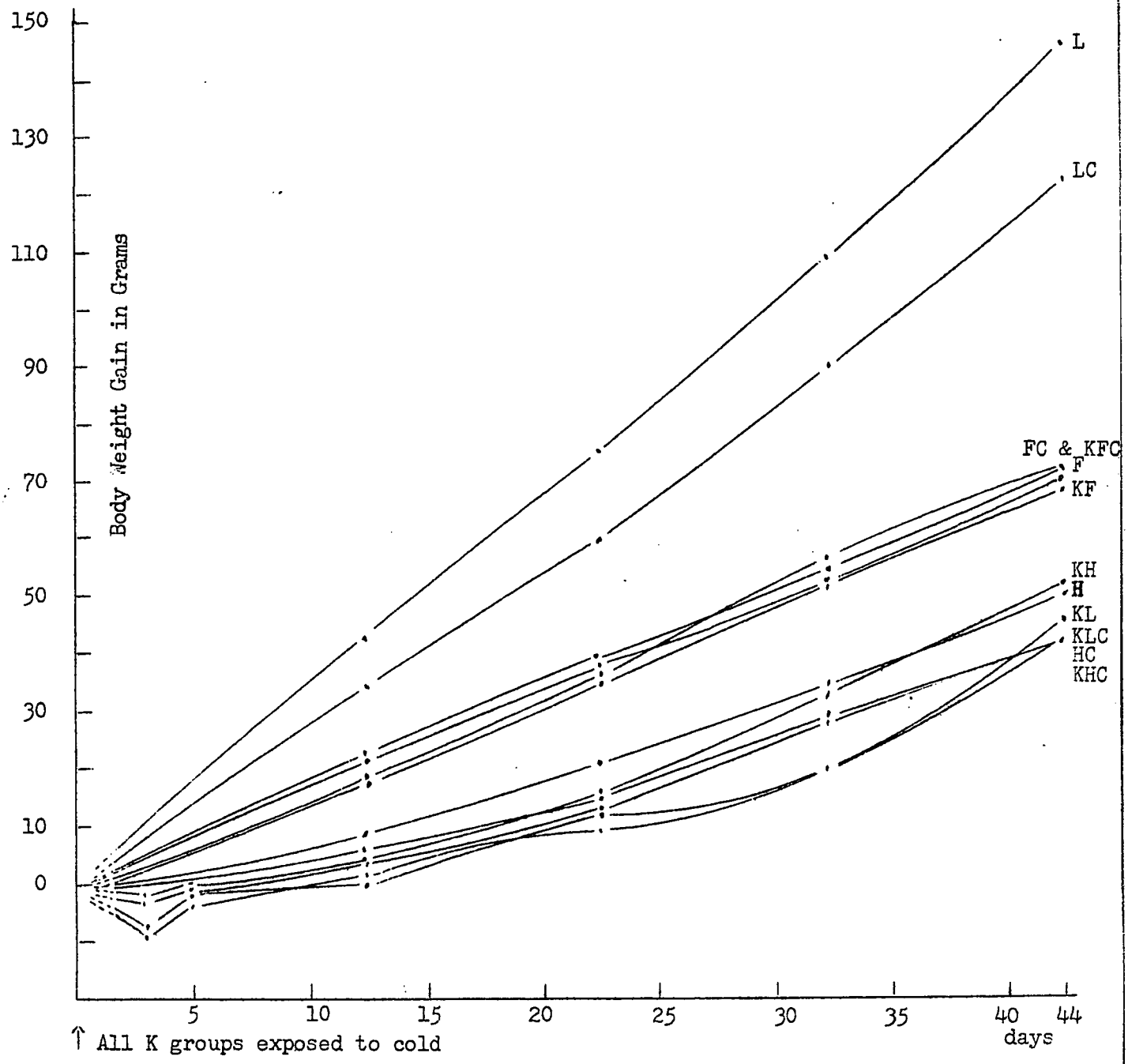

FIGURE 20 (Experiment X)

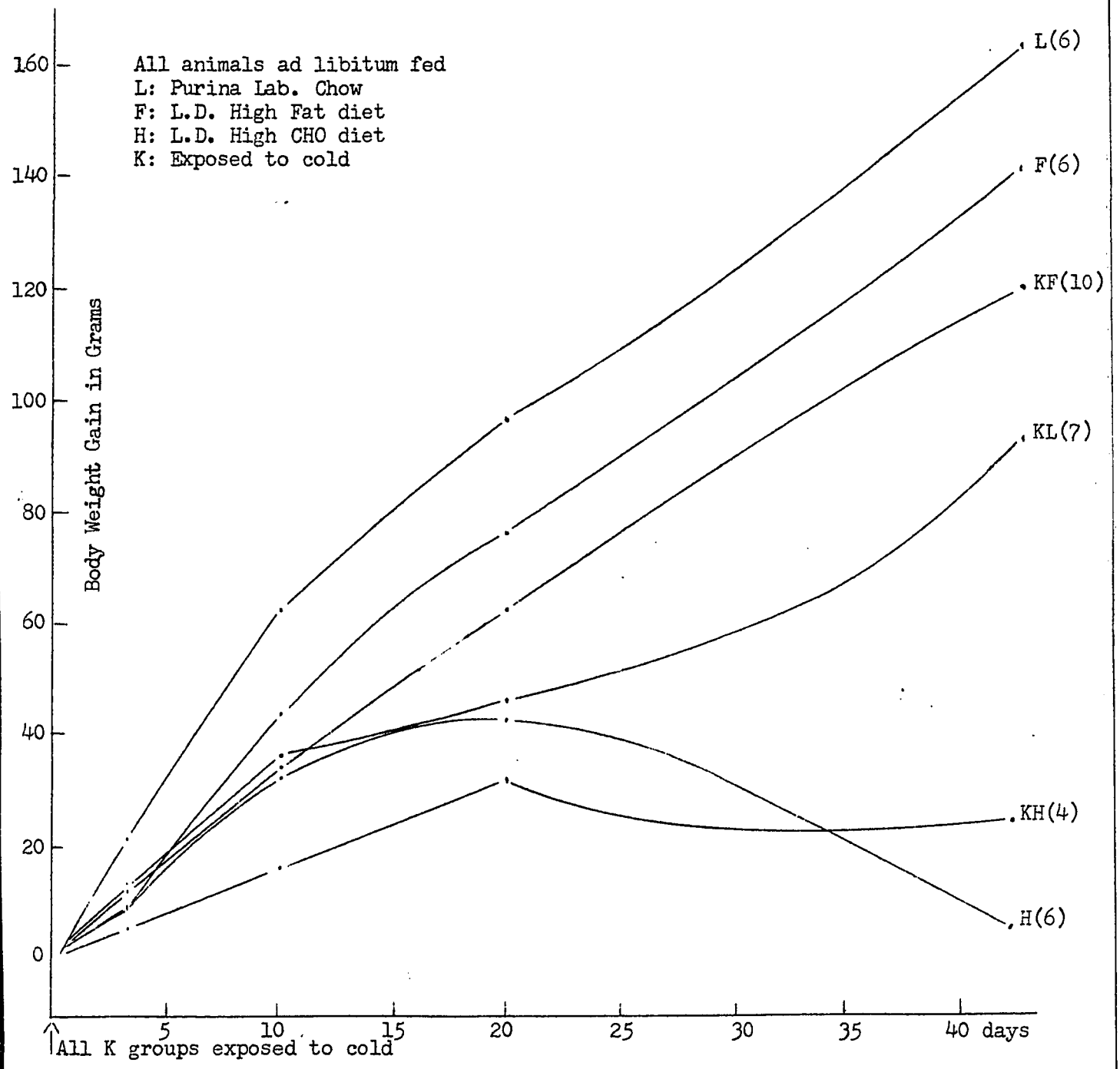

FIGURE 21 (Experiment XI)