To the Graduate School:

I am submitting herewith a thesis written by Gregory Stephen Lewis entitled "Effects of Exogenous Vitamin C on Maintenance of Pregnancy in Diet-Restricted Rats." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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EFFECTS OF EXOGENOUS VITAMIN C ON MAINTENANCE OF PREGNANCY IN DIET-RESTRICTED RATS

A Thesis

Presented for the

Master of Science

Degree

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Gregory Stephen Lewis

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ABSTRACT

Colony-bred four to five month old female rats (average weight 240 g, S.D. 20 g) of Sprague-Dawley origin were used to determine the effects of adding ascorbic acid to the diet of restricted fed pregnant rats on: (1) body weight gains of the dams, (2) organ weights, and (3) tissue biochemistry of the dams during gestation, and (4) fetal weights. A minimum of 20 bred females were assigned to each of the following treatments: (1) ad libitum fed plus 25 mg vitamin C (U.S.P.-crystals) administered per os daily via stomach tube attached to a 1 ml plastic syringe; (2) ad libitum fed; (3) restricted fed (15 g/day) plus 25 mg vitamin C per os daily; (4) restricted fed (15 g/day); (5) restricted fed (15 g/day) plus 12.5 mg vitamin C per os daily; (6) restricted fed (15 g/day).

Females were assigned to treatment groups on the day sperm were found in the vaginal smears. Body weights were recorded on day 1 and day 22 of pregnancy (prior to sacrifice). Livers and uteri were removed from each animal and weighed, and approximately 2 ml of blood for glucose determination were taken via cardiac puncture. The placentas were freed from the fetuses and analyzed for glycogen, and the fetuses were individually weighed.

Supplementing the ration with vitamin C did not effect any of the parameters studied except placental weight per fetus ($P<.05$). The major differences were associated with level of feeding.

Maternal weight gain during pregnancy, maternal liver weights and

uteri of ad libitum fed females were significantly greater ($P<.001$) than those of the limited fed (15 g day) females.

There was no evidence that vitamin C or level of feeding affected litter size.

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CHAPTER I

INTRODUCTION

Adequate food intake by pregnant females is necessary to support pregnancy and normal development of the young (Nelson and Evans, 1954). A subnormal food intake during gestation can result in retarded fetal development, small and weak young at birth or fetal death (Everson, Northrop, Chung and Getty, 1954; Nelson and Evans, 1954; Berg, 1965, 1967). It has, however, been reported that pregnancy can be maintained in females on protein deficient rations by daily administration of estrogen and progesterone (Nelson and Evans, 1954); this finding suggests than an adequate food intake is necessary for steroid hormone synthesis (Paul and Duttagupta, 1974). The role of vitamin C in steroid hormone synthesis and maintenance of pregnancy is not clear.

Recently it was reported that ascorbic acid supplementation of restricted fed (15 g/day) rats increased the reproductive performance of the dams and weight of the offspring (Paul and Duttagupta, 1974).

Restricting the daily feed intake of gestating sows to conserve feed and prevent excessive fat deposition during gestation is an accepted practice in swine husbandry. Should sows on a restricted diet respond to ascorbic acid supplementation in the same way as the female rat, birth weights, viability of the newborn pigs, and the milk production by the sow during early lactation might be greatly improved.

The following study was designed to determine the effects of adding ascorbic acid to the diet of intake-restricted pregnant rats

on (1) body weight gains of the dams, (2) organ weights, (3) tissue biochemistry of the dams during gestation, and (4) fetal weights.

CHAPTER II

REVIEW OF LITERATURE

As early as 1915, Ingier reported that vitamin C depletion during human pregnancy resulted in resorption or abortion of fetuses. Draft and Schwarz (1952) reported that rats fed pantothenic acid or riboflavin deficient diets developed typical deficiency symptoms and died, but their litter-mates, receiving identical diets supplemented with 2% ascorbic acid, appeared to grow and mature normally. Hundley and Ing (1953), in studies with rats on the relationship of ascorbic acid to pantothenic acid, found that pantothenic acid-free diets containing either glucuronolactone, glucuronic acid, D-isoascorbic acid, calcium diketogluonate, gluconolactone, gluconic acid, galacturonic acid, or ascorbic acid produced 60% to 300% greater growth than animals on the unsupplemented diet. Ascorbic acid and related compounds usually prevented adrenal hemorrhage, thymus atrophy and death (Hundley and Ing, 1953).

Citing the work by Draft and Schwarz (1952) and Hundley and Ing (1953) as the basis for their studies, Everson, Northrop, Chung and Getty (1954) looked at the sparing action of ascorbic acid on pantothenic acid in relation to reproduction in the rat. They found a striking improvement in reproductive performance of the females on the pantothenic acid-free diet when the ration was supplemented with vitamin C at the rate of 2% of the daily ration. Everson et al. (1954) reported that litters born to females receiving a diet deficient in pantothenic

acid and ascorbic acid averaged 3.7 g per fetus while females fed the same diet supplemented only with ascorbic acid produced litters with an average fetal weight of 4.3 g. The young from ascorbic acid-supplemented females were superior at birth in pantothenic acid content of the blood, ascorbic acid content in serum and generally were hardier than those from pantothenic acid and ascorbic acid deficient dams.

Sigal and King (1936) stated that in guinea pig blood, glucose increased as ascorbic acid decreased, and Banerjee and Ghosh (1947) found that as ascorbic acid in guinea pig blood decreased, the liver glycogen decreased. Glucose tolerance was shown to be lowered as blood ascorbic acid was lowered, and they postulated that the role of ascorbic acid in carbohydrate metabolism is somehow involved with insulin production by the pancreas (Banerjee and Ghosh, 1947).

Ascorbic acid is known to play a role in the hydroxylation of proline to hydroxyproline which is found in collagen and a few collagen-like proteins (White, Handler and Smith, 1968).

The injection of ACTH has been shown to cause a rapid depletion of ascorbic acid from the adrenal cortex with some temporary increase in synthesis and discharge of adrenal cortical steroids (Oser, 1965). This ascorbic acid-steroid synthesis interaction may have a significant role in the beneficial effects of ascorbic acid on reproduction (Paul and Duttagupta, 1974).

In addition to the somewhat general deficiencies that have been shown to effect reproduction adversely, many workers have described specific deficiencies having detrimental effects on reproduction.

Berg (1965) found that feeding a protein-free ration or limitation to 25% of a normal intake caused resorption of a majority of the fetuses. He also reported that transitory feeding of a normal ration during early (day 0 through 4) or mid-gestation maintained pregnancy.

Nelson and Evans (1954) found that pregnancy could be maintained on a protein deficient diet by exogenous estrogen and progesterone therapy. Successful maintenance of pregnancy in protein-depleted rats by steroid injections was not associated with increased food intake or excessive mobilization of hepatic or uterine protein; they suggested that protein was mobilized from skeletal muscles since both muscle size and protein concentration decreased in response to the steroid regimen (Hazelwood and Nelson, 1965). Muscle and liver glycogen and blood glucose was decreased by the steroid injections, while uterine glycogen was increased due to the steroid treatment (Hazelwood and Nelson, 1965). Yang (1970) found that blood glucose increased in virgin rats given a single dose of progesterone.

Working with rats, Paul (1971) found that estrogen (0.5 μg/day) treatment for 21 days reduced uterine glycogen in pregnant females; however, during normal gestation uterine weight and glycogen content increased with advance of pregnancy. Estrogen treatment for 14 to 21 days in intact nonpregnant female rats reduced liver glycogen (Paul, 1972). During normal pregnancy total liver glycogen increased after the first week of gestation, but had returned to the normal level (140 ± 19.4 mg per liver) on day 21 (Paul, 1972). Estrogen treatment had no effect on liver or uterine glycogen in ovariectomized animals (Paul, 1971, 1972). Connally, Bitman, Cecil and Wrenn (1962)

showed that in pregnant rats interdecidual glycogen increased (110 ± 34.4 mg/100 ml wet wt to 595 ± 196.9 mg/100 ml wet wt) from day 9 to 21 of gestation, and glycogen in the maternal placenta quadrupled (262 ± 33 mg/100 g wet wt to 748 ± 346 mg/100 ml wet wt) during the last 8 days, while glycogen in the fetal placenta diminished to one-third of its earlier value (723 ± 272 mg/100 g wet wt to 234 ± 94 mg/100 g wet wt).

Herrera, Knopp and Freinkel (1969) observed that liver glycogen ranged from 5.61 mg in fed animals to 0.27 mg per liver in fasted animals; the liver weight varied inversely with level of feed intake.

Paul and Duttagupta (1974) reported that vitamin C supplementation of rats on a restricted diet had a propitious effect on maintenance of pregnancy. They found that restriction of the daily feed intake to 15 g during the first 14 days of pregnancy did not cause fetal death, but restriction of feed for 21 days caused abortion in about 80% of the females. Fetal and placental weights, maternal liver glycogen, placental glycogen, blood glucose and body weight gains by the dams on the restricted diet were significantly ($P<.001$) less than those in ad libitum fed rats. When, however, they supplemented restricted fed animals with 25 mg of vitamin C daily, all parameters studied were comparable to those of the ad libitum fed females.

CHAPTER III

EXPERIMENTAL PROCEDURE

I. ANIMALS

Healthy colony-bred four to five month old rats of Sprague-Dawley origin (average weight 240 g, S.D. 20 g) were used in this study. They were maintained under a 12:12 light-dark schedule in a temperature controlled room (average 27°C). Prior to assignment to treatment groups they received a commercial pelleted ration (Purina Rat Chow) and water ad libitum. The proximate analysis of the ration was 22.0% crude protein, 4.0% crude fat, 5.0% fiber and 9.0% ash. The caloric value of the ration was 4165 calories per kilogram.

To determine date of breeding, groups of four females were caged with two males and vaginal smears examined each day between 8 and 10 a.m. Vaginal smears were obtained with cotton swabs moistened in an isotonic saline solution; a swab was inserted into the vagina and rotated to collect cells. The swab was immediately rolled onto a clean 7.62 cm x 2.54 cm glass slide and the slide examined under a microscope for sperm. Pregnancy was assumed on finding sperm in the vaginal smear. The day sperm were found was counted as day 1 of gestation.

As the bred females were identified they were assigned to the treatment groups in sequential order, that is, the first sperm positive feamle was assigned to treatment 1, the second female to treatment 2, and the third to treatment 3, etc., until a minimum of 20 females had been assigned to each group. The treatments were as follows:

1. Ad libitum intake plus 25 mg vitamin C (U.S.P.-crystals) administered per os daily via stomach tube attached to a 1 ml plastic syringe.
2. Ad libitum intake.
3. Restricted intake (15 g/day) plus 25 mg vitamin C per os daily.
4. Restricted intake (15 g/day).
5. Restricted intake (15 g/day) plus 12.5 mg vitamin C per os daily.
6. Restricted intake (15 g/day).

The females were housed individually in cages measuring 24 cm deep x 21 cm wide x 18 cm high. The cages were arranged in four tiers of six each on both sides of the cage racks. Three racks were used; that is, animals in treatments 1 and 2 were in one rack, animals in treatments 3 and 4 were in a second rack, and animals in treatments 5 and 6 were in a third rack. Thus the females were paired as to position (i.e., animal 1 in treatment 1 and animal 1 in treatment 2 occupied the same relative position in the rack). The restricted groups had continuous access to water and were offered their daily allowances of 15 g of feed at 10 a.m.

The vitamin C was administered prior to feeding each day. A fresh solution of vitamin C was prepared daily in concentrations that provided the desired quantity (i.e., 25 or 12.5 mg) for each rat in 0.2 ml of distilled water.

Body weights were recorded on day 1 and day 22 of pregnancy (prior to sacrifice). Immediately after weighing, the females were

moved from the colony room to the laboratory to be sacrificed. Females were anesthetized by an intraperitoneal injection of 0.4 ml of a commercial preparation of sodium secobarbital and mephenesin (Myothesia, S. E. Massengill Company). The abdominal wall was opened by a ventral midline incision to expose the internal organs. Approximately 2 ml of blood was obtained via cardiac puncture using a 2.54 cm 22 gauge needle attached to a 3 ml heparinized syringe.

Blood samples were immediately transferred to 15 ml polypropylene tubes containing 3 drops (delivered from a syringe with a 22 gauge needle) of 4% sodium fluoride solution (w/v) to inhibit glycolysis. Mixing of blood and glycolytic inhibitor was achieved by gentle swirling of the tubes. Samples were stored at 3°C until they were centrifuged (no later than 3 hours after collection) at 2000 rpm for 10 minutes in a non-refrigerated centrifuge (International Centrifuge, size 1). The plasma was transferred to clean 15 ml polypropylene tubes and stored at -20°C until analyzed for blood glucose.

Immediately after collection of blood samples, livers were excised and weighed. The uteri were excised, trimmed free of fat, extraneous tissue, and cervices, and weighed. The discoidal placentas, free of fetal membranes, were weighed and immersed in 15 ml of 30% potassium hydroxide (KOH) solution. The tubes containing the discoidal placentas and KOH solution were immersed in boiling water until all tissue had been digested. After digestion, the tubes were cooled at room temperature and capped with cork stoppers. They were then stored at -20°C until analyzed for glycogen. The fetuses were dissected free of fetal membranes and stored in 10% formalin solution for 24 hours.

Subsequently they were rinsed in tap water, blotted with paper towels, and individually weighed.

The final body weights of the females were taken as the difference between initial weight (day 1) and weight at sacrifice (day 22) less the weight of the uterus and its contents. Gains during pregnancy were taken as the differences between initial and final body weights. Uterine weights were calculated by subtracting the products of conception from the total weight of the uterus. All tissues were weighed to the nearest 0.1 g on a top loading Metler balance.

II. BIOCHEMICAL DETERMINATIONS

Glycogen

Placental glycogen was estimated using the technique of Good, Kramer and Somogyi (1933) as modified by Dubois, Gilles, Hamilton, Rebers and Smith (1956) and Montgomery (1957). The procedure was as follows:

1. Immediately after being weighed the discoid placentas were transferred to the tubes containing 15 ml of 30% aqueous potassium hydroxide (KOH) solution (w/v) (a minimum of 2 ml per g of tissue). The tubes were immediately placed in a boiling water bath until digestion had been completed.
2. After removal from the water bath the tubes were held at room temperature for about 30 minutes prior to storage at -20°C. Due to the caustic nature of the KOH solution, the tubes were capped with cork stoppers.

11. Triplicate 2 ml aliquots of glycogen solutions and standards were transferred to 19 x 150 mm clean, dry, matched cuvettes.

12. To the standards and unknown, 1 ml of phenol solution (w/v) was added. The phenol previously stored at 3°C was used at this temperature.

13. Five milliliters of H_2SO_4 were added to the samples from a 100 ml fast delivery buret. The stream of acid was directed against the surface of the liquid, instead of the side of the cuvette, to facilitate mixing.

14. The tubes were allowed to stand for 10 minutes at room temperature. They were then swirled and returned to the cuvette rack until they had cooled for 20 to 30 minutes.

15. The transmittance of the solution (which was a yellow-orange color) was measured at 490 nm in a Model 350 Turner Spectrophotometer. Prior to recording light transmittance the spectrophotometer was adjusted to 100% transmission using the blank sample. (The color developed in the cuvettes after the addition of H_2SO_4 and was stable for several hours. Neither time nor temperature was critical in reproducibility of the readings among the triplicate samples.)

16. The concentration of glycogen was read directly from the standard curve and multiplied by the appropriate dilution factor(s) to obtain the total placental glycogen. Example:

if the concentration per ml of unknown glycogen sample was 0.03 mg and the dilution factor was 5000 then 0.03 x 5000 = 150 mg glycogen in the placentas.

Blood Glucose

Glucose in blood plasma was estimated using a glucose reagent kit (Sigma Chemical Company) specific for beta-D-glucose. The procedure was as follows:

1. A 10 mg/ml glucose stock solution was prepared by adding 2.5 g glucose to 250 ml of 0.1% benzoic acid solution (w/v). Ten milliliters of the stock solution plus 90 ml distilled water were used to make the glucose standard (1 mg/ml).
2. Twenty milliliters of distilled water were added to one vial of the color reagent (O-dianisidine dihydrochloride), and the solution was stored at 3°C.
3. The contents of one PGO enzyme capsule (glucose oxidase, peroxide and buffer) was dissolved in 100 ml of distilled water in an amber bottle. This solution was used immediately after preparation.
4. The enzyme-color reagent solution was prepared fresh as needed by adding 1.6 ml O-dianisidine dihydrochloride solution to 100 ml PGO enzyme solution; the solution was mixed by inverting several times.
5. Prior to deproteinization of plasma samples, 0.5 ml of distilled water (for the blank), 0.5 ml of standard glucose solution (for the standard) or 0.5 ml of plasma were added to the tubes containing 5.5 ml of distilled water.

6. For deproteinization 2.0 ml of 0.3 N barium hydroxide ($Ba(OH)_2 \cdot H_2O$) solution (w/v) were added to each tube and mixed. Then 2.0 ml of zinc sulfate ($ZnSo_4 \cdot 7H_2O$) solution (w/v) were added and thoroughly mixed (the pH of 2 ml of $Ba(OH)_2$ and 2 ml of $ZnSO_4$ was 6.96).
7. The tubes were centrifuged at 2000 rpm for 15 minutes.
8. One half milliter of the standard glucose solution (10 mg/ml), 0.5 ml of the deproteinized plasma solution or 0.5 ml of the blank solution were added to clean, dry, matched, 19 x 150 mm cuvettes. The glucose standard and plasma samples were run in duplicate.
9. Then 5.0 ml of the enzyme color reagent were added and mixed (total volume in cuvette equaled 5.5 ml).
10. The tubes were incubated in a water bath at 39°C for 30 minutes.
11. Groups of 20 tubes (10 duplicated samples, i.e., the number that could be handled within 10 minutes) were removed from the water bath; the tubes were wiped with disposable tissues and placed in a cuvette holder. A variable amoung of a floculant material developed in the cuvettes, but on standing for about 5 minutes it settled to the bottom of the cuvettes. With gentle handling the cuvettes could be transferred to the spectrophotometer without disturbing this material. The absorbance of the glucose solutions were read in a Model 350 Turner spectrophotometer at a wavelength of 450 nm.

12. A blank containing distilled water (5.5 ml) was used to set the absorbance at zero on the spectrophotometer.
13. A fresh blank and standard were prepared each time a fresh enzyme color reagent was prepared.
14. Milligrams percent blood glucose was calculated using the following formula:

$$mg\ \% = \frac{\text{absorbance of unknown - absorbance of blank}}{\text{absorbance of standard - absorbance of blank}} \times 100.$$

III. STATISTICAL ANALYSIS

The data were first analyzed using orthogonal comparisons. Since a Bartlett's test for homogeneity of variances (Steel and Torrie, 1960) showed that the least squares means for the orthogonal comparisons were heterogeneous, a least squares analysis of variance (Harvey, 1960) fitting treatment and position was executed. The model used was:

$$Y_{ij} = \mu + T_i + P_j + E_{ij};$$

$$T_i = i^{th} \text{ treatment},$$

$$P_j = j^{th} \text{ position}.$$

In order to remove the "position effects" the comparisons were made among cages within racks.

CHAPTER IV

RESULTS AND DISCUSSION

The treatment means are shown in Table I. Least squares means for the orthogonal comparisons are summarized in Table II and the analysis of variance for the comparisons are presented in Table III. Since many of the F-statistics for the orthogonal comparisons were considerably less than one, heterogeneity of sub-group variables was suspected. The least squares means were tested for homogeneity of variances using Bartlett's test (Steel and Torrie, 1960). Inflated error terms caused all but one (placental glycogen) of the seven parameters to be heterogeneous. The error terms were large, possibly because the treatments were confounded with the location of the cage racks in the room.

The comparisons were then made within racks of cages. Since the "position effects" were limited to the two ad libitum fed groups (see Table IV), it seems plausible that it was due to the varied feed intake among females in these two groups.

The only significant differences associated with vitamin C treatment was heavier placental weights per fetus ($P<.05$). The major differences were associated with level of feeding (Table IV). Ad libitum fed females gained an average of 52 g, whereas, the limited fed females lost an average of 4 g during pregnancy. Livers of limited fed females weighed about 37% less than those of ad libitum fed females. This agrees with the findings of Hazelwood and Nelson (1965) and Herrera et al. (1969); they stated that liver weights of rats varied

Table I. Treatment Means and Coefficients of Variation

Treatment Groups[a]	Maternal Body Weight[b] (g)	Maternal Weight Gain[c] (g)	Maternal Liver Weight (g)	Uterine Weight (g)	Placental Weight Per Fetus (g)	Glycogen (mg) Per Gram of Placenta	Blood Glucose mg/100 ml
1	296.6	51.1	12.5	18.3	0.60	1.30	91.5
	9.3	29.3	11.2	31.7	28.9	60.9	30.9
2	299.3	53.3	12.5	19.7	0.57	1.00	110.2
	4.3	3.10	9.8	34.3	27.5	66.3	38.4
3	237.9	-0.7	8.0	16.0	0.56	1.18	85.9
	5.5	1920.9	8.9	19.7	10.0	6.00	25.0
4	231.8	-4.0	7.8	17.3	0.53	1.22	71.4
	5.1	233.9	6.6	14.2	8.72	54.7	32.8
5	243.3	1.3	8.0	15.9	0.63	1.58	109.8
	8.7	930.4	8.4	30.9	33.0	59.7	44.4
6	227.6	-5.3	7.5	13.0	0.47	1.29	93.6
	9.3	375.7	13.6	40.8	37.9	54.5	50.5

[a]The treatments were: (1) Ad libitum fed plus 25 mg vitamin C per os daily; (2) Ad libitum fed; (3) Restricted fed (15 g/day) plus 25 mg vitamin C per os daily; (4) Restricted fed (15 g/day); (5) Restricted fed (15 g/day) plus 12.5 mg vitamin C per os daily; (6) Restricted fed (15 g/day).

[b]Maternal weight is the weight at time of sacrifice minus the uterus and its contents.

[c]Maternal body weight minus initial weight. [d]Uterine weight minus the products of conception.

[e]Means and coefficients of variation.

Table II. Least Squares Means for the Orthogonal Comparisons

Comparisons[a]	Treatments Making up the Comparisons[b]	Maternal Body Weight (g)	Maternal Weight Gain (g)	Maternal Liver Weight (g)	Uterine Weight (g)	Placental Weight (g) Per Fetus (g)	Glycogen (mg) Per Gram of Placenta (g)	Blood Glucose mg/100 ml
1	1,3,5	259.3	17.3	9.5	16.7	0.60	1.35	95.7
	2,4,6	252.9	14.7	9.3	16.7	0.52	1.19	91.7
2	1,2	277.0	34.1	11.0	17.8	0.57	1.22	97.2
	3,4,5,6	235.2	-2.2	7.8	15.6	0.55	1.32	90.2
3	1 os, 3,5	253.8	14.8	9.3	16.3	0.55	1.29	88.0
	2 os, 4,6	258.4	17.2	9.4	17.1	0.57	1.25	99.4
4	3	253.4	15.0	9.4	16.8	0.52	1.07	81.8
	5	258.8	17.0	9.4	16.6	0.60	1.47	105.7
5	4	258.1	16.6	9.6	18.8	0.59	1.24	82.6
	6	254.0	15.4	9.2	14.6	0.53	1.30	104.8

[a]The comparisons were: (1) Vitamin C vs no vitamin C; (2) Ad libitum intake vs restricted intake; (3) Ad libitum intake plus vitamins vs restricted intake plus vitamin C x ad libitum intake no vitamin C vs restricted intake no vitamin C; (4) Restricted intake plus 25 mg vitamin C vs restricted intake plus 12.5 mg vitamin C; (5) Treatment 4 (restricted intake) vs treatment 6 (restricted intake].

[b]The treatments and variables are described in Table I.

Table III. Analyses of Variance for the Orthogonal Comparisons

Variable/ Source	Degrees of Freedom	Mean Square
Maternal Body Weight (g)	5	18237.40
Treatment 1[a]	1	1044.5
2	1	87134.5[d]
3	1	1033.6
4	1	240.8
5	1	165.8
Error	98	349.81
Maternal Weight Gain (g)	5	13314.2
Treatment 1	1	173.3
2	1	65376.9[d]
3	1	284.5
4	1	33.8
5	1	14.5
Error	98	216.68
Maternal Liver Weight (g)	5	99.0
Treatment 1	1	1.8
2	1	487.6[d]
3	1	0.6
4	1	0.0
5	1	1.0
Error	98	0.92
Uterine Weight (g)	5	93.7
Treatment 1	1	0.1
2	1	263.0[c]
3	1	25.2
4	1	0.1
5	1	180.5[b]
Error	98	23.82
Placental Weight (g) Per Gram Fetus	5	0.06
Treatment 1	1	0.15
2	1	0.04
3	1	0.02
4	1	0.05
5	1	0.03
Error	98	0.02

Table III (continued)

Variable/ Source	Degrees of Freedom	Mean Square
Glycogen (mg) Per Gram of Placenta	5	0.49
Treatment 1	1	0.71
2	1	0.42
3	1	0.08
4	1	1.28
5	1	0.05
Error	98	0.57
Blood Glucose, mg/100 ml	5	3896.8
Treatment 1	1	414.6
2	1	2498.7
3	1	6430.5[b]
4	1	4714.8
5	1	4797.5
Error	98	1352.29

[a]The comparisons are described in Table III, and the variables are described in Table I.

[b]$P<.05$; [c]$P<.01$; [c]$P<.001$.

Table IV. Analyses of Variance Fitting Treatment and Position

Variable/ Source	Treatment[a] 1 vs 2 DF[b]	1 vs 2 MS[c]	3 vs 4 DF	3 vs 4 MS	5 vs 6 DF	5 vs 6 MS
Maternal Body Weight						
Treatment	1	17.9	1	286.6	1	2280.6[e]
Position	17	9345.3	20	3931.7	20	10717.9
Error	13	354.1	15	97.2	13	325.0
Maternal Weight Gain						
Treatment	1	64.8[e]	1	8.6	1	51.7
Position	17	6391.7	20	2431.0	20	6405.7
Error	13	82.5	15	135.9	13	222.7
Maternal Liver Weight						
Treatment	1	0.14	1	0.18	1	1.29
Position	17	40.2[d]	20	8.5	20	14.6
Error	13	0.91	15	0.30	13	0.84
Uterine Weight						
Treatment	1	17.6	1	7.4	1	33.4
Position	17	979.6[d]	20	161.5	20	667.2
Error	13	16.3	15	7.6	13	15.5
Placental Weight per Fetus						
Treatment	1	0.01[f]	1	0.005	1	0.15
Position	17	0.79	20	0.06	20	0.33
Error	13	0.003	15	0.002	13	0.07

Table IV (continued)

Variable/ Source	Treatment					
	1 vs 2		3 vs 4		5 vs 6	
	DF	MS	DF	MS	DF	MS
Glycogen per g of Placenta						
Treatment	1	0.47	1	0.00005	1	0.66
Position	17	13.2[d]	20	9.4	20	12.9
Error	13	0.28	15	0.48	13	0.72
Blood Glucose						
Treatment	1	1236.9	1	1474.5	1	2250.4
Position	17	30418.2[d]	20	12511.0	20	63429.4[d]
Error	13	650.8	15	353.3	13	954.3

[a]The treatments and variables are described in Table I.

[b]Degrees of freedom.

[c]Mean square.

[d]$P<.05$; [e]$P<.01$; [f]$P<.001$.

inversely with level of feed intake. Uterine weights of limited fed females averaged about 30% less than those of ad libitum fed females. Paul and Duttagupta (1974) reported similar findings; the uterine weight of their limited fed females were 25% less than those of ad libitum fed females. However, in the current study vitamin C supplementation did not produce uteri from limited fed females comparable in weight to those from ad libitum fed females. This does not agree with the findings of Paul and Duttagupta (1974).

There was no evidence of an effect of either vitamin C or level of feeding on litter size (Table V). A total of 1265 live fetuses, as indicated by color and vascularity, were recovered. Only 22 placental sites did not have associated fetuses. These were distributed as follows:

Treatment group 1: None

Treatment group 2: Two resorptions in one litter

Treatment group 3: None

Treatment group 4: One resorption in one litter;
three resorptions in another litter

Treatment group 5: One resorption in one litter;
two resorptions in a second litter;
twelve resorptions in a third litter

Treatment group 6: One resorption in one litter.

The average weight of fetuses from limited fed females was only 9% less than the weight of fetuses from ad libitum fed females. There was, however, a significant regression of fetal weight on litter size (Table V). This relationship is well recognized (Berg, 1965).

The results reported here do not agree with the report of Paul

Table V. Effects of Treatment on Litter Size and Fetal Weights

Treatment Groups[a]	Number of Litters	Mean Litter Size	Mean Fetal Weight (g)	Regression of Fetal Weight on Litter Size
1	15	12.1[b] ±.6	4.9 ±.1	
				-0.79[cd] ±.17
2	15	13.4 ±.3	4.8 ±.1	
3	17	12.3 ±.5	4.8 ±.1	
				-0.23[d] ±.04
4	20	13.40 ±.9	4.4 ±.1	
5	16	11.8 ±1.0	4.3 ±.1	
				-0.1 ±.06
6	17	12.4 ±.8	4.4 ±.1	

[a]The treatments are described in Table I.

[b]Means ± SEM.

[c]Regression coefficient.

[d]P<.001.

and Duttagupta (1974). They reported that daily subcutaneous injections of 25 mg of vitamin C into females restricted to 15 g feed per day prevented body weight loss during pregnancy, whereas, females not receiving vitamin C lost an average of 24 g. Since subcutaneous injections of vitamin C in preliminary studies of the experiment reported here produced lesions approximately 2 cm in diameter within two days, the ascorbic acid was administered via stomach tube.

Paul and Duttagupta (1974) reported that vitamin C supplementation prevented a reduction in weight of livers, uteri, placentas and fetuses; liver and placental glycogen and blood glucose were maintained at near the levels of ad libitum fed control females. Also, they reported that 81% of their females not receiving vitamin C lost all fetuses between 14 and 21 days of gestation. In the study reported here, 15 of the total resorptions occurred in the groups receiving vitamin C. Eighteen females (5, 4, 3, 1, 2, and 3 in groups 1 through 6, respectively) were not pregnant as determined by palpation on day 14 after breeding. No female called pregnant on day 14 failed to produce a litter.

Two females littered during the night prior to scheduled autopsy the next morning. Two females littered prematurely, one at 14 days and the other at 17 days after sperm were found in the vaginal smears. The most plausible explanation is that they were pregnant, but had been remated during pregnancy.

Berg (1965) reported that 10 of 12 pregnant rats which were maintained on a restricted diet (14 g of a 20% protein ration for the first 11 days of gestation and 16 g of the same ration until sacrifice

at 19 days of pregnancy) bore litters (13.4 fetuses averaging 3.3 ± .1 g) identical to that of ad libitum fed controls (11.9 fetuses averaging 3.8 ± .1 g).

A possible explanation for the discrepancies in the results of the present study and those reported by Paul and Duttagupta (1974) may be in the difference in the energy levels of the two rations. Based on the quoted caloric values, their animals received about 25% fewer calories per day than did the animals in the current study. Possibly, a more severe limitation of feed might have allowed the vitamin C to exert the beneficial action described by Paul and Duttagupta (1974).

It is suggested that future studies on this problem are needed. One approach would be to determine the daily feed intake of ad libitum fed females throughout pregnancy and to limit the feed intake of others to a constant percentage of the ad libitum group. Furthermore, a level of feeding which results in a definite weight loss during pregnancy and fetal deaths should be determined. Then this could be used as the basal ration to which vitamin C would be supplemented.

CHAPTER V

SUMMARY AND CONCLUSIONS

The effects of supplementing pregnant rats, on ad libitum or restricted (15 g/day) feed consumption, with vitamin C on maternal body weight body changes; liver, placental and uterine weights, placental glycogen and blood glucose; and fetal weights were studied.

Supplementing the ration with 12.5 or 25 mg vitamin C administered per os daily via stomach tube did not effect any of the parameters except placental weight. The major differences observed were due to level of feed consumption by the pregnant rats.

Ad libitum fed females gained an average of 52 g during pregnancy, whereas, the limited fed females lost an average of 4 g. Livers and uteri of the limited fed females were about 37% and 30%, respectively, less than those from the ad libitum fed females.

There was no evidence that vitamin C or level of feeding effected litter size. Fetal weights, however, were shown to be a function of litter size. When the litters were increased by one fetus, the weight of each fetus changed by -0.79 ± .17 g, -0.23 ± .04 g and -0.01 ± .06 g, respectively, for combined treatment groups, 1 and 2, 3 and 4, and 5 and 6.

From these results it can be concluded that vitamin C does not have a sparing effect on energy in the pregnant rat, and, therefore, does not improve reproductive performance.

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VITA

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