EFFECTS OF HIGH ASCORBIC ACID INTAKE DURING PREGNANCY AND LACTATION IN MICE

by

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INTRODUCTION

Large doses of ascorbic acid have many effects in humans and animals. In humans, large doses have been reported to reduce the frequency and severity of the common cold (1,2), decrease cholesterol and triglyceride levels (3,4), and have even been suggested as a treatment for cancer (5). Undesirable effects reported from high ascorbic acid intake include nausea and diarrhea (6), absorption of excessive amounts of food iron (7), destruction of vitamin B-12 (8), and interference with diagnostic tests (9).

Effects of large doses of ascorbic acid have also been tested in species not requiring this vitamin in their diets. Interestingly, there is some evidence that high ascorbic acid intake may be beneficial for these animals during various forms of stress. Large doses of ascorbic acid have been found to reduce mineral toxicities in chicks (10), reduce toxicity to organophosphorus insecticides in the rat (11), decrease liver damage caused by carbon tetrachloride in mice (12), and stimulate circulating interferon levels in mice injected with murine leukemia virus (13).

There is very little known about the effects of large doses of ascorbic acid on growth. Since laboratory mice grow quite rapidly and reproduce frequently they should be excellent test subjects. Female mice nearly double in weight during an average 21-day gestation period. Following gestation, there is a 3-week lactation period during

which pups increase in size from approximately 1.5 to 10 grams.

The purpose of this research is to document how large doses of ascorbic acid administered during pregnancy and lactation influence the tissue ascorbic acid concentration and growth of the offspring. Measurements of growth include weight gain, tissue weights, and cell number and cell size of selected tissues.

REVIEW OF LITERATURE

Ascorbic Acid Metabolism

Chemistry

Ascorbic acid (AsA), also known as vitamin C, is a hexose derivative with an empirical formula of $C_6H_6O_8$. It is a white, crystalline substance highly soluble in water, but insoluble in organic solvents such as benzene and chloroform (14).

Ascorbic acid is readily oxidized to dehydroascorbic acid by the removal of hydrogen from its enediol group (figure 1). Both forms are physiologically active and both are found in body fluids. Further oxidation of dehydroascorbic acid results in formation of diketogulonic acid and loss of vitamin activity.

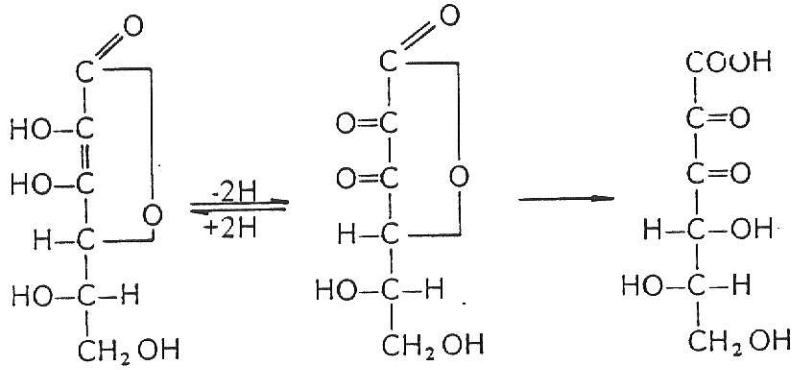

Fig. 1 Oxidation of ascorbic acid

Ascorbic acid is stable in an acid environment, and labile in the presence of heat, alkali, and light. It is easily oxidized and is thus a powerful reducing agent (14). Ascorbic acid has a molecular weight of 176.12 daltons and a melting point between 190-192^{o} (14).

Synthesis of ascorbic acid

Many plant and animal species are able to synthesize AsA. Plants produce AsA using glucose, galactose, and other carbohydrate precusors, while animals synthesize AsA mainly by means of the glucuronic acid pathway shown in figure 2 (15).

The ability to synthesize AsA is believed to have evolved first in the kidneys of early amphibians. Reptiles retained the biosynthetic capacity in the kidneys, but in mammals the site of synthesis shifted to the liver. Although most modern day mammals retain the capacity to endogenously synthesize AsA, a few, including man and other primates, the guinea pig, the Indian fruit bat, and certain fishes, have lost that ability (15).

Burns (16) and Chatterjee et al. (17) stated that the inability of these species to synthesize AsA is due to a lack of the enzyme L-gulonolactone oxidase (EC 1.1.3.8.). This enzyme is an essential catalyst in the terminal step of the conversion of glucose to AsA.

In rats the biosynthesis of AsA is believed to occur in the microsomal fraction of the liver cells. Chatterjee and coworkers (18) found that L-gulonolactone oxidase was located almost entirely in the cellular fraction sedimented between 8,500 xg and 88,700 xg, the microsomal layer. They also found this enzyme to be absent in the

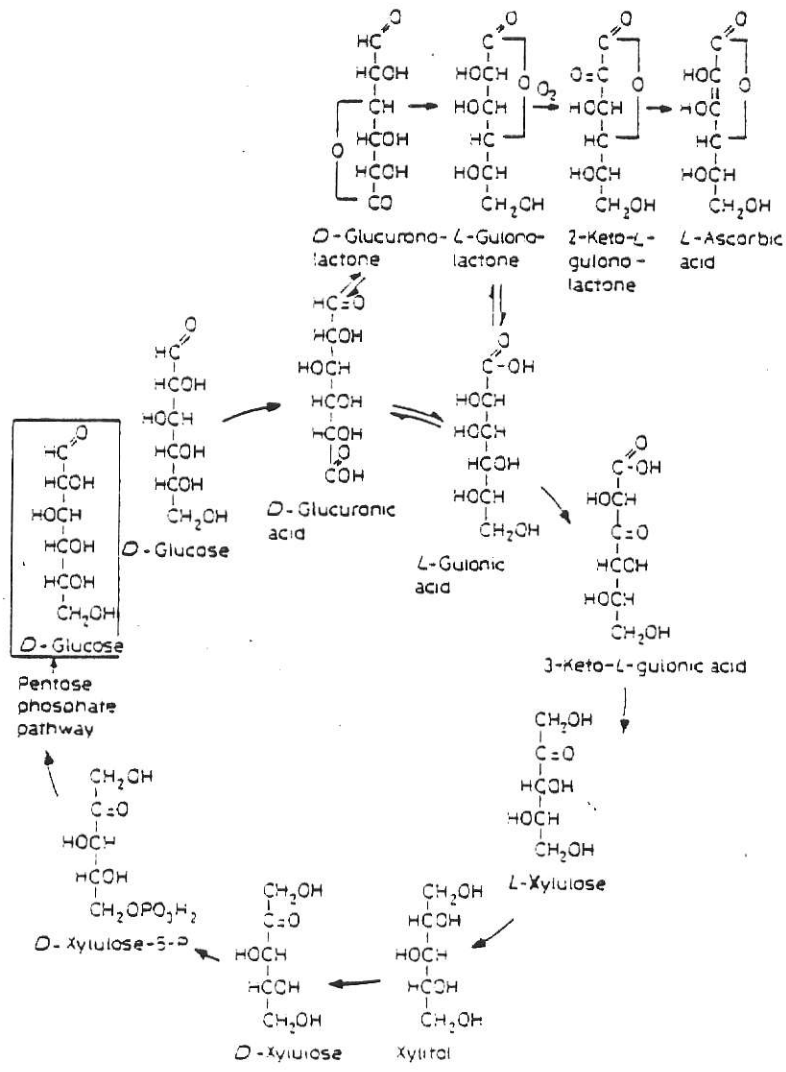

Fig. 2 Biosynthesis of ascorbic acid in animals via the glucuronic acid pathway (15).

microsomal layer in the livers of fetal rats prior to 20 days gestation, and that its activity increased following birth.

Several researchers have shown that testosterone may influence AsA metabolism. Chinoy and Seethalakshimi (19) found that testosterone increased AsA synthesis and AsA concentration in plasma and tissues. Khandwekar and coworkers (20) found that castration of male rats resulted in decreased L-gulonolactone oxidase activity in the liver but that AsA levels were restored by testosterone. Khandwekar and associates further found activity of dehydroascorbatase, an enzyme involved in the oxidation of AsA, to significantly increase in castrated rats. A decrease in dehydroascorbatase activity was observed in both the liver and kidneys after testosterone was administered.

Hornig et al. (21) found that in the rat hypophysectomy results in decreased AsA synthesis. He postulated that this resulted from a metabolic defect which impaired gulonolactone hydrolase activity.

Catabolism of ascorbic acid

In rats and guinea pigs, AsA is oxidized to respiratory CO_2 (22). The first step in this catabolism is the oxidation of AsA to dehydroascorbic acid through enzymatic and nonenzymatic processes. The lactone group on dehydroascorbic acid is then enzymatically removed to form diketogulonic acid which is then decarboxylated to CO_2 and L-xylonic acid and L-lyxonic acid (23,24,25). In the rat, diketogulonic acid is degraded to L-lyxonic acid and L-xylonic acid in the kidney (24). Chan et al. (26) found that in guinea pigs the degradation of dehydroascorbic acid does not need to proceed via diketogulonic acid but can be decarboxylated

to L-xylose. The conversion of AsA to diketogulonic acid and then to respiratory CO_2 apparently does not occur in man. Figure 3 shows the catabolism of L-AsA to L-xylonic acid and L-lyxonic acid and intermediates. Another pathway of AsA catabolism in animals is through conversion of ascorbic acid to oxalate and a 4-carbon intermediate. The two carbons of oxalate come from the C_1 and C_2 carbons of the ascorbic acid molecule (15). In man a small amount of ascorbic acid is converted to urinary oxalate (27).

Absorption and transport of ascorbic acid

Although both L-ascorbic acid and dehydroascorbic acid have vitamin activity, they may have different transport mechanisms into various cells. Martin and Mecca (28) concluded that in the rat dehydroascorbic acid is the form most readily taken up by the tissues. Hammarstrom (29), however, discovered that rats were unable to accumulate dehydroascorbic acid in the adrenal cortex, but that AsA was rapidly taken up by this tissue. Results from Hornig (31) indicate that in the rat AsA is the favored form of transport in the pituitary, adrenal gland, lungs, kidney, bones, and skin. Although Bigley and Stankova (32) found that dehydroascorbic acid was preferentially transported into neutrophils, erythrocytes, and lymphocytes, Hornig (31) found that in erythrocytes the dehydroascorbic acid is rapidly reduced to AsA.

Hornig (31) reported that active transport is necessary for the uptake of AsA by various tissues, but that dehydroascorbic acid can be absorbed through simple diffusion. Earlier work by Hornig et al. (33) illustrated that intestinal absorption of AsA occurred by passive

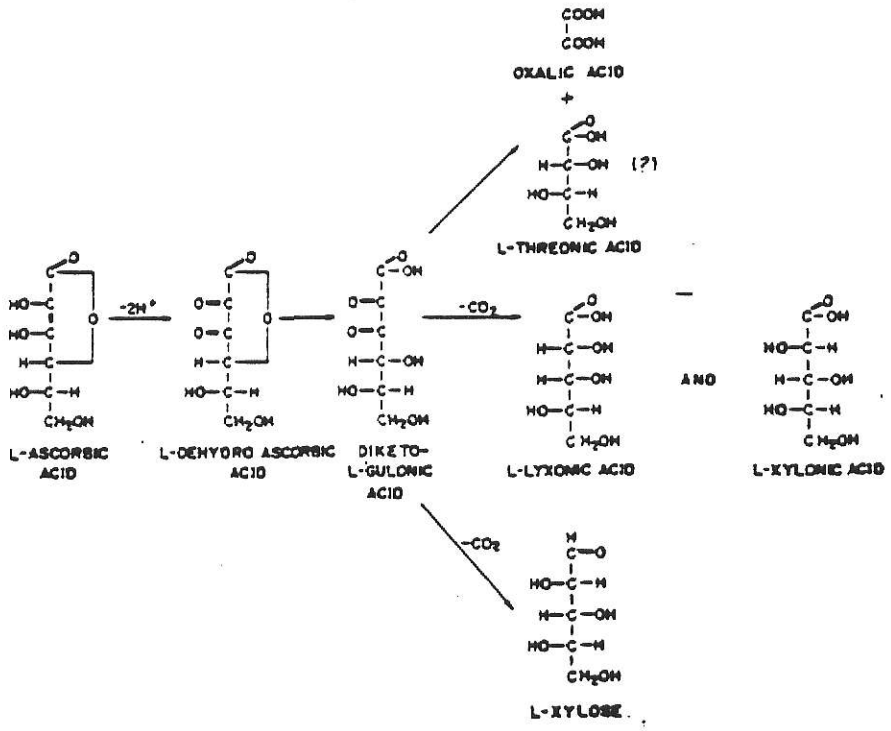

Fig. 3 Ascorbic acid catabolism (30).

diffusion in the rat and by active transport in the guinea pig.

Hornig et al. (21) suggested that the pituitary regulates the uptake and transport of AsA by the tissues. In rats, hypophysectomy was followed by a decreased uptake of AsA in the adrenal gland, ovaries, pancreas, spleen, lungs, paratid glands, cerebrum, cerebellum, and eyes, but not the kidneys, liver, skeletal and heart muscles, skin, and adipose tissues. Hornig also postulated that the pituitary influences transport kinetics for AsA. Without the pituitary there was a linear relationship between the uptake of labeled AsA and the elapsed time after dosage, suggesting absorption by passive diffusion. He concluded that the pituitary gland is in some way necessary for active transport of AsA.

Tissue distribution of ascorbic acid

Ascorbic acid is present in most tissues of the body, but it is especially concentrated in tissues with high levels of biosynthetic and metabolic activity. In humans, the highest concentrations of AsA are found in the adrenal glands and pituitary gland. Other tissues with high AsA levels are the brain, liver, spleen, pancreas, eye lens, kidney, and heart (34). The tissue concentration of AsA in guinea pigs and rats follow a pattern similar to that of humans, except in rats the AsA concentrations are much greater (31). Table 1 illustrates the tissue concentrations in the rat.

TABLE 1

Tissue concentrations of ascorbic acid in rats (31)

Tissue	Ascorbic Acid (mg/100 g tissue)
Adrenal glands	280-400
Pituitary gland	100-130
Liver	25-40
Spleen	40-50
Lungs	20-40
Kidneys	15-20
Testes	25-30
Thyroid	22
Thymus	40
Brain	35-50
Eye lens	8-10
Skeletal muscle	5
Heart muscle	5-10
Bone marrow	12
Plasma	1.6
Blood	0.9

Hornig (31) observed significant differences in the tissue concentrations between male and female rats. Male rats had significantly greater AsA content in the tissues attributed primarily to the greater hepatic enzyme activities involved in AsA synthesis.

Factors that influence the synthesis of AsA also affect its concentration in tissues. Such factors include decreased dietary intake, undernourishment, adrenalectomy, hypophysectomy, and castration (31). Age also influences AsA concentration in rats. AsA content increases in the rat brain during fetal development and reaches the highest concentration about 4 days after birth. Then AsA concentrations steadily decline to adult levels (35).

The adrenal cortex, and to a lesser extent the kidneys, are the storage sites for AsA in the rat (19). The predominant storage form is

L-AsA with smaller amounts of dehydroascorbic acid and 2,3-diketogulonic acid (31). Hornig (31) suggested that the presence of the latter two metabolites might be due to metabolism continuing after decapitation or possibly decomposition during analysis.

Functions of Ascorbic Acid

Collagen synthesis

Ascorbic acid has an important role in the synthesis of collagen. Collagen is a fibrous protein found in the skin, bones, tendons, cartilage, blood vessels and teeth. Collagen has a structural role in mature tissues and a directive role in the growth of developing tissues (36).

Glycine, proline, hydroxyproline, and hydroxylysine are the main amino acids present in collagen. In the first step of collagen synthesis, prolyl and lysyl residues are incorporated into the peptide chain linkage. Then hydroxylation of the prolyl and lysyl residues to hydroxyproline and hydroxylysine occurs concurrently with translation while the polypeptide chain is still attached to the ribosomes (37). After hydroxylation, three chains join to form a helical unit known as "procollagen" which then form tropocollagen fibrils. These fibrils, in turn, are converted into insoluble, extracellular collagen fibers.

Ascorbic acid does not influence the first step, synthesis of the polypeptide chain, but all succeeding steps rely upon the second step, hydroxylation of proline and lysine, which is dependent upon AsA (38,39). The enzyme necessary to convert proline to hydroxyproline is prolyl hydroxylase. This enzyme has a ferrous ion at its active site,

which requires a reducing agent such as AsA to keep it in its ferrous state (36). Without hydroxylation of proline, normal collagen cannot be synthesized and symptoms of scurvy can occur.

Iron absorption, transport, and storage

Ascorbic acid and ATP are required to incorporate plasma-bound iron into ferritin in the liver tissues (40). Most iron present in the plasma is in the form of an iron-protein complex, transferrin. In this complex, the iron atoms are tightly bound to the protein and can be released only by reduction from the ferric state to the ferrous state. Mazur (40) suggested that ATP, AsA and iron forms an activated complex which increases the flow of electrons, causing the reduction of ferric to ferrous iron. Once iron in the ferrous state is released from its linkage to transferrin it is subsequently incorporated into tissue ferritin (41).

To supply iron for body processes, the iron in ferritin must be released. Although most of the iron in ferritin is believed to be located inside the protein molecule, some iron is located at or near the molecule surface. The release of ferritin-bound iron in the liver involves an interaction between the surface iron and the reduced form of the enzyme xanthine oxidase. The iron is then available to bind to plasma transferrin (40).

Lipschitz and coworkers (42) showed that AsA deficiency resulted in changes in iron stores in tissues. In guinea pigs deprived of AsA, non-heme iron concentration decreased in the liver and it increased in the spleen. In addition to the redistribution of iron, the proportion

ferritin decreased relative to hemosiderin, another storage form of iron. When AsA nutriture was brought to normal, tissue contents of these iron complexes were similar to those of control animals.

Ascorbic acid and stress

Ascorbic acid has been shown to be involved in the physiological responses to stress in animals. A particularly interesting feature about AsA is that under stress conditions its intake appears to benefit animals that do not ordinarily require this vitamin in their diets. Growth retardation caused by mineral toxicities in chicks was reduced by large doses of AsA (10). Siegel (13) found that mice supplemented with AsA exhibited higher levels of circulating interferon after injection with murine leukemia virus than those not receiving AsA.

Subramarian et al. (43,44), Nandi (45), and Chatterjee (15) suggested that AsA could be beneficial under stress conditions through its effects on histamine metabolism. Nandi and coworkers (45) found that rats supplemented with large doses of AsA and exposed to various stresses such as temperature extremes, toxoid administration, or inadequate diets, excreted less urinary histamine than the control rats not receiving AsA. Chatterjee (15) found that the increased histamine formation induced by a drug or toxin in the rat liver was paralleled by an increased AsA biosynthesis. He postulated that the increased AsA synthesis was a response to the greater need for histamine detoxification.

There are differences between the antihistaminic properties of AsA and antihistamine drugs. Nandi and colleagues (45) found that in

vivo auto-oxidation of L-AsA in the presence of histamine inactivated the histamine by rupturing its imidazole ring. Antihistamine drugs, on the other hand, competitively bind to the histamine receptor on the cell membrane.

Effects of High Ascorbic Acid Intake During Pregnancy and Lactation

The relationship between vitamin C and mammalian reproduction is not well understood. However, ovaries and testes contain a fairly high level of AsA (14), suggesting that it may be involved in reproduction. Changes in ovarian and uterine AsA have been noted during pregnancy in Wistar albino rats and Swiss albino mice (46).

Maintenance of pregnancy

Results from several studies indicate that large doses of AsA have either no effect or detrimental effects on pregnancy maintenance. Sambroskaya and Ferdman (47) found that rats receiving large daily doses of vitamin C (833 mg/kg sc) had a higher rate of abortion than non-supplemented rats. These findings were corroborated by Fahim and coworkers (48) using doses of 250 mg/kg po. No effects on abortion rate in guinea pigs, rats, and hamsters were found by Alleva and coworkers (49) using oral dosages of 50-450 mg/kg/day. Fromberg and associates (50) reported that no effects on pregnancy were found in rats given oral dosages of 50-1000 mg/kg/day, or in mice given oral dosages of 250-1000 mg/kg/day.

Others reported beneficial effects of high AsA intake on pregnancy. Paul and Duttagupta (51) showed that when the diet of pregnant rats

was restricted to non-pregnant levels, they failed to remain pregnant. Injection of the diet-deficient pregnant rats with AsA (25 mg/rat/day) was followed by an improved ability to maintain pregnancy, and more optimal levels of glycogen and blood glucose. They postulated that although the exact role of vitamin C during pregnancy is unknown, it seems to be involved in regulating carbohydrate metabolism in the liver, uterus, and blood. They further suggested that since AsA supplementation is able to maintain carbohydrate stores in underfed rats, endogenous AsA synthesis may be affected by diet restriction. Paul and Sarathchandran (52) found that pregnant rats receiving AsA (25 mg/rat/day) consumed less feed but had similar weight gains when compared to non-supplemented pregnant rats. The effects of high AsA intake during lactation were not tested.

Placental transfer of ascorbic acid

Although the adult rat is able to endogenously synthesize AsA, the fetal rat does not have the necessary enzymatic activity until the 20th day of gestation. Adult levels of tissue ascorbic acid are not attained until two weeks after birth (18). Because of this inability to synthesize AsA, the fetus must obtain the vitamin from its mother through placental transfer.

In an attempt to determine the rate of placental transfer of AsA, Rosso and Norkus (53) surmised that AsA requirements should parallel changes in fetal growth and metabolic rate. To examine this possibility, they designed a study to determine whether changes in fetal AsA requirements parallel changes in placental transfer of AsA. Pregnant

rats were injected with L-(1-^{14}C) AsA (3-4 mCi/mole) and samples of the maternal blood, one placenta, and one fetus were removed immediately after injection. This procedure was repeated at several stages of pregnancy. Results obtained showed that throughout gestation the placenta was able to concentrate labeled AsA better than the fetus. The transfer of AsA from the placenta to the fetus remained nearly constant per gram of fetal body weight throughout gestation, indicating an increased uptake of AsA by the growing fetus as gestation progressed.

Hammarstrom (29) injected labeled AsA intravenously (9-18 mg/kg) into pregnant rats and mice and measured AsA concentration in various tissues of the mothers and fetuses 4 hours later. He found a very low concentration of the vitamin in the fetal blood, and that fetal uptake was greatest in the adrenal and retina. Fetal distribution was similar to maternal distribution except that the fetus had a slightly higher AsA concentration in the bones and cartilage. He attributed this difference to the prominent role AsA plays in collagen synthesis and in facilitating rapid growth of tissues.

Effects of high ascorbic acid intake on litter size

Ascorbic acid supplementation prior to and during pregnancy seems to have no effect on litter size in rats. Nandi (54) divided male and female rats into a control group and an AsA-supplemented group (100 mg/100g body wt/day). AsA supplemented rats received vitamin C for two weeks prior to and during mating. Once pregnant, the females were separated from the males and those in the supplemented group continued to receive AsA during gestation and lactation. No significant

differences were found when comparing litter size or birth weights of pups born to AsA-supplemented mothers to those of control mothers. In a similar study, Alleva and coworkers (49) supplemented the diets of 3-month-old Holtzman rats with 0, 50, 150, or 450 mg of AsA/kg daily from the first through the 19th day of pregnancy. No differences were found between the groups for litter size, abortion rate, or mortality of offspring.

MATERIALS AND METHODS

Animals and Their Care

Forty-eight female and sixteen male random-bred Swiss albino mice were obtained from our departmental breeding colony. Virgin females 8 to 9 weeks of age and weighing 25 to 30 g were randomly assigned to breeding cages with a ratio of three females to one male. Room conditions were maintained at 21 to 24^{o} with a 12-hour light-dark cycle. Throughout the study animals had free access to a commercial stock diet[1] and deionized drinking water unless otherwise stated.

At the time of mating (day 0), female mice were assigned to one of four treatment groups:

CON: Control; no supplementary ascorbic acid.

PREG: Ascorbic acid supplementation during pregnancy only (days 0 to 22).

LAC: Ascorbic acid supplementation during lactation only (days 22 to 45).

P & L: Ascorbic acid supplementation during pregnancy and lactation (days 0 to 45).

As shown in figure 4, the cages were arranged within the battery so that the different treatment groups would have uniform exposure to variables such as light intensity, ventilation, and disruption. Each cage contained one male and three females.

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[1]Purina® Rodent Laboratory Chow 5001, St. Louis, MO.

P & L	Lac	Preg	Con
Preg	Con	P & L	Lac
Lac	Preg	Con	P & L
Con	P & L	Lac	Preg

Fig. 4 Random allocation of female breeders to treatment groups.

Ascorbic acid[1], when administered, was given in the drinking water at a concentration of 250 mg per 100 ml water during the late afternoon and evening hours. That treatment had been used previously for mice (13,55), and we found in preliminary studies that it did not restrict water intake.

Females were removed from breeding cages one week following matings and transferred to individual solid-bottom polypropylene cages, where they remained until their pups were weaned. Females were weighed at least twice weekly throughout pregnancy and lactation; they were also weighed daily for 3 days prior to the anticipated day of birth to obtain weight at term. Feed consumption and water intake were recorded for each female over 3-day periods during the third week of pregnancy and the third week of lactation.

Pups were born approximately 3 weeks following matings. The majority of females had their pups within a 48-hour period; these were

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[1]L-Ascorbic acid, ICN Nutritional Biochemicals, Cleveland, OH.

the pups used in our study. On the day of birth each litter was adjusted to 8 pups, because this size was previously shown to be optimal for the growth of mice (56). The litter size adjustment was also necessary to provide a uniform nutrient supply among pups. Because our litter size ranged from 4 to 15 pups, we randomly selected 8 mice from larger litters using a table of random numbers (57). If a litter size was less than 8 we added an orphan of similar age obtained from another litter. Pups were identified by toe clipping procedures on the day of birth and later during the third week of age by ear punch procedures. Orphans were not included for the measurements performed on the pups. The mouse pups were weighed twice weekly from birth to 45 days of age.

After a 24-day lactation period, the pups were separated from their mothers and two male pups from each litter were randomly selected and sacrificed. The tissues obtained from one pup were analyzed for ascorbic acid contents and the tissues obtained from the other pup were analyzed for DNA, RNA, and protein contents. The remaining male pups received the stock diet and deionized water ad libitum until they were 45 days of age, when they were sacrificed. The tissues from the 45-day-old mice were analyzed for DNA, RNA, and protein.

Tissue Preparation

Mice were fasted 12-14 hours before sacrificing by cervical dislocation. Each mouse was then weighed and its liver, kidneys, and brain were removed, blotted, weighed, wrapped in aluminum foil, frozen in liquid nitrogen, and stored at -18^{o} for later analysis. In animals sacrificed at 24 days, adrenals were also removed and immersed in 1.5 ml

cold 5% trichloroacetic acid for immediate analysis of ascorbic acid. Tissues were homogenized in a Potter-Elvejem-type tissue homogenizer (Wheaton Instruments, Millville, N.J.).

Determination of Total Ascorbic Acid (58)

Principle. Tissue protein is precipitated by trichloroacetic acid (TCA) during homogenizing procedures. After centrifugation, the protein-free supernatant is diluted and dinitrophenylhydrazine-thiourea-copper sulfate reagent (DTC) is added. The copper ions oxidize ascorbic acid to dehydroascorbic acid, which then forms an orange complex with 2,4-dinitrophenylhydrazine during a 4-hour incubation period. The orange complex is dissolved in sulfuric acid and is measured spectrophotometrically. This procedure measures both L-ascorbic acid and dehydroascorbic acid and therefore provides an estimate of total ascorbic acid.

Procedure. The flow chart for the determination of ascorbic acid in liver, kidney, brain, and adrenal tissues is shown in figure 5. Eight samples were homogenized at a given time including at least one sample from each treatment group. A standard (0.04 mg ascorbic acid/ml) and a blank (5% TCA) were included with each run to correct for variation between experimental runs. Absorbance of the sample homogenates, standard, or blank, were read at 515 nm on a Bausch and Lomb Spectronic 20 Spectrophotometer. Ascorbic acid contents of the homogenates were determined by linear regression using the blank and standard concentration and absorbance values as reference points. These values were then multiplied by dilution factors employed in the initial

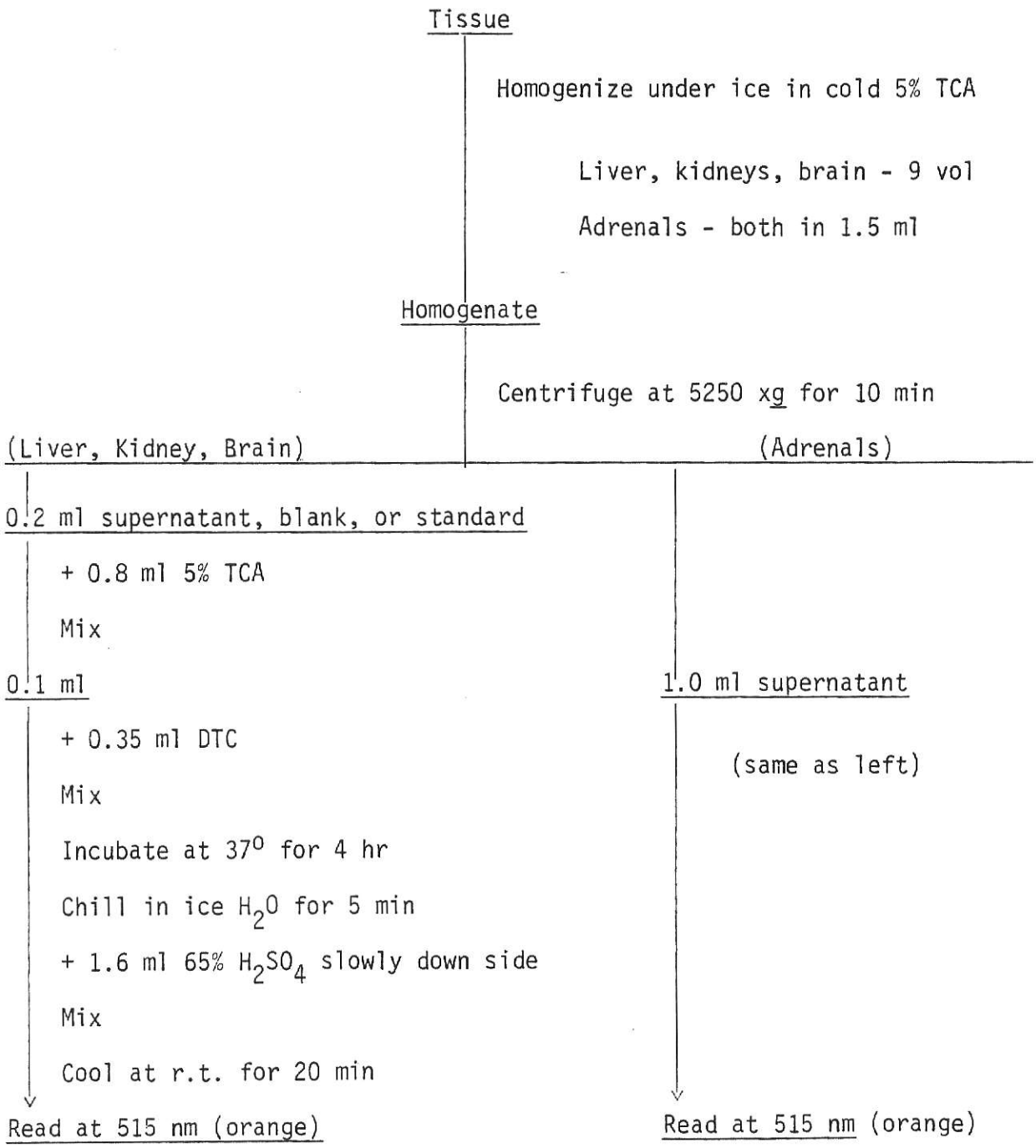

Fig. 5 Flow chart for the determination of ascorbic acid in tissues of mice. Composition of reagents is listed in appendix table 1.

homogenization procedures. Liver, kidney and brain values were multiplied by a dilution factor of 10 to get mg/100 g tissue. Adrenal values were multiplied by 0.6 to get µg ascorbic acid in the total tissue.

Determination of Protein by the Biuret Procedure (59)

Principle. Under alkaline conditions copper sulfate in the biuret reagent reacts with compounds containing two or more peptide bonds to give a violet-colored complex.

Procedure. The biuret assay for protein is shown in figure 6. A standard (5 mg bovine serum albumin/ml) and a blank (deionized water) were included in each experimental run. In tubes containing brain homogenates we observed a turbidity which interferred with the measurement of the violet-colored complex. To correct for this, a blank containing 0.1 ml homogenate, 0.9 ml 1 N NaOH and 1.5 ml biuret reagent without the $CuSO_4$ was prepared for each brain sample and subtracted from sample readings.

Estimation of the Nucleic Acids

Initially deoxyribonucleic acid (Type 1: calf thymus[1]) and ribonucleic acid (Type IV: calf liver[1]) were used as the nucleic acid standards. Unfortunately we found them very expensive for routine inclusion in the experimental runs; also they were not easily solubilized in water. Because our methods actually analyze for the pentose contents,

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[1]Sigma Chemical Company, St. Louis, MO.

deoxyribose and ribose were substituted for the DNA and RNA respectively. One mg deoxyribose was found to give the same absorbance reading as 6.67 mg calf thymus DNA; and one mg ribose gave the same absorbance reading as 5.87 mg calf liver RNA after they were run in parallel through the analytical procedures. These factors were used to estimate the nucleic acid contents of tissues after pentose contents were determined.

Extraction of Nucleic Acids. Nucleic acids were extracted from the homogenate by a modification of the method of Zile et al. (60). The proteins and nucleic acids were precipitated with 10% TCA and heated at 95^{o} to hydrolyze the precipitate. The soluble fraction containing the nucleic acids was then assayed for deoxyribose by the diphenylamine reaction (59), and ribose by the orcinol reaction (61).

Estimation of DNA by the Diphenylamine Reaction (59)

Principle. Deoxyribose is assayed under acid conditions by its conversion to α-hydroxylevulinaldehyde, which reacts with diphenylamine to give a blue complex.

Procedure. The assay for deoxyribose is shown in figure 6. A pentose standard and a blank were run parallel with each run of samples. Deoxyribose contents were determined by linear regression and multiplied by the appropriate dilution factors (kidney-11, liver-7, and brain-4). Deoxyribonucleic acid contents (mg/g wet wt) were estimated by multiplying the deoxyribose contents by 6.67.

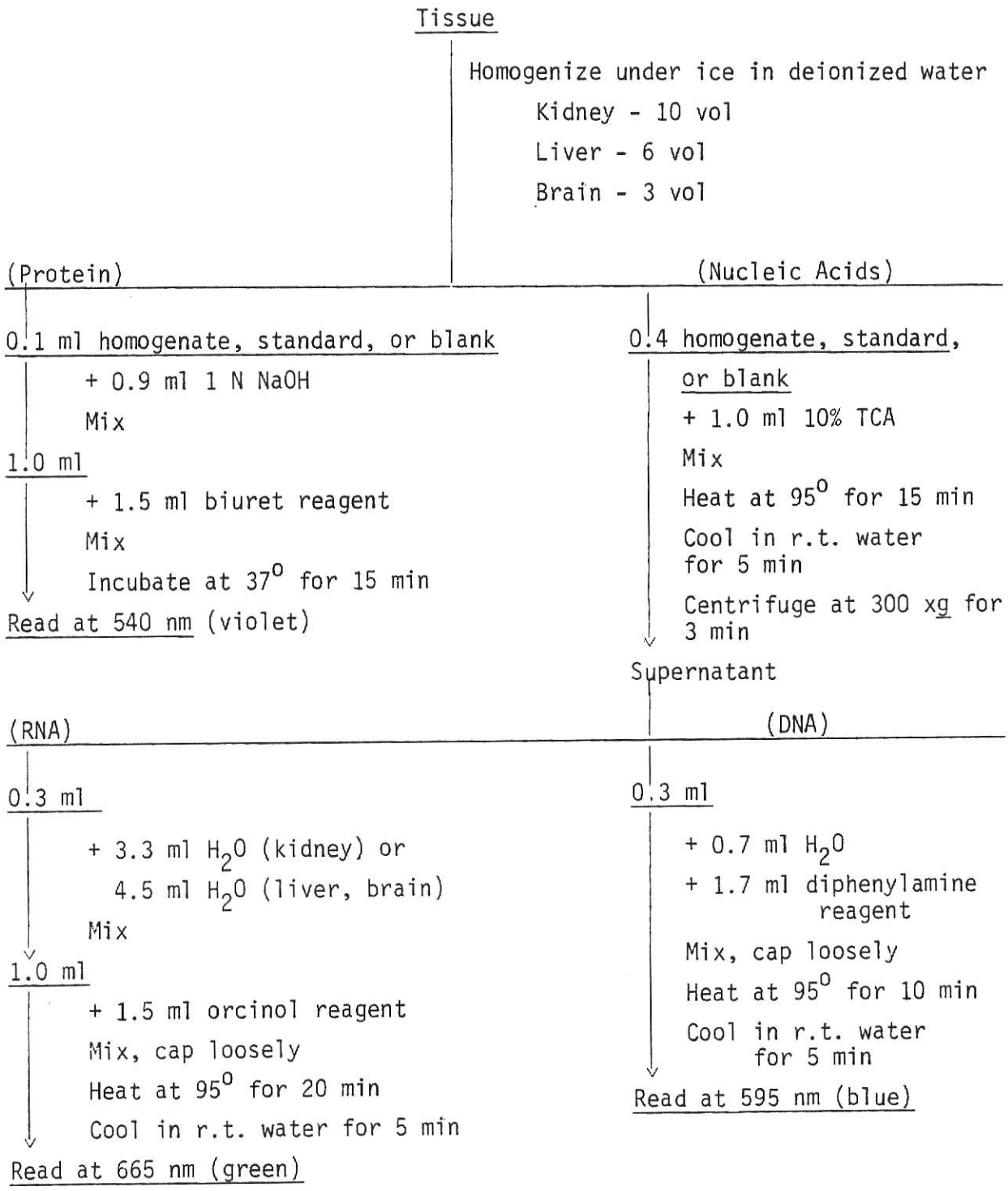

Fig. 6 Flow chart for the determination of protein, DNA, and RNA in tissues of mice. Composition of reagents is listed in appendix table 2.

Estimation of RNA by the Orcinol Reaction (61)

Principle. The soluble fraction used in the deoxyribose assay was also used for ribose determination. In the presence of HCl, ribose is converted to furfural. Orcinol reacts with furfural in the presence of ferric chloride to give a green color measureable spectrophotometrically.

Procedure. The assay for ribose is shown in figure 6. Kidneys were diluted differently than brains and livers because they had lower ribose contents. A pentose standard and a blank were run parallel with each run of samples. Ribose contents were determined by linear regression and multiplied by the appropriate dilution factors (kidney-11, liver-7, and brain-4). Ribonucleic acid contents (mg/g wet wt) were estimated by multiplying ribose contents by 5.87.

Statistical Analyses

Ascorbic acid effects were determined by Least Significant Difference Tests following significant ($P < 0.05$) Analysis of Variance procedures (62). The sources of variation in the anova table were:

Source	df
AsA	3
Female (AsA)	20

Female (AsA) was included as a source of variation because of normal variations between females. For example, larger females were likely to have larger pups and it is necessary to correct for this source of

variation. Our statistical analyses were run using the computerized SAS (Statistical Analysis System) programs (63). A sample computer program for data collected from mouse pups is shown in appendix table 3.

RESULTS AND DISCUSSION

Maternal Feed Intake and Weight Gain During Pregnancy and Lactation

Maternal responses to ascorbic acid supplementation are presented in table 2. Ascorbic acid supplementation during gestation did not appear to affect weight gain, feed consumption, or water intake of the pregnant females. In contrast to our results, Paul and Sarathchandran (52) reported that excess ascorbic acid administered during pregnancy significantly reduced food intake in rats. Lack of agreement may be attributed to the different dosages of AsA used in that study.

Administration of ascorbic acid during pregnancy had no apparent effect on the length of gestation, average litter size, or average birth weight of the pups. In previous studies with rats, litter size, body weight, abortion rate, and mortality rate were not influenced by high ascorbic acid intake (49, 54). Other researchers, however, found that ascorbic acid-fed rats had higher abortion rates (47,48). It is probable, although not proven, that the pregnant mice excreted the excess ascorbic acid so that fetal exposure was minimized.

Administration of ascorbic acid during the period of lactation did not significantly affect the weight gain of the nursing females. However, females receiving the AsA regimen had significantly lower ($P < 0.05$) water consumption than the non-supplemented mice during this period of time. The supplemented mice consumed approximately 25 ml less water over a three-day period than the control animals. A

TABLE 2

Maternal responses to ascorbic acid-supplemented diets[1]

Measurements	Control	AsA-supplementation		
		Preg.	Lac.	P & L
Pregnancy	(7)[2]	(5)	(6)	(7)
Weight at mating (g)	28.4±0.4	28.6±0.5	27.6±0.4	28.5±0.4
Weight at term (g)	51.9±1.2	53.5±1.5	52.3±1.3	52.2±1.2
Weight gain (g)	23.5±1.1	24.9±1.3	24.7±1.2	23.7±1.1
3-Day feed consumption (g)	18.5±0.8	18.1±1.0	17.6±0.9	18.9±1.1
3-Day water consumption (ml)	40.0±2.2	36.6±2.6	42.3±2.3	43.0±2.1
Length gestation (days)	21.2±0.4	21.0±0.0	21.0±0.0	21.4±0.5
Mean birth weight of pups (g)	1.40±0.4	1.40±.05	1.38±.04	1.40±.04
Lactation				
Weight after birth (g)	33.8±0.7	33.1±0.8	32.6±0.7	34.1±0.7
Weight at weaning (g)	36.9±0.7	33.1±0.8	32.6±0.7	34.1±0.7
Weight gain (g)	3.0±0.7	3.9±0.8	2.2±0.7	2.4±0.7
3-Day feed consumption (g)	40.9±2.9	48.2±3.3	40.1±3.0	44.3±2.8
3-Day water consumption (ml)	102.5±5.2	110.8±6.1	84.5±5.6[a]	78.7±5.2[a]

[1]Values are mean ± SEM. [2]Number of animals per treatment group.

[a]$P < 0.05$ when compared to control mice.

numerically lower, but not significant, weight gain was observed in the nursing females during the lactation period.

Feed consumption during the 3rd week was approximately double that during the 3rd week of pregnancy, owing to the greater provision of milk nutrients by the nursing females.

Weight Gain and Tissue Growth

Growth responses in mouse pups from control and ascorbic acid-supplemented females are presented in table 3. Ascorbic acid administration during pregnancy and/or lactation did not appear to have any effect on the weight gain of pups during weaning or during a later stage of growth, between 24 and 45 days of age.

No differences in weight gain, feed intake, or feed efficiency were noted between treatment groups during the post-weaning period.

The effects of ascorbic acid-supplementation on the growth of various organs during weaning and post-weaning are presented in table 4. The weight of the liver, kidneys, and brain was measured, and recorded as both actual weight and as a percentage of body weight. Tissue weights were not significantly different among treatment groups when taken from mice at 24 and 45 days of age.

Tissue Ascorbic Acid Contents

Table 5 illustrates the tissue ascorbic acid contents of weanling pups from control and ascorbic acid-supplemented females. No significant differences were noted between treatment groups. Rosso and Norkus (53) have shown that rats do not attain adult levels of ascorbic acid until about two weeks after birth; so much of that which is present is likely

TABLE 3

Growth of pups from control or ascorbic acid-supplemented females[1]

Measurements	Control	AsA-supplementation		
		Preg.	Lac.	P & L
Pre-weaning	(25)[2]	(21)	(19)	(26)
Body weight (g)				
Birth weight	1.43±.04	1.43±.06	1.42±.05	1.44±.05
Weaning weight	8.78±.67	8.54±.96	7.88±.72	8.81±.70
Weight gain	7.35±.67	7.06±.95	6.38±.69	7.38±.69
Post-weaning	(13)	(11)	(8)	(14)
Body Weight (g)				
Begin. wt. (day 24)	9.08±.71	9.45±.83	8.51±.93	9.11±.70
End wt. (day 45)	25.1±.10	25.4±1.1	25.0±1.3	23.9±0.9
Weight gain	16.0±0.5	15.9±0.6	16.5±0.6	14.8±0.5
3-Day feed intake (g)	11.5±0.8	13.3±1.5	12.5±1.6	14.8±1.3
Feed efficiency (g wt gain/g feed)	0.28±.35	0.27±.03	0.28±.37	0.27±.47

[1]Values are mean ± SEM. [2]Number of animals per treatment group. There were no significant differences between treatment groups.

TABLE 4

Tissue weights of pups from control or ascorbic acid-supplemented females[1]

		AsA-supplementation		
Tissues	Control	Preg.	Lac.	P & L
Age, 24 days	(12)[2]	(10)	(11)	(12)
Liver (g)	0.35±.04	0.35±.04	0.29±.05	0.36±.04
% of body wt.	4.20±.19	3.90±.20	3.88±.21	4.06±.19
Kidney (g)	0.11±.01	0.13±.01	0.10±.01	0.11±.01
% of body wt.	1.30±.06	1.44±.06	1.30±.06	1.36±.06
Brain (g)	0.37±.01	0.37±.01	0.36±.01	0.38±.01
% of body wt.	4.68±.36	4.49±.38	5.24±.39	4.86±.36
Age, 45 days	(13)	(11)	(8)	(14)
Liver (g)	1.25±.06	1.30±.07	1.32±.08	1.22±.06
% of body wt.	5.27±.12	5.40±.13	5.50±.14	5.46±.11
Kidney (g)	0.36±.03	0.41±.06	0.38±.04	0.36±.05
% of body wt.	1.52±.06	1.68±.06	1.60±.07	1.59±.05
Brain (g)	0.44±.01	0.44±.01	0.43±.01	0.43±.01
% of body wt.	1.86±.07	1.85±.07	1.82±.08	1.93±.06

[1]Values are mean ± SEM. [2]Number of animals per treatment group. There were no significant differences between treatment groups.

TABLE 5

Tissue ascorbic acid contents in 24-day-old mouse pups[1]

Tissue	Control	AsA-supplementation		
		Preg.	Lac.	P & L
	(5)[2]	(5)	(5)	(5)
Liver (mg/100 g wet wt.)	25.7±1.9	25.6±1.9	30.0±1.9	32.2±1.9
Kidney (mg/100 g wet wt.)	20.4±1.8	18.0±1.8	24.9±1.9	22.1±1.8
Brain (mg/100 g wet wt.)	42.6±1.5	42.7±1.5	45.7±1.6	43.5±1.5
Adrenals (µg total)	11.1±1.5	10.9±1.3	9.3±1.2	12.9±1.2

[1]Values are mean ± SEM. [2]Number of animals per treatment group. There were no significant differences between treatment groups.

from the mother's milk. Assuming this is also true for mice, one might expect that excess amounts of ascorbic acid in female mice may also be transferred to the offspring via the milk. Our data indicated that if excess ascorbic acid is transferred to the offspring it does not accumulate in their tissues. Perhaps the females had an effective means of disposing excess ascorbic acid without transferring it to the pups, or possible, the pups disposed of excess levels received. Another possibility is that since females receiving ascorbic acid during lactation had a restricted water intake, they may not have been receiving as large a dosage as expected.

Cellular Measurements in Brain Tissue

Measurements of DNA, RNA, and protein content were taken to determine the effects of high ascorbic acid intake on cellular growth during the critical developmental periods of pregnancy and lactation. Since DNA is constant within a single diploid cell in any species, measurements of DNA can be used to determine cell number (64). A mammalian cell contains 6 pg of DNA per cell (65). A measurement of DNA per gram of tissue can be used to determine cellularity, and a ratio of protein to DNA content can be used to determine cell size (65).

Tissue weight, total DNA, DNA per gram of tissue, total protein, protein per gram of tissue, protein/DNA ratio and RNA per gram of brain tissue of mouse pups from different treatment groups are illustrated in table 6. No major differences were noted between treatment groups at weaning (24 days) or post-weaning (45 days). A general comparison,

TABLE 6

Cellular measurements in brains of pups from control or ascorbic acid-supplemented female mice[1]

Measurements	Control	AsA-supplementation		
		Preg.	Lac.	P & L
Age, 24 days	(7)[2]	(6)	(5)	(7)
Tissue wt. (g)	0.37±.01	0.37±.01	0.36±.01	0.38±.01
Total DNA, mg/tissue	1.26±.10	1.29±.11	1.18±.12	1.17±.10
DNA, mg/g wet wt.	3.41±.24	3.38±.26	3.25±.29	3.11±.24
Protein, mg/g wet wt.	144±11	156±11	144±12	130±11
Protein/DNA	42.0±2.3	46.8±2.5	45.2±2.7	42.1±2.3
RNA, mg/g wet wt.	8.16±.34	7.94±.37	7.34±.40	7.66±.34
Age, 45 days	(13)	(11)	(8)	(14)
Tissue wt. (g)	0.44±.01	0.44±.01	0.42±.01	0.43±.01
Total DNA, mg/tissue	1.52±.06	1.49±.07	1.41±.08	1.39±.06
DNA, mg/g wet wt.	3.47±.13	3.37±.14	3.34±.16	3.23±.12
Protein, mg/g wet wt.	126±5	126±6	135±7	136±5
Protein/DNA	37.1±1.9	38.0±2.3	39.4±2.5	40.6±1.9
RNA, mg/g wet wt.	7.95±.28	8.08±.33	7.34±.37	7.77±.28

[1]Values are mean ± SEM. [2]Number of animals per treatment group. There were no significant differences between treatment groups.

however, between brains of 24-day-old mice and those of 45-day-old mice reveals that brain size increased approximately 17% and DNA, or cell number, increased 19%. In the rat, DNA synthesis and thus cell division ceases at about 20 days postnatally, and in the mouse most cell division ceases one or two days earlier (65). This is corroborated by our data which indicated that most, but not all, cell division had stopped by this time. A somewhat lower protein/DNA ratio was observed at day 45, than at day 24. This finding might be explained by the fact that in the brain cell enlargement is also due to deposition of myelin and other lipid materials which were not measured by our methods.

Cellular Measurements in Kidney Tissue

The cellular measurements of the kidney in the control and supplemented mouse pups are shown in table 7. These measurements were performed in kidneys since kidneys of rodents have a fairly high content of ascorbic acid (31) and also excrete ascorbic acid and its metabolites. Statistical analyses of the measurements for tissue weight, total DNA, DNA per gram of tissue, protein content, DNA/protein and RNA content, indicated no differences between treatment groups in either 24-day-old or 45-day-old mice. Between days 24 and 45 the average weight of the kidneys increased approximately 228%. During this time the total DNA (cell number) and protein/DNA content (cell size) increased 137% and 74% respectively. In rats the DNA in the kidney increases rapidly from birth to about 30 days, and thereafter, increases at a slower rate (64). Cellular growth of visceral organs such as kidneys has been previously studied in mice (67).

TABLE 7

Cellular measurements in kidneys of pups from control or ascorbic acid-supplemented female mice[1]

Measurements	Control	AsA-supplementation		
		Preg.	Lac.	P & L
Age, 24 days	(7)[2]	(6)	(5)	(7)
Tissue wt. (g)	0.11±0.1	0.13±.01	0.10±.01	0.12±.01
Total DNA, mg/tissue	1.29±.09	1.40±.11	1.13±.01	1.21±.10
DNA, mg/g wet wt.	12.9±1.9	10.8±1.0	11.8±0.9	11.4±0.8
Protein, mg/g wet wt.	272±19	264±23	233±20	253±19
Protein/DNA	21.6±2.3	24.8±2.7	20.4±2.4	23.2±2.3
RNA, mg/g wet wt.	16.9±1.4	14.4±1.6	14.6±1.5	16.6±1.4
Age, 45 days	(13)	(11)	(8)	(14)
Tissue wt.	0.36±.03	0.41±.03	0.38±.04	0.36±.03
Total DNA, mg/tissue	2.86±.17	3.32±.21	2.98±.22	2.75±.17
DNA, mg/g wet wt.	7.94±.39	8.22±.48	7.95±.50	7.51±.39
Protein, mg/g wet wt.	322±11	305±14	306±15	318±11
Protein/DNA	40.9±3.7	37.8±2.5	39.6±4.7	38.5±3.5
RNA, mg/g wet wt.	14.2±0.5	14.2±0.6	13.2±0.6	13.8±0.5

[1]Values are mean ± SEM. [2]Number of animals per treatment group. There were no significant differences between treatment groups.

Cellular Measurements in Liver Tissues

Table 8 shows the cellular measurements in the livers of the control and ascorbic acid-supplemented mouse pups.

Analysis of our data indicated that there were no significant differences in the liver weight, cell number and cell size between treatment groups; thus it appears that large intakes of ascorbic acid during pregnancy and lactation does not affect the growth and development of the liver in the offspring of mice.

Liver weights were approximately 282% heavier in 45-day-old mice than in 24-day-old mice. Total DNA (cell number) was 201% higher in the older mice. The measurement of DNA in liver tissues may not accurately reflect the number of hepatocytes since liver cells may contain more than one nucleus (68), and hence more DNA. Others, however, have used these measurements to indicate number of liver cells (69,70). Winick (64) reported that rats continue to develop new liver cells until about 120 days after birth; this study suggests that the number of liver cells also increase post-weaning in mice.

TABLE 8

Cellular measurements in livers of pups from control or ascorbic acid-supplemented female mice[1]

Measurements	Control	AsA-supplementation		
		Preg.	Lac.	P & L
Age, 24 days	(7)[2]	(6)	(5)	(7)
Tissue wt. (g)	0.36±.03	0.35±.03	0.29±.13	0.33±.03
Total DNA, mg/tissue	2.38±.28	2.54±.30	2.49±.33	2.46±.27
DNA, mg/g wet wt.	7.12±.48	7.97±.51	8.54±.57	7.70±.48
Protein, mg/g wet wt.	247±11	276±13	278±14	282±12
Protein/DNA	35.4±2.5	35.5±2.7	33.7±2.9	36.9±2.5
RNA, mg/g wet wt.	23.4±1.6	20.2±1.7	21.5±1.9	19.1±1.6
Age, 45 days				
Tissue wt. (g)	1.25±.07	1.30±.07	1.32±.08	1.23±.06
Total DNA mg/tissue	7.51±.35	7.55±.41	7.71±.46	6.96±.34
DNA, mg/g wet wt.	5.94±.23	5.80±.22	5.94±.31	5.73±.23
Protein, mg/g wet wt.	335±11	315±13	329±14	311±11
Protein/DNA	53.4±2.6	55.2±3.0	56.3±3.4	55.8±2.6
RNA, mg/g wet wt.	23.4±1.1	23.2±1.3	25.2±1.4	24.6±1.1

[1]Values are mean ± SEM. [2]Number of animals per treatment group. There were no significant differences between treatment groups.

SUMMARY

The effects of high ascorbic acid intake during pregnancy and lactation were studied in mice. Forty-eight female mice were randomly assigned to one of four treatment groups: a control group, given no supplementary ascorbic acid; a pregnancy group, given ascorbic acid during pregnancy only; a lactation group, given ascorbic acid during lactation only; and a pregnancy and lactation group, given ascorbic acid during both pregnancy and lactation. Ascorbic acid, when administered, was given in the drinking water at a concentration of 250 mg/100 ml water.

The mice receiving the ascorbic acid during lactation consumed significantly less ($P < 0.05$) water than the control group. However there were no significant differences in weight gain, feed consumption or feed efficiency between females in treatment groups when compared during pregnancy or lactation. Average litter size and average birth weight of mouse pups were not different among treatment groups.

Tissue ascorbic acid contents and growth of male mouse pups from supplemented or nonsupplemented females were also studied. Measurements of growth included body weight, and a determination of tissue weights, cell number (total DNA), and cell size (protein/DNA) in brains, kidneys and livers of 24-day-old and 45-day-old mice. Ascorbic acid, when administered during pregnancy and/or lactation, did not influence any of these measurements in the offspring.

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APPENDIX

APPENDIX TABLE 1

Reagents for Analysis of Total Ascorbic Acid

1. Diluting solution: Dissolve 5 g metaphosphoric acid in 10 ml concentrated glacial acetic acid and adjust volume to 100 ml with deionized water.

2. Working ascorbic acid standard (0.04 mg/ml): Dry 1 g L-ascorbic acid (ICN Nutritional Biochemicals) in a 100° forced-air draft oven for 1 hr and cool in a desiccator for 3-4 hr. Prepare stock standard by dissolving 50 mg ascorbic acid in 50 ml diluting solution. Prepare working standard (0.04 mg/ml) by diluting 2.0 ml stock standard with 48 ml diluting solution. Store 0.5 ml aliquots of working standards at -18° for not more than 1 wk.

3. 0.06% $CuSO_4$: Dissolve 0.06 g $CuSO_4$ in 100 ml deionized water.

4. 9 N H_2SO_4: Add 62.5 ml concentrated H_2SO_4 carefully to 187.5 ml deionized water in the sink.

5. 2,4-dinitrophenylhydrazine reagent (DNP): Dissolve 5.5 g 2,4-dinitrophenylhydrazine reagent (J.T. Baker Chemical Co., Phillipsburg, N.J.) in 250 ml 9 N H_2SO_4, and age overnight at 4°. Filter the following morning through Whatman #1 filter paper.

6. 5% thiourea: Dissolve 5 g thiourea in 100 ml deionized water and store at 4° more than a month.

7. Dinitrophenylhydrazine-thiourea-copper sulfate reagent (DTC): Ten ml 5% thiourea is combined with 10 ml 0.6% $CuSO_4$ and 200 ml DNP reagent. Store at 4° for not more than 1 wk.

8. 5% Trichloroacetic acid (TCA): Dissolve 5 g trichloracetic acid in 100 ml deionized water and store at 4°.

9. 65% H_2SO_4: Add 700 ml concentrated H_2SO_4 carefully to 300 ml deionized water in the sink and store at 4°.

APPENDIX TABLE 2

Reagents for Analysis of Protein, DNA, and RNA

Protein

1. Protein standard: Dissolve 5 g bovine serum albumin[1] in 100 ml deionized water and store at 4° not more than 1 wk.

2. Biuret reagent: Dissolve 3 g copper sulfate ($CuSO_4 \cdot 7H_2O$) and 9 g sodium potassium tartrate in 500 ml of 0.2 N NaOH. Add 5 g potassium iodide to the solution and make up to 1 liter with 0.2 N NaOH. Filter through Whatman #1 filter paper.

Deoxyribose and Ribose

3. Pentose standard: Dissolve 40 mg deoxyribose and 40 mg ribose in 100 ml deionized water. Store at 4°.

4. Diphenylamine reagent: Dissolve 0.5 g diphenylamine[1] in 50 ml glacial acetic acid, mix, and add 1.25 ml concentrated H_2SO_4. Prepare fresh daily.

5. Orcinol reagent: Dissolve 150 mg ferric chloride ($FeCl_3 \cdot 6H_2O$) in 150 ml concentrated HCl and add 5.2 ml of 6% orcinol. Prepare 6% orcinol reagent by dissolving 0.6 g orcinol in 10 ml ethyl alcohol.

6. 10% trichloroacetic acid (TCA): Dissolve 10 g trichloroacetic acid in 100 ml deionized H_2O. Store at 4°.

[1] Sigma Chemical Co., St. Louis, MO.

APPENDIX TABLE 3

Sample SAS[1] Computer Program for LSD Procedure

```
//TISSASA JOB (391601257, XXXXXXXX), MITCHELL
//STEP 1 EXEC SAS
//SAS.SYSIN DD *
DATA WEIGHT;
INPUT CARD 1-2 ASA 4 MALE 6 FEMALE 6-8 PUP 6-9 BIRWT 11-13
ENDWT 15-18 WTCHAN 20-23;
CARDS;
PROC SORT; BY ASA; PROC PRINT; BY ASA;
PROC GLM;
CLASSES FEMALE ASA;
MODEL BIRWT ENDWT WTCHAN = ASA FEMALE (ASA);
TEST H = ASA E = FEMALE (ASA);
LSMEANS ASA/STDERR PDIFF;
/*
```

[1] Statistical Analysis System

APPENDIX TABLE 4

Maternal Responses to Ascorbic Acid-Supplemented Diets During Pregnancy

Female No.	Wt. at Mating	Wt. at Term	Weight Gain*
	(g)	(g)	(g)
	Control[1]		
1	28.9	50.3	21.4
2	29.0	52.9	23.9
3	26.9	52.1	25.2
4	29.5	54.5	25.0
5	28.1	50.3	22.2
6	28.8	53.5	24.5
7	27.5	49.9	22.2
	Pregnancy[2]		
1	29.6	57.8	28.2
2	27.9	53.8	25.9
3	29.5	50.6	21.1
4	29.5	54.6	25.1
5	26.5	50.9	24.4
	Lactation[3]		
1	28.5	57.6	29.1
2	27.2	49.4	22.2
3	27.1	54.2	27.1
4	28.4	55.5	27.1
5	25.7	46.5	20.8
6	28.8	50.8	22.0
	Pregnancy & Lactation[4]		
1	29.1	49.1	20.0
2	28.8	50.4	21.6
3	28.9	51.6	22.7
4	27.7	55.8	28.1
5	28.8	58.5	29.7
6	27.2	47.5	20.3
7	28.8	52.5	23.7

[1] Control - No ascorbic acid supplementation.
[2] Pregnancy - Ascorbic acid supplementation during pregnancy only.
[3] Lactation - Ascorbic acid supplementation during lactation only.
[4] Pregnancy & Lactation - Ascorbic acid supplementation during both pregnancy and lactation.

APPENDIX TABLE 5

Maternal Responses to Ascorbic Acid-Supplemented Diets During Lactation

Female No.	Wt. After Birth	Wt. at Weaning	Weight Gain
	(g)	(g)	(g)
		Control[1]	
1	33.4	36.8	2.4
2	31.4	31.3	-0.1
3	31.5	34.4	2.9
4	34.4	41.9	7.5
5	33.2	38.6	5.4
6	36.6	37.8	1.2
7	36.1	37.8	1.7
		Pregnancy[2]	
1	35.1	40.0	4.9
2	34.5	39.9	5.5
3	32.1	36.4	2.3
4	31.7	36.2	4.5
5	32.3	34.8	2.5
		Lactation[3]	
1	33.8	37.6	3.8
2	31.8	33.1	1.3
3	33.1	35.0	1.9
4	34.4	37.1	2.7
5	32.2	34.4	2.2
6	30.0	31.5	1.5
		Pregnancy & Lactation[4]	
1	31.4	35.1	3.7
2	34.7	38.0	3.3
3	36.7	36.6	-0.1
4	34.7	37.7	3.0
5	33.7	36.4	2.7
6	32.6	35.6	3.0
7	34.9	36.5	1.6

[1] Control - No ascorbic acid supplementation
[2] Pregnancy - Ascorbic acid supplementation during pregnancy only.
[3] Lactation - Ascorbic acid supplementation during lactation only.
[4] Pregnancy & Lactation - Ascorbic acid supplementation during both pregnancy and lactation.

APPENDIX TABLE 6

Three-day Water Intake and Feed Consumption During the Third Week of Pregnancy in Control and Ascorbic Acid-Supplemented Female Mice

Female No.	Feed Consumption	Water Intake
	(g)	(ml)
	Control[1]	
1	21.5	47.0
2	17.3	43.0
3	18.8	40.0
4	18.8	39.0
5	14.9	36.0
6	20.1	38.0
7	18.4	37.0
	Pregnancy[2]	
1	18.2	37.0
2	19.8	42.0
3	17.7	31.0
4	16.9	36.0
5	18.0	37.0
	Lactation[3]	
1	17.9	40.0
2	16.4	37.0
3	16.3	42.0
4	19.2	40.0
5	17.5	46.0
6	18.1	49.0
	Pregnancy & Lactation[4]	
1	14.3	46.0
2	23.0	54.0
3	19.6	32.0
4	17.6	54.0
5	17.9	41.0
6	16.6	37.0
7	23.0	37.0

[1] Control - No ascorbic acid supplementation.
[2] Pregnancy - Ascorbic acid supplementation during pregnancy only.
[3] Lactation - Ascorbic acid supplementation during lactation only.
[4] Pregnancy & Lactation - Ascorbic acid supplementation during both pregnancy and lactation.

APPENDIX TABLE 7

Three-day Water Intake and Feed Consumption During the Third Week of Lactation in Control and Ascorbic Acid-Supplemented Female Mice

Female No.	Feed Consumption	Water Intake
	(g)	(ml)
	Control[1]	
1	38.2	112.0
2	30.7	103.0
3	35.6	108.0
4	44.9	107.0
5	53.2	121.0
6	35.0	74.0
7	48.8	93.0
	Pregnancy[2]	
1	51.1	103.0
2	55.8	94.0
3	43.3	141.0
4	49.3	96.0
5	41.4	120.0
	Lactation[3]	
1	39.1	82.0
2	47.6	89.0
3	26.5	71.0
4	47.2	96.0
5	35.9	87.0
6	44.1	82.0
	Pregnancy & Lactation[4]	
1	38.4	73.0
2	51.6	76.0
3	45.6	62.0
4	35.1	88.0
5	41.7	93.0
6	43.3	74.0
7	54.2	85.0

[1] Control - No ascorbic acid supplementation.
[2] Pregnancy - Ascorbic acid supplementation during pregnancy only.
[3] Lactation - Ascorbic acid supplementation during lactation only.
[4] Pregnancy and Lactation - Ascorbic acid supplementation during both pregnancy and lactation.

APPENDIX TABLE 8

Length of Gestation, Litter Size and Average Birth Weight of Pups from Control and Ascorbic Acid-Supplemented Female Mice

Female No.	Gestation	Litter Size	Ave. Birth Wt.
	(days)		(g)
		Control[1]	
1	21	11	1.4
2	21	12	1.3
3	21	12	1.4
4	21	11	1.5
5	21	8	1.3
6	22	10	1.5
7	21	9	1.4
		Pregnancy[2]	
1	21	13	1.3
2	21	10	1.5
3	21	12	1.4
4	21	14	1.3
5	21	11	1.5
		Lactation[3]	
1	21	15	1.3
2	21	10	1.4
3	21	12	1.3
4	21	10	1.7
5	21	8	1.3
6	21	8	1.3
		Pregnancy & Lactation[4]	
1	22	11	1.3
2	21	9	1.5
3	22	11	1.4
4	21	13	1.3
5	21	15	1.4
6	22	9	1.5
7	21	12	1.4

[1] Control - No ascorbic acid supplementation.
[2] Pregnancy - Ascorbic acid supplementation during pregnancy only.
[3] Lactation - Ascorbic acid supplementation during lactation only.
[4] Pregnancy & Lactation - Ascorbic acid supplementation during both pregnancy and lactation.

APPENDIX TABLE 9

Pre-weaning Weight Gain of Mouse Pups From Control or Ascorbic-Acid-Supplemented Female Mice

Pup No.	Birth Wt.	Weaning Wt.	Weight Gain
	(g)	(g)	(g)
	Control[1]		
1	1.5	6.6	5.1
2	1.4	6.9	5.5
3	1.6	7.6	6.0
4	1.5	8.9	7.4
5	1.5	7.7	6.2
6	1.3	9.1	7.8
7	1.4	8.6	7.2
8	1.4	7.5	6.0
9	1.5	7.5	6.0
10	1.5	6.4	4.9
11	1.5	10.1	8.6
12	1.5	9.7	8.2
13	1.5	10.4	9.9
14	1.6	11.2	9.6
15	1.3	9.7	8.4
16	1.2	11.6	10.4
17	1.3	11.9	10.6
18	1.4	10.3	8.9
19	1.3	6.8	5.5
20	1.4	7.0	5.6
21	1.4	7.5	6.1
22	1.5	10.4	8.9
23	1.2	8.2	6.0
24	1.5	10.7	9.2
25	1.5	10.7	9.2
	Pregnancy[2]		
1	1.4	10.4	9.4
2	1.2	9.9	7.7
3	1.4	10.0	8.6
4	1.3	10.9	9.6
5	1.2	10.8	9.6
6	1.2	9.9	7.7
7	1.6	12.4	10.8
8	1.5	11.7	10.2
9	1.6	12.2	10.6
10	1.7	13.3	11.6
11	1.4	5.5	4.1
12	1.3	8.7	7.0

APPENDIX TABLE 9

(Continued)

Pup No.	Birth Wt.	Weaning Wt.	Weight Gain
	(g)	(g)	(g)
	Pregnancy (Cont.)		
13	1.4	6.4	5.0
14	1.4	7.1	5.7
15	1.4	8.2	6.8
16	1.4	7.8	6.4
17	1.5	8.6	7.1
18	1.6	9.5	7.9
19	1.6	8.4	6.8
20	1.4	6.8	5.4
21	1.4	8.4	7.0
	Lactation[3]		
1	1.3	9.0	7.7
2	1.3	7.9	6.6
3	1.3	8.5	7.2
4	1.2	8.1	6.9
5	1.5	10.2	8.7
6	1.5	10.9	9.4
7	1.6	10.9	9.3
8	1.6	11.6	10.0
9	1.5	6.8	5.3
10	1.4	8.6	6.2
11	1.2	7.6	5.4
12	1.3	7.3	6.0
13	1.7	9.0	7.4
14	1.8	9.1	7.3
15	1.6	7.6	6.0
16	1.6	7.7	6.1
17	1.3	7.1	5.8
18	1.5	7.3	5.8
19	1.3	6.0	4.7
	Pregnancy & Lactation[4]		
1	1.5	7.7	6.2
2	1.5	6.6	5.1
3	1.5	7.3	5.8
4	1.6	6.2	4.6
5	1.6	7.0	5.4
6	1.4	7.4	6.0
7	1.3	6.1	4.8
8	1.2	7.9	6.7

APPENDIX TABLE 9

(Continued)

Pup No.	Birth Wt.	Weaning Wt.	Weight Gain
	(g)	(g)	(g)
	Pregnancy & Lactation (Cont.)		
9	1.4	9.7	8.5
10	1.5	10.2	8.7
11	1.5	8.1	6.6
12	1.5	10.1	8.6
13	1.4	11.0	9.6
14	1.4	10.3	8.9
15	1.4	12.0	10.6
16	1.5	9.4	7.9
17	1.3	7.2	5.9
18	1.4	7.6	6.2
19	1.4	9.9	8.5
20	1.5	10.4	8.9
21	1.3	9.9	8.6
22	1.5	10.6	9.1
23	1.6	8.4	6.8
24	1.6	11.5	9.9
25	1.5	9.4	7.9
26	1.4	8.4	7.0

[1] Control - No supplementary ascorbic acid
[2] Pregnancy - Ascorbic acid supplementation during pregnancy only.
[3] Lactation - Ascorbic acid supplementation during lactation only.
[4] Pregnancy & Lactation - Ascorbic acid supplementation during both pregnancy and lactation.

APPENDIX TABLE 10

Post-weaning Weight Gain of Mouse Pups From
Control or Ascorbic Acid-Supplemented Female Mice

Pup No.	Wt. at Day 24	Wt. at Day 45	Wt. Gain
	(g)	(g)	(g)
	Control[1]		
1	8.9	26.0	17.1
2	6.2	22.9	16.7
3	9.2	25.6	16.4
4	8.6	23.7	15.1
5	7.0	21.2	14.2
6	9.5	27.5	18.0
7	10.6	27.8	17.2
8	10.2	26.3	16.1
9	10.9	25.8	14.9
10	7.0	21.1	14.1
11	8.1	23.9	15.7
12	10.8	27.6	16.8
13	11.2	26.0	14.8
	Pregnancy[2]		
1	10.6	27.6	17.0
2	10.0	27.1	17.1
3	11.1	27.4	16.3
4	10.3	27.5	17.2
5	12.7	30.4	17.7
6	11.7	28.5	16.8
7	9.1	23.3	14.2
8	6.5	21.2	14.7
9	7.7	23.3	15.6
10	9.2	25.6	16.4
11	9.5	24.5	16.0
	Lactation[3]		
1	9.3	28.2	18.9
2	8.3	25.2	16.9
3	10.8	27.6	16.8
4	11.7	26.7	15.0
5	7.2	22.2	15.0
6	8.3	27.2	18.9
7	6.0	19.4	13.4
8	8.0	24.4	16.4
	Pregnancy & Lactation[4]		
1	7.1	22.7	15.6
2	6.4	23.0	16.6

APPENDIX TABLE 10

(Continued)

Pup No.	Wt. at Day 24	Wt. at Day 45	Wt. Gain
	(g)	(g)	(g)
	Pregnancy & Lactation (Cont.)		
3	6.3	21.3	15.0
4	5.8	20.8	15.0
5	7.6	22.8	15.2
6	10.3	24.2	14.1
7	10.8	26.9	16.1
8	10.7	25.4	14.7
9	10.0	27.6	17.6
10	7.9	20.5	12.6
11	10.8	24.8	14.0
12	10.6	28.2	17.6
13	11.8	25.1	13.3
14	10.1	23.6	13.6

[1] Control - No supplementary ascorbic acid.
[2] Pregnancy - Ascorbic acid supplementation during pregnancy only.
[3] Lactation - Ascorbic acid supplementation during lactation only.
[4] Pregnancy & Lactation - Ascorbic acid supplementation during both pregnancy and lactation.

APPENDIX TABLE 11

Brain Weights of 24-day-old Pups From Control or Ascorbic Acid-Supplemented Female Mice

Pup No.	Brain Wt.	Brain Wt., % Body Wt.
	(g)	
	Control[1]	
1	0.40	3.70
2	0.39	3.68
3	0.36	4.86
4	0.38	5.21
5	0.37	5.61
6	0.40	4.65
7	0.37	4.93
8	0.36	5.71
9	0.34	5.57
10	0.37	5.57
11	0.41	3.72
12	0.35	3.47
	Pregnancy[2]	
1	0.39	3.86
2	0.40	3.54
3	0.43	3.91
4	0.36	2.77
5	0.31	6.20
6	0.33	5.89
7	0.35	4.86
8	0.38	4.58
9	0.37	4.93
10	0.36	4.34
	Lactation[3]	
1	0.34	6.93
2	0.31	6.32
3	0.37	4.51
4	0.35	4.27
5	0.38	6.44
6	0.37	4.35
7	0.37	5.25
8	0.36	4.68
9	0.35	3.61
10	0.36	5.29
11	0.40	5.95

APPENDIX TABLE 11

(Continued)

Pup No.	Brain Wt.	Brain Wt., % Body Wt.
	(g)	
	Pregnancy & Lactation[4]	
1	0.34	5.23
2	0.41	4.02
3	0.41	3.66
4	0.38	4.81
5	0.36	5.00
6	0.40	4.04
7	0.35	6.48
8	0.37	5.44
9	0.37	5.00
10	0.37	4.62
11	0.41	4.27
12	0.39	5.13

[1] Control - No supplementary ascorbic acid.
[2] Pregnancy - Ascorbic acid supplementation during pregnancy only.
[3] Lactation - Ascorbic acid supplementation during lactation only.
[4] Pregnancy & Lactation - Ascorbic acid supplementation during both pregnancy and lactation.

APPENDIX TABLE 12

Kidney Weights of 24-day-old Pups from Control or Ascorbic Acid-Supplemented Female Mice

Pup No.	Kidney Wt. (g)	Kidney Wt., % Body Wt.
	Control[1]	
1	0.15	1.38
2	0.14	1.32
3	0.10	1.35
4	0.10	1.37
5	0.08	1.21
6	0.11	1.28
7	0.12	1.60
8	0.09	1.43
9	0.06	0.98
10	0.12	1.42
11	0.13	1.18
12	0.11	1.09
	Pregnancy[2]	
1	0.15	1.49
2	0.15	1.34
3	0.18	1.64
4	0.21	1.62
5	0.06	1.20
6	0.09	1.60
7	0.09	1.25
8	0.12	1.44
9	0.10	1.33
10	0.12	1.44
	Lactation[3]	
1	0.06	1.22
2	0.05	1.02
3	0.10	1.22
4	0.10	1.22
5	0.07	1.18
6	0.12	1.41
7	0.09	1.28
8	0.10	1.30
9	0.16	1.62
10	0.08	1.18
11	0.12	1.43
	Pregnancy & Lactation[4]	
1	0.07	1.08

APPENDIX TABLE 12

(Continued)

Pup No.	Kidney Wt.	Kidney Wt., % Body Wt.
	(g)	
	Pregnancy & Lactation (Cont.)	
2	0.14	1.18
3	0.16	1.42
4	0.11	1.39
5	0.08	1.11
6	0.15	1.52
7	0.08	1.48
8	0.08	1.18
9	0.12	1.62
10	0.13	1.63
11	0.17	1.77
12	0.10	1.32

[1] Control - No supplementary ascorbic acid.
[2] Pregnancy - Ascorbic acid supplementation during pregnancy only.
[3] Lactation - Ascorbic acid supplementation during lactation only.
[4] Pregnancy & Lactation - Ascorbic acid supplementation during both pregnancy and lactation.

APPENDIX TABLE 13

Liver Weights of 24-day-old Pups From
Control or Ascorbic Acid-Supplemented Female Mice

Pup No.	Liver Wt.	Liver Wt., % Body Wt.
	(g)	
	Control[1]	
1	0.49	4.53
2	0.56	5.48
3	0.31	4.19
4	0.31	4.24
5	0.26	3.94
6	0.35	4.07
7	0.30	4.00
8	0.22	3.49
9	0.25	4.10
10	0.35	4.17
11	0.51	4.64
12	0.39	3.86
	Pregnancy[2]	
1	0.43	4.23
2	0.49	4.38
3	0.52	4.73
4	0.60	4.62
5	0.13	2.60
6	0.18	3.21
7	0.27	3.75
8	0.29	3.49
9	0.30	4.00
10	0.33	3.98
	Lactation[3]	
1	0.14	2.86
2	0.17	3.46
3	0.34	4.15
4	0.32	3.90
5	0.21	3.56
6	0.33	3.88
7	0.29	4.14
8	0.28	3.64
9	0.42	4.32
10	0.28	4.12
11	0.38	4.52

APPENDIX TABLE 13

(Continued)

Pup No.	Liver Wt.	Liver Wt., % Body Wt.
	(g)	
	Pregnancy & Lactation[4]	
1	0.23	3.54
2	0.42	4.12
3	0.46	4.11
4	0.38	4.81
5	0.30	4.28
6	0.41	4.14
7	0.20	3.70
8	0.25	3.68
9	0.28	3.78
10	0.34	4.25
11	0.38	3.96
12	0.34	4.47

[1] Control - No supplementary ascorbic acid.
[2] Pregnancy - Ascorbic acid supplementation during pregnancy only.
[3] Lactation - Ascorbic acid supplementation during lactation only.
[4] Pregnancy & Lactation - Ascorbic acid supplementation during both pregnancy and lactation.

APPENDIX TABLE 14

Brain Weights of 45-day-old Pups From Control or Ascorbic Acid-Supplemented Female Mice

Pup No.	Brain Wt. (g)	Brain Wt., % Body Wt.
	Control[1]	
1	0.42	1.76
2	0.48	2.32
3	0.43	1.87
4	0.48	1.85
5	0.45	1.83
6	0.46	1.88
7	0.43	1.96
8	0.44	1.78
9	0.42	1.89
10	0.41	2.05
11	0.43	1.66
12	0.41	1.57
13	0.42	1.86
	Pregnancy[2]	
1	0.47	2.08
2	0.48	1.82
3	0.47	1.77
4	0.46	1.78
5	0.42	1.60
6	0.46	1.61
7	0.48	1.80
8	0.42	1.90
9	0.39	1.87
10	0.41	1.88
11	0.42	1.76
	Lactation[3]	
1	0.43	1.60
2	0.47	1.94
3	0.42	2.48
4	0.43	2.01
5	0.45	1.61
6	0.48	1.74
7	0.43	1.63
8	0.36	1.76

APPENDIX TABLE 14

(Continued)

Pup No.	Brain Wt.	Brain Wt., % Body Wt.
	(g)	
	Pregnancy & Lactation[4]	
1	0.40	2.11
2	0.43	1.81
3	0.44	1.71
4	0.47	2.20
5	0.38	1.91
6	0.41	1.97
7	0.44	1.83
8	0.46	1.73
9	0.42	2.10
10	0.42	1.90
11	0.44	1.92
12	0.43	1.96
13	0.46	1.84
14	0.45	1.97

[1] Control - No supplementary ascorbic acid.
[2] Pregnancy - Ascorbic acid supplementation during pregnancy only.
[3] Lactation - Ascorbic acid supplementation during lactation only.
[4] Pregnancy & Lactation - Ascorbic acid supplementation during both pregnancy and lactation.

APPENDIX TABLE 15

Kidney Weights of 45-day-old Pups from Control or Ascorbic Acid-Supplemented Female Mice

Pup No.	Kidney Wt. (g)	Kidney Wt., % Body Wt.
	Control[1]	
1	0.33	1.39
2	0.30	1.44
3	0.36	1.43
4	0.37	1.43
5	0.39	1.58
6	0.39	1.60
7	0.32	1.46
8	0.43	1.74
9	0.37	1.67
10	0.27	1.35
11	0.41	1.58
12	0.39	1.49
13	0.38	1.51
	Pregnancy[2]	
1	0.37	1.63
2	0.48	1.82
3	0.47	1.77
4	0.47	1.82
5	0.46	1.75
6	0.55	1.93
7	0.49	1.82
8	0.35	1.58
9	0.30	1.44
10	0.35	1.60
11	0.37	1.55
	Lactation[3]	
1	0.46	1.71
2	0.39	1.61
3	0.25	1.48
4	0.38	1.78
5	0.51	1.82
6	0.50	1.82
7	0.40	1.52
8	0.28	1.36
	Pregnancy & Lactation[4]	
1	0.28	1.48
2	0.44	1.85
3	0.44	1.71

APPENDIX TABLE 15

(Continued)

Pup No.	Kidney Wt.	Kidney Wt., % Body Wt.
4	0.31	1.45
5	0.29	1.45
6	0.29	1.45
7	0.38	1.58
8	0.44	1.65
9	0.28	1.40
10	0.39	1.36
11	0.37	1.61
12	0.37	1.69
13	0.45	1.80
14	0.41	1.79

[1] Control - No supplementary ascorbic acid.
[2] Pregnancy - Supplementation of ascorbic acid during pregnancy only.
[3] Lactation - Supplementation of ascorbic acid during lactation only.
[4] Pregnancy & Lactation - Supplementation of ascorbic acid during both pregnancy and lactation.

APPENDIX TABLE 16

Liver Weights of 45-day-old Pups From Control or Ascorbic Acid-Supplemented Female Mice

Pup No.	Liver Wt. (g)	Liver Wt., % Body Wt.
	Control[1]	
1	1.22	5.12
2	1.05	5.07
3	1.28	5.56
4	1.29	4.98
5	1.32	5.36
6	1.23	5.04
7	1.28	5.84
8	1.36	5.51
9	1.21	5.45
10	1.00	5.00
11	1.39	5.37
12	1.39	5.32
13	1.31	5.47
	Pregnancy[2]	
1	1.32	5.84
2	1.47	5.57
3	1.42	5.36
4	1.37	5.31
5	1.42	5.40
6	1.46	5.12
7	1.28	4.78
8	1.33	6.02
9	1.22	5.84
10	1.10	5.04
11	1.12	4.70
	Lactation[3]	
1	1.58	5.87
2	1.39	5.74
3	0.86	5.09
4	1.15	5.37
5	1.67	5.96
6	1.51	5.49
7	1.46	5.55
8	1.08	5.19
	Pregnancy & Lactation[4]	
1	1.02	5.49
2	1.30	5.46

APPENDIX TABLE 16

(Continued)

Pup No.	Liver Wt.	Liver Wt., % Body Wt.
	(g)	
	Pregnancy & Lactation (cont.)	
3	1.37	5.33
4	1.26	5.91
5	0.99	4.97
6	1.08	5.19
7	1.29	5.38
8	1.52	5.71
9	1.21	6.05
10	1.20	5.43
11	1.25	5.45
12	1.15	5.25
13	1.38	5.52
14	1.22	5.35

[1] Control - No Supplementary ascorbic acid
[2] Pregnancy - Ascorbic acid supplementation during pregnancy only.
[3] Lactation - Ascorbic acid supplementation during lactation only.
[4] Pregnancy & Lactation - Ascorbic acid supplementation during both pregnancy and lactation.

APPENDIX TABLE 17

Ascorbic Acid (AsA) Contents in Tissues of 24-day-old Pups From Control or Ascorbic Acid-Supplemented Female Mice

Pup No.	Liver AsA	Kidney AsA	Spleen AsA	Brain AsA	Adrenal AsA
	(mg/100 g)	(mg/100 g)	(mg/100 g)	(mg/100 g)	(mg total)
		Control[1]			
1	28.72	25.64	40.00	45.85	11.2
2	18.46	16.41	35.00	34.15	12.3
3	24.86	15.61	37.89	42.03	12.5
4	28.29	25.26	32.93	46.67	13.4
5	28.29	18.95	54.54	44.44	8.4
		Pregnancy[2]			
1	21.54	16.41	35.00	43.90	17.1
2	25.64	14.36	24.60	41.95	7.8
3	34.59	25.37	39.48	38.97	8.8
4	24.86	13.66	35.15	44.12	10.2
5	26.34	20.00	43.64	44.44	9.9
		Lactation[3]			
1	34.87	30.77	33.33	47.80	11.2
2	27.03	22.44	41.06	45.13	8.2
3	33.51	20.49	42.63	41.02	11.0
4	29.27	25.26	41.82	42.22	9.9
5	26.67	23.59	30.75	48.20	7.5
		Pregnancy and Lactation[4]			
1	31.79	24.64	28.34	44.88	13.4
2	28.11	23.41	28.42	45.13	9.6
3	37.07	22.11	43.64	42.22	14.1

APPENDIX TABLE 17

(Continued)

Pup No.	Liver AsA (mg/100 g)	Kidney AsA (mg/100 g)	Spleen AsA (mg/100 g)	Brain AsA (mg/100 g)	Adrenal AsA (µg total)
		Pregnancy and Lactation (Cont.)			
4	33.17	20.00	50.91	43.33	14.1
5	30.77	20.51	26.16	42.05	13.5

[1] Control - No supplementary ascorbic acid
[2] Pregnancy - Ascorbic acid supplementation during pregnancy only.
[3] Lactation - Ascorbic acid supplementation during lactation only.
[4] Pregnancy and Lactation - Ascorbic acid supplementation during both pregnancy and lactation.

APPENDIX TABLE 18

Brain DNA, RNA, and Protein Contents in 24-day-old Pups From Control or Ascorbic Acid-Supplemented Female Mice

Pup No.	DNA (mg/g)	DNA (mg/tissue)	RNA (mg/g)	Protein (mg/g)	Protein DNA
			Control[1]		
1	3.56	1.35	8.00	168.0	47.1
2	4.00	1.36	8.00	178.0	44.5
3	2.75	0.96	7.75	115.5	42.0
4	3.55	1.42	9.60	157.5	44.4
5	3.35	1.24	7.50	134.5	40.2
6	3.35	1.24	8.00	134.5	40.0
7	3.35	1.24	8.30	120.0	35.8
			Pregnancy[2]		
1	4.45	1.78	8.50	192.5	43.2
2	4.00	1.32	7.75	168.0	42.0
3	3.55	1.53	9.25	178.0	50.1
4	2.75	1.04	7.20	125.0	45.4
5	3.15	1.13	8.80	139.0	44.1
6	2.40	0.91	6.15	134.5	56.0
			Lactation[3]		
1	3.40	1.19	6.60	178.0	52.3
2	4.45	1.78	8.00	118.5	37.8
3	3.15	1.13	8.00	111.5	35.2
4	2.40	0.82	7.25	120.5	50.0
5	2.85	1.00	7.50	144.0	50.5
			Pregnancy and Lactation[4]		
1	4.45	1.60	8.25	187.5	42.1
2	2.35	0.87	6.70	92.5	39.4
3	2.75	1.13	8.30	129.5	47.1
4	3.15	1.29	7.75	111.0	35.2
5	2.85	1.00	6.10	130.0	45.6
6	2.85	0.97	8.55	149.0	52.3
7	3.35	1.31	8.00	110.0	32.8

[1] Control - No supplementary ascorbic acid.
[2] Pregnancy - Ascorbic acid supplementation during pregnancy only.
[3] Lactation - Ascorbic acid supplementation during lactation only.
[4] Pregnancy and Lactation - Ascorbic acid supplementation during both pregnancy and lactation.

APPENDIX TABLE 19

Kidney DNA, RNA, and Protein Contents in 24-day-old Pups From Control or Ascorbic Acid-Supplemented Female Mice

Pup No.	DNA (mg/g)	DNA (mg/tissue)	RNA (mg/g)	Protein (mg/g)	Protein DNA
			Control[1]		
1	12.43	1.23	14.96	264.0	21.24
2	15.18	0.91	18.37	254.1	16.74
3	13.75	1.51	22.44	302.5	22.00
4	10.78	1.62	14.96	353.1	32.76
5	10.78	1.29	11.66	183.7	17.23
6	11.77	1.41	13.31	284.9	24.20
7	15.62	1.09	22.55	265.1	16.97
			Pregnancy[2]		
1	10.45	1.57	14.96	295.9	28.38
2	11.33	1.02	13.53	201.3	17.77
3	9.46	1.70	13.53	264.0	27.90
4	10.78	1.29	17.05	302.5	28.06
5	11.77	1.41	12.98	259.6	22.06
			Lactation[3]		
1	10.45	1.04	14.96	222.2	21.26
2	10.45	1.25	10.89	264.0	25.26
3	14.74	1.47	22.57	321.2	21.79
4	15.62	0.94	14.96	193.6	12.37
5	12.76	1.02	15.84	244.2	19.12
6	6.82	1.04	8.36	152.9	22.42
			Pregnancy & Lactation[4]		
1	14.19	1.13	19.03	264.0	18.60
2	9.79	1.17	15.62	207.9	21.23
3	9.79	1.66	14.96	301.4	30.77
4	8.80	1.41	20.35	322.2	37.75
5	10.78	0.86	12.54	193.6	17.95
6	12.76	0.89	18.37	244.2	19.14
7	13.75	1.38	14.96	234.3	17.04

[1] Control - No supplementary ascorbic acid.
[2] Pregnancy - Ascorbic acid supplementation during pregnancy only.
[3] Lactation - Ascorbic acid supplementation during lactation only.
[4] Pregnancy & Lactation - Ascorbic acid supplementation during both pregnancy and lactation.

APPENDIX TABLE 20

Liver DNA, RNA, and Protein Contents in 24-day-old Pups From Control or Ascorbic Acid-Supplemented Female Mice

Pup No.	DNA (mg/g)	DNA (mg/tissue)	RNA (mg/g)	Protein (mg/g)	Protein/DNA
			Control[1]		
1	6.79	2.10	21.42	259.0	38.14
2	7.35	1.84	23.45	142.8	19.43
3	7.28	2.84	28.77	278.6	38.27
4	5.35	2.92	26.53	272.3	50.90
5	7.49	2.25	21.42	259.0	34.58
6	7.49	2.62	19.32	272.3	36.36
7	8.12	2.11	22.61	246.4	30.34
			Pregnancy[2]		
1	6.79	3.32	22.96	266.0	39.17
2	9.66	1.74	12.46	291.9	30.22
3	6.79	3.53	15.96	272.3	40.10
4	6.65	1.93	23.10	272.3	40.95
5	7.98	2.63	22.61	259.0	32.46
6	9.94	2.09	23.87	298.2	30.00
			Lactation[3]		
1	7.35	2.35	20.93	252.7	34.38
2	7.91	3.00	23.94	298.2	37.70
3	9.38	2.63	24.15	304.5	32.46
4	11.20	1.57	18.06	252.7	22.56
5	6.86	2.88	20.23	284.9	41.53
			Pregnancy & Lactation[4]		
1	7.35	2.20	14.98	272.3	37.04
2	7.28	2.04	25.20	272.3	37.40
3	8.68	3.30	25.69	317.1	36.53
4	9.38	4.31	21.07	310.8	33.13
5	6.86	1.37	12.74	278.6	48.61
6	7.49	1.72	14.84	259.9	34.69
7	6.86	2.33	19.32	266.0	38.78

[1] Control - No supplementary ascorbic acid.
[2] Pregnancy - Ascorbic acid supplementation during pregnancy only.
[3] Lactation - Ascorbic acid supplementation during lactation only.
[4] Pregnancy and Lactation - Ascorbic acid supplementation during both pregnancy and lactation

APPENDIX TABLE 21

Brain DNA, RNA, and Protein Contents in 45-day-old Pups From Control or Ascorbic Acid-Supplemented Female Mice

Pup No.	DNA (mg/g)	DNA (mg/tissue)	RNA (mg/g)	Protein (mg/g)	Protein/DNA
			Control[1]		
1	2.56	1.08	7.00	104.0	40.62
2	2.96	1.42	8.52	110.4	37.30
3	3.68	1.58	8.32	125.6	34.13
4	2.96	1.27	6.60	111.2	45.14
5	3.32	1.43	8.40	125.6	37.83
6	4.04	1.78	9.16	140.8	34.85
7	3.80	1.56	9.12	131.2	34.53
8	3.44	1.44	8.88	131.2	38.14
9	4.48	2.15	7.48	131.2	29.28
10	3.32	1.49	7.12	125.6	37.83
11	3.68	1.69	7.56	148.0	40.22
12	3.32	1.36	7.68	132.0	39.76
13	3.32	1.36	7.92	98.0	29.51
			Pregnancy[2]		
1	2.56	1.20	7.20	118.4	46.25
2	3.32	1.59	8.96	133.6	40.24
3	3.68	1.73	8.12	104.0	28.26
4	3.32	1.39	7.36	125.6	37.83
5	3.32	1.53	8.88	125.6	37.83
6	3.80	1.75	10.44	116.0	30.52
7	3.80	1.48	9.40	131.2	35.53
8	3.80	1.56	10.20	152.8	40.21
9	3.32	1.39	6.64	118.4	35.66
10	3.68	1.54	6.20	133.6	36.27
11	3.68	1.77	7.28	120.4	32.72
			Lactation[3]		
1	2.96	1.27	6.56	125.6	42.43
2	3.32	1.46	7.20	125.6	37.83
3	2.96	1.39	5.84	133.6	45.14
4	3.32	1.39	8.12	111.2	33.49
5	3.44	1.55	10.72	123.2	35.81
6	3.32	1.59	7.56	155.2	46.75
7	4.04	1.45	6.88	155.2	38.42
8	3.00	1.29	6.84	133.2	44.40
			Pregnancy and Lactation[4]		
1	2.96	1.18	6.76	118.4	40.00
2	3.32	1.43	7.00	133.6	24.24

APPENDIX TABLE 21

(Continued)

Pup No.	DNA (mg/g)	DNA (mg/tissue)	RNA (mg/g)	Protein (mg/g)	Protein/DNA
		Pregnancy & Lactation (cont.)			
3	3.32	1.46	9.40	133.6	40.24
4	2.96	1.30	7.60	133.6	37.57
5	2.96	1.36	8.64	133.6	45.14
6	3.44	1.62	10.20	145.6	42.33
7	4.12	1.73	9.40	131.2	31.84
8	3.12	1.40	8.08	131.2	42.05
9	2.96	1.24	5.72	125.6	42.74
10	2.96	1.12	7.80	155.2	52.43
11	3.68	1.62	8.04	155.2	42.17
12	3.32	1.43	7.56	104.0	31.33
13	3.00	1.23	6.84	101.6	33.87
14	3.32	1.53	7.28	119.2	35.90

[1] Control - No supplementary ascorbic acid.
[2] Pregnancy - Ascorbic acid supplementation during pregnancy only.
[3] Lactation - Ascorbic acid supplementation during lactation only.
[4] Pregnancy & Lactation - Ascorbic acid supplementation during both pregnancy and lactation.

APPENDIX TABLE 22

Kidney DNA, RNA, and Protein Contents in 45-day-old Pups From Control or Ascorbic Acid-Supplemented Female Mice

Pup No.	DNA (mg/g)	DNA (mg/tissue)	RNA (mg/g)	Protein (mg/g)	Protein/DNA
		Control[1]			
1	7.59	2.50	14.96	306.4	40.43
2	9.46	2.84	17.38	295.9	31.28
3	9.46	3.03	16.39	327.8	34.65
4	8.58	3.09	13.75	324.4	37.81
5	6.60	2.71	13.75	356.4	54.00
6	6.60	2.84	11.44	356.4	54.00
7	7.59	3.42	12.10	275.0	32.26
8	7.59	2.81	13.20	306.9	40.43
9	8.88	3.18	18.48	467.3	54.46
10	6.82	2.66	13.20	290.4	42.58
11	8.58	2.32	12.42	284.9	33.21
12	7.59	2.96	12.98	275.0	36.23
13	8.26	3.01	11.84	291.5	35.26
		Pregnancy[2]			
1	7.59	2.80	14.41	275.0	36.23
2	7.59	3.64	15.40	337.7	44.49
3	7.59	3.57	10.89	323.4	42.61
4	8.58	3.00	10.34	269.5	31.41
5	8.58	4.72	14.16	344.3	40.12
6	5.79	3.53	14.19	327.8	43.19
7	6.60	1.98	14.19	306.9	46.50
8	10.45	3.66	16.83	327.8	31.37
9	6.82	3.14	12.65	300.3	44.03
10	7.59	3.72	13.97	284.9	37.54
11	9.26	3.54	12.95	301.2	35.64
		Lactation[3]			
1	8.58	3.95	17.38	295.9	34.49
2	7.59	3.34	9.90	190.3	25.07
3	6.60	2.57	10.34	284.4	39.17
4	10.45	2.61	16.61	301.4	28.84
5	6.60	3.37	9.46	316.8	48.00
6	4.95	2.48	12.65	269.5	54.44
7	9.79	2.74	13.20	321.2	32.81
8	7.59	3.04	14.52	380.6	50.14
		Pregnancy & Lactation[4]			
1	9.46	2.65	15.40	275.0	29.07
2	7.59	3.34	13.86	316.8	41.74

APPENDIX TABLE 22

(Continued)

Pup No.	DNA (mg/g)	DNA (mg/tissue)	RNA (mg/g)	Protein (mg/g)	Protein/DNA
		Pregnancy & Lactation (cont.)			
3	10.45	3.97	18.37	316.8	30.32
4	6.60	2.90	12.65	301.4	45.67
5	6.60	2.90	12.65	334.5	50.67
6	6.60	2.05	15.29	316.8	48.00
7	7.59	2.12	14.19	295.9	38.99
8	6.60	2.71	13.20	306.9	46.50
9	6.02	2.05	13.20	342.1	50.16
10	6.82	1.98	13.20	300.3	44.03
11	8.54	3.17	12.65	342.1	39.87
12	6.82	2.52	11.44	311.3	45.65
13	8.58	2.57	13.97	327.8	38.21
14	6.60	2.97	12.98	369.6	56.00

[1] Control - No supplementary ascorbic acid.
[2] Pregnancy - Ascorbic acid supplementation during pregnancy only.
[3] Lactation - Ascorbic acid supplementation during lactation only.
[4] Pregnancy & Lactation - Ascorbic acid supplementation during both pregnancy and lactation.

APPENDIX TABLE 23

Liver DNA, RNA, and Protein Contents in 45-day-old Pups From Control or Ascorbic Acid-Supplemented Female Mice

Pup No.	DNA (mg/g)	DNA (mg/tissue)	RNA (mg/g)	Protein (mg/g)	Protein/DNA
			Control[1]		
1	5.11	6.23	24.01	390.6	26.4
2	6.79	7.13	28.42	289.1	42.5
3	6.23	7.97	27.58	303.1	48.6
4	6.86	8.78	22.98	324.1	47.2
5	5.60	7.78	21.42	291.9	52.1
6	6.23	8.47	22.12	324.1	52.0
7	5.67	7.26	22.26	404.6	71.3
8	5.67	6.86	25.76	350.0	61.7
9	6.23	8.04	24.92	370.3	59.4
10	5.46	7.21	18.43	309.4	56.6
11	5.46	6.71	22.96	350.0	64.1
12	6.23	6.23	26.32	316.4	50.7
13	6.23	9.73	17.22	316.4	50.7
			Pregnancy[2]		
1	5.11	6.75	25.34	289.1	56.5
2	5.11	7.51	23.52	343.0	67.1
3	5.60	7.84	22.96	330.4	59.0
4	7.49	9.96	16.80	259.0	34.5
5	6.86	10.02	24.50	363.3	52.9
6	5.67	7.77	26.60	316.4	55.8
7	4.97	6.06	16.94	296.1	59.5
8	6.23	6.95	30.24	302.4	48.5
9	6.02	8.54	19.18	363.3	60.3
10	5.46	6.12	23.66	316.4	57.9
11	5.60	7.17	22.96	363.3	64.8
			Lactation[3]		
1	5.67	8.96	24.01	323.4	57.0
2	6.23	5.36	22.26	329.7	52.9
3	5.67	7.77	21.42	350.0	61.7
4	6.86	7.89	22.12	350.0	51.0
5	4.34	7.25	27.88	309.4	71.2
6	4.83	7.29	24.50	309.4	64.0
7	6.65	7.18	26.74	323.4	48.6
8	6.23	9.09	28.00	336.7	54.0

APPENDIX TABLE 23

(Continued)

Pup No.	DNA (mg/g)	DNA (mg/tissue)	RNA (mg/g)	Protein (mg/g)	Protein/DNA
		Pregnancy & Lactation[4]			
1	6.79	6.93	26.67	282.8	41.6
2	6.23	8.10	23.52	282.8	45.3
3	6.23	7.97	29.33	214.9	34.4
4	6.23	8.54	24.50	336.7	54.0
5	4.97	7.55	25.20	350.0	73.0
6	4.97	6.26	25.76	350.0	70.4
7	4.97	6.01	20.44	296.1	59.5
8	4.97	6.06	23.44	350.0	70.4
9	4.83	5.58	23.66	302.4	62.6
10	5.46	5.46	27.58	323.4	59.2
11	6.65	8.31	23.66	323.4	48.6
12	5.46	6.28	22.99	350.0	64.1
13	6.23	6.72	28.00	323.4	51.9
14	4.97	6.86	19.74	323.4	65.0

[1] Control - No supplementary ascorbic acid.
[2] Pregnancy - Ascorbic acid supplementation during pregnancy only.
[3] Lactation - Ascorbic acid supplementation during lactation only.
[4] Pregnancy & Lactation - Ascorbic acid supplementation during both pregnancy and lactation.

EFFECTS OF HIGH ASCORBIC ACID INTAKE DURING PREGNANCY AND LACTATION IN MICE

by

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B. S., University of Oregon, 1980

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The effects of high ascorbic acid intake during pregnancy and lactation were studied in mice. Forty-eight female mice were randomly assigned to one of four treatment groups: a control group, given no supplementary ascorbic acid; a pregnancy group, given ascorbic acid during pregnancy only; a lactation group, given ascorbic acid during lactation only; and a pregnancy and lactation group, given ascorbic acid during both pregnancy and lactation. Ascorbic acid, when administered, was given in the drinking water at a concentration of 250 mg/100 ml water.

The mice receiving the ascorbic acid during lactation consumed significantly less ($P < 0.05$) water than the control group. However there were no significant differences in weight gain, feed consumption or feed efficiency between females in treatment groups when compared during pregnancy or lactation. Average litter size and average birth weight of mouse pups were not different among treatment groups.

Tissue ascorbic acid contents and growth of male mouse pups from supplemented or nonsupplemented females were also studied. Measurements of growth included body weight, and a determination of tissue weights, cell number (total DNA), and cell size (protein/DNA) in brains, kidneys and livers of 24-day-old and 45-day-old mice. Ascorbic acid, when administered during pregnancy and/or lactation, did not influence any of these measurements in the offspring.