RELATIONSHIPS OF ASCORBIC ACID TO PREGNANCY, AND ORAL CONTRACEPTIVE STEROIDS *

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The human requirement for vitamin C is increased during pregnancy. It also appears to be increased in persons ingesting oral contraceptive steroids. The rationale for increased requirements is based primarily on the findings of a downward trend in plasma ascorbic acid levels in successive trimesters of pregnancy [1-4] and decreased plasma, white cell, and platelet ascorbic acid levels in women taking oral contraceptives.[5-8]

Oral Contraceptive Steroids

The estrogenic and progestational components of oral contraceptives are given either in combination or in sequence. Progestational compounds in common use are derivatives of either testosterone (19-norsteroids) or 17 α-hydroxyprogesterone. The commonly used estrogenic components are mestranol and ethynylestradiol. The metabolic effects of oral contraceptive steroids have been reviewed.[9] The decrease in plasma, leukocyte, and platelet ascorbic acid in women taking oral contraceptives is well documented.[1-4] The question of whether ascorbic acid supplementation can restore plasma and tissue concentrations to control levels needs further study.

Present information suggests that supplementation will increase plasma and leukocyte concentrations. However, the increase is not comparable to that seen in control subjects, even with large quantities of supplemental ascorbic acid.[5, 8]

Results from both human and animal studies suggest that it is the estrogenic component of the oral contraceptive that causes depressed ascorbic acid concentrations.[6, 8, 10, 11] Serum levels of copper have been shown to increase in women ingesting oral contraceptives [10-12] and under estrogen influence.[10, 13, 14] Ceruloplasmin has ascorbic acid oxidase activity,[15] and several investigators have suggested that an increased catabolism of ascorbic acid may account for the decreased plasma and tissue levels in humans and animals treated with estrogen or oral contraceptive steroids.[6-8, 10, 16] Other suggestions include decreased absorption,[7] changes in tissue distribution,[7, 8] and decreased levels of reducing compounds such as reduced glutathione.[10] Although increased urinary excretion has been mentioned as a possible explanation,[16] this does not appear to be true.[5]

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We are currently studying the catabolism and tissue uptake of ascorbic acid in rats and guinea pigs that have been given ethynylestradiol, norgestrel, a mixture of ethynylestradiol and norgestrel, and progesterone. The preliminary data suggest that the steroids have no effect on the rate of ascorbic acid breakdown but do alter tissue uptake patterns. Thus, the changes in blood levels of ascorbic acid in oral contraceptive users may be due to changes in tissue distribution.

Pregnancy

Human Beings

In support of a marked increase in the requirement for vitamin C during human pregnancy are the reports of large quantities of ascorbic acid being utilized when the ovum is released,[17] decreased tissue levels after fertilization,[18] and external signs of vitamin C deficiency in pregnant women not being supplemented with ascorbic acid.[19]

Although plasma levels of vitamin C decrease during pregnancy, a specific relationship between low levels and either complications of pregnancy or health of the offspring has not been demonstrated in human subjects. Several reports suggest an association between low plasma levels of ascorbic acid and increased neonatal mortality,[20] stillbirths,[21] premature births,[22, 23] decreased birth weight,[23, 15] preeclamptic toxemia,[24, 25] and deformities of the newborn.[26] Other reports show either no association [4] or, at best, only a contributory role for ascorbic acid in relation to several of these complications.[2] In a study of more than 300 pregnant women, we also were unable to show a definite relationship between low plasma levels of ascorbic acid and the outcome of pregnancy.[27] Enumerable factors contribute to poor obstetrical outcome; observations on pregnant women in uncontrolled experiments can, at best, provide only suggestions of associations.

The effect of large doses of ascorbic acid on human fertility and pregnancy is of current concern. The reports are controversial. Some workers have reported increased fertility with large doses,[18] while others suggest infertility [28] or no effect.[29]

Animals

Low dietary intake of ascorbic acid causes marked detrimental effects in both the dam and offspring. The investigations of Ingier [30] showed that when ascorbic acid deficiency was induced during the embryonic stages of fetal development, fetal deaths were high; whereas fetuses of dams made deficient later in gestation were born alive and apparently fully developed. We studied the effect of ascorbic acid deficiency induced during day 20 to day 35 on collagen hydroxyproline in the uterus and fetus.[31] A vitamin C deficiency during this period leads to marked reduction in collagen hydroxyproline in both the uterus and fetus. This abnormality was apparent without any obvious signs of scurvy in the dam. Other investigators have reported that animals fed low levels of ascorbic acid either fail to conceive or the pregnancy terminates in abortion or resorption.[32, 33] Thus, it appears that the lack of ascorbic acid for

collagen synthesis is basic to the increased abortions and resorptions that occur in severe deficiency.

Large intakes of ascorbic acid during pregnancy have also been reported to be detrimental. Reported effects include *in utero* conditioning of offspring for a greater than normal requirement,[34] increased fetal mortality and decreased fertility,[35] increased abortions,[36] and increased stillbirths.[37] None of these reports have been confirmed.

In an attempt to quantitate the ascorbic acid requirement in the pregnant guinea pig, Pye et al.[38] fed either 2, 4, 6, or 8 mg of ascorbic acid per day. Some females failed to produce young at each level of ascorbic acid intake, but the largest number of nonproductive females fell within the group fed 2 mg. The rate of reproduction was highest and the number of young born was greatest in the group fed 8 mg. Others[39, 40] have reported that 5 mg of ascorbic acid daily will maintain growth and support reproduction over a long

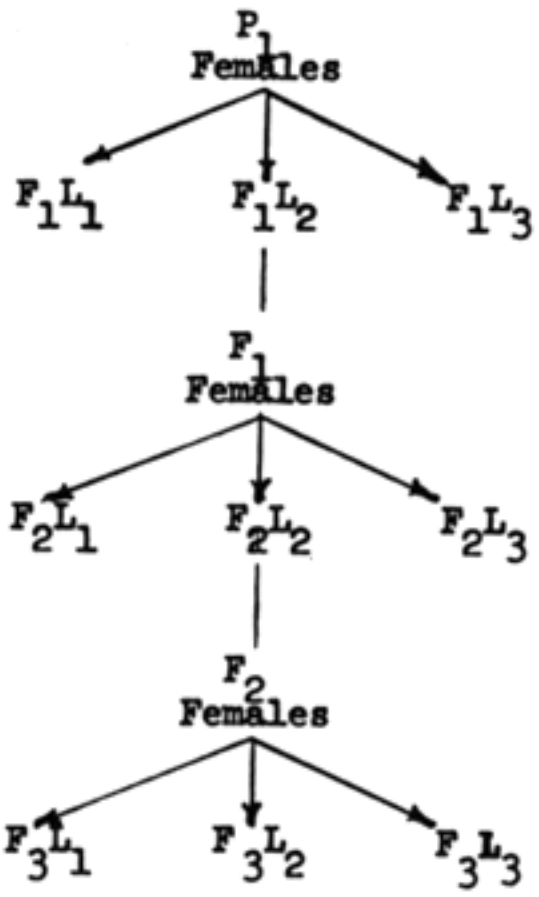

FIGURE 1. Design of the study. P_1, first generation; F_1, second generation; F_2, third generation; F_3, fourth generation. L_1, L_2, L_3 refers to first, second, and third litters, respectively. Male offspring were killed at 14 days of age and dams of each generation were killed after weaning the third litter. Dams that failed to produce three litters were killed if they did not conceive after five or more mating trials.

period of time. We have carried out a similar study to that reported by Pye et al. to determine the effects of chronically low and high intakes of the vitamin on reproductive performance in guinea pigs.

MATERIALS AND METHODS

Overall Design

The overall design of the study is depicted in FIGURE 1. Animals were fed one of three levels of ascorbic acid for three generations. Dams were given the opportunity to produce 3 litters within each generation. Data on the third generation of Group 1 were not obtained because of an insufficient number of animals. The female offspring of all 3 litters were kept for breeding and the male offspring were killed at 14 days of age. After producing 3 litters, the dams of each generation were also killed.

Animals and Diet

The original female guinea pigs were obtained at 14 days of age from a colony derived from the Rockland Farms strain cross-bred with strain 13 from the National Institutes of Health. Littermates were placed in different experimental groups and were matched as closely as possible on the basis of body weight. The basic experimental diet was an ascorbic-acid-deficient diet prepared according to the method of Krehl.† It contained 40% ground rolled oats, 15% wheat bran, 8% alfalfa leaf meal, 20% whole milk powder, 10% casein, 5% cottonseed oil, 0.5% sodium chloride, 1% calcium carbonate, and 0.5% magnesium sulfate. This diet was chosen in preference to a totally purified diet because Crampton and Bell [39] reported that dietary fiber decreased the incidence of reproductive anomalies in control animals. Vitamins and minerals were added to the powdered diet in amounts estimated to be adequate for growth, pregnancy, and lactation.[38, 40–46] Based on calculations of the nutrient composition of the diet [47–49] and the weighed additions of selected nutrients, the composition of the diet as fed is shown in TABLE 1. Diet was made fresh weekly and was fed ad libitum in powdered form.

Ascorbic Acid Intake

Three groups of animals were given daily either 0.15, 0.40, or 10.00 mg ascorbic acid per 100 g of body weight, by gavage. These 3 levels represent a chronically low, adequate, and high intake of ascorbic acid. During the 14-day lactation period, the dam was fed the same amount of ascorbic acid as on the last day of pregnancy. That is, the dose was not adjusted downward to correspond with weight decrease at parturition. Offspring were not given an ascorbic acid supplement until after weaning, at 14 days of age. At this time, the female offspring were given the same level of ascorbic acid as the respective parent animal.

Mating

The females were mated at 90 days of age or as soon thereafter as they came into estrus. The male animals used for breeding were from the same colony and were fed the experimental diet and given 3.0 mg of ascorbic acid per 100 g of body weight, daily. In order to minimize possible differences due to the male breeding animals, an attempt was made to have a particular male sire the same number of first, second, and third litters in each group. Deviations from this procedure occurred when a female animal failed to conceive after several breeding attempts with a male that had previously serviced a female of another group.

Copulation date was determined by daily vaginal smears from each animal whose vaginal membrane was open. If sperm was found in the vaginal smear, or if a plug was found in the cage, conception was assumed and the female

† Laboratory Manual at Yale Nutritional Laboratory, New Haven, Conn. Obtained from Teklad Mills, Madison, Wis.

TABLE 1

COMPOSITION OF THE DIET

Ingredient	Form of Added Nutrient	Diet * as Purchased (per kg)	Additions (per 960 g)	Diet as Fed (per kg)
Protein (g)		240.8	—	231.2
Vitamin A (I.U.)†	Crystalline vitamin A acetate (gelatin coated), 500,000 I.U. per gram	1,136	21,500	22,191
Vitamin D (I.U.)†	Crystalline vitamin D_3, 1 I.U. per .025 μg	Trace	1,200	1,200
Vitamin E (mg)†	DL alpha-tocopherol acetate 250 I.U. per gram	33	250	282
Vitamin K (mg)†	Menadione	0.43	10.00	10.40
Thiamine (mg)		4.60	—	4.40
Riboflavin (mg)†	Riboflavin	4.33	2.00	6.15
Niacin (mg)		37.2	—	35.7
Choline (g)†	$C_5H_{14}ClNO$	0.5	4.5	5.0
Folic acid (mg)†	Crystalline folic acid	2.6	6.0	6.5
Pantothenic acid (mg)		15.03	—	14.43
Para-aminobenzoic acid (mg)†	Crystalline *p*-amino-benzoic acid	—	1.00	1.00
Vitamin B_6 (μg)		3,290	—	3,158
Vitamin B_{12} (μg)		3.20	—	3.07
Vitamin C (mg)		12.0	—	11.5
Magnesium (g)		2.6	—	2.5
Potassium (g)‡	CH_3COOK	6.4	5.1	11.2
Calcium (g)‡		8.8	3.2	11.7
Phosphorus (g)		6.4	—	6.1
Manganese (mg)‡	$MnSO_4 \cdot H_2O$	35	120	153
Sodium (g)§	CH_3COONa	3.6	4.0	7.4
Iron (g)¶	$FeC_6H_5O_7 \cdot 5H_2O$	0.09	0.15	0.24
Copper (mg)‡	$CuSO_4 \cdot 5H_2O$	4.4	15.0	19.2
Cobalt (mg)‡	$CoCl_2 \cdot 6H_2O$	0.015	0.100	0.115
Zinc (mg)		86.0	—	82.6
Chlorine (g)†	$C_5H_{14}ClNO$	6.1	4.1	10.0
Iodine (g)‡	KI	—	0.01	0.01

* By calculation from tables of feed composition.

† Nutritional Biochemicals Company, Cleveland, Ohio.

‡ Mallinckrodt Chemical Works, St. Louis, Mo.

§ Baker and Adamson, Allied Chemical, Industrial Chemicals Division, Morristown, N.J.

¶ Fisher Scientific Company, Chemical Manufacturing Division, Fair Lawn, N.J.

was removed from the cage. Otherwise the female was removed from the male when the vaginal membrane closed.

Determinations

Records were kept of body weights, mating, the results of pregnancy, (defined here as the condition that ended in either abortion or birth), survival of offspring, and mortality of adult animals.

Total ascorbic acid and dehydroascorbic acid plus diketogulonic acid were determined in the cerebrum, remaining brain tissue, hypothalamus, pituitary, adrenal gland, uterus, and ovary by the 2,4-dinitrophenylhydrazine method[50] as modified by Mitchell.[51] The hydrogen sulfide treatment was omitted since diketogulonic acid was not determined separately. Animals were killed by decapitation and the tissues quickly excised, weighed, and homogenized in cold 5% trichloroacetic acid. Following centrifugation at 4° C the supernatant solution was frozen and stored at —20° C until analyzed.

Differences between and within groups were tested for significance by Student's t-test for unequal sample sizes. The selected significance level was 5% or less.

RESULTS

Body Weight

Growth of the offspring from birth to 14 days of age for each generation are given in FIGURES 2, 3, and 4. Although there were weight differences between the sexes, these were inconsistent and not statistically significant. Therefore, the growth curves represent the combined weights of both sexes.

In the F_1 generation, animals whose dams were fed the lowest level of ascorbic acid were heavier at birth than those in the other two groups, but the weight gains were comparable among the groups. In the F_2 generation the birth weights of animals whose dams were fed the lowest level of ascorbic acid were significantly lower than those of the other groups. Animals in the control group were also heavier at birth than those of dams fed 10 mg ascorbic acid/100 g body weight. Again, as in the F_1 generation, the rate of weight gain was comparable for all groups. In the F_3 generation there were no significant differences between Groups 2 and 3 in either birth weight or rate of weight gain.

Growth of animals from 14 to 92 days of age within each generation are given in FIGURES 5, 6, and 7. Animals fed 0.15 mg ascorbic acid/100 g of body weight had significantly less weight gain than did those fed the higher levels. The decreased rate of weight gain became obvious immediately after weaning in the P_1 and F_1 generations (FIGURES 5 and 6). Since the decreased rate of weight gain in Group 1 did not occur until after weaning, it appears that during nursing ascorbic acid in the milk was sufficient to promote normal growth. The high intake of ascorbic acid neither depressed nor accelerated weight gain in comparison with the control group. Comparisons of weight gains across generations show no differences within the ascorbic acid groups.

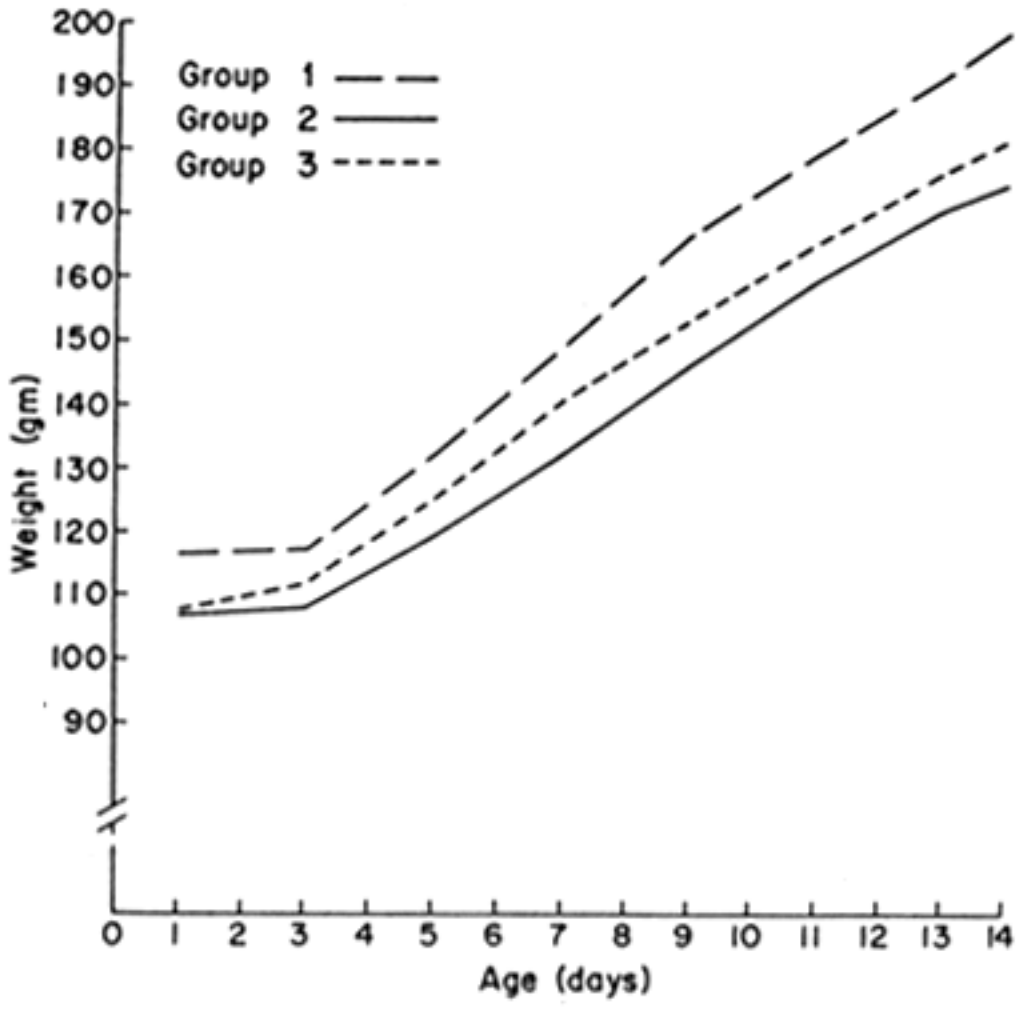

FIGURE 2. Growth of F_1 generation offspring from dams fed three levels of ascorbic acid. The levels of ascorbic acid fed in mg/100 g body weight per day were: Group 1, 0.15; Group 2, 0.40; and Group 3, 10.0.

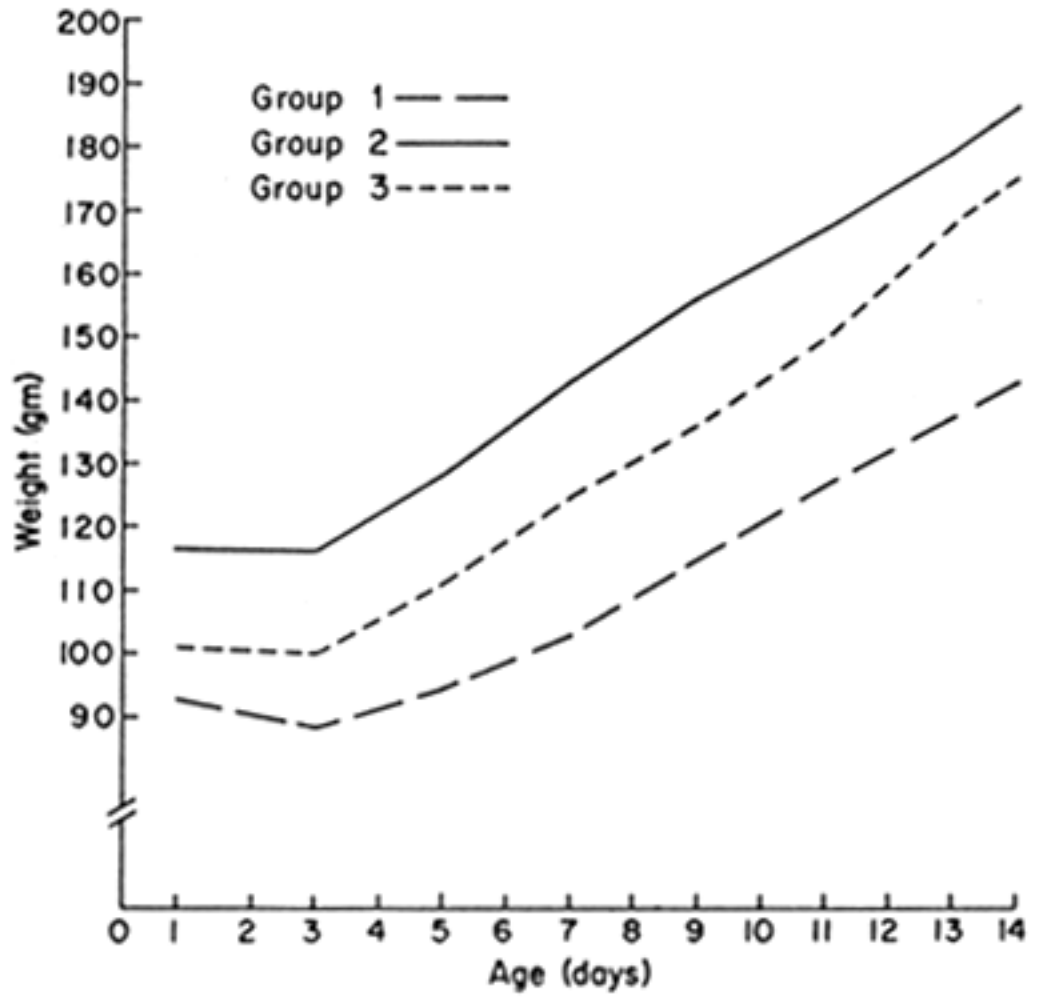

FIGURE 3. Growth of F_2 generation offspring from dams fed three levels of ascorbic acid. The levels of ascorbic acid fed in mg/100 g body weight per day were: Group 1, 0.15; Group 2, 0.40; and Group 3, 10.0.

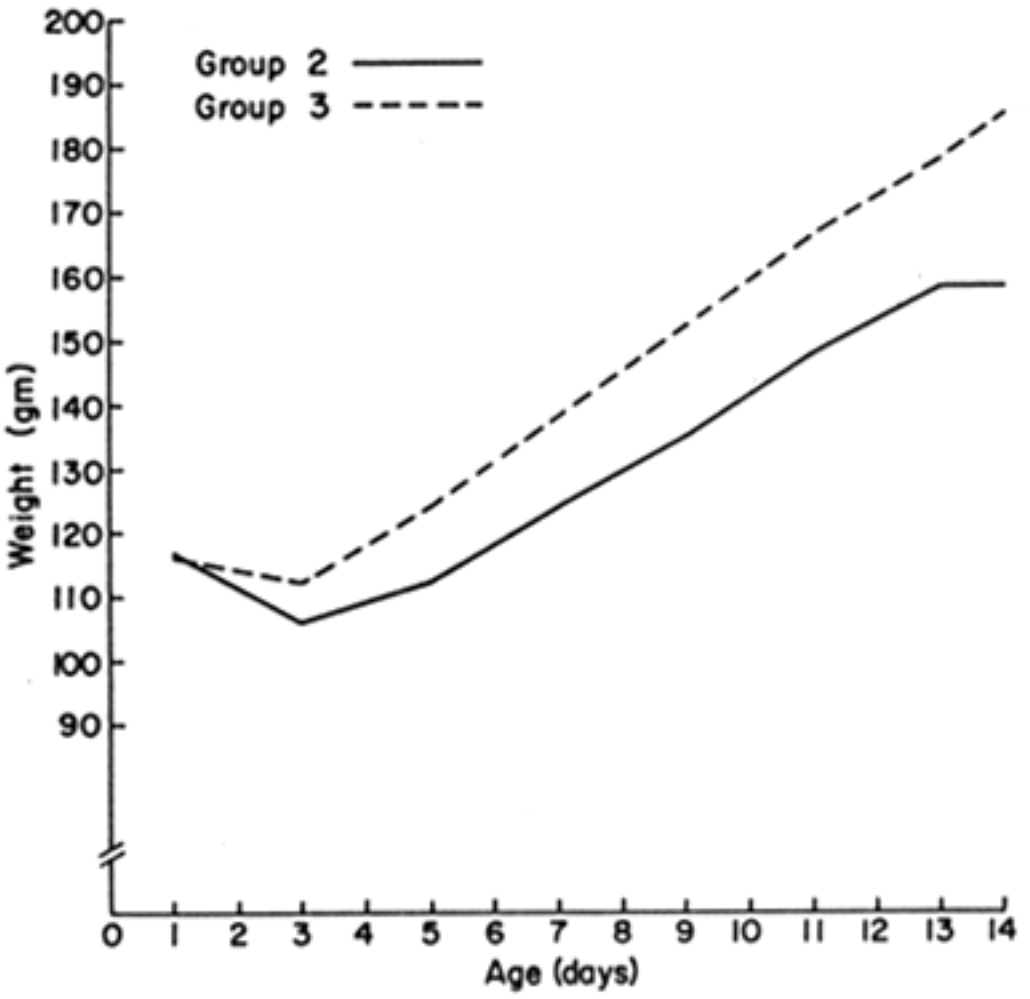

FIGURE 4. Growth of F_3 generation offspring from dams fed two levels of ascorbic acid. The levels of ascorbic acid fed in mg/100 g body weight per day were: Group 2, 0.40; Group 3, 10.0.

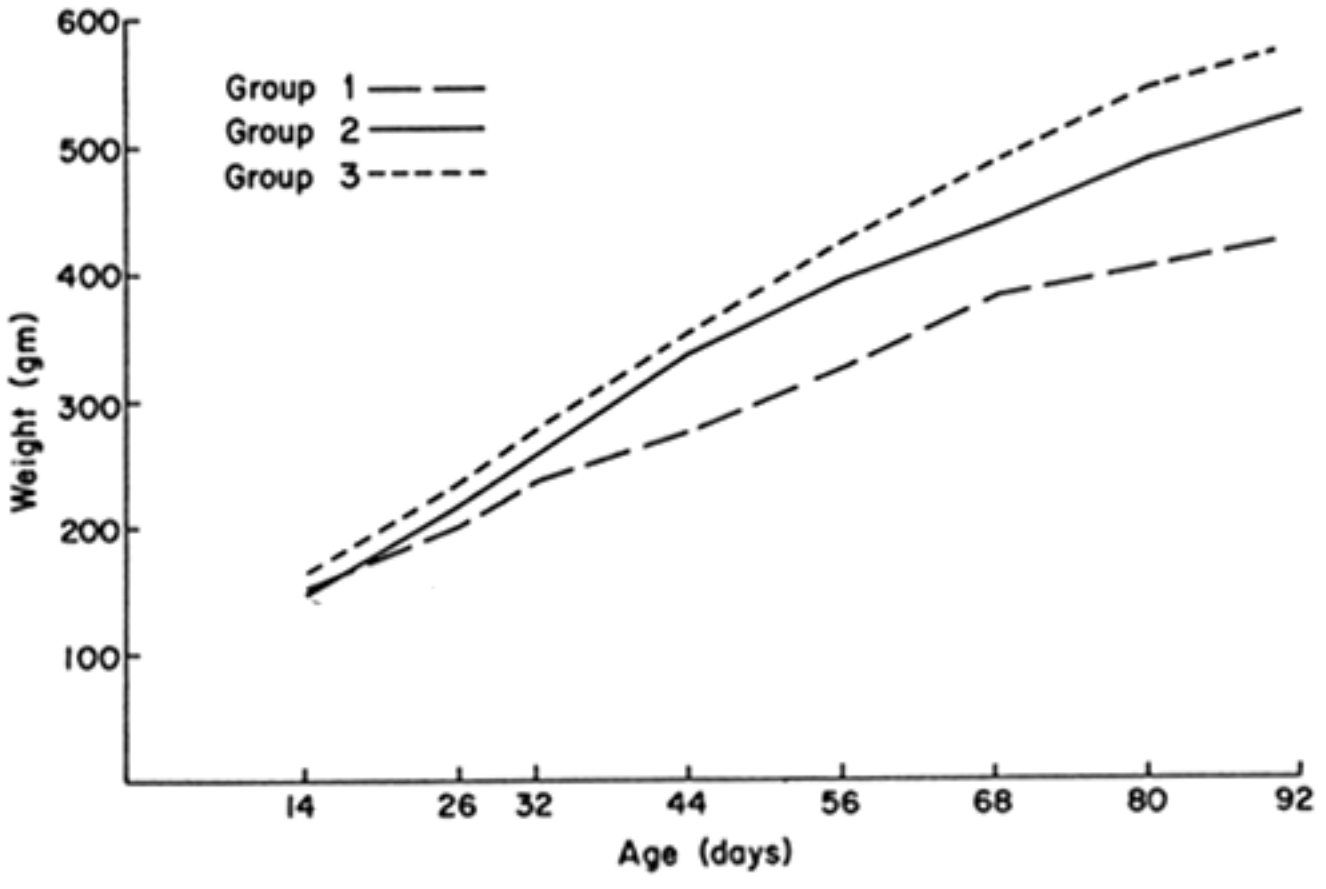

FIGURE 5. Growth of P_1 generation dams fed three levels of ascorbic acid. The levels of ascorbic acid fed in mg/100 g body weight per day were: Group 1, 0.15; Group 2, 0.40; and Group 3, 10.0.

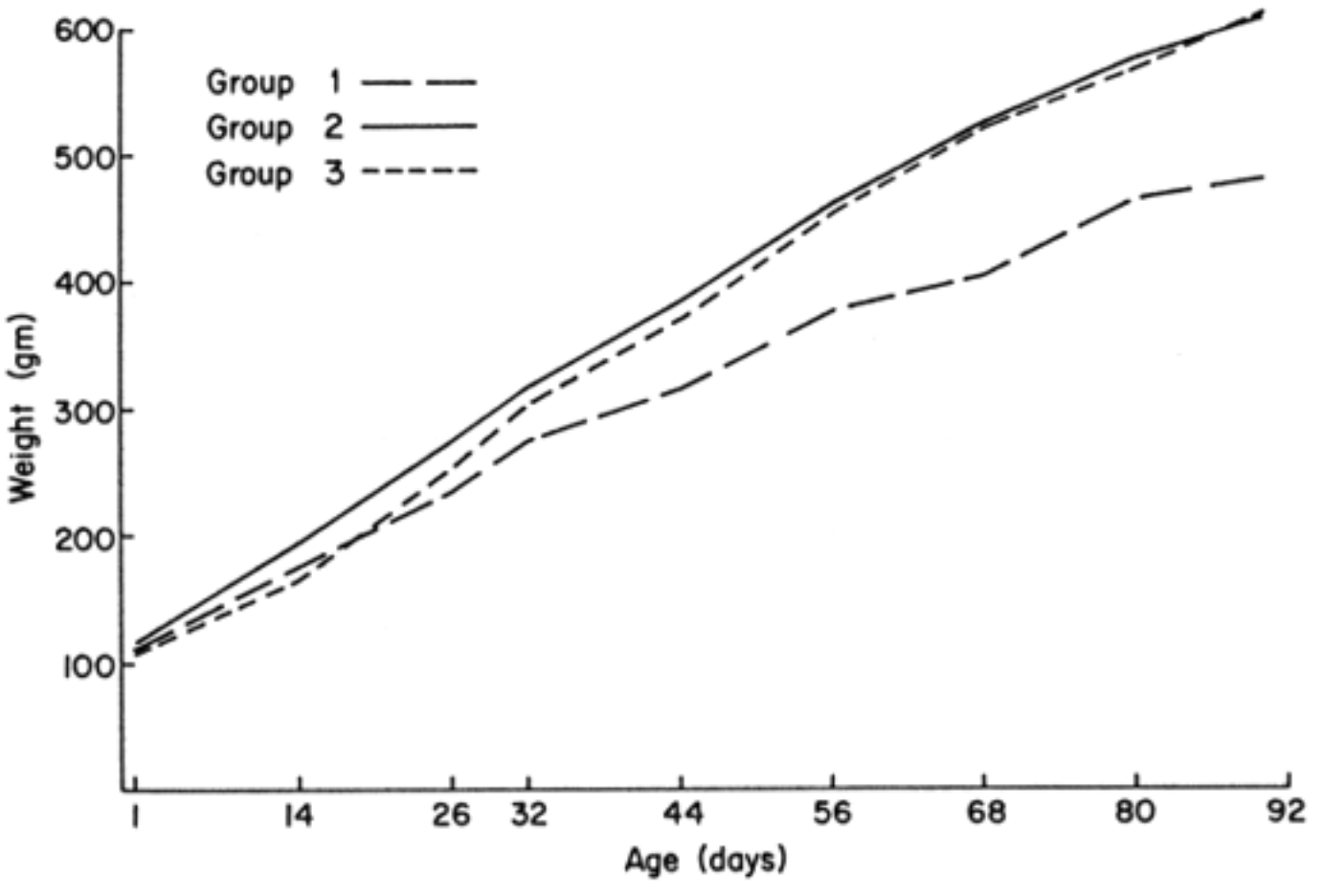

FIGURE 6. Growth of F_1 generation dams fed three levels of ascorbic acid. The levels of ascorbic acid fed in mg/100 g body weight per day were: Group 1, 0.15; Group 2, 0.40; and Group 3, 10.0.

Reproduction

Reproduction results are presented in TABLE 2. The mating data were combined for all three generations since there were no differences among generations within a group. If more than five mating attempts were required to produce a pregnancy, the data were not included in the tabulation. This excludes mating data corresponding to 3 of the 32 pregnancies in Group 1, 6 of the 60 preg-

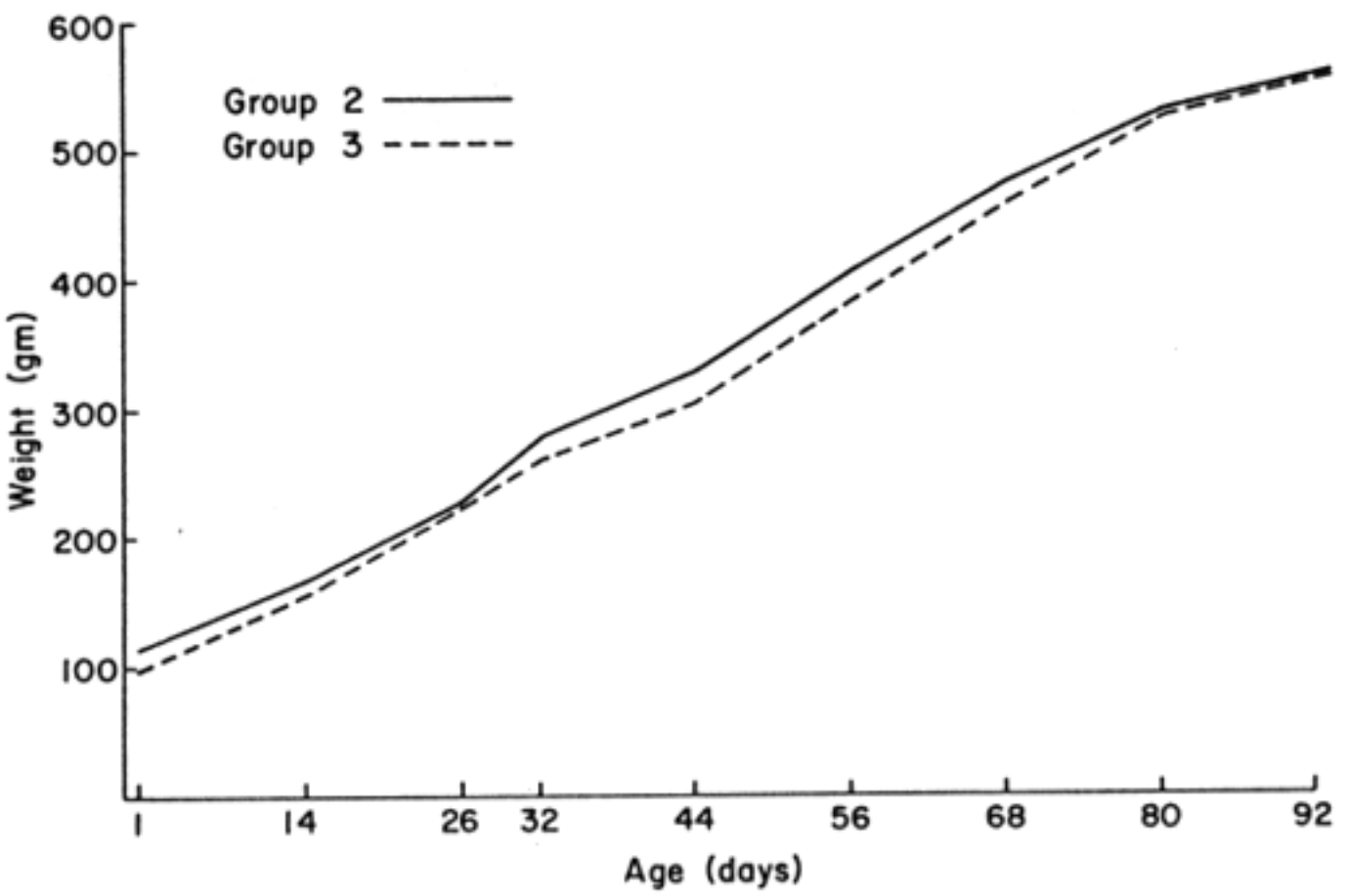

FIGURE 7. Growth of F_2 generation dams fed two levels of ascorbic acid. The levels of ascorbic acid fed in mg/100 g body weight per day were: Group 2, 0.40; Group 3, 10.0.

TABLE 2

SUMMARY OF REPRODUCTION FOR ALL THREE GENERATIONS *

	Ascorbic Acid Intake (mg/100 g body weight/day)		
	Group 1 0.15	Group 2 0.40	Group 3 10.00
Females	25	33	34
Matings per pregnancy	2.13	2.00	1.67
Litters produced	24	50	72
Abortions	8	10	12
Litters per female	0.96	1.51	2.12
Abortions per 10 pregnancies	2.50	1.67	1.43
Young born per litter	2.46	2.66	2.65
Viable young per litter	1.54	1.44	1.64

* All values are average for each group.

nancies in Group 2, and 8 of the 84 pregnancies in Group 3. The number of matings required for these pregnancies varied from 6 to 13. Group 3 dams required fewer matings per pregnancy, produced more litters per female, and had fewer abortions on the basis of number of pregnancies than did those in Groups 1 and 2. The number of young born per litter and viable young at weaning was similar for all three groups.

The reproductive performance of dams within each generation is given in TABLE 3. A higher percentage of the dams in Group 3 produced first, second, and third litters within each generation than did those in Group 2; the difference in the third generation is particularly striking. Likewise, more dams in Group

TABLE 3

REPRODUCTION PERFORMANCE WITHIN EACH GENERATION

Ascorbic Acid Intake (mg/100 g body weight/day)	Generation	Number of Females	Number of Litters	Percentage of Females Producing: Litters 1	2	3	Number of Abortions
Group 1 (0.15)	P_1	14	15	64	36	7	6
	F_1	11	9	54	18	9	2
Group 2 (0.40)	P_1	10	18	90	50	40	4
	F_1	14	27	86	64	43	4
	F_2	9	5	44	11	0	2
Group 3 (10.00)	P_1	8	20	100	88	63	3
	F_1	14	28	100	64	36	7
	F_2	12	24	100	67	33	2

2 produced first, second, and third litters than did those in Group 1. In Group 1 there were only five viable F_2 females at weaning, two of which died before 90 days of age. Therefore this group was not continued for the third generation.

Death of dams occurred in each group and each generation. Some of the animals died while pregnant and a few died immediately after giving birth. There was no apparent relationship between these observations and the level of ascorbic acid intake.

Tissue Ascorbic Acid Concentrations

The tissue ascorbic acid data for dams were analyzed on the basis of number of litters produced and age at sacrifice. No statistically significant differences were found due to these variables. The tissue ascorbic acid data for the 14-day-old offspring were analyzed on the basis of sex; no statistically significant differences were found. Therefore, the data for all dams and all offspring within groups were combined.

Tissue concentrations of total ascorbic acid are given in TABLE 4 and of dehydroascorbic acid plus diketogulonic acid (oxidized) in TABLE 5. Dams and offspring in Group 2 had tissue levels significantly less than those in Group 3. With the exception of the ovary and pituitary of dams and the brain and pituitary of offspring, tissue levels of Group 2 were significantly greater than those of Group 1.

Offspring in Groups 1 and 2 had lower concentrations in the pituitary than did dams. Levels in other tissues of dams and offspring in Groups 1 and 2 were comparable, except in Group 2, where the offspring had higher concentrations than dams in the uterus and cerebrum. In Group 3, the offspring had higher concentrations than dams in all tissues except the ovary.

Tissues of offspring had higher levels of oxidized ascorbic acid than dams. With the exception of the cerebrum and brain in Group 3, the differences were all significant. Differences were particularly marked in the uterus, adrenal, and ovary. Group 3 offspring had greater oxidized ascorbic acid concentrations in the adrenal than Group 2 offspring. The oxidized levels in tissues of dams were also significantly greater in Group 3 than in Group 2, except for the ovary.

DISCUSSION

The growth rate of offspring during the nursing period and the number of viable young per litter at 14 days of age were comparable among the 3 groups. The ascorbic acid in the secreted milk of dams on the lowest intake was apparently adequate for normal growth. During lactation, the dams were given ascorbic acid on the basis of body weight prior to parturition. This procedure increased the ascorbic acid intake during lactation by about 20–30%. Birth weights were not consistently related to ascorbic acid intake, and the number of young born per litter was similar for all groups. The data suggest that if pregnancy is sustained, the ascorbic acid intake is adequate for growth and development of the fetus. Depressed growth was observed between 14 and 92 days of age in animals fed the lowest level of ascorbic acid. The highest dietary level of ascorbic acid neither accelerated nor depressed weight gain.

The best reproduction performance was observed in animals fed ascorbic

TABLE 4

TOTAL ASCORBIC ACID CONCENTRATIONS IN TISSUES OF DAMS AND 14-DAY-OLD OFFSPRING

	Group 1			Group 2			Group 3		
	Ascorbic Acid Intake (mg/100 g body weight/day)								
		0.15			0.40			10.00	
Tissue	(n)	Mean	±SEM	(n)	Mean	±SEM	(n)	Mean	±SEM
Cerebrum									
Dams	—	—	—	12	7.15	0.62	9	17.66	1.23
Offspring	7	2.02	0.35	11	13.30	1.23	30	23.93	1.28
Brain component									
Dams	—	—	—	11	7.08	0.51	10	14.96	0.53
Offspring	6	9.12	2.66	10	9.49	1.00	34	23.25	1.02
Hypothalamus									
Dams	6	5.78	0.38	9	9.30	1.31	7	20.56	1.51
Offspring	6	5.60	0.92	5	14.86	3.30	13	25.41	2.68
Uterus									
Dams	10	3.58	0.24	16	4.99	0.46	17	11.51	1.17
Offspring	6	5.67	0.70	6	8.67	0.72	16	21.51	2.16
Adrenal									
Dams	13	21.44	2.19	18	38.26	2.28	20	78.31	6.23
Offspring	20	20.99	1.87	25	35.13	3.68	45	106.09	5.63
Ovary									
Dams	8	8.68	0.58	14	15.23	3.01	18	36.11	2.60
Offspring	5	7.82	0.97	4	18.11	2.79	12	42.57	3.52
Pituitary									
Dams	9	27.17	3.09	5	47.92	5.83	6	65.06	3.59
Offspring	4	12.89	4.36	3	21.44	2.53	6	99.22	4.45

Significant differences (confidence limits 5% or less)
Between groups:
Groups 2 and 3: Dams, all tissues; offspring, all tissues
Groups 1 and 2: Dams, all tissues except ovary and hypothalamus; offspring, all tissues except brain and pituitary
Between dams and offspring within groups:
Group 1: Pituitary

Tis	0.15 (n)	0.15 Mean	0.15 ±SEM	0.40 .)	0.40 Mean	0.40 ±SEM	10.00 (n)	10.00 Mean	10.00 ±SEM
Cerebrum									
Dams	—	—	—	12	7.15	0.62	9	17.66	1.23
Offspring	7	2.02	0.35	11	13.30	1.23	30	23.93	1.28
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Offspring	4	12.89	4.36	3	21.44	2.53	6	99.22	4.45

Significant differences (confidence limits 5% or less)
Between groups:
Groups 2 and 3: Dams, all tissues; offspring, all tissues
Groups 1 and 2: Dams, all tissues except ovary and hypothalamus; offspring, all tissues except brain and pituitary
Between dams and offspring within groups:
Group 1: Pituitary
Group 2: Cerebrum, uterus, pituitary
Group 3: All tissues except ovary

acid at levels considerably higher than what has been presumed to be adequate for reproduction in the guinea pig.[38-40] The young born per litter and viable young at weaning were similar for all 3 groups, but dams on the highest intake of ascorbic acid required fewer matings per pregnancy, produced more litters per female, and had fewer abortions. These findings are contrary to the suggestions that large quantities of ascorbic acid are detrimental during pregnancy.[34-37]

TABLE 5

TOTAL OXIDIZED ASCORBIC ACID (DEHYDROASCORBIC ACID PLUS DIKETOGULONIC ACID) CONCENTRATIONS IN TISSUES OF DAMS AND 14-DAY-OLD OFFSPRING

	Group 2			Group 3		
	Ascorbic Acid Intake (mg/100 g body weight)					
	0.40			10.00		
	(n)	Mean	±SEM	(n)	Mean	±SEM
Cerebrum						
Dams	10	0.49	0.04	4	0.71	0.09
Offspring	11	1.18	0.16	28	1.02	0.10
Brain Component						
Dams	9	0.68	0.16	9	1.38	0.16
Offspring	9	1.34	0.22	28	1.38	0.12
Hypothalamus						
Dams	7	1.29	0.19	6	2.12	0.47
Offspring	5	3.08	0.73	14	4.73	0.61
Uterus						
Dams	8	1.52	0.18	9	1.99	0.22
Offspring	4	5.81	1.30	15	5.13	0.69
Adrenal						
Dams	10	1.55	0.15	10	2.93	0.35
Offspring	17	6.62	0.58	32	10.25	0.69
Ovary						
Dams	7	2.97	0.86	11	3.60	0.78
Offspring	4	8.37	2.19	12	11.18	1.59

Significant Differences (confidence limits 5% or less)
- Between groups:
  - Dams: All tissues except ovary
  - Offspring: Adrenal
- Between dams and offspring within groups
  - Group 2: All tissues
  - Group 3: All tissues except cerebrum and brain

The tissue ascorbic acid concentrations in dams fed 10 mg ascorbic acid/100 g body weight daily were lower than expected. In previous work[31, 51, 52] pregnant guinea pigs fed 0.75, 1.2, or 1.5 mg of ascorbic acid/100 g body weight and killed at midgestation had tissue levels considerably higher than those found for Group 3 in this study. Based on a previous long-term feeding study, the pituitary and adrenal glands of Group 3 animals were considerably below

saturation levels.[53] Based on these observations, the requirement for ascorbic acid is markedly increased during the last half of pregnancy and lactation. Tissue saturation is not maintained in dams even with what may be considered an excessive intake of ascorbic acid. The tissue levels in the offspring of Group 3 were higher than in dams and were near saturation except for the pituitary, adrenal, and ovary, which can be further increased in young animals with massive doses of ascorbic acid.[54] Thus, it appears that when tissue concentrations of ascorbic acid are high in the dam, the offspring are born with even higher tissue concentrations that are sustained during the nursing period.

Dams and offspring in Group 2 had tissue levels significantly below those of Group 3, and far below saturation levels. The relationship between tissue levels in the dams and offspring in Groups 1 and 2 were similar. The results show that when ascorbic acid intake is insufficient to maintain high tissue concentrations in the dam, the offspring do not concentrate the vitamin at the expense of the maternal organism. Thus, the fetus and neonate, because of a more rapid metabolism of ascorbic acid, are more vulnerable than the dam to low intakes of the vitamin. In the pituitary of both Groups 1 and 2, the offspring had lower levels of ascorbic acid than did the dams. We have previously found indirect evidence for a rapid metabolism of ascorbic acid in the pituitary of young, growing guinea pigs. This tissue may possibly be a more sensitive indicator of ascorbic acid nutriture than others.

The oxidized ascorbic acid levels were higher in tissues of offspring than in dams. This is probably due to a faster rate of metabolism in the young, growing animal. In general, the amount of oxidized ascorbic acid is increased when tissues have high concentrations of ascorbic acid. This accounts for the higher levels of oxidized ascorbic acid in Group 3 than in Group 2.

Summary

The 1974 RDA is 60 mg per day for pregnant women and 80 mg per day for lactating women. In the present study an attempt was made to simulate this intake in the guinea pig and study reproduction performance in relation to guinea pigs fed chronically low and high levels.

In animals that conceived and carried the young to term, all 3 dietary levels of ascorbic acid appeared to be adequate for maintaining viability of fetuses and of offspring, and for growth of offspring during the nursing period. The chronically low intake level was not adequate for growth after weaning. The control group was superior to the chronically low-intake group but inferior to the high-intake group in conceiving, producing litters, and carrying litters to term. The level of intake in the control group was inadequate to maintain tissue stores. Even the high intake was inadequate to maintain some tissues at saturation levels. The results suggest that the requirement for ascorbic acid during pregnancy and lactation has been markedly underestimated.

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Discussion

Dr. M. Winick: I am a little confused about the two experiments that were presented this morning and the one discussed just now. It seems to me that in making these comparisons we are comparing apples to oranges. The experiments are totally different and in no way conflict. I did not understand that there was a difference in birth weight reported in Dr. Rosso's paper, so his finding was precisely what you found.

Dr. Rivers: Yes, our findings agreed with Dr. Rosso's.

Dr. Winick: The point that should be open for discussion is the measurement used to determine whether or not a particular intake is safe, high, or not high enough. In one case, looking at reproduction from the standpoint of Dr. Rosso, a detriment results. At this stage of our understanding we must question whether or not these kinds of studies must be carried a lot farther before we can decide whether we should or should not use very high doses of ascorbic acd.

Dr. Rivers: I agree. If you recall, Dr. Rosso was feeding orally twice as much ascorbic acid as administered to our high-intake group.

Dr. J. J. Kamm: Were the male pigs that were used for mating matched with the dam with respect to ascorbic acid intake?

Dr. Rivers: No, males that were used for mating were given 3 mg of ascorbic acid per 100 gram of body weight.

Dr. Kamm: At the time of mating, were all groups given the same amount?

Dr. Rivers: All breeding males were given 3 mg of ascorbic acid per 100 grams of body weight. We attempted to have the same male sire a litter for each female within each group.

Question: How much information is there on reproductive performance in the range between 0.4 and 10 mg ascorbate per day?

DR. RIVERS: There is an excellent study by Pye where dietary intakes varied from 2 to 8 mg per day. There is also the work of Crampton and Bell; they reported that 5 mg of ascorbic acid per day was adequate for reproduction in the guinea pig.

DR. KAMM: Did you observe any differences as a function of the ascorbic acid intake in the number of successful pregnancies or the number of aborted pregnancies in the 3 groups?

DR. RIVERS: We presented the data on the basis of abortions per 10 pregnancies. I'm defining pregnancy here as that condition that either terminated normally or in abortion. We have no data on resorption. We did find that animals on high-intake level had fewer abortions per pregnancy than did animals on the low-intake level.