Annu. Rev. Nutr. 2004. 24:79–103
doi: 10.1146/annurev.nutr.24.012003.132150

First published online as a Review in Advance on January 16, 2004

DEVELOPMENTAL ASPECTS AND FACTORS INFLUENCING THE SYNTHESIS AND STATUS OF ASCORBIC ACID IN THE PIG

D.C. Mahan,[1] S. Ching,[2] and K. Dabrowski[3]
[1]*Department of Animal Sciences, College of Food, Agriculture, and Environmental Sciences; The Ohio State University, Columbus, Ohio 43210; email: mahan.3@osu.edu*
[2]*Department of Oral Biology, College of Dentistry, The Ohio State University, Columbus, Ohio 43210; email: ching.6@osu.edu*
[3]*School of Natural Resources, The Ohio State University, Columbus, Ohio; email: dabrowski.1@osu.edu*

Key Words ascorbic acid, vitamin C, dehydroascorbic acid, pigs

■ **Abstract** Ascorbic acid synthesis in the pig occurs at mid-pregnancy, but activity of the enzyme L-gulono-γ-lactone oxidase (GLO) declines thereafter during gestation and remains low when the pig nurses the sow. During late gestation the ascorbic acid concentration in the fetus increases, but serum and liver ascorbic acid concentration in the sow declines without affecting the dam's liver GLO activity. It is presumed that as gestation progresses an increased amount of maternal ascorbic acid is transferred to the fetus and to the mammary gland. Colostrum and milk are rich sources of the vitamin and supply the nursing pig with ascorbic acid. The available data suggest that high amounts of ascorbic acid appear to suppress liver GLO activity in the pig. Upon weaning, when exogenous vitamin C is generally not provided, liver GLO activity and serum ascorbic acid increases. During the initial periods postweaning, some reports have indicated growth benefits of supplemental vitamin C. Body tissues differ in their concentrations of ascorbic acid, but tissues of high metabolic need generally have greater concentrations. The corpus luteum in the female, the testis in the male, and the adrenal glands in all pigs contain greater concentrations of the vitamin. Knockout genes preventing ascorbic acid synthesis in pigs have demonstrated poor skeletal and collagen formation and poor antioxidant protection. Under periods of stress ascorbic acid declines in the adrenal, but the pig rapidly recovers to its resting state once the stressor agent is removed. Although there are periods when supplemental vitamin C has been shown to promote pig performance (e.g., during high environmental stress and early postweaning), supplemental vitamin C has not been shown to routinely enhance pig performance.

CONTENTS

INTRODUCTION

Because the pig can synthesize vitamin C it cannot be considered as a dietary essential for this species, but clearly it is a metabolic essential. The key enzyme L-gulono-γ-lactone oxidase (GLO) necessary for ascorbic acid synthesis is found in pig liver microsomes with D-glucose as the precursor. The body's principal need for vitamin C is as an antioxidant and collagen synthesis, hydroxylation processes, and hormone secretion. Its antioxidant role involves trapping reactive oxygen species (ROS) and making them less reactive, but it is also involved in fetal skeletal development and gonad tissue growth and maintenance. The pig regulates the amount of the vitamin synthesized (via GLO activity), for excesses, as well as deficiencies, can be harmful. The enzyme's activity thus depends upon the source (endogenous or exogenous) and the total quantity of the vitamin provided. This review evaluates the development and changes of the GLO activity during life-cycle changes in the pig, factors affecting its status and synthesis, and the effect of exogenous supplies. When one considers the developmental changes of ascorbic acid status in the pig, critical periods of need, and factors regulating its synthesis, its metabolic need may justify periodic supplementation.

Prenatal Development

MID TO LATE PERINATAL PERIOD The gene for the synthesis of the GLO [C 1.13.8] is found on chromosome 14 in the pig (42). This enzyme catalyzes the terminal reaction converting L-gulono-γ-lactone to L-keto-gulono-γ-lactone, whereupon L-ascorbic acid is produced through isomerizations (14). The enzyme is found in the liver of higher vertebrate species and in the kidney of lower classes of vertebrates (birds, turtles, and some fish), but not in man, primates, or guinea pigs. Other enzymes responsible for the conversion of D-glucose to ascorbic acid precursors are found in several body tissues (39), but it is the GLO that is lacking in those species that cannot ultimately synthesize the vitamin.

The synthesis of ascorbic acid in the pig has been considered to occur postnatally (10), a conclusion that is based on tissue concentrations and urinary excretion of ascorbic acid. The identity of the critical GLO for ascorbic acid synthesis in species lacking this ability (39, 82) established it as a more reliable indicator for

determining ascorbic acid synthesis than tissue or urinary ascorbic acid values. Tissue accumulation and excretion of the vitamin in young pigs is doubly confounded because colostrum and the later milk consumed by young pigs are rich in this vitamin (7–9).

Recent evidence using liver GLO activity as the determinant demonstrated that the pig fetus has a high rate of ascorbic acid synthesis by mid gestation (i.e., day 60), but its activity declined as gestation progressed (18). This is consistent with rat data where fetuses showed no enzyme activity on day 14 and 15 of gestation, but high activities on day 16, whereupon its activity subsequently declined by the end of gestation (61).

Fetal plasma and tissue have a greater concentration of ascorbic acid than the dam's tissue at comparable stages of gestation (6, 13, 18). Although fetal synthesis may be partially derived from in vivo synthesis, the data imply an active transport mechanism for the vitamin through the maternal-fetal barrier. This is consistent with species that synthesize ascorbic acid (58, 60) or those unable to synthesize the vitamin (67, 91, 95). The greater tissue ascorbic acid concentration in the developing pig fetus compared with the dam implies a greater metabolic activity in the fetus, which most probably is needed for skeletal and collagen formation (24, 45).

Although GLO activity prior to day 60 postcoitum has not been established in the pig, it is clear that ascorbic acid is needed during early pregnancy for fetal growth and skeletal formation. At the latter stages of gestation the fetus continues to have the capability to synthesize the vitamin, but the maternal supply effectively seems to cross the placental barrier and subsequently to suppress the activity of fetal GLO.

Fetuses from sows genetically incapable of synthesizing ascorbic acid have demonstrated several pathological conditions in fetal tissue (e.g., edema, subcutaneous bleeding, and abnormal skeletal development) that have contributed to the understanding about the role of ascorbic acid. These conditions are attributed to damage in the placenta, where hemorrhages of the capillary system were extensive (82).

Placental ascorbic acid in the pig becomes increasingly concentrated as gestation progresses, growing sixfold from day 60 to 80 of gestation and another twofold from day 80 to 100 (18). These responses are comparable to the rat (95), sheep (58), human (85), and guinea pig (25, 79). Placental ascorbic acid is readily transported in the oxidized form (dehydroascorbic acid) via the glucose transport system (51), but because this form has a potentially toxic effect on young developing tissue (4), it is readily converted to the reduced (ascorbate) form (21, 79). The placenta may thus have a role in regulating the ratio of both ascorbic acid forms to the fetus and in maintaining a redox balance.

LATE GESTATION TO PARTURITION During the last two weeks of gestation, or the period of most rapid quantitative growth in fetal pigs, liver GLO activity increases while adrenal gland, liver, and kidney ascorbic acid concentrations decline (13, 18).

The rapid growth rate of of both muscle and skeletal tissue in the fetus during late gestation not only increases the need for all nutrients, but concurrently increases the antioxidant need for ascorbic acid. The marked decline in placental ascorbic acid during late gestation (18) suggests a greater transfer of placental ascorbic acid to the fetus. The small increase in sow liver GLO activity with the corresponding decline in liver, spleen, and adrenal gland ascorbic acid concentration is consistent with these conclusions (18).

Because the pig is a litter-bearing animal, the duration of the parturition process is often several hours, resulting in a lower blood and oxygen supply to the fetus, depending on its birth order. Under such conditions hypoxia has been shown to exist (5), thus potentially affecting the ascorbic acid status of the neonate. Pigs born late in the birth sequence were found to have lower liver and kidney ascorbic acid concentrations (18), a finding that suggests greater metabolism of the vitamin, but liver GLO activity was similar to those born earlier.

It is common on many commercial swine farm units to induce parturition one to three days prepartum by administering prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Early delivery of piglets, however, has often resulted in greater pig mortalities postnatally (13). Neonatal pigs from sows administered $PGF_{2\alpha}$ have lower liver and kidney ascorbic acid concentrations, which supports the above observations, but GLO activities were similar to neonates from sows not administered $PGF_{2\alpha}$ (18).

Following parturition liver ascorbic acid concentration declines from its prenatal level, but kidney ascorbic acid increases. This suggests an increasing metabolic use and mobilization of ascorbic acid, and its subsequent excretion through the kidney (18).

Postnatal Development

NURSING PERIOD The neonatal pig is born with a moderate body reserve and blood concentration of ascorbic acid, but with a low serum and tissue α-tocopherol concentration (19, 69). Colostrum has a rich supply of both nutrients (7–9, 19, 69), thus supplying the pig with an immediate source of both antioxidants. The ascorbic acid concentration of sow colostrum and milk is greater than that of other domestic animals (10). With antioxidant demands imposed from metabolism and stress, the immediate supply of both ascorbic acid and α-tocopherol becomes extremely important to quench oxidative activity in the young neonate (65). Serum and tissue α-tocopherol concentrations increase in the young pig following the consumption of these milks (69), but ascorbic acid concentration declines (19). Liver GLO activity also declines from its prenatal activity within day 3 of age and remains at a low activity while the pig nurses the sow (19). This suggests that the exogenous supply of ascorbic acid from mammary fluid may suppress liver GLO activity, with the ascorbic acid need completely met by the maternal milk supply. It has been demonstrated (104) that an exogenous source of ascorbic acid, when provided in large quantities, suppresses GLO activity in mice. Recently it has been demonstrated that high dietary levels of vitamin C also suppress GLO activity in the weaned pig (S. Ching, unpublished data).

Liver and adrenal gland ascorbic acid concentrations decline in the nursing pig from its prenatal concentration, but kidney ascorbic acid concentration increases; the latter reflecting excretion of the vitamin (19). The pig is a rapidly growing animal during the initial postnatal weeks, doubling its weight each week until weaning (∼week 3). The antioxidant demands for tissue metabolism in the young pig would be great. The decline in tissue ascorbic acid during the nursing period and the increased kidney ascorbic acid concentrations would be consistent with this conclusion.

Navel bleeding of neonates in commercial swine herds has been reported to be responsive to supplementation of vitamin C when added to the dam's prepartum diet. The quantity of 1 g/day for a 10-day period prior to farrowing has prevented the condition in problem herds (97). Although the results demonstrated a positive effect in some herds, no mechanism of action or cause of the vitamin C deficiency in the herds have been reported. The condition is not widespread.

WEANING At the onset of weaning, when the milk and its ascorbic acid supply are eliminated from the pig's diet, a stimulation of liver GLO activity occurs in young pigs. In a recent study (19), pigs weaned at 10, 17, or 24 days of age (industry average $\leq$21 days) were found to have liver GLO activities that increased three- to fivefold within seven days of weaning in each group. The relative rate of increase was greater initially for the group weaned at the later age, with GLO activity continuing to increase at weekly intervals in all groups, but was lower at each subsequent measurement period (19). The greater rate of increase for the group weaned later may have occurred because of the greater amount of metabolism occurring and a greater metabolic need, as these pigs had a larger body size and would have been consuming more feed. They therefore would have a greater need for antioxidant protection. These results clearly demonstrate that ascorbic acid synthesis was stimulated in weaned pigs at the onset of weaning, not at a particular age or body weight. Until ascorbic acid synthesis reached a rate that met the pig's need, tissue depletion of endogenous ascorbic acid and subsequent excretion increases were found to occur. A decline in plasma, liver, adrenal gland, and kidney ascorbic acid concentrations occurs in pigs upon weaning and for the ensuing weeks postweaning (19, 40, 110). It has been suggested that the pig has inadequate synthesis of ascorbic acid and cannot meet its ascorbic acid need until approximately 8 weeks of age (11, 110).

It is also during the initial weeks postweaning that serum and tissue concentrations of other antioxidants, particularly α-tocopherol and Se, also decline (69). Postweaning pig mortalities are frequently reported during this period by commercial swine producers and veterinarians. These mortalities occur with the largest and fastest growing pigs. Although vitamin E and Se deficiencies are frequently implicated as the causative agent, supplemental vitamin C, vitamin E, and Se have not completely resolved the problem. It has also been reported that various management stressors (59) may exacerbate oxidative reactions in the young pig. Consequently, it is possible that ROS accumulate during the period when GLO

activity is low in the weaned pig while α-tocopherol and Se concentrations are declining. These factors, combined with stressor agents (e.g., crowding, environment, etc.), may exacerbate the mortality problem.

When neonatal pigs were weaned and fed supplemental vitamin C in liquid form for a 21-day period, performance benefits were not attained nor were blood parameters improved (102). Supplementation of dietary ascorbic acid to pigs weaned after a normal nursing period has produced variable performances. Published trials conducted with nursery pigs are summarized in Table 1. These data suggest a positive gain response in nine trials and no gain or a loss in gain in ten trials. The overall mean response to supplemental vitamin C was approximately 17 g/day above that of an average daily gain of ~500 g/day.

GROWER-FINISHER PIG Vitamin C is important for collagen synthesis. Because the growing pig is rapidly increasing this tissue, the need for vitamin C may be greater during early growth phases. Riker (92) showed that growing pigs fed supplemental vitamin C exhibited fewer abnormalities in the forelegs. Serum ascorbic acid normally increases during the grower-finisher period without supplemental supplies (70), but no studies have evaluated the changes in GLO activity. Performance responses have been variable (Table 2). These responses currently do not justify the dietary addition of vitamin C for the market pig.

Reproduction

The mature pig synthesizes ascorbic acid when dietary starch (i.e., glucose units) or body glycogen stores are in ample supply. Because both the gestating female pig and male pig are generally fed a restricted quantity of feed in order to restrict body fat deposition, the quantity of simple carbohydrates available to the liver may become a limiting factor in ascorbic acid synthesis and thus antioxidant protection. There may be periods or conditions during the reproductive cycle where the biological need for the vitamin is greater than what the body can provide. Feed restriction lowers blood glucose and body glycogen stores, thus potentially reducing ascorbic acid synthesis and possibly some of the steroids essential for reproduction.

FEMALE PIG (SOW) Pregnant rats with a diet restricted to approximately 40% ad libitum did not maintain their pregnancy during the latter part of gestation, but the addition of vitamin C reversed this response (84). Glycogen reserves in the liver, placenta, and uterus were lower in the restricted group fed no vitamin C, but glycogen was greater when vitamin C was supplemented. This suggested that vitamin C has an important role in carbohydrate metabolism and fetal survival, at least in rats fed restricted diets. Conversely, when excess glucose was consumed by diabetic rats, the quantity of ROS produced increased along with congenital malformations and resorptions of fetuses (99). The combination of vitamins C and E, or vitamin E or vitamin C singly, prevented these congenital abnormalities (16).

TABLE 1 Effect of dietary ascorbic acid on nursery pig performance

		Vitamin C			Response		
Initial weight (kg)	**No. pigs (total)**	**Vitamin C source**	**Added vitamin C (mg/kg)**	**Trial length (d)**	**Daily gain (g)**	**Daily feed (g)**	**Reference**
11	36	L-ascorbic acid	0, 500, 1000/H_2O	28	+60	NR[a]	11
7	256	L-ascorbic acid	0, 150, 300, 450	28	+13	+19	20
8	140	L-ascorbic acid	0, 150, 300, 450	28	−5	+12	20
5	72	L-ascorbyl-2-P	0, 75, 150	42	+20	+8	27
7	120	L-ascorbyl-2-P	0, 75, 150	31	+21	+3	27
6	64	L-ascorbic acid	0, 330, 990	28	+40	+66	115
	40	L-ascorbic acid	0, 300	84	+100	NR	83
6	288	Mg-L-ascorbyl-2-P	0, 50, 500	35	+17	+1	70
7	1296	L-ascorbic acid	0, 625	28	−2	−11	80
8	240	L-ascorbic acid	0, 700	35	0	+0	59
6	236	L-ascorbic acid	0, 350	28	−5	−8	72
6	366	L-ascorbic acid	0, 450, 900	28	+10	+10	72
6	128	L-ascorbic acid	0, 900	28	0	+10	72
7	72	L-ascorbic acid	0, 350, 700	44	0	−10	77
6	48	L-ascorbic acid	0, 660	27	−5	+45	117
8	216	L-ascorbic acid	0, 660	28	−13	−6	119
8	144	L-ascorbic acid	0, 660	28	+4	+3	119
7	48	L-ascorbic acid	0, 660	28	−3	−88	118
7	64	L-ascorbic acid	0, 660	28	−5	—	118

[a]NR, not reported.

TABLE 2 Effect of supplemental dietary ascorbic acid on grower-finisher pig performance

		Vitamin C			Response		
Initial weight (kg)	**No. pigs (total)**	**Vitamin C source**	**Added vitamin C (mg/kg)**	**Trial length (d)**	**Daily gain (g)**	**Daily feed (g)**	**Reference**
24	30	L-ascorbic acid	0, 220	49	+80	NR[a]	71
32	160	L-ascorbic acid	0, 150, 300, 450	28	+66	+83	20
55	160	L-ascorbic acid	0, 150, 300, 450	28	−24	+40	20
26	132	L-ascorbic acid	0, 1100	79	+23	−187	50
22	216	Mg-L-ascorbyl-2-P	0, 50, 500	35	−1	−30	70
27	64	L-ascorbic acid	0, 200, 440	82	+48	+66	21
18	64	L-ascorbic acid	0, 200, 440	103	−58	−194	21
15	110	L-ascorbic acid	0, 220	80	+12	+21	21
23	72	L-ascorbic acid	0, 350, 700	120	+30	−40	77
35	80	L-ascorbic acid	0, 250, 500	NR	−10	−96	76

[a]NR, not reported

The results suggest that fetal abnormalities can be produced under conditions of extreme high blood glucose concentrations (e.g., diabetes), but they may be reduced or prevented with vitamin C supplementation. Generally, the adult female pig is not diabetic and when fed a starch diet in excessive amounts its body fat content increases and other problems (e.g., management and infertility) occur. Consequently, the gestating sow and adult boar are normally fed restricted quantities of feed to prevent parturition and future breeding problems. Although severely restricted feed intakes can apparently have an effect on rats that can be mediated by vitamin C, no congenital abnormalities or abortions have been noted with the adult female pig. Even though low feed intakes frequently occur in sows during lactation and/or prior to breeding, congenital defects have not been observed in pigs. The reason for the discrepancy between these species is unclear, but may be the result of the greater buffering capability of energy reserves in the adult female pig from body glycogen and fat depots. Feeding 1 to 2 g of ascorbic acid during the late gestation period of sows has no advantage on reproductive performance during either gestation or lactation periods (68, 116), but the number of stillbirths may be lower (68). Under periods of thermal stress supplemental vitamin C has not improved conception rate or other reproductive parameters (37). Most experiments have thus shown no benefit to supplemental vitamin C when fed to reproducing sows.

The reproductive need for ascorbic acid seems to be largely involved in its role as an antioxidant. The corpus luteum loses its functionality when ROS increases, thus destroying the ova. Conversely, the functional corpus luteum during the estrus cycle and gestation has an extremely high concentration of ascorbic acid, thus quenching the ROS that is produced. This permits the maintenance of the corpus luteum (88). The ascorbic acid may prevent apoptosis of the corpus luteum, subsequently enhancing the developing blastocyst by protecting it against oxidative damage. Toward the end of pregnancy, when $PGF_{2\alpha}$ and luteinizing hormone are normally released, the ascorbic acid in the corpus luteum is released into the blood, the corpus luteum is destroyed, and parturition occurs (87). Although ascorbic acid concentration is high in the corpus luteum of the pig during gestation, it declines within a few days of parturition and remains low during early lactation (18).

Ascorbic acid also seems to be involved in protecting the developing conceptus during implantation. Zavy et al. (120) reported a high concentration of ascorbic acid in the lumen of the uterus of the pig during early pregnancy, which suggests its having a protective role.

It has also been demonstrated that ascorbic acid may inhibit the release of prostaglandins (94) and is involved in cholesterol synthesis (36).

Sow plasma ascorbic acid concentration and liver GLO activity seem to be relatively constant until the latter part of gestation, whereupon liver and plasma ascorbic acid concentrations decline (18, 110). During the latter period of gestation there is a concurrent increase in fetal liver and plasma ascorbic acid concentration (18), with the colostrum having a high ascorbic acid concentration. Both factors undoubtedly contribute to the declining ascorbic acid status of the dam during late gestation. However, within one to five days postpartum, sow liver GLO activity

approximately doubles from late-gestation activity levels, probably reflecting the larger feed intake and greater quantity of ascorbic acid being synthesized and transferred to the milk (18). Consequently, the liver and the adrenal gland in the sow have a greater ascorbic acid concentration during lactation than during late gestation.

MALE PIG (BOAR) Because the structural maintenance of lipoproteins is dependent upon antioxidants, the need for vitamins E and C is greater during periods of testicular development and sperm production. The essential fatty acids are incorporated into the structural lipids of cellular testicular membranes and the spermatozoa. Steroidgenesis in the testis results in a greater production of ROS, which suggests that the metabolic need for vitamin C and vitamin E is greater. Ascorbic acid is perhaps the best antioxidant in the aqueous phase of body cells. This can have important implications for the male reproductive tract. A minimal amount of research has been conducted with the boar, but important physiological conclusions from research on other species may be applied to the boar. In all species studied testicular tissue and seminal plasma have one of the greater ascorbic acid concentrations of the body. Semen ascorbic acid concentration is high in the rat, in the human, and in poultry (66). Surai et al. (101) demonstrated an approximate equal distribution of ascorbic acid between the spermatozoa and seminal plasma in the avian species, suggesting it has an important function in both compartments. In contrast, most of the ascorbic acid in the semen of the boar is present in the seminal plasma, not in the spermatozoa (89). Because of the high concentration of ascorbic acid in gonad tissue, a high ascorbic acid need would be expected. Studies done with other species suggest that ascorbic acid affects the integrity of the tubular structure of the testis, maintaining the layers of collagen that form the complex basal lamina and for the maintenance of the Leydig cells (66). Seminal plasma ascorbic acid concentration in human seminal plasma was found to be correlated negatively with ROS accumulation and positively correlated with spermatozoa of normal morphology (103). Humans deficient in ascorbic acid have had lower sperm concentrations and greater incidences of oxidative damage to sperm DNA (33). Consequently, sperm production, sperm motility, and morphology can be negatively affected under conditions of ascorbic acid deficiency.

The ascorbic acid concentration of the testis of the young growing boar is greater during early life, with the total quantity increasing to adult age (30). The accessory glands (prostate, Cowper's gland, and seminal vesicle) have a lower ascorbic acid concentration than the testis, with the vitamin concentration greatest in the fluids of the epididymis (30). Concentration of ascorbic acid in boar semen was thus moderately high, but greater than that found in blood serum (30).

Under conditions of heat stress, Lin et al. (64) demonstrated greater sperm concentrations with fewer spermatozoa abnormalities when boars were fed 300 mg ascorbic acid per day. Although stress conditions may increase the response to supplementary vitamin C and justify supplementation under such conditions, the routine addition of the vitamin to boar diets does not currently seem warranted.

TISSUE DISTRIBUTION IN THE PIG

Although the liver is the site of ascorbic acid synthesis in the pig, its concentration in and between different tissues varies considerably. Pig tissues of highest ascorbic acid concentration (>1 mg/g wet tissue) are the pituitary and adrenal glands and the aqueous humor in the eye; moderate concentrations (0.25 to 0.75 mg/g wet tissue) are found in the spleen, thymus, thyroid, parathyroid, brain, and eye lens; lesser concentrations (<0.25 mg/g wet tissue) are in the liver, kidney, lungs, heart, loin muscle, and blood plasma (19, 112). These relative distributions are generally consistent with those of cattle (57), guinea pigs (121), and mice and rats (41).

When a mutant strain of pigs, unable to synthesize ascorbic acid, was fed vitamin C (50 mg/kg diet) and then the vitamin C was withdrawn from the diet of half the animals, all tissue had a lowered concentration of the vitamin, but the rate of decline differed between tissues. Ascorbic acid concentrations of most tissues of a lesser concentration group (i.e., muscle, tendon), moderate concentration group (i.e., liver, plasma), or the aqueous humor had ascorbic acid concentrations that were more rapidly depleted than the endocrine glands (i.e., adrenal, pituitary, parathyroid) with the brain and eye lens having a minimal decline (112).

An assessment of the priority of distribution after ^{14}C-labeled ascorbic acid was intravenously injected demonstrated a greater concentration in the endocrine glands, but lower distributions in the brain, muscles, and connective tissues (46). The liver, spleen, kidney, pancreas, thymus gland, and lungs had intermediate retention rates (112), responses generally similar to those of the guinea pig (48). The lung has a low ascorbic acid concentration, but a genetically mutant strain of pigs unable to synthesize ascorbic acid demonstrated that the concentration in this tissue was rapidly depleted once vitamin C was withdrawn (112).

Pig reproductive tissue contains a moderate to high ascorbic acid concentration. The testis and corpus luteum have greater tissue ascorbic acid concentrations than other body tissue, with the exception of the adrenal and pituitary glands. During administration, ascorbic acid was more rapidly incorporated into pig testis than into other tissue, but upon ascorbic acid withdrawal the testis retained its ascorbic acid more tenaciously (112). As indicated above, the functional corpus luteum retains ascorbic acid during preovulatory and postovulatory periods, but when $PGF_{2\alpha}$ was administered the corpus luteum released its ascorbic acid into the circulatory system (88).

These findings suggest that although tissue has a relatively high ability to accumulate ascorbic acid, the final concentrations differ. The brain tissue accumulates ascorbic acid at a lesser rate, but the rate of depletion is slower than other tissues, responses that are generally consistent with the guinea pig (48, 121). Reproductive tissue has a high metabolic need for ascorbic acid during the reproductive cycle in both the male and female. The withdrawal of ascorbic acid from various tissues seems to identify those tissues of high and low priority, with the tissues having high metabolic activity tenaciously retaining ascorbic acid for a longer period.

ROLE OF ASCORBIC ACID IN ANTIOXIDANT FUNCTION

Ascorbic acid rapidly loses its electrons and is an excellent reducing agent. Ascorbate is the reduced and biologically active form; dehydroascorbic acid is the oxidized form of vitamin C. In most body tissue dehydroascorbic acid is rapidly converted to ascorbate because of its potential oxidizing effect. Because ascorbate provides electrons rapidly to electron receptors, it produces oxidized compounds that are essential for body development, or its electrons are involved in activating various enzymes. Generally, ascorbate's properties are primarily those of an electron donor.

Although the pig synthesizes ascorbic acid, the circulating blood level is relatively low compared with concentrations in tissue. Recent results indicate that dehydroascorbic acid in pig serum is between 30% and 50% of total ascorbic acid concentrations (17). Supplemental vitamin C elevates total serum ascorbic acid concentration, but by relatively small increments (70, 72).

The subsequent transport of ascorbic acid into a cellular compartment is rapid and by active transport. Ascorbate is transported into the cell via a protein carrier that is Na^+- and energy dependent. Tissue saturation and ascorbate metabolites inhibit further transport of ascorbate into the cell. In contrast, dehydroascorbate is more rapidly transported into cellular tissue and is Na^+- and energy independent (63, 114). Once inside the cell, dehydroascorbate is rapidly reduced to ascorbate by glutathione. Because of the rapid uptake of dehydroascorbate and its subsequent conversion to the reduced form, in addition to other factors preventing the transport of ascorbate into the cell, cells maintain ascorbate concentrations characteristic of that tissue.

Within the cell, ascorbate acts as a scavenger of reactive oxygen molecules, superoxides, and hydroxyl free radicals, largely produced from normal metabolism. Ascorbate loses an electron in the presence of an oxidizing agent (i.e., electron receptor), forming an ascorbate free radical. Although the ascorbate free radical can be reversibly reduced to ascorbate and reduced nicotinamide adenine dinucleotide semidehydroascorbate reductase (exclusively intracellular enzyme), dehydroascorbic acid or semidehydroascorbic acid are formed (Figure 1). Either form can be reversibly converted to ascorbate, or the dehydroascorbic acid's ring structure can be broken, with 2,3 diketo-1-gulonic acid formed and excreted through the kidney (63).

The lipid-soluble antioxidant in cell membranes is α-tocopherol that upon peroxidation is converted to a tocopherol quinone, whereupon it is reconverted to its active form by the electron donation from ascorbate or glutathione. If located in the membrane bilayer, electrons can be transferred from ascorbate to the extracellular space. There is no evidence that lipid peroxidation occurs from any ascorbic acid analogs (3). Consequently, ascorbic acid readily loses its electrons and is one of the body's most effective and rapid-acting water-soluble antioxidants.

Because the accumulation of ROS may also affect DNA stability, the transcription process, or membrane integrity, vitamin C has a central role in controlling

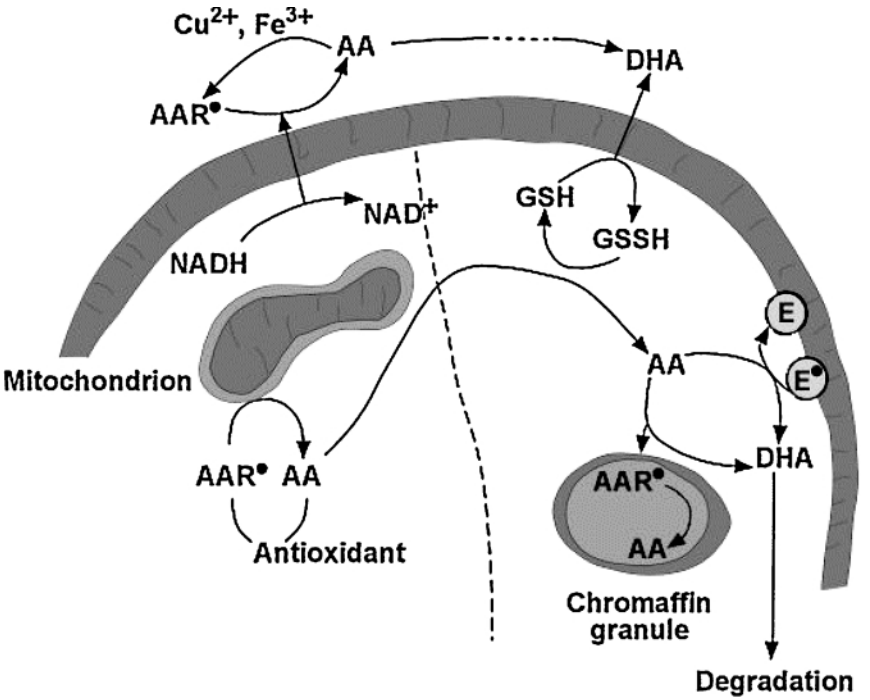

Figure 1 Schematic representation of vitamin C's antioxidant role and factors influencing the formation of oxidized and reduced forms of ascorbic acid. Abbreviations: AA, ascorbic acid; AAR, ascorbic acid radical (monodehydroascorbic acid radical); DHA, dehydroascorbic acid; E, vitamin E (α-tocopherol); GSH, glutathione (reduced); GSSH, glutathione (oxidized); NAD, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide, reduced (electron donor).

oxidative reactions in the cell. Extracellular ascorbate may prevent low-density lipoprotein oxidation (62). In the presence of free Fe^{3+} or Cu^{2+}, ascorbate reduces each of these metals to Fe^{2+}, or Cu^{1+}, respectively (100). These elements are normally sequestered organically (e.g., Fe in ferritin), thus preventing the more oxidized form from having detrimental effects on body tissue.

Enzyme Activation

Ascorbate is the biochemically active form of vitamin C, and although semidehydroascorbic acid and dehydroascorbic acid are biologically inactive, they are rapidly converted to the reduced form prior to being functionally active (62). Eight known enzymes require ascorbate for their activation. These reactions involve hydroxylation of proline, hydroxyproline or lysine in collagen synthesis. Ascorbate is essential for carnitine synthesis, the amidation of the carboxyl group for the synthesis of several peptide hormones, or for tyrosine metabolism (62).

BONE METABOLISM Collagen is abundant in connective tissue and provides an extracellular matrix to the body. Several types of collagen are characterized by their unique helical conformations. Ascorbic acid is involved in the synthesis of collagen, principally by its action as a cofactor in the hydroxylation of proline and lysine residues. It regulates collagen polypeptide synthesis and posttranslational hydroxylation and activates two hydroxylases (98).

A mutant strain of pigs unable to produce GLO and thus ascorbic acid was discovered by Danish workers (53), but similar genetic defects have also been observed in rats (75). Research results showed that ascorbic acid deficiencies impaired the synthesis of collagen, osteoid tissue, and the vasculature system in developing pig fetuses within 30 days after withdrawal of the vitamin from the dam's diet (82). Pathological damage to the fetus occurred prior to any evidence of the deficiency in the sow. Degeneration of capillary endothelium in pig fetal placenta was ascribed to defective collagen synthesis (113). Osteoblast cells were fewer in number and unable to synthesize sufficient quantities of extracellular matrix for the subsequent calcification of bone tissue. Subperiosteal hemorrhages can be observed in some, but not all, fetuses, and in growing pigs, sows, and boars deficient in vitamin C (53, 82, 111). Although subcutaneous hemorrhages are characteristic of scorbutic animals and were observed in the fetuses of sows unable to synthesize vitamin C, it was not generally observed in growing pigs, sows, and boars genetically incapable of synthesizing the vitamin (113).

Leg weakness is frequently encountered in both the forelegs and rear legs of growing pigs (56), but leg structure is particularly important in boars, who use their rear legs in the mating process. Boars with a genetic predisposition for the deficient condition have been identified (28, 55). Leg abnormalities ascribed to vitamin C deficiency (78), effects on bone metabolism (90), evidence of osteochondrosis (38, 77), or ulna dyschondroplasic lesions (107) occur when boars are vitamin C–deficient. However, blood vitamin C concentrations have not been correlated with deformation of the forelegs of pigs (2). High dietary levels of vitamin C also have had no effect on bone metabolism or any bone characteristics (38, 90). In cell culture, pig bone cell growth was not affected by adding vitamin C to the medium (26). These combined results indicate that the leg weakness frequently encountered in growing pigs that are capable of synthesizing vitamin C is generally not attributable to vitamin C inadequacy. However, because the abnormality can be produced by a genetic predisposition to inadequate synthesis of ascorbic acid, factors that may increase the metabolic need for vitamin C may exacerbate the condition and the need for the vitamin.

TRACE MINERAL METABOLISM Because ascorbic acid donates electrons rapidly, several trace minerals are affected in the digestive tract. Vitamin C may also alleviate the effects of several transition metals in the body post absorption.

Because the body has a limited capacity to excrete Fe, control of homeostasis occurs primarily at the gastrointestinal level. Several factors affect the absorption of Fe, with inorganic Fe being sensitive to conditions in the gastrointestinal tract.

Gastric pH and ascorbic acid can form soluble Fe–ascorbic acid complexes or convert the dietary Fe salt to the ferrous (Fe^{2+}) form, where both are subsequently absorbed in the duodenum. Upon absorption the Fe^{2+} is converted in the mucosal cell to the ferric (Fe^{3+}) form via ceruloplasmin, sequestered with transferrin, and released into the bloodstream as ferri-transferrin (35). Ascorbic acid is necessary for the subsequent release of Fe from ferri-transferrin by converting Fe^{3+} to Fe^{2+} prior to its incorporation into essential proteins (e.g., hemoglobin) or its storage in liver or spleenic tissue (35, 74). Under conditions of ascorbic acid deficiency in humans, guinea pigs, and rats, supplemental ascorbic acid increased Fe absorption, hemoglobin levels, and liver stores of the element (106).

When the Fe status of a vitamin C–deficient mutant strain of boars was evaluated, both plasma Fe and Fe saturation of plasma transferrin were less when the diet was devoid of vitamin C compared with a genetically similar group that was fed vitamin C (112). Hemoglobin levels were greater as well as liver and spleen Fe contents when the mutant vitamin C–deficient pig had been fed vitamin C.

The classical interactions between Fe and vitamin C reported above are somewhat contrary to that found in pigs capable of synthesizing the vitamin. The neonatal pig has a low body Fe reservoir, at least in relation to its postnatal need; it would normally develop anemia during the initial postnatal weeks unless administered an exogenous source of Fe. Feeding ascorbic acid to neonatal pigs increases the serum and spleenic ascorbic acid concentrations, but not liver or adrenal gland concentrations, nor was there any effect on hemoglobin or hematocrit (54). Because ascorbic acid concentration is high in colostrum and milk (9, 19) its supplementation is not deemed necessary while the pig nurses the dam.

Supplemental ascorbic acid when fed to weanling pigs has not influenced serum Fe concentrations, except during the last week of one trial where a decline in serum Fe occurred with supplemental vitamin C (115). Another study incorporated dietary ascorbic acid to 900 ppm and demonstrated a small, but nonsignificant, increase in hemoglobin and hematocrit values and a small increase in liver Fe (72). Another study evaluated weanling pigs fed diets devoid of supplemental Fe and 500 ppm vitamin C; this research demonstrated increased plasma Fe and an increase in the Fe saturation level of transferrin with added vitamin C (106). These results suggest that the Fe status of pigs capable of synthesizing ascorbic acid is not greatly affected by supplemental ascorbic acid when adequate Fe is present in the diet of young pigs.

Copper is a transient element which, if present in the oxidized form, can be toxic and cause the death of the animal. Ascorbate or glutathione can donate an electron, thus reducing the oxidizing capability of free Cu. However, this may be physiologically irrelevant in the pig because of the sequestering of Cu with organic proteins. Dietary ascorbic acid inhibits Cu absorption in rats by the same mechanism that enhances Fe absorption. Consequently, the reduction of Fe^{3+} to Fe^{2+} increases the absorption of Fe while converting Cu^{2+} to Cu^{1+} reduces the absorption of Cu.

Zinc and Se metabolism are influenced by dietary ascorbic acid. Plasma Zn and Se increase when pigs are fed ascorbic acid (23, 113) by presently unidentified mechanisms.

Carnitine Synthesis

Carnitine is required for the formation of acyl carnitine derivatives needed for the transport of long-chain fatty acids into the mitochondria for their subsequent oxidation. Peptide-linked trimethyllysine and methionine are needed for the synthesis of carnitine. Its synthesis involves two hydroxylation reactions with the reduction of Fe. Ascorbate is considered the best reducing agent, at least in vitro, for this reaction (63). When the precursors for carnitine biosynthesis are present in excess, carnitine is stored in tissue. Consequently, a deficiency would not be expected unless the precursor supply is deficient (63). Carnitine in animal products is the major dietary source for animals. Although its synthesis is considered adequate for the pig it seems to be low during the immediate period postweaning, concurrent with the period when GLO activity is low and ascorbic acid concentration is declining (19). Consistent with the factors regulating the synthesis and storage of carnitine, some studies have shown improved performance responses to dietary carnitine when fed to weanling pigs (43, 93), whereas other reports have not reported beneficial responses (44, 81). Improved utilization of fatty acids (from soybean oil) has been linked to the addition of carnitine (93).

Immune Function

The synthesis of ascorbic acid under conditions of disease and/or infection has not been widely investigated with swine. A few studies have been conducted with other species that may give some insight into the physiological responses in the pig. The immune response consists of cellular and humerol functions and both have shown responses to ascorbic acid therapy (47). Although there are several antioxidants, ascorbate is probably the primary antioxidant in plasma that quenches aqueous peroxyl radicals and/or lipid peroxidation products (34). Consequently, serum ascorbic acid concentrations decline in animals with infections and in those experimentally injected with a bacterium promoting the immune system (22, 52, 96). Pigs infested with parasites or infected with erysipelas bacteria show a reduced tissue concentration of ascorbic acid (15). Vaccination of pigs reduced the ascorbic acid concentration in the adrenals, but when supplemented with ascorbic acid (50 to 70 mg/kg) it induced an increase in plasma ascorbic acid and a 7% weight gain (1).

When ascorbic acid was fed to infected animals, the decline in serum ascorbic acid and phagocytosis activity was prevented while tissue ascorbic acid concentrations increased (85). This finding suggests that extracellular ascorbic acid may be important in immune stimulation by protecting leukocyte membranes from oxidative damage. Leukocytes have a very high ascorbic acid concentration and may be responsible for transporting ascorbic acid to damaged tissue or to the site of an

infection where its reducing capability can be used. Ascorbic acid thus seems to have a crucial role in the immune function of the body.

Stress

Stressors are present daily, but are increased under certain conditions. For pigs these are generally recognized to be at birth, weaning, movement and mixing of pigs, extreme environmental conditions, disease onset, prior to and during slaughter, and at farrowing. Stress assessment is difficult because there are no absolute measurements for this physiological state, but it is generally recognized in pigs by behavioral changes along with increased respiration rates. Changes in various blood metabolites, enzymes, and/or hormones, particularly increased adrenocorticotrophins and glucocorticoids, characterize stress conditions in the pig (29). Although these and other blood parameters have been used to measure the effects of stress, they are also influenced by other factors, including diurnal variation. At the onset of stress there is a rapid decline of ascorbic acid concentration in the adrenal gland. Upon removal of the stressor and subsequent restoration of homeostasis, the ascorbic acid content of this gland is restored to its resting state (109). Increased urinary excretion of ascorbic acid has been demonstrated in stressed pigs (29). A reduction of adrenal ascorbic acid content has therefore frequently been used as one of the measurement tools to evaluate stress on an animal (109).

During the latter part of gestation, late births, or after prolonged parturition the ascorbic acid content of the adrenal glands and tissue are lower in fetal or neonatal pigs, respectively (18). The administration of Fe to young pigs during the initial postnatal days can exacerbate the pigs' antioxidant status and thus increase the need for these vitamins. However, because of high colostrum and milk ascorbic acid and tocopherol concentrations, the young pigs' tissues become rapidly saturated with vitamins C and E. Weaning and the resulting inappetence produce a lowered ascorbic acid status in the animal. However, with a dietary supply of highly digestible carbohydrates (12), the precursor for ascorbic acid synthesis, and the increasing liver GLO activity in the weaned pig that occurs postweaning (19), ascorbic acid synthesis is endogenously increased to meet the pigs' need. Although placing weanling pigs in a cold environment is a stressor that reduces adrenal ascorbic acid concentration, older pigs do not exhibit the same magnitude of response (31).

Preslaughter conditions involving the loading and transporting of animals, perhaps for extended periods, mixing pigs with unfamiliar animals, and fasting for 48 hours prior to slaughter have all been identified as stressful situations. Wariss (108) demonstrated that fasting had a minimal effect on adrenal ascorbic acid concentrations, which suggests that other body stores were adequate to restore ascorbic acid in the adrenal. Loading and transporting pigs for short distances ($<$1 hr) produced a 20% lower ascorbic acid content in the adrenal gland compared with pigs not transported. When pigs were transported for longer periods ($>$6 hr), adrenal ascorbic acid concentrations were greater than in the group transported

for a short period. The authors concluded that the ascorbic acid concentration was partially, but not fully, restored in pigs that were more rested at the end of a long haul (109). The fighting of pigs that generally ensues when unfamiliar pigs are mixed also results in a lower adrenal ascorbic acid concentration, a lower glycogen reserve in the muscle, and a higher ultimate (i.e., 24-hr postslaughter) pH of the loin muscle; this combination of factors is detrimental to meat quality (29). Pigs that were more involved in establishing their dominance in the herd by fighting had lower adrenal ascorbic acid contents than those that were less aggressive.

The effects of high temperature and humidity can be extremely stressful because pigs do not sweat and, as a consequence, have higher respiration rates to rid the body of extra heat. In reproducing sows the addition of dietary ascorbic acid during high-temperature months did not seem to be effective in altering seasonal infertility (37). Feeding vitamin C to reproducing boars during the hotter months resulted in higher concentrations of sperm with fewer abnormalities (64), responses similar to those reported for fowl (86).

Although stress conditions can in some cases be of benefit to the animal, more often they are detrimental. The pig has the ability to synthesize ascorbic acid and unless the stress is immediate, the pig seems to have the ability to adapt to stressor agents and restore tissue ascorbic acid.

EFFECT OF MEGADOSES

Beneficial responses to supplemental vitamin C have been reported for specific periods of the growth cycle and reproductive phase in animals capable of synthesizing ascorbic acid. On the other hand, when dietary vitamin C is provided in great excess, detrimental responses are demonstrated. Tissue damage that causes the release of Fe would provoke free radical generation via the ascorbate-Fe system (100). The limiting factor is the availability of the free metal ions because these elements are generally tightly sequestered in various body protein complexes. The balance between ascorbate and free transition elements is therefore important. Clearly, the main role of vitamin C in body tissue is to serve as an antioxidant, but when provided in excess it may serve as a prooxidant (100).

The pig apparently adjusts its synthesis of the vitamin based on the supply of ascorbic acid to the various tissues. For example, in fetal pigs GLO synthesis is high during early gestation, but declines at the latter stages of gestation, presumably because the dam is transferring ascorbic acid to the fetus during late gestation. Postnatally, the pig's synthesis of the vitamin is suppressed by the ample supply of ascorbic acid provided in colostrum and milk until weaning occurs, whereupon synthesis is increased (19). These results suggest that regulation of vitamin synthesis may be important for the animal in maintaining its oxidation/reduction balance. Feeding megadoses of vitamin C to young pigs increases urinary excretion of ascorbic acid. Liver GLO activity also declined when up to 9000 ppm vitamin C was fed to weanling pigs (S. Ching, unpublished data). These results suggest

that the pig adjusts its synthesis of the vitamin and when an excess is provided, it attempts to excrete it in the urine, but also to reduce the rate of synthesis.

The *Annual Review of Nutrition* is online at http://nutr.annualreviews.org

LITERATURE CITED

1. Alekseev VA. 1979. Synthetic water soluble vitamins in diets of young pigs in relation to nitrogen metabolism. *Trucy Gorkovskogo Selskokh. Inst.* 135:87–92
2. Armocida A, Beskow P, Amcoff P, Kaliner A, Ekman S. 2001. Vitamin C plasma concentrations and leg weakness in the forelegs of growing pigs. *J. Vet. Med. Physiol. Pathol. Clin. Med.* 48:165–78
3. Berger TM, Polidori MC, Dabbagh A, Evans PJ, Halliwell B. 1997. Antioxidant activity of vitamin C in iron-overloaded human plasma. *J. Biol. Chem.* 272:15656–60
4. Bianchi J, Wilson FA, Rose RC. 1986. Dehydroascorbic acid and ascorbic acid transport systems in the guinea pig ileum. *Am. J. Phys.* 250:G461–68
5. Bielski HK, Frank J. 1995. Antioxidants in nutrition and their importance in the antioxidative balance in the immune system. *Immun. Infekt.* 23:166–73
6. Birk H, Kolb E, Liebaug F, Siebert P, Gollnitz L, et al. 1993. The content of ascorbic acid in tissues of swine fetuses and newborn piglets and in the blood plasma and tissue of swine of different ages with regard to the effect of the restriction of suckling time. *Dtsch. Tierarztl. Wochenschr.* 100:309–13
7. Bowland JP, Grummer RH, Phillips PH, Bohstedt G. 1949. The vitamin A and vitamin C content of sows' colostrum and milk. *J. Anim. Sci.* 8:98–106
8. Braude R, Kon SK, Thompson SY. 1946. A note on certain vitamins in sow's colostrum. *J. Dairy Res.* 14:414–19
9. Braude R, Coates ME, Henry KM, Kon SK, Rowland SJ, et al. 1947. A study of the composition of sow's milk. *Br. J. Nutr.* 1:64–77
10. Braude R, Kon SK, Porter JWG. 1950. Studies in the vitamin C metabolism of the pig . *Br. J. Nutr.* 4:186–99
11. Brown RG, Buchanan-Smith JG, Sharma VD. 1975. Ascorbic acid metabolism in swine: the effects of frequency of feeding and level of supplementary ascorbic acid on swine fed various energy levels. *Can. J. Anim. Sci.* 55:353–58
12. Brown RG, Sharma VD, Young UG. 1970. Ascorbic acid metabolism in swine. Interrelationships between the level of energy intake and serum ascorbate levels. *Can. J. Anim. Sci.* 50:605–9
13. Brown RG, Harris WH, Cummings JN. 1972. Ascorbate metabolism in swine. Influence of maternal hypoxia on fetal tissue ascorbate levels. *Can. J. Physiol. Pharmacol.* 50:407–10
14. Burns JJ, Mosbach EH. 1956. Further observations on the biosynthesis of L-ascorbic acid from D-glucose in the rat. *J. Biol. Chem.* 227:107–11
15. Butyanov DD, Zhakov MS. 1972. Histochemical study of the ascorbic acid content of some organs of pigs inoculated with combined and separate vaccines against swine fever and erysipelas. *Uch. Zap. vitebskogo Vet. Inst.* 25:19–22
16. Cederberg J, Simán CM, Eriksson UJ. 2001. Combined treatment with vitamin E and vitamin C decreases oxidative stress and improves fetal outcome in experimental diabetic pregnancy. *Pediatr. Res.* 49:755–62
17. Ching S. 2000. Ontogeny of liver L-gulonolactone oxidase activity and tissue ascorbic acid concentration in fetal,

nursing, and weaned pigs, and factors influencing its synthesis. Ph.D thesis. Ohio State Univ., Columbus

18. Ching S, Mahan DC, Ottobre JS, Dabrowski K. 2001a. Ascorbic acid synthesis in fetal and neonatal pigs and in pregnant and postpartum sows. *J. Nutr.* 131:1997–2001
19. Ching S, Mahan DC, Dabrowski K. 2001b. Liver L-gulonolactone oxidase activity and tissue ascorbic acid concentrations in nursing pigs and the effect of various weaning ages. *J. Nutr.* 131:2002–6
20. Chiang SH, Pettigrew JE, Moser RL, Cornelius SG, Miller KP, Heeg TR. 1985. Supplemental vitamin C in swine diets. *Nutr. Rep. Int.* 31:573–81
21. Choi JL, Rose RC. 1989. Transport and metabolism of ascorbic acid in human placenta. *Am. J. Physiol. Cell Physiol.* 257:C110–13

21a. Cromwell GL, Hays VW, Overfield JR. 1970. Effect of dietary ascorbic acid on performance and plasma cholesterol levels of growing swine. *J. Anim. Sci.* 31:63–66

22. Curca D, Cambir S, Mihai M. 1977/1978. Changes in blood ascorbic acid in lambs experimentally infected with *Salmonella* and *Escherichia coli*. *Lucr. Stint. Inst. Agron.* "*N Bakcescu*" *Seria C* 20/21:55–60
23. Cupp MS, Combs GF, Corradino RA. 1982. Ascorbic acid and selenium interaction in the prevention of exudative diathesis in chicks. *Fed. Proc.* 41:530 (Abstr.)
24. Damron CM, Monier MM, Roe JH. 1952. Metabolism of L-ascorbic acid, dehydro-L-ascorbic acid, and diketo-L-gulonic acid in the guinea pig. *J. Biol. Chem.* 195:599–606
25. Das S, Powers HJ. 1998. The effects of maternal intake and gestational age on materno-fetal transport of vitamin C in the guinea-pig. *Br. J. Nutr.* 80:485–91
26. Denis I, Pointillart A, Lieberherr M. 1994. Cell-stage-dependent effects of ascorbic acid on cultured porcine bone cells. *Bone Miner.* 25:149–61
27. de Rodas BZ, Maxwell CV, Davis ME, Mandali S, Broekman E, Stoecker BJ. 1998. L-ascorbyl-2-polyphosphate as a vitamin C source for segregated and conventionally weaned pigs. *J. Anim. Sci.* 76:1636–43
28. Draper DD, Rothchild MF, Christian LI, Goedegebuure SA. 1988. Effects of divergent selection for leg weakness on angularity of joint in Duroc swine. *J. Anim. Sci.* 66:1636–42
29. Dvořak M. 1966. Ascorbic acid concentration in the blood serum and urine in piglets under normal conditions and under acute stress. *Docum. Vet. Brno.* 6:13–20
30. Dvořak M, Podany J. 1966. Changes in the ascorbic acid content of testes during the development of boar. *Docum. Vet. Brno.* 5:25–30
31. Dvořak M. 1973. The relation of starvation to hypoglycemia and adrenocortical activity in newborn and six-week old piglets. *Zbl. Vet. Med.* 20:370–81
32. Ellender G, Gazelakis T. 1996. Growth and bone remodelling in a scorbutic rat model. *J. Aust. Dent.* 41:97–106
33. Fraga CG, Motchnic PA, Shigenaga MK, Helbock HJ, Jacob RA, Ames BN. 1991. Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. *Proc. Natl. Acad. Sci. USA* 88: 11003–6
34. Frei B, Stocker R, England I, Ames BN. 1990. Ascorbate: the most effective antioxidant in human blood plasma. *Adv. Exp. Med. Biol.* 264:155–63
35. Frieden E. 1973. The ferrous to ferric cycles in iron metabolism. *Nutr. Rev.* 31:41–44
36. Ginter E. 1998. Ascorbic acid in cholesterol metabolism and in detoxification of xenobiotic substances: problem of optimum vitamin C intake. *Nutrition* 5:369–74
37. Greer EB, Gardner IA, Wright GL. 1987. Failure of dietary vitamin C supplementation to prevent seasonal infertility in pigs. *Aust. J. Exp. Agric.* 27:343–47

38. Grødalen T, Hanssen I. 1984. Effect of megadoses of vitamin C on osteochondrosis in pigs. In *Ascorbic Acid in Domestic Animals*, ed. I Wegger, FJ Tagwerker, J Moustgaard, pp. 114–15. Copenhagen: Royal Danish Agric. Soc.
39. Grollman AP, Lehninger AL. 1957. Enzyme synthesis of L-ascorbate acid in different animal species. *Arch. Biochem. Biophys.* 69:458–67
40. Grummer RH, Whitehair CK, Bohstedt G, Phillips PH. 1948. Vitamin A, vitamin C and niacin levels in the blood of swine. *J. Anim. Sci.* 7:222–27
41. Hammarström L. 1966. Audoradiographic studies on the distribution of ^{14}C-labelled ascorbic acid and dehydroascorbic acid. *Acta Physiol. Scand.* 70(Suppl.):3–83
42. Hasan L, Vögeli P, Neuenschwander S, Stoll P, Meijerink E, et al. 1999. The L-gulono-gamma-lactone oxidase gene (GULO) which is a candidate for vitamin C deficiency in pigs maps to chromosome 14. *Anim. Gen.* (Short Commun.) 30:309–12
43. Heo K, Odle J, Han K, Cho W, Seo S, et al. 2000. Dietary L-carnitine improves nitrogen utilization in growing pigs fed low energy fat-containing diets. *J. Nutr.* 130:1809–14
44. Hoffman LA, Ivers DJ, Ellersieck MR, Veum TL. 1993. The effect of L-carnitine and soybean oil on performance and nitrogen and energy utilization by neonatal and young pigs. *J. Anim. Sci.* 71:132–38
45. Hornig D, Weber F, Wiss O. 1972a. Tissue distribution of labeled ascorbic acid in normal and hypophysectomized rats. *Intern. J. Vit. Nutr. Res.* 42:487–96
46. Hornig D, Weber F, Wiss O. 1972b. Tissue distribution of labelled material in vitamin C-deficient guinea pigs after intravenous injection of [1-^{14}C] ascorbic acid or [1-^{14}C] dehydroascorbic acid. *Intern. J. Vit. Nutr. Res.* 42:511–23
47. Hornig D, Gatthar B, Moser U. 1984. General aspect of ascorbic acid function and metabolism. In *Ascorbic Acid in Domestic Animals*, ed. I Wegger, FJ Tagwerker, J Moustgaard, pp. 3–24. Copenhagen: Royal Danish Agric. Soc.
48. Hughes RE, Hurley RJ, Jones PR. 1971. The retention of ascorbic acid by guinea-pig tissues. *Br. J. Nutr.* 26:433–38
49. Hulse JD, Henderson LM. 1980. Carnitine biosynthesis. Purification of 4-N′-trimethylaminobutyraldehyde dehydrogenase from beef liver. *J. Biol. Chem.* 255:1146–51
50. Hutagalung RI, Cromwell GL, Hays VW, Chaney CH. 1969. Effect of dietary fat, protein, cholesterol and ascorbic acid on performance, serum and tissue cholesterol levels and serum lipid levels of swine. *J. Anim. Sci.* 29:700–5
51. Ingermann RL, Stankova L, Bigley RH, Bissonnetts JM. 1988. Effect of monosaccharide on dehydroascorbic acid uptake by placental membrane vesicles. *J. Clin. Endocrinol. Metab.* 67:389–94
52. Jaescle G, Keller H. 1982. Beitrag zum ascorbin-saurestatus des pierdes. 4. Mittelung: Das verhalten von serumwerten nach intravenöser. Applikation. *Berl. Munch. Tierarzl. Wschr.* 95:71–79
53. Jensen PT, Basse A, Nielsen DH, Larsen H. 1983. Congenital ascorbic acid deficiency in pigs. *Acta Vet. Scand.* 24:393–402
54. Jewell DE, Siwecki JA, Veum TL. 1981. The effect of dietary vitamin C on performance and tissue vitamin C levels in neonatal pigs. *J. Anim. Sci.* 53(Suppl. 1): 249 (Abstr.)
55. Jørgensen B, Verstegen T. 1990. Genetics of leg weakness in boars at the Danish pig breeding stations. *Acta Agric. Scand.* 40:59–69
56. Jørgensen B. 1995. Effect of different energy and protein levels on leg weakness and osteochondrosis in pigs. *Livest. Prod. Sci.* 41:171–81
57. Kolb E, Warren M, Dobeleit G, Grundel G. 1989. The content of ascorbic acid in

different tissue of cattle, normally developed piglets, splay-legged piglets, adult swine and dogs. *Arch. Exp. Veterina. Med.* 43:327–34

58. Kolb E, Wahren ML, Siebert P, Volker L. 1991. Ascorbic acid concentrations in plasma, in amniotic and allantoic fluids, in the placenta and in 13 tissues of sheep fetuses and newborn lambs. *Dtsch. Tierarztl. Wochenschr.* 98:424–27 (From German)
59. Kornegay ET, Meldrum JB, Schurig G, Lindemann MD, Gwazdauskas FC. 1986. Lack of influence of nursery temperature on the response of weanling pigs to supplemental vitamins C and E. *J. Anim. Sci.* 63:484–91
60. Kratzing CC, Kelly JD. 1982. Tissue levels of ascorbic acid during rat gestations. *Intern. J. Vit. Nutr. Res.* 52:326–32
61. Kratzing CC, Kelly JD. 1986. Ascorbic acid synthesis by the mammalian fetus. *Intern. J. Vit. Nutr. Res.* 56:101–3
62. Levine M, Rumsey S, Wang Y, Park J, Kwon O, et al. 1996. Vitamin C. In *Present Knowledge in Nutrition*, pp. 146–59. Washington, DC: Intl. Life Sci. Inst. 7th ed.
63. Levine M, Rumsey S, Wang Y, Park J, Kwon O, et al. 2000. Vitamin C. In *Biochemical and Physiological Aspects of Human Nutrition*, pp. 541–67. Philadelphia, PA: Saunders. 1st ed.
64. Lin HK, Chen SY, Huang CY, Wung LC. 1990. Studies on improving semen quality of working boars fed diets with addition of vitamin C in summer. *Proc. Symp. Ascorbic Acid in Domestic Animals, 2nd*, ed. C Wenk, R Fenster, L Völker, pp. 249–50. Kartause Ittingen, Switzerland
65. Loudenslager MJ, Ku JP, Whetter PA, Ullrey DE, Whitehair CK, et al. 1986. Importance of diet of dam and colostrum to the biological antioxidant status and parenteral iron tolerance of the pig. *J. Anim. Sci.* 63:1905–14
66. Luck M, Jeyaseelan RI, Scholes RS. 1995. Minireview: ascorbic acid and fertility. *Biol. Reprod.* 52:262–66
67. Lund C, Christensen ID, Wegger I. 1980. Ascorbic acid metabolism in swine. Studies on ascorbic acid content in blood, milk and tissues. *Am. Rep. Steril. Res. Inst.* 23:48–61
68. Lynch PB, O'Grady JF. 1981. Effect of vitamin C (ascorbic acid) supplementation of sows in late pregnancy on piglet mortality. *Ir. J. Agric. Res.* 20:217–19
69. Mahan DC. 1971. Assessment of the influence of dietary vitamin E on sows and offspring in three parities: reproductive performance, tissue tocopherol, and effects on progeny. *J. Anim. Sci.* 69:2904–17
70. Mahan DC, Lepine AJ, Dabrowski K. 1994. Efficacy of magnesium-L-ascorbyl-2-phosphate as a vitamin C source for weanling and growing-finishing swine. *J. Anim. Sci.* 72:2354–61
71. Mahan DC, Pickett RA, Perry TW, Curtin TM, Featherstone WR, Beeson WM. 1966. Influence of various nutritional factors and physical form of feed on esophagogastric ulcers in swine. *J. Anim. Sci.* 25:1019–23
72. Mahan DC, Saif LJ. 1983. Efficacy of vitamin C supplementation for weanling swine. *J. Anim. Sci.* 56:631–39
73. Deleted in proof
74. Mazur A, Green S, Carlson A. 1960. Mechanism of plasma iron incorporation into hepatic ferritin. *J. Biol. Chem.* 235:595–603
75. Mizushima Y, Harauchi T, Yoshizaki T, Makino S. 1984. A rat mutant unable to synthesize vitamin C. *Experientia* 40:359–61
76. Mourot J, Aumaitre A, Wallet P. 1990. Effect of a dietary supplement of vitamin C on growth and pig meat quality. In *Proc. Symp. Ascorbic Acid in Domestic Animals, 2nd*, ed. C Wenk, R Fenster, L Volker, pp. 176–85. Kartause Ittingen, Switzerland
77. Nakano T, Aherne FX, Thompson JR. 1983. Effect of dietary supplementation

of vitamin C on pig performance and the incidence of osteochondrosis in elbow and stifle joints in young growing swine. *Can. J. Anim. Sci.* 63:421–28

78. Nielsen NC, Vinter K. 1984. Influence of dietary vitamin C supplement on leg weakness in pigs. In *Ascorbic Acid in Domestic Animals*, ed. I Wegger, FJ Tagwerker, J Moustgaard, pp. 39–41. Copenhagen: Royal Danish Agric. Soc.
79. Norkus EP, Bassi J, Rosso P. 1979. Maternal-fetal transfer of ascorbic acid in the guinea pig. *J. Nutr.* 109:2205–12
80. NCR-89 Comm. Confine. Manage. Swine. 1989. Effect of vitamin C and space allowance on performance of weanling pigs. *J. Anim. Sci.* 67:624–27
81. Owen KQ, Nelssen JL, Goodband RD, Tokach MD, Friesen KG. 2001. Effect of dietary L-carnitine on growth performance and body composition in nursery and growing-finishing pigs. *J. Anim. Sci.* 79:1509–15
82. Palludan B, Wegger I. 1990. Ascorbic acid and fetal development in swine. In *Proc. Symp. Ascorbic Acid in Domestic Animals, 2nd*, ed. C Wenk, R Fenster, L Volker, pp. 176–85. Kartause Ittingen, Switzerland
83. Partridge IG, Brown RG. 1971. Role of ascorbic acid in stress relief. *J. Anim. Sci.* 33:1153 (Abstr.)
84. Paul PK, Duttagupta PN. 1974. Maintenance of pregnancy and tissue carbohydrate levels by vitamin C in rats on restricted diets. *Fertil. Steril.* 25:68–73
85. Pavlov VP. 1970. Effect of vitamin C and cobalt chloride on the resistance of hens to tuberculosis. *Veterinarija* (*Moskva*) 4:67–68
86. Perek M. 1984. Ascorbic acid and the endocrine system with emphasis on the stress problem in poultry. In *Proc. Symp. Ascorbic Acid in Domestic Animals, 2nd*, ed. C Wenk, R Fenster, L Volker, pp. 185–93. Kartause Ittingen, Switzerland
87. Petroff BK, Dabrowski K, Ciereszko RE, Ottobre AC, Pope WF, Ottobre JS. 1998. Depletion of vitamin C from pig corpus luteum by prostaglandin $F_{2\alpha}$-induced secretion of the vitamin. *J. Reprod. Fertil.* 112:243–47
88. Petroff BK, Ciereszko RE, Dabrowski K, Ottobre JS. 1997. Ascorbate and dehydroascorbate concentrations in porcine corpora lutea, follicles, and ovarian stroma throughout the estrous cycle and pregnancy. *Theriogenology* 47:1265–73
89. Podany J, Dvořak M. 1966. The relation of ascorbic acid to sperm concentrations in boar semen and its fractions. *Docum. Vet. Brno.* 5:157–64
90. Pointillart A, Denis I, Colin C, Lacrois H. 1997. Vitamin C supplementation does not modify bone mineral concentrations or mineral absorption in growing pigs. *J. Nutr.* 127:1514–18
91. Raiha N. 1959. On the placental transfer of vitamin C. *Acta Physiol. Scand.* 45(Suppl. 155):7–53
92. Riker JT, Perry TW, Pickett RA, Heidenreich CJ. 1967. Influence of controlled temperatures on growth rates and plasma ascorbic acid values in swine. *J. Nutr.* 92:99–103
93. Rincker MJ, Carter SD, Real DE, Nelssen JL, Tokach MD, et al. 2003. Effects of increasing dietary L-carnitine on growth performance of weanling pigs. *J. Anim. Sci.* 81:2259–69
94. Rosenkrans CF Jr, Paria BC, Davis DL, Milliken G. 1990. In vitro synthesis of prostaglandin E and $F_{2\alpha}$ by pig endometrium in the presence of estradiol, catechol estrogen, and ascorbic acid. *J. Anim. Sci.* 68:435–43
95. Rosso P, Norkus E. 1976. Prenatal aspects of ascorbic acid metabolism in the albino rat. *J. Nutr.* 106:767–70
96. Salageanu C, Curca D, Ursu I, Batrinu A. 1971. A:C hypovitaminosis in newly weaned calves with enteropathies. *Rev. Zootechn. Med. Vet.* 21:57–63
97. Sandholm M, Honkanen-Buzalski T. 1979. Prevention of navel bleeding in

piglets by preparturient administration of ascorbic acid. *Vet. Rec.* 104:337–38
98. Schwartz ER. 1984. Effect of ascorbic acid on collagen structure and metabolism in normal and osteoarthritic tissues. In *Ascorbic Acid in Domestic Animals*, ed. I Wegger, FJ Tagwerker, J Moustgaard, pp. 32–38. Copenhagen: Royal Danish Agric. Soc.
99. Simán CM, Eriksson UJ. 1997. Vitamin C supplementation of the maternal diet reduces the rate of malformation in the offspring of diabetic rats. *Diabetologia* 40:1416–24
100. Stadtman ER. 1991. Ascorbic acid and oxidative inactivation of proteins. *Am. J. Clin. Nutr.* 54:1125–28S
101. Surai PF, Cerolini S, Wishart GJ, Speake BK, Noble RC, Sparks NHC. 1998. Lipid and antioxidant composition of chicken semen and its susceptibility to peroxidation. *Poult. Avian Biol. Rev.* 9:11–23
102. Swecki JA. 1985. Dietary ascorbic acid for neonatal pigs reared artificially. *Intern. J. Vit. Nutr. Res.* 5:413–19
103. Thiele JJ, Friesieben HJ, Fuchs J, Ochsendorf FR. 1995. Ascorbic acid and urate in human seminal plasma—determination and interrelationships with chemiluminiscence in washed semen. *Hum. Reprod.* 10:110–15
104. Tsao CS, Young M. 1989. Effect of exogenous ascorbic acid intake on biosynthesis of ascorbic acid in mice. *Life Sci.* 45:1553–57
105. Deleted in proof
106. Völker L, Weiser H, Schulze J, Streff K. 1984. Ascorbic acid and iron metabolism in pigs. In *Ascorbic Acid in Domestic Animals*, ed. I Wegger, FJ Tagwerker, J Moustgaard, pp. 91–101. Copenhagen: Royal Danish Agric. Soc.
107. Walker T, Fell BF, Jones AS, Boyne R, Elliott M. 1966. Observations on leg weakness in pigs. *Vet. Rec.* 79:472–79
108. Wariss PD. 1979. Adrenal ascorbic depletion as an index of pre-slaughter stress in pigs. *Meat Sci.* 3:281–85
109. Wariss PD. 1984. Monitoring preslaughter stress in pigs by adrenal ascorbic acid depletion. In *Ascorbic Acid in Domestic Animals*, ed. I Wegger, FJ Tagwerker, J Moustgaard, pp. 107–13. Copenhagen: Royal Danish Agric. Soc.
110. Wegger I, Palludan B. 1984. Ascorbic acid status of swine, genetic and developmental variations. In *Ascorbic Acid in Domestic Animals*, ed. I Wegger, FJ Tagwerker, J Moustgaard, pp. 68–79. Copenhagen: Royal Danish Agric. Soc.
111. Wegger I, Palludan B. 1986. Ascorbic acid in swine nutrition: pre-, neo-, and postnatal aspects. *Proc. 9th Int. Pig Vet. Soc. Congr.* Barcelona: Am. Assoc. Swine Practitioners. 429 pp.
112. Wegger I, Palludan B. 1990. Iron, zinc, and copper status of ascorbic acid deficient boars. In *Proc. Symp. Ascorbic Acid in Domestic Animals, 2nd*, ed. C Wenk, R Fenster, L Volker, pp. 114–22. Kartause Ittingen, Switzerland
113. Wegger I, Palludan B. 1994. Vitamin C deficiency causes hematological and skeletal abnormalities during fetal development in swine. *J. Nutr.* 124:241–48
114. Welch RW, Wang Y, Crossman A, Park JB, Kirk KL, Levine M. 1995. Accumulation of vitamin C (ascorbate) and its oxidized metabolite dehydroascorbic acid occurs by separate mechanisms. *J. Biol. Chem.* 270:12584–92
115. Yen JT, Pond WG. 1981. Effect of dietary vitamin C addition on performance, plasma vitamin C and hematic iron status in weanling pigs. *J. Anim. Sci.* 53:1292–96
116. Yen JT, Pond WG. 1983. Response of swine to periparturient vitamin C supplementation. *J. Anim. Sci.* 56:621–24
117. Yen JT, Ku PK, Pond WG, Miller ER. 1985. Response to dietary supplementation of vitamin C and vitamin E in weanling pigs fed low vitamin E-selenium diets. *Nutr. Rep. Intern.* 31:877–85
118. Yen JT, Pond WG. 1984. Responses of weanling pigs to dietary supplementation

with vitamin C or carbadox. *J. Anim. Sci.* 58:132–37

119. Yen JT, Pond WG. 1987. Effect of dietary supplementation with vitamin C or carbadox on weanling pigs subjected to crowding stress. *J. Anim. Sci.* 64:1672–81
120. Zavy MT, Clark WR, Sharp DC, Roberts RM, Bazer FW. 1982. Comparison of glucose, fructose, ascorbic acid, and glucosephosphate isomerase enzymatic activity in uterine flushings from nonpregnant and pregnant gilts and pony mares. *Biol. Reprod.* 27:1147–58
121. Zlock Z, Ginter E. 1988. Influence of age on the kinetics of vitamin C catabolism in guinea-pigs. *Physiol. Bohemoslov.* 37:459–66

Annual Review of Nutrition
Volume 24, 2004

CONTENTS