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Ascorbic Acid and the Growth and Development of Insects

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The structural requirements for vitamin C activity in insects were comparable to those observed in guinea pigs. A dietary level of 0.5 mM L-*ascorbic acid was necessary for normal development of the tobacco hornworm* (Manduca sexta); *magnesium 2-O-phosphono-*L-*ascorbate, sodium 6-O-myristoyl-*L-*ascorbate, and* L-*dehydroascorbic acid were equally potent.* D-*Ascorbic acid, 6-bromo-6-deoxy-*L-*ascorbic acid, and* D-*isoascorbic acid were approximately one-half, one-fifth, and one-tenth as effective, respectively. Tissues from* M. sexta *lacked* L-*gulono-γ-lactone oxidase, the biosynthetic enzyme usually absent from ascorbate-dependent species. Vitamin C was found in eggs, larval labial gland, hemolymph, gut, muscle, cuticle, adult nervous tissue, and gonads at concentrations ranging from < 10–170 mg/100 g of wet tissue. No ascorbate was detected in larval fat body, Malpighian tubules, or adult salivary gland. Insects reared on an* L-*ascorbate-deficient diet contained no detectable* L-*ascorbic acid. Some possible physiological actions of the vitamin in insects are discussed.*

Considerable data are available on the insect's requirement for L-ascorbic acid. Dietary vitamin C is needed for normal growth, molting, and fertility of many insects, and vitamin C, or another compound with similar biological properties, is probably an essential growth

factor for this class of animals. Although most insects subsisting on green plants need L-ascorbate to develop fully (*1–6*), it was proposed that some species may dispense with the vitamin or may synthesize it either de novo or rely on symbiotic organisms (*7–10*). However, the ability of certain insects (or their symbionts) to synthesize ascorbic acid has not been adequately demonstrated. This chapter reviews some of the previous work on the role of ascorbic acid in insects and includes results of efforts to develop a bioassay for vitamin C using an insect, measure the growth-promoting activity of compounds structurally related to L-ascorbic acid, determine diet and tissue levels of L-ascorbate in insects, and ascertain whether specific tissues in insects are capable of converting a putative precursor, L-gulono-γ-lactone, into vitamin C.

Experimental

Animals. *Manduca sexta* larvae were reared on an agar-based diet (*11*) at 28°C and 60% relative humidity with a 16-h photophase. The Indian meal moth, *Plodia interpunctella* Hubner, and American cockroach, *Periplaneta americana* L., were taken from laboratory cultures. Dissection was performed under anesthesia by cooling to 5°C (*12*).

Biological Assay. Prior to bioassay, the hot diet was cooled to 60°C, L-ascorbic acid or a related compound was added, and the mixture was thoroughly blended. Labile derivatives were applied to the surface of the gelled diet at room temperature. Neonate larvae were used in all tests and the growth of larvae on the control diet was compared with that of larvae on test diets. At 1–4-d intervals, up to 40 d, the mean weight of ten to twenty animals was determined. Fecal matter was removed at each observation. Test compounds were obtained or prepared as described previously (*5*).

Paper Chromatography. One-tenth of a gram of tissue was homogenized in 0.25 mL of 2% (w/v) metaphosphoric acid at 4°C. Descending paper chromatography was done on Whatman #1 paper using ethyl acetate:acetic acid: water (6:3:2) as developing solvent. Ascorbic acid was detected by dipping the chromatogram sequentially in 0.10 mL of saturated silver nitrate mixed with 20 mL of acetone containing 0.1 mL of concentrated ammonium hydroxide, 1 *M* NaOH in 95% ethanol, 0.2 *M* aqueous sodium thiosulfate, and water (*13*). The detection limit was 2 μg after chromatography.

High Performance Liquid Chromatography. Tissue extracts were analyzed with a Varian model 5020 liquid chromatograph equipped with a Rheodyne model 7120 loop injector valve, a Tracor 970 variable wavelength detector set at 257 nm, an automated Hewlett-Packard 3385A printer–plotter system for determining retention times and peak areas, and a Waters μ Bondapak column (3.9 mm i.d. × 300 mm) for carbohydrate analysis. The buffer was eluted isocratically at 1 mL/min with a 1:4 (v/v) mixture of 0.01 *M* monobasic sodium phosphate (pH 4.46) and methanol. The minimum amount detectable was 10 ng.

L-Gulonolactone Oxidase Assay. Tissues were assayed for L-gulonolactone oxidase by the method of Azaz et al. (*14*). Weighed portions of tissue (50–200 mg) were homogenized in 2 mL of 50 m*M* sodium phosphate (pH 7.4) containing 0.2% sodium deoxycholate. Homogenates were centrifuged at

5000 *g* for 10 min at 4°C, and 1-mL aliquots of the supernatant were incubated with 2 m*M* L-gulono-γ-lactone (Sigma Chemical Company) for 60 min at 35°C. Ascorbate was measured by the 2,4-dinitrophenylhydrazine method of Roe and Kuether (*15*) as modified by Geshwind et al. (*16*). Chicken kidney was assayed as a control tissue rich in L-gulonolactone oxidase.

Results

Bioassay. The effect of L-ascorbic acid on the development rate of *M. sexta* is shown in Figure 1 (*5*). All neonate larvae developed into adults on the artificial diet that contained 0.5 m*M* L-ascorbic acid. Also, larvae raised on that diet exhibited a normal growth curve, and were robust and bright blue-green in color. Normal development occurred in 35 d with larval–pupal ecdysis occurring at day 15 and pupal–adult ecdysis at day 35. Higher levels of L-ascorbic acid were not more effective, but lower ones were inadequate for the hornworm. As the amount of L-ascorbic acid was decreased in the diet, pathological effects appeared after a feeding period that depended on the vitamin concentration. Animals reared on an ascorbate-deficient diet were reduced in size and colored a dull yellowish-green. Abnormalities in cuticle soon became apparent. Extremities such as mouth parts and tarsi exhibited premature darkening of cuticle. Navon (*6*) observed similar effects in the cotton leafworm, *Spodoptera littoralis*. In all tests, larvae appeared normal to the second instar, probably because of an amount of L-ascorbic acid derived from parent insects. In larvae on diets lacking in L-ascorbic acid, pathological consequences occurred at the third instar. At the beginning of the third molting period, the insects began to shrivel and 1 d later became moribund. Larvae fed medium containing 0.05 m*M* L-ascorbic acid were similarly affected, but at one stadium later. Fifty percent of the larvae fed diet supplemented with 0.25 m*M* vitamin C died in the prepupal stage; the other half underwent pupal and adult eclosion 3–6 d later than the control group.

We have used the growth effects and pathologies associated with L-ascorbic acid deficiency as a basis for the determination of the biological potency of related compounds (Table I). At a dietary concentration of 0.5 m*M*, *L*-ascorbic acid and dehydroascorbic acid were fully active, as well as some ester derivatives including the 6-myristate and 2-phosphate compounds. The insect may be metabolically like the guinea pig because both were able to utilize those esters (*17*). Carboxylesterases and phosphatases probably converted those derivatives to the free vitamin (*18*). The 6-bromo compound was less active and apparently cannot be metabolized to L-ascorbic acid or only poorly so.

One of the least active compounds in the insect bioassay was the 2-sulfate ester of L-ascorbic acid. To develop normally the hornworm

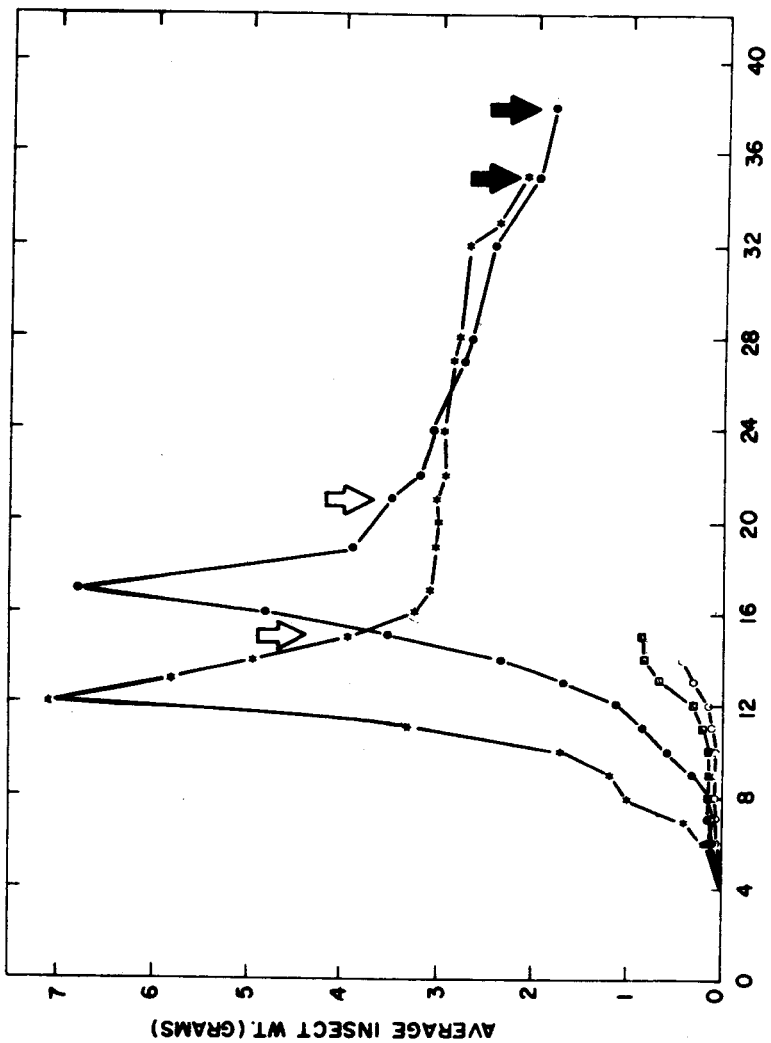

Figure 1. Growth curves of M. sexta *fed diet containing added 0.50 mM (–*–), 0.25 mM (–●–), 0.05 mM (–■–), or 0.00 mM (–○–) vitamin C. Open arrow and closed arrow denote the time of pupal and adult ecdysis, respectively (5).

Table I. Effect of L-Ascorbic Acid and Related Compounds on Growth of *M. sexta* and *Cavia cobaya*

Compound	*Relative Activity*[a]	
	Hornworm	*Guinea Pig*
L-*threo*-Hex-2-enonic acid γ-lactone (L-ascorbic acid)	100	100
Sodium 6-*O*-myristoyl-L-ascorbate	100	100
Magnesium 2-*O*-phosphono-L-ascorbate	100	100
D-*threo*-Hex-2-enonic acid γ-lactone (D-ascorbic acid)	40 ± 10	0
6-Bromo-6-deoxy-L-ascorbic acid	20 ± 10	not available
D-*erythro*-Hex-2-enonic acid γ-lactone (D-isoascorbic acid)	10 ± 10	5
Potassium 2-*O*-sulfo-L-ascorbate	5	0
L-*erythro*-Hex-2-enonic acid γ-lactone (L-isoascorbic acid)	0	0
L-*threo*-Hex-2,3-diulosic acid γ-lactone (L-dehydroascorbic acid)	100	100

[a] Insect growth activity is expressed as the amount of compound relative to L-ascorbic acid (0.50 m*M*) required for >80% of the test animals to attain a weight of 1 g in 10 d (*5*).

required a twenty times greater concentration of this conjugate (10 m*M*). Apparently, *M. sexta* does not metabolize the sulfate ester back to L-ascorbic acid because it probably lacks a sulfohydrolase enzyme. Preliminary results indicated that the 2-sulfate derivative was about half as active as L-ascorbic acid in the southwestern corn borer, *Diatraea grandiosella* Dyar (*19*). That species required a dietary supplement of 21 m*M* L-ascorbic acid for optimal growth (*20*), approximately forty times higher than the level required by *M. sexta*. These differences may express the metabolic needs of individual species.

Three stereoisomers of L-ascorbic acid were also bioassayed using the tobacco hornworm (Table I). Configurational changes at C4 and C5 affected activity and indicated that the geometry of C5 was more critical for activity than that of C4. The enantiomer, D-ascorbic acid, had approximately 40% activity, while the C5 epimer, D-isoascorbic acid, had 10% activity. The relative potency of those isomers is reversed in vertebrate and invertebrate animals. With D-isoascorbic, 2–10% activity in other insects was reported (*20–22*), but this compound did not promote development of the cotton leafworm (*23*). L-Isoascorbic acid had no activity in the hornworm or guinea pig.

L-Dehydroascorbic acid, a derivative with potent vitamin activity in vertebrates, was inactive in our bioassay when it was mixed with hot diet prior to gelation. However, when we repeated the bioassay by applying dehydroascorbic acid to the surface of the gelled diet, the

oxidized form proved to be as effective as L-ascorbic acid in promoting insect growth. Apparently, dehydroascorbic acid was destroyed at the elevated temperature (*24*).

The possibility that L-ascorbic acid was exerting its growth-promoting effect on the hornworm as a nonspecific reducing agent was tested. Organic and inorganic agents such as reductones, tocopherol, hydroquinone, pyrocatechol, thiols, ferrous sulfate, and sodium dithionite exhibited no activity. The carbon ring analog, reductic acid, was also inactive. Obviously, the tobacco hornworm displayed stereoselectivity for L-ascorbic acid and is a good model for the study of structure–activity relationships.

Ascorbate Levels in Tissues. Several tissues were dissected from *M. sexta* and analyzed for L-ascorbic acid by high performance liquid chromatography (HPLC) (Figure 2), paper chromatography, or the dinitrophenylhydrazine method (*5*). As anticipated, L-ascorbic acid was

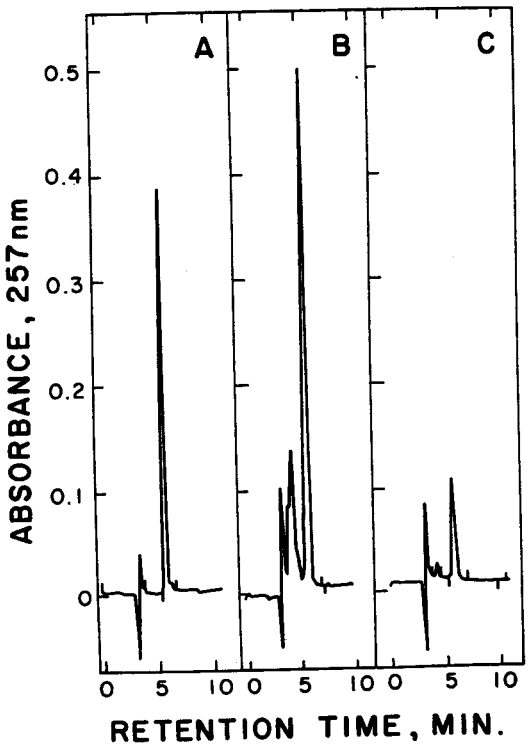

Figure 2. HPLC of L-*ascorbic acid from insect tissues* (65). *A,* L-*ascorbic acid, 1.7 μg; B,* M. sexta *hemolymph (0.01 mL) extract; C,* M. sexta *labial gland extract, 1.4 mg wet weight.*

present in nearly all tissues (Table II), although it was most abundant in larval labial gland and hemolymph, ranging from 1 to 10 m*M*. L-Ascorbic acid was also present at varying levels in eggs, larval gut, muscle, cuticle, adult nervous tissue, and gonads. For comparison, L-ascorbic acid was assayed in diet and fecal matter at 24 and 5 mg/100 g, respectively. This result indicated that approximately 80% of the vitamin was absorbed and/or metabolized by tissues. No ascorbate was detected in larval fat body, Malphigian tubule, or adult salivary gland. Thus, insects appear to be different from vertebrates, where the highest levels of L-ascorbic acid occur in the adrenals and nervous tissue (25).

L-Ascorbic acid was also analyzed in tissues from hornworms fed a vitamin-deficient diet. Without L-ascorbic acid neonate larvae grew into the third instar, but died before the next molt. These larvae retained little or no vitamin in tissues (Table II). A similar result was characteristic of fifth instar larvae reared on an ascorbate-deficient diet beginning at the mid fourth instar. These larvae failed to complete pupation. Apparently, the diet was the sole source of L-ascorbic acid and when tissues became depleted, major pathological consequences ensued.

Table II. L-Ascorbic Acid Content of Tissues from *M. sexta*

Tissue	*Stage*[a]	L-*Ascorbate Content*[b]
Labial gland	L5	86 ± 84 (24)
	L3	69 ± 10 (4)
Hemolymph	L5	48 ± 40 (24)
Brain and nerve cord	A	41 ± 30 (4)
Gonad	A	63 ± 8 (2)
	A	60 ± 14 (2)
Egg	—	43 ± 3 (4)
Gut	L5	39 ± 8 (6)
Muscle	L5	27 ± 11 (4)
Cuticle	L5	22 ± 15 (4)
Mouth exudate	L5	15 ± 4 (2)
Fat body	L5	< 1 (4)
Malphigian tubule	L5	< 1 (3)
Salivary gland	A	< 1 (2)
	L-ascorbate deficient diet	
Labial gland	L5[c]	< 1 (6)
	L3[d]	< 1 (6)
Hemolymph	L5	< 1 (4)

[a] Key: L, larva; A, adult; 3, third instar; and 5, fifth instar.
[b] Units are mg of L-ascorbic acid/100 g of wet tissue or 100 mL hemolymph ± sd. Amounts of tissue or hemolymph analyzed were 20–300 mg or 0.3–0.5 mL, respectively. Number of determinations listed in parentheses.
[c] Hornworm reared on ascorbate deficient diet from middle of fourth larval instar.
[d] Hornworm reared on ascorbate deficient diet from neonate stage.

The titer of L-ascorbic acid in tissues was examined during development. During the fifth instar, L-ascorbate increased about eightyfold in the labial gland and ten-fold in the hemolymph, where millimolar levels were measured (Figure 3). Regression analysis of the tissue kinetics revealed that the labial gland accumulated L-ascorbic acid about twice

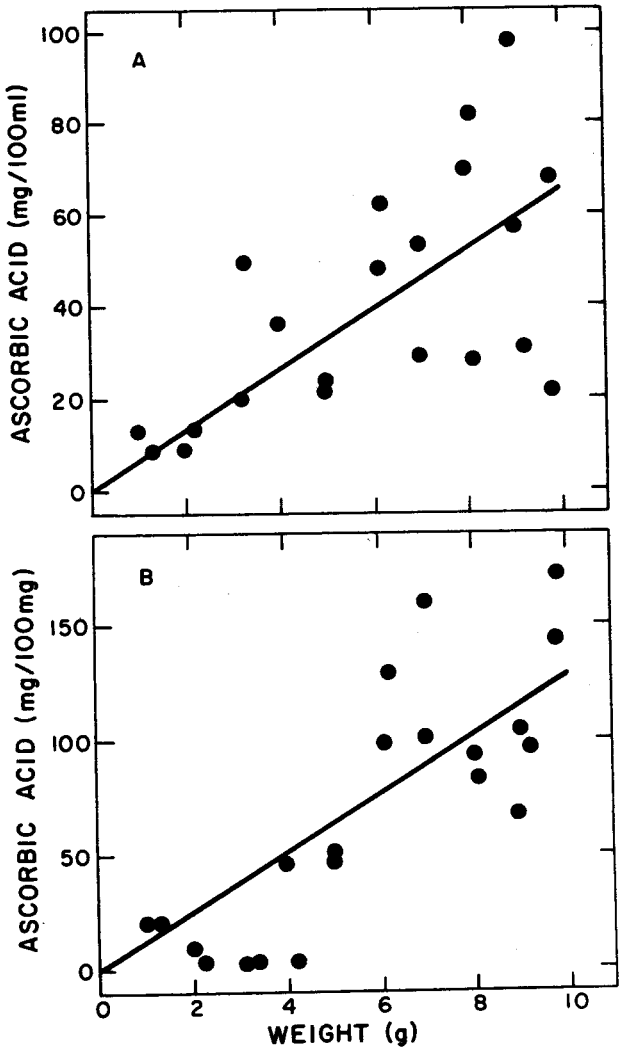

Figure 3. Changes in the content of L-*ascorbic acid in hemolymph and labial gland during larval development of* M. sexta (65). *A, Hemolymph: regression analysis yielded line defined as larval weight = 6.5 [ascorbic acid] at $\alpha = 0.01$ level and $R^2 = 0.83$; B, labial gland: regression analysis yielded line defined as larval weight = 12.7 [ascorbic acid] at $\alpha = 0.01$ level and $R^2 = 0.87$.*

as fast as did hemolymph. Labial glands removed from a third instar larva also contained a high titer of L-ascorbate. Vitamin C may be depleted during the intermolt period, after which feeding recommenced and tissue accumulation occurred. Three other phytophagus species, *S. littoralis* (*6*); the silkworm, Bombyx mori (*8*); and the locust, *Schistocerca gregaria* (*16*) showed high ascorbic acid titers in various tissues during the instar and low titers at the molting stage.

Absence of L-Gulono-γ-lactone Oxidase in Insect Tissues. There is no conclusive evidence that any species of insect can synthesize L-ascorbic acid. As a first test to determine whether *M. sexta* could synthesize the vitamin, L-gulono-γ-lactone, a well-known precursor in animals, was tested in the bioassay (*27*). At 0.5 m*M* L-ascorbic acid in the diet or when injected into the hemocoel of larvae, no activity was observed. We also surveyed insect tissues for L-gulono-γ-lactone oxidase, the enzyme catalyzing the final step in animal biosynthesis of L-ascorbic acid from glucose. Chicken kidney and liver were control tissues; the former synthesized 10 μg L-ascorbate/mg/h, while the latter was inactive (*28*). Within the limits of the assay, no evidence for L-gulono-γ-lactone oxidase was detected in tissue homogenates from *M. sexta, P. interpunctella,* and *P. americana.* The latter two species were cultured on L-ascorbic-acid-deficient media. Apparently, certain insects may not require L-ascorbic acid for growth, may synthesize the vitamin or a similar factor at a rate too slow to measure, may use a synthetic pathway that the assay procedure (which was developed for vertebrate tissue) failed to detect, or may rely on a symbiotic organism to produce L-ascorbic acid. More work concerning biosynthesis needs to be done.

Discussion

Review of Literature. An obvious question is why use insects instead of vertebrate animals to study ascorbic acid biochemistry. Primarily, it is more convenient. Insects are relatively small and have a rapid generation time. Large numbers can be used to get valid statistical data. Biological effects can be analyzed using a synchronous population where stages of development can be timed with accuracy (*29*). Small amounts of test material (mg) can be used in most cases.

Although many insects nutritionally require ascorbic acid, numerous species have apparently been reared on artificial or synthetic diets without ascorbic acid or related nutrients. These include Diptera and assorted roaches, crickets, beetles, and moths, whose normal food comprises detritus, seeds, carrion, and dry stored products that are deficient in ascorbate for certain vertebrate animals. The general presumption has been that the diets lack vitamin C and that certain insects can biosyn-

thesize it (Table III). Whether these diets are deficient for invertebrate animals is unknown. Thus, one must be careful about which insect is chosen as the experimental animal.

Insects and ascorbic acid have been studied for a relatively long time. A listing of such studies is presented in Table III. The earliest paper was that Girond et al. (*30*) who, over 40 years ago, detected L-ascorbic acid in the gonads and endocrine glands of a predaceous diving beetle, *Dytiscus marginalis*. A novel report concerned a patent obtained in Japan for preparing ascorbic acid from silkworm pupae (*31*). Dadd (*32*) first discovered a dietary requirement for vitamin C in insects using a grasshopper, *Locusta migratoria*. Overall, approximately seventy papers have been published concerning dietary requirements, tissue levels, biosynthesis, physiological effects, and structure–activity relationships involving one or more of fifty different species. In nearly all cases, L-ascorbic acid in the diet had a positive effect on growth and development. In two species, the ambrosia beetle, *Xyleborus ferrugineus* (*33*), and the sawtoothed grain beetle, *Oryzaephilus surinamensis* (*34*), L-ascorbic acid decreased the rate of development, survival, or progeny production. L-Ascorbic acid was proposed to cause browning of dietary protein that, in turn, led to amino acid deficiencies (*33*). Another study using *O. surinamensis* reported that L-ascorbic acid was beneficial in larval development (*35*). Since vitamin C is essentially nontoxic to other animals, it is most likely innocuous to insects as well.

Physiological Function. The mechanism by which L-ascorbic acid benefits an insect is unknown. The vitamin is found in many tissues where it probably plays a variety of roles related to its redox potential. Besides the possible general function of detoxifying superoxide and hydrogen peroxide, L-ascorbic acid may be involved in metabolic processes such as tyrosine metabolism, collagen formation, steroid synthesis, detoxification reactions, phagostimulation, or neuromodulation. At this time one can only speculate about the function of vitamin C in some specific tissues.

Table III. Ascorbic Acid and Its Effects in Insects

Species	*Comments*	*References*
Alabama argillacea	levels in tissues decreased during development	*41*
Anthonomus grandis	dietary requirement	*42*
Apis indica	no biosynthesis detected	*43*
Apis mellifera	tissue levels at 5–600 μg/g	*44*
Argyrotaenia velutinana	no dietary requirement	*45*

Table III. (Continued)

Species	*Comments*	*References*
Auripennis lepel	no biosynthesis detected	*43*
Bombyx mori	dietary requirement	*46–48*
	synthesis detected in pupal fat body from D-mannose	*8*
	detected in eggs	*49*
	patent for isolation from pupae	*49*
	phagostimulant	*50*
	D-isoascorbic acid slightly active, dehydroascorbic acid fully active	*21, 22*
	no biosynthesis detected	*43*
Chorthippus sp.	phagostimulant	*51*
Corcyra cephalonica	biosynthesis (?)	*52, 53*
	no biosynthesis	*43*
Culex molestus	beneficial in diet	*54*
Cutelia sedilotti	whole body levels at 1 mg/100 g	*38*
Dasycolletes hirtipes	whole body levels at 2 mg/100 g	*38*
Diatraea grandiosella	dietary requirement	*55*
	D-isoascorbic acid slightly active	*20*
Dichomeris marginalis	no vitamin detected in whole body	*56*
Dytiscus marginalis	~ 500 μg/g in gonads and endocrine glands	*30, 57, 58*
Ectomyelois ceratoniae	no dietary requirement	*59*
Ephestia (Caudra) cautella	beneficial effect due to prevention of oxidative rancidity in diet	*60*
Estigmene acrea	dietary requirement, vitamin accumulated in tissues during development	*41, 42*
Eurygoster integriceps	dietary requirement (0.4%)	*61*
Graphosoma lineatum	detrimental effect in diet	*61*
Heliothis zea	dietary requirement, vitamin accumulated in tissues during growth	*41*
Heliothis virescens	dietary requirement	*62*
Laspeyresia pomonella	dietary requirement	*63*
Leptinotarsa decemlimeata	dietary requirement	*64*

Continued on next page.

Table III. (Continued)

Species	*Comments*	*References*
Leucophaea maderae	synthesis by fat body and by symbionts	*9*
Locusta migratoria	vitamin detected in tissues by histochemical staining	*7*
	dietary requirement, vitamin accumulated in hemolymph during development	*26, 32*
Lucilia sp.	vitamin detected in tissues by histochemical staining	*7*
Manduca sexta	dietary requirement (0.5 m*M*); dehydroascorbic acid, Mg 2-*O*-phosphonoascorbate, Na 6-*O*-myristoylascorbate fully active; D-ascorbic acid 50% active; 6-bromoascorbic acid 20% active; D-isoascorbic acid 10% active; K 2-*O*-sulfoascorbate, L-isoascorbic acid, L-gulonic acid γ-lactone inactive	*5*
	vitamin accumulated in hemolymph and larval labial gland (also present in eggs, gut, muscle, cuticle, nervous tissue and gonads), no L-gulonolactone oxidase detected	*65*
Melampsalata cingulata	whole body levels at 1 mg/100 g	*38*
Melanoplus biovittatus	dietary requirement	*66*
Musca domestica	whole body contained 1.5 mg/100 g, synthesis from hexose	*38*
Myzus persicae	dietary requirement, D-isoascorbic acid fully active	*67*
Neomyzus circumflexus	dietary requirement	*68*
Neotermes sp.	dietary requirement	*69*
Oryzaephilus surinamensis	detrimental effect in diet	*34*
	vitamin C replaced pantothentic acid requirement in diet	*35*
Ostrinia nubilalis	dietary requirement	*70*
Pectinophora gossypiella	no dietary requirement	*71*
	synthesized and accumulated vitamin C during development	*41*

Table III. (Continued)

Species	*Comments*	*References*
Periplaneta americana	whole body at 10 mg/g, synthesis	*72*
	present in Malpighian tubules at 0.6–1.0 mg/g	*73*
	biosynthesis from hexose?	*74*
	synthesized by symbionts in fat body	*75*
	synthesized in gut (by symbionts?)	*10*
	no biosynthesis from hexose, glucuronate, L-gulono-γ-lactone, L-galactono-γ-lactone	*43*
Plebliogrylus guttiventri	no biosynthesis	*43*
Polistes herbraeus	no biosynthesis	*43*
Rhycacionia buoliana	dietary requirement	*76*
Schistocerca gregaria	hemolymph titer at 100–500 μg/mL, hemolymph titer increased during growth	*26, 32*
Sphingomorpha chlorae	no biosynthesis	*43*
Spodoptera littoralis	dietary requirement (0.5%); D-araboascorbic acid, D-glucurono-γ-lactone, L-glulono-γ-lactone inactive	*77*
	D-glucoascorbic acid (0.05–0.3%) in diet produced deformed spermatophores	*78*
	vitamin C present in hemolymph and molting fluid	*6*
	Na ascorbate, Ca ascorbate, L-dehydroascorbic acid active, D-isoascorbic acid inactive	*23*
Tenebrio molitor	none detected in whole body	*56*
Trichoplusia ni	dietary requirement, Na L-ascorbate and Ca L-ascorbate active	*79*
	dietary requirement	*80*
Tryporyza incertulas	no synthesis	*43*
Xyleborus ferrugineus	L-ascorbic acid, D-isoascorbic acid, and L-dehydroascorbic acid inhibited progeny by producing diet nutritionally deficient in protein (browning)	*33*

Ascorbic acid may be involved in molting because the titer decreased in the hemolymph and increased in the molting fluid during apolysis (*6, 26*). The old exoskeleton is digested by proteases and chitinases while the new exoskeleton is formed by tanning enzymes, chitin synthase, and protein synthase. The earliest pathology in vitamin-deficient insects was observed in the cuticle, which tanned abnormally and exhibited lesions (*5, 6*). In certain insects the absence of L-ascorbic acid may allow cuticle tanning reactions such as tyrosine hydroxylation and oxidation to occur prematurely. Reductants such as ascorbic acid have been implicated in enzymatic hydroxylation reactions (*36, 37*). However, evidence for this involvement is ambiguous. Navon (*6*) observed that catecholamine oxidation was inhibited in the cotton leafworm, while Briggs (*38*) reported that tyrosine oxidation in flies was stimulated by L-ascorbic acid. There may be a species dependence where oxidation of phenols is activated in Diptera and inhibited in Lepidoptera.

Another possible function of L-ascorbic acid in the cuticle is to promote collagen formation. No evidence for this has been obtained using insects, but L-ascorbic acid deficiency disease in penaeid shrimp, termed "black death," was related to collagen hypohydroxylation (*39, 40*). Melanized lesions of loose connective tissue occurred in endocuticle at intersegmental spaces. Perhaps insects also underhydroxylate collagen when deficient in ascorbic acid.

L-Ascorbic acid is plentiful in the larval labial gland of the tobacco hornworm. Whereas many lepidopterans use the gland for the production of silk fibroin, the hornworm uses the gland contents for "body wetting" (*29*). Prior to pupal apolysis, the larva wets itself all over with a proteinaceous fluid. This secretion may be used as an external lubricant for burrowing behavior or it may be involved in cuticle degradation. What role ascorbic acid plays in the labial gland is unknown.

The last tissue to be discussed is the hemolymph. Perhaps L-ascorbic acid has no particular function there, except to maintain a highly reducing environment that serves as a reservoir of L-ascorbic acid for other tissues.

Research Needs. Over the years L-ascorbic acid has been shown to be an essential nutrient for many insects including species of Lepidoptera, Orthoptera, Coleoptera, and Diptera. Others such as cockroaches, houseflies, and mealworms are reared on simple diets without added ascorbic acid. Perhaps those insects require very low levels of vitamin C in their diets. A sensitive analytical method is needed to measure levels of L-ascorbic acid and dehydroascorbic acid in insect tissue and food. Such a method, which is likely to be developed using HPLC with electrochemical detection, could be used to monitor vitamin C levels in feed ingredients as well as in tissues during an insect's life cycle. This information is needed to determine whether ascorbic acid is used to

regulate the activity of enzymes, such as those involved in molting. Vitamin C appears to have a varied and almost ubiquitous role in insects. Much more research is required to determine whether ascorbic acid is an essential nutrient for all insects and to define its mechanism of action in insect development.

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