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REVIEWS

Tomato Glycoalkaloids: Role in the Plant and in the Diet

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Tomatoes, a major food source for humans, accumulate a variety of secondary metabolites including phenolic compounds, phytoalexins, protease inhibitors, and glycoalkaloids. These metabolites protect against adverse effects of hosts of predators including fungi, bacteria, viruses, and insects. Because glycoalkaloids are reported to be involved in host-plant resistance, on the one hand, and to have a variety of pharmacological and nutritional properties in animals and humans, on the other, a need exists to develop a better understanding of the role of these compounds both in the plant and in the diet. To contribute to this effort, this integrated review presents data on the history, composition, and nutrition of tomatoes, with special focus on the assessment of the chemistry, analysis, composition, nutrition, microbiology, and pharmacology of the tomato glycoalkaloids comprising α-tomatine and dehydrotomatine; their content in different parts of the tomato plant, in processed tomato products, and in wild and transgenic tomatoes; their biosynthesis, inheritance, metabolism, and catabolism; plant−microbe relationships with fungi, bacteria, viruses, insects, and worms; interactions with ergosterol and cholesterol; disruption of cell membranes; tomatine-induced tomatinases, pantothenate synthetase, steroid hydroxylases, and cytokines; and inhibition of acetylcholinesterase. Also covered are tomato−human pathogen relationships and tomatine-induced lowering of plasma cholesterol and triglycerides and enhancement of the immune system. Further research needs in each of these areas are suggested. The overlapping aspects are discussed in terms of general concepts for a better understanding of the impact of tomato glycoalkaloids in the plant in general and in food in particular. Such an understanding can lead to the creation of improved tomatoes and to improved practices on the farm and in the consumption of tomatoes.

Keywords: α-Tomatine; β_1-tomatine; β_2-tomatine; γ-tomatine; δ-tomatine; dehydrotomatine; tomatidine; tomatidenol; lycopene; tomatoes; transgenic tomatoes; chemistry; analysis; composition; biosynthesis; plant physiology; antibiotic effects; tomatinases; plant pathogens; host-plant resistance; disease resistance; bacteria; fungi; insects; viruses; protozoa; nutrition; cytochemistry; cell membrane structures; tomatine−cholesterol complex; cholesterol-lowering effects; human health; cancer chemotherapy; malaria vaccine adjuvant

INTRODUCTION

Glycoalkaloids are N-containing secondary plant metabolites found in numerous solanaceous plant species including eggplants, potatoes, and tomatoes. α-Tomatine, a glycoside in which four carbohydrate residues are attached to the 3-OH group of the aglycon tomatidine, occurs naturally in tomatoes (*Lycopersicon esculentum*). Immature green tomatoes contain up to 500 mg of α-tomatine/kg of fresh fruit weight. The compound is largely degraded as the tomato ripens until, at maturity, it reaches levels in red tomatoes of ∼5 mg/kg of fresh fruit weight. Consumers of green tomatoes, high-tomatine red tomatoes, and tomato products such as pickled green and green fried tomatoes consume significant amounts of tomatine (*1*−*3*).

The glycoalkaloid known as tomatine, first isolated by Fontaine et al. (*4*), actually consists of a mixture of two glycoalkaloids, α-tomatine and dehydrotomatine (*1*). Both compounds are present in all parts of the tomato plant (*3*). Other seminal observations originating from this laboratory include characterization of the tomatine degradation product allopregnenolone formed during tomato ripening and development of a radioligand assay for tomatine by Heftmann and Schwimmer (*5*, *6*), studies on the pharmacology of tomatine by Wilson et al. (*7*), and isolation of new compounds from tomatoes by Buttery et al. (*8*).

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Table 1. Tomatine Concentration and Proximate Composition of Freeze-Dried Green and Red Tomatoes (Adapted from Reference *24*)

tomato component	green tomatoes	red tomatoes
tomatine, mg/kg of fresh wt	48	0.4
tomatine, mg/kg of dry wt	743	0.7
solid content, %	6.4	5.8
N, %	2.49	2.11
fat, %	3.24	3.20
ash, %	10.31	10.02
insoluble fiber, %	19.76	17.03
soluble fiber, %	4.26	4.91

Concurrent with the discovery of tomatine, studies were undertaken which showed that the molecule possessed antibiotic properties against a variety of fungi and the human pathogens *Escherichia coli* and *Staphylococcus aureus* (*4*, *9*−*13*). These observations suggested that tomatine may play a major role in disease resistance in the tomato plant and may be biologically active in animals and humans. This suggestion then stimulated worldwide interest in the role of tomatine in the plant and in the diet. The main objective of this paper is to integrate the widely scattered literature on the multifaceted aspects of tomatine chemistry, analysis, and biological functions in plants and animals from a basic, mechanistic standpoint and to delineate the practical benefits of the wide-ranging studies for plant sciences and the diet. Furthermore, we offer suggestions for future research in all of these areas. As part of this effort I present capsule summaries of the experimental findings on which the cited authors based their assessment of the role of tomato glycoalkaloids in different milieus.

THE TOMATO AS FOOD

The Aztecs of Central America cultivated the tomato plant, which they called *xitomatl* at around 700 A.D. Spanish conquistadors named it *tomate*. Tomato seeds were transported from the Andes to Spain and from there to other European countries around 1520. In Italy, tomatoes were named *pomodoro* or "golden apple" and in France, "pomme d'amour" or apple of love. In 1781, Thomas Jefferson mentioned that tomatoes were grown in Virginia; the use of tomato soups and sauces in the United States began around 1830 (*14*, *15*). Because the tomato plant belongs to the Solanaceae plant family, members of which produce toxic alkaloids such as nicotine, the fruits were assumed to be poisonous and their consumption was frowned upon. For this reason, most tomato plants were grown as ornaments. The widespread use of tomatoes in the United States as a food began during the second half of the 19th century. Current world production is estimated at ∼24 million tons (*16*). Tomatoes are used in many processed foods such as canned and sun-dried tomatoes, juices, ketchup, pastes, purees, salads, sauces, and soups. Although the tomato is widely assumed to be a vegetable, it is actually a fruit, often called tomato fruit. Humans consume tomato glycoalkaloids as a part of the diet, so to place the following discussion in the proper perspective, we will briefly describe the composition, nutrition, and health benefits of tomatoes.

Tomatoes contribute antioxidants, carbohydrates, fiber, flavor compounds, minerals, proteins, vitamins, calistegines, and glycoalkaloids to the diet (*17*−*23*). Some of the nutrients and non-nutritive compounds that could modulate the biological actions of tomatine are mentioned below.

Tables 1 and **2** show the proximate and amino acid compositions of dehydrated green and red tomatoes (*24*). The compositions of green and red tomatoes are similar except that the former contain chlorophyll but no lycopene and ∼100 times more tomatine than the latter. Free amino acids contribute significantly to the total amino acid profiles of both green and red tomatoes, for example, free lysine constitutes ∼20−25% of the total and free methionine, ∼12−18%. The lysine/arginine ratio of the total amino acid profile is more than twice that reported for cereal proteins (rice and wheat gluten) and is similar to that reported for legume (pea and soy) proteins; the methionine/glycine ratio is greater than that for legume proteins and is similar to that of animal proteins (casein and ovalbumin). The essential amino acid content of tomatoes represents a good-quality protein, being similar to that of soy protein (*25*).

Table 2. Amino Acid Composition of Dehydrated Tomatoes (Adapted from Reference *24*)

	green tomatoes		red tomatoes	
amino acid	g/100 g	g/16 g of N	g/100 g	g/16 g of N
free amino acids				
aspartic acid	0.35	2.5	0.88	6.3
threonine	0.19	1.3	0.11	0.80
serine	0.30	2.1	0.16	1.1
glutamic acid	0.29	2.0	2.4	16.9
glycine	0.034	0.24	0.016	0.11
alanine	0.073	0.52	0.045	0.32
valine	0.091	0.64	0.023	0.16
methionine	0.022	0.16	0.033	0.24
isoleucine	0.089	0.63	0.031	0.22
leucine	0.071	0.50	0.030	0.21
tyrosine	0.11	0.78	0.028	0.20
phenylalanine	0.17	1.2	0.12	0.86
lysine	0.13	0.90	0.067	0.48
histidine	0.15	1.0	0.13	0.92
arginine	0.124	0.87	0.076	0.54
total amino acids				
aspartic acid	1.4	9.6	1.7	12
threonine	0.39	2.7	0.33	2.4
serine	0.49	3.5	0.36	2.6
glutamic acid	3.6	26	5.3	38
proline	0.28	2.0	0.24	1.7
glycine	0.33	2.3	0.32	2.3
alanine	0.36	2.5	0.29	2.1
valine	0.35	2.5	0.26	1.9
isoleucine	0.30	2.1	0.24	1.7
leucine	0.46	3.2	0.39	2.8
tyrosine	0.30	2.1	0.22	1.6
phenylalanine	0.41	2.9	0.35	2.5
lysine	0.52	3.7	0.42	3.0
histidine	0.37	2.6	0.29	2.1
arginine	0.47	3.3	0.42	3.0
cystine	0.12	0.85	0.17	1.2
methionine	0.19	1.3	0.18	1.3
tryptophan	0.12	0.85	0.12	0.86

Epidemiological studies indicate that plasma levels of the tomato red pigment lycopene are inversely related to the risk of breast, colon, lung, and prostate cancer (*26*). The protective effect may be due to the ability of lycopene to trap reactive hydroxyl and nitroxyl radicals that damage DNA, cells, and tissues. Because tomatoes contain other antioxidants such as ascorbic acid, β-carotene, chlorogenic acid, rutin, plastoquinones, tocopherol, and xantophylls, as well as trace elements such as copper, iron, and chromium (*27*, *28*) that can also participate in redox reactions, it is possible that these tomato ingredients can act synergistically with lycopene in preventing cell damage. This latter possibility seems to be plausible because lycopene-containing tomato diets appear to be more effective in cancer prevention than is pure lycopene. Individual lycopene isomers differ in their antioxidative potencies (*29*−*31*). It is also relevant that olive oil, which contains phenolic antioxidants, but not sunflower oil, which does not, enhances the antioxidative

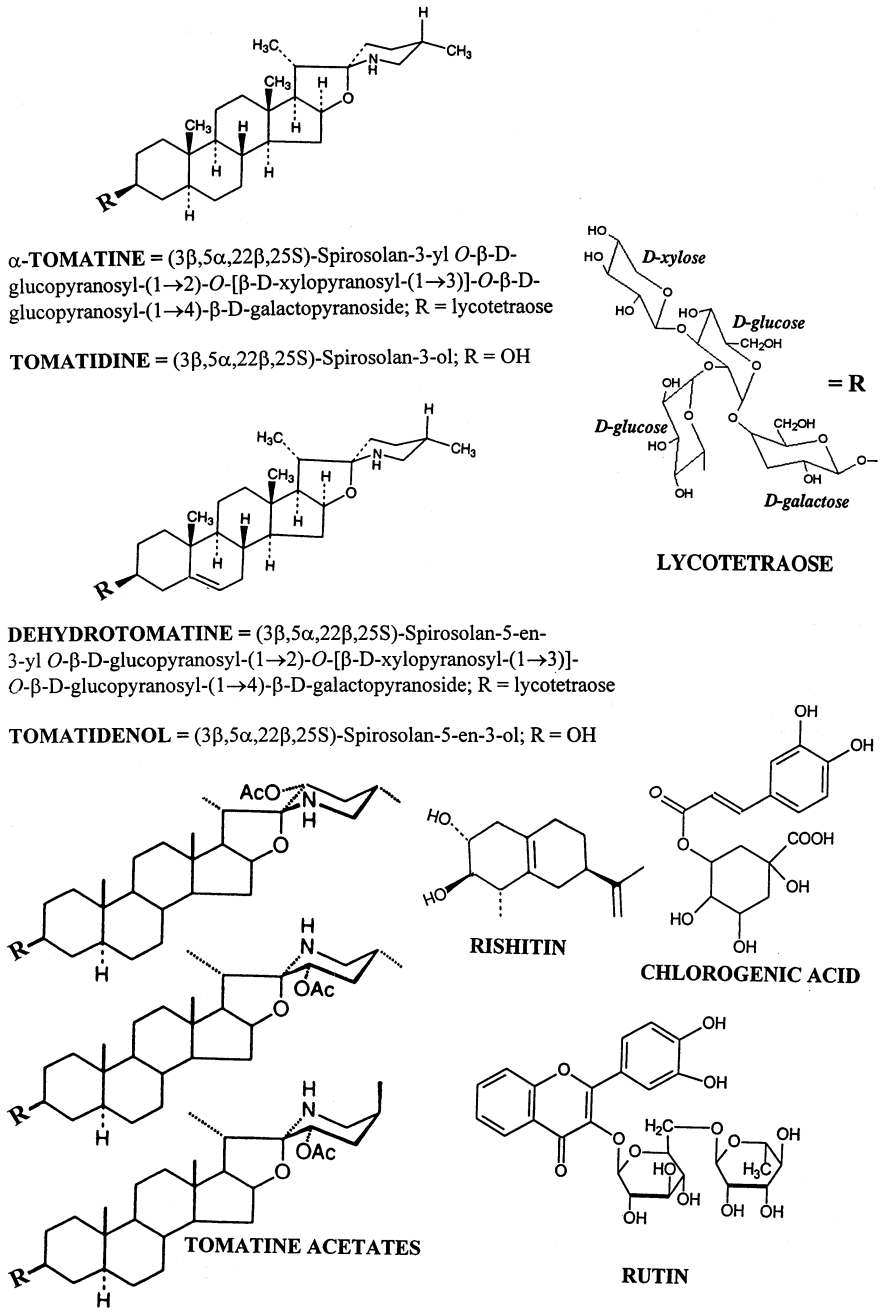

Figure 1. Structures of tomato glycoalkaloids and three other secondary metabolites of the tomato plant.

activity of lycopene in plasma (*32*). Other studies showed that tomato diets significantly reduced LDL cholesterol and triglyceride plasma levels of hamsters, as described below.

CHEMISTRY

Nomenclature. Irving et al. (*12*) isolated a fungistatic compound from tomato plants, which they named "lycopersicin". It was then noted that this name was already in use as a synonym for the red tomato pigment "lycopene". The compound was therefore renamed "tomatine". VanEtten et al. (*33*) suggested the name "phytoanticipin" for naturally occurring antimicrobial compounds such as α-tomatine, to distinguish those from compounds such as rishitin, which are induced following exposure of the plant to stress including phytopathogens. It is not clear, however, how the name "phytoanticipin" would accommodate stress-induced increases in the biosynthesis of naturally occurring compounds such as tomatine already present in the plant.

The discovery that all parts of the tomato plant contain a second glycoalkaloid, dehydrotomatine, is significant because all previous studies with the so-called "α-tomatine" are based not on the pure compound but rather on a mixture of two glycoalkaloids having different biological potencies and which can act synergistically or antagonistically both in the plant and in the diet, as discussed below. Unless otherwise indicated, the term "α-tomatine" will be used interchangeably with the generic name "tomatine" for both compounds and "pure α-tomatine" for the compound separated from the mixture.

Structure of α-Tomatine. Fontaine et al. (*4*) are credited with the isolation of crystalline tomatine from the tomato plant *Lycopersicon pimpinellifolium.* These USDA scientists also demonstrated that the glycoalkaloid consisted of the aglycon

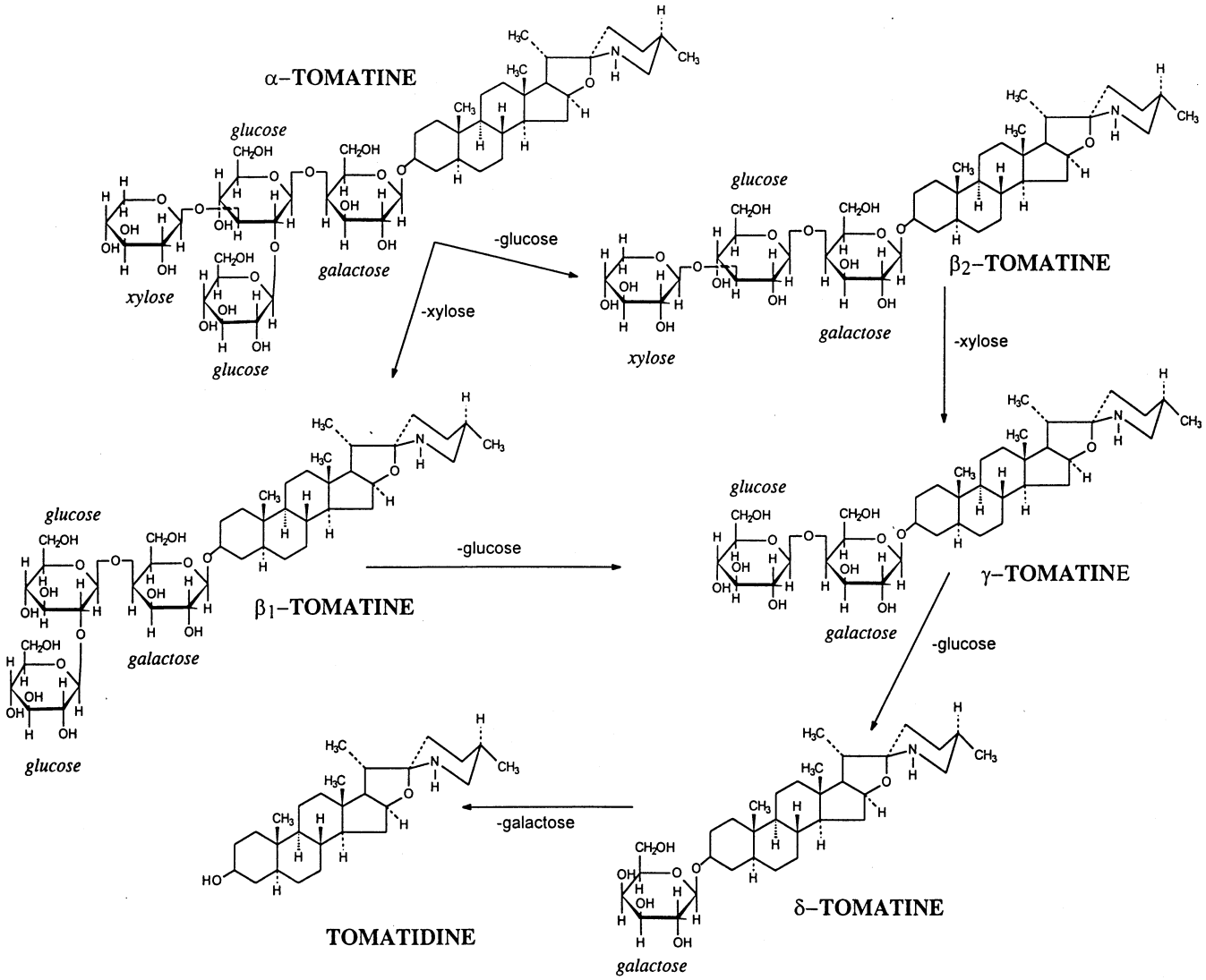

Figure 2. Acid- and fungal-tomatinase-catalyzed hydrolysis of the lycotetraose side chain of α-tomatine. A similar scheme can be written for the glycolysis of dehydrotomatine.

tomatidine and a tetrasaccharide side chain composed of xylose, galactose, and two glucose units. On the basis of the preparation of several tomatidine derivatives and infrared spectra of tomatidine, *N*,*O*-diacetyltomatidine, cholesterol, and acetylcholesterol, Fontaine et al. (*34*) were able to assign the correct structure to tomatidine. Concurrent studies by Sato et al. (*35*) supported the structure of tomatidine proposed by Fontaine and colleagues. Earlier, Kuhn et al. (*10*) incorrectly concluded that tomatine has a double bond in ring B of the steroid part of the molecule. Ironically, the proposed structure corresponds to that of dehydrotomatine (*36*).

The composition and stereochemistry of the lycotetraose tetrasaccharide of tomatine are shown in **Figure 1** (*9*). The tomatine carbohydrate side chain was partially hydrolyzed in sulfuric acid solutions to β_1-, β_2-, γ-, and δ-tomatines **(Figure 2)**. The liberated sugars were characterized as the permethylated derivatives. Additional X-ray diffraction studies of tomatidine hydroiodide and ^{13}C NMR studies of α-tomatine allowed complete assignments to the stereochemistry of the anomeric carbon atoms of the aglycon and lycotetraose moiety as well as to the *spiro* junction between rings E and F of the aglycon moiety (*37*−*39*).

The structure of tomatine consists of a hydrophilic part (the tetrasaccharide side chain), a hydrophobic part (the steroidal moiety), and a polar −NH group, which can participate in acid−base equilibria. These unique features of the molecule govern its biological activities, outlined below.

Structure of Dehydrotomatine. The discovery of dehydrotomatine (*1*, *40*) and the demonstration that it was present in all parts of the tomato plant (*3*) were followed by chemical and analytical studies designed to elucidate the exact structure of dehydrotomatine (*36*). The mass spectra of pure α-tomatine and dehydrotomatine exhibited molecular ion peaks that were in agreement with reported values. **Figure 3** shows an HPLC chromatogram, UV absorption data, and mass spectra for dehydrotomatine and pure α-tomatine separated from commercial tomatine. Experiments were then carried out to establish the structure of the carbohydrate side chain of dehydrotomatine. This was accomplished by (a) isolating α-tomatine and dehydrotomatine from commercial tomatine; (b) derivatizing the monosaccharides formed on acid hydrolysis of commercial tomatine, α-tomatine, and dehydrotomatine to alditol acetates and methylated alditol acetates; and (c) determining the structures of the galactose, glucose, and xylose formed by GC-MS. The results revealed that both α-tomatine and dehydrotomatine have the same carbohydrate side chain. They differ only by the presence in dehydrotomatine of a double bond due to removal of 2 H's at C-atoms 5 and 6 of ring B and its absence

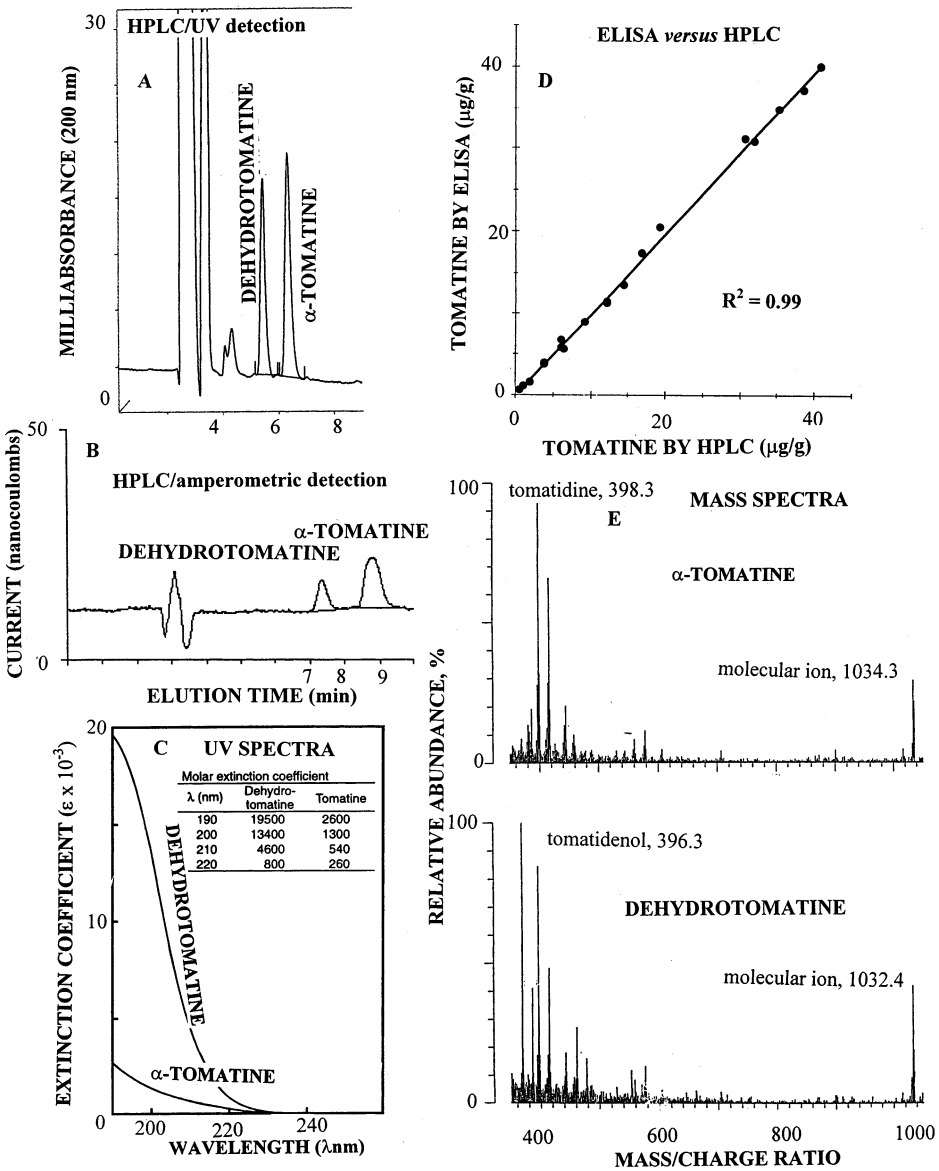

Figure 3. Analytical data: (A, B) HPLC with UV and amperometric detection (adapted from refs *2* and *3*); (C) UV spectra (*41*); (D) correlation between ELISA and HPLC for samples listed in **Table 7** (adapted from ref *84*); (E) mass spectra (adapted from ref *36*).

in α-tomatine. Dehydrotomatine (tomatidenol-3β-lycotetraose) represents a new class of glycoalkaloids in tomatoes. The proposed structure was confirmed by Ono et al. (*41*) with the aid of ^{13}C NMR.

Other Tomato Glycoalkaloids. Tomatine and three additional glycoalkaloids from leaves and fresh fruits of the tomato plant *Lycopersicon esculentum* were isolated by Yahara et al. (*42*) (**Figure 1**). The yield of γ-tomatine from tomato leaves was 0.00093% compared to 0.11% for α-tomatine. The yield of the three new glycoalkaloids from tomato fruits ranged from 0.00015 to 0.0005% of fresh weight. The three new glycoalkaloids are tomatine derivatives containing acetoxy groups in the F-ring of the molecule. They are reminiscent of leptines, analogous OH derivatives of potato glycoalkaloids. Nagaoka et al. (*43*) isolated low amounts of several new glycoalkaloids in the roots of a *L. esculentum* × *L. hirsutum* hybrid highly resistant to soil fungi. The yields of these compounds ranged from 0.00002 to 0.00011% of the root fresh weight. Two other new alkaloids, pimpifolidine and 22-isopimpifolidine, were discovered in the roots of the wild tomato *L. pimpinellifolium* (*44*). *Solanum arboretum* contains two tomatines with one and two sugars, respectively, tomatidine 3-*O*-β-glucopyranoside and 3-*O*-[*O*-β-xylopyranosyl-(1→6)]-β-D-glucopyranoside (*45*). The root glycoalkaloids may be involved in protecting roots against undesirable soil fungi.

Acid Hydrolysis Products of α-Tomatine. Several investigators evaluated conditions for the partial and complete acid hydrolysis of the sugar side chain of glycoalkaloids (*46*−*51*). Hydrolysis of saccharides with H_2SO_4 has a drawback because of the necessity of its removal using $BaCO_3$ to precipitate sulfate prior to derivatization because some of the carbohydrate is probably adsorbed during the precipitation step. We selected HCl as the hydrolysis medium for our kinetic and preparative studies.

As part of an effort to define the biological roles of carbohydrate groups of the tomato glycoalkaloid α-tomatine in plants and animals, studies were carried out to optimize the acid hydrolysis of the tetrasaccharide side chain of α-tomatine to products with three, two, one, and zero sugar groups (**Figure 2**) (*36*, *50*). This was accomplished by following the time course for hydrolysis in 1 N HCl at 100 °C, isolating the hydrolysis products by chromatography on an aluminum oxide column, and determining the number and nature of hydrolysis products, including free sugars, with the aid of TLC and GC-MS of alditol

acetate sugar derivatives. A 20-min hydrolysis time appears to be optimal for the formation of a mixture of the mono-, di-, trisaccharides, and a 70-min exposure achieved complete hydrolysis of α-tomatine to the tomatidine. α-Tomatine was stable to hydrolysis at 37 °C, suggesting that it may also be stable at acid pH values of the gut of insects, animals, and humans. Efforts to isolate the other possible trisaccharide, β_2-tomatine, were unsuccessful. [In principle, β_2-tomatine could be made by enzyme (tomatinase) hydrolysis of α-tomatine (see below) and/or by synthesis from tomatidine.] This approach and use of ethanol to control the partial hydrolysis of glycoalkaloids (*49*, *51*) should be generally useful for preparing hydrolysis products of glycoalkaloids to facilitate assessing the role of the carbohydrate groups of these compounds in disease resistance, microbiology, and nutrition.

Tomatine and Tomatidine Derivatives. The carbohydrate side chain and the NH functionalities of tomatine and tomatidine can be chemically modified to derivatives useful for structural and metabolic studies. Reported modifications include the formation of acid hydrolysis products mentioned above, *N*-nitroso (−N−NO) tomatine (*52*), ^{14}C- and ^{15}N-labeled tomatines (*53*), and *N*-methylated (−N−CH_3) (*54*) and deuterium-labeled tomatidines (*55*).

Tomatine—Sterol Interactions. Tomatine forms strong, insoluble complexes with both plant and animal sterols. Such interactions contribute significantly to the biological and structural effects of tomatine. This aspect of tomatine chemistry is examined in some detail below.

Tomatine forms a 1:1 insoluble complex with cholesterol (*56*). Addition of acetic acid to a solution of the cholesterol−tomatine complex in DMF resulted in dissociation of the complex to starting materials. The method can be used to separate tomatine from tomatidine because the latter is not precipitated by cholesterol. Roddick (*57*) studied the rate of complex formation between α-chaconine, α-solanine, and α-tomatine and several sterols (camposterol, cholesterol, ergosterol, sitosterol, and stigmasterol), using scintillation spectrometry to analyze ^{14}C-labeled cholesterol and sitosterol and GLC to measure the other steroids. The data show that temperature did not influence complex formation, the sterol-complexing ability of tomatine was greater than that of the potato glycoalkaloids, tomatine had a lower affinity for cholesterol than for the other sterols, and tomatidine did not bind the steroids.

In a subsequent study, Roddick and Drysdale (*58*) examined the effect of α-tomatine concentration and pH on the leakage of peroxidase from liposomes. At the optimum pH of 7.2, the extent of membrane disruption increased in the concentration range of 10−100 μM tomatine. The extent of membrane disruption also correlated with the concentration of cholesterol in the liposomes and indirectly with pH in the range of 5−8. These results suggest that the unprotonated form of tomatine is the active form and that the variable effect of tomatine on membranes of fungal cells, erythrocytes, mitochondria, etc., is due to differences in the sterol content of the membranes. The nature of the steroid moiety, that is, cholesterol, ergosterol, and stigmasterol, did not significantly affect the tomatine-induced membrane disruption.

The plant sterols β-sitosterol and fucosterol had a greater affinity for the glycoalkaloids than did cholesterol and the fungal sterol ergosterol (*59*). The higher potency of α-tomatine compared to the potato glycoalkaloids is likely due to the lycotetraose carbohydrate side chain compared to the triose side chains of α-chaconine and α-solanine and to differences in the structures of the aglycon part of the molecules. Subsequent studies (*47*, *60*) revealed that (a) the α-tomatine−cholesterol complex forms spherical structures; (b) the planar ring structure of the sterol is paramount for efficient α-tomatine-sterol binding; (c) hydrogen-bonding between tomatine and membrane sugar groups contributes to the relative stabilities of structurally different glycoalkaloid−sterol complexes; (d) the glycoalkaloid-induced membrane disruption of naturally occurring cells (Caco-2 human cancer cells, human erythrocytes, mitochondria) was similar to those observed with membranes of vesicles artificially prepared from egg yolk phosphatidylcholine; and (e) hydrolysis products with fewer than the four sugar groups (β_1-tomatine-without xylose, β_2-tomatine-without glucose, γ-tomatine-without xylose and glucose, and δ-tomatine-without xylose, and both glucoses) did not disrupt cholesterol-containing membranes. These observations provide a rationale for a proposed model for glycoalkaloid-induced membrane disruption.

Table 3. Dehydrotomatine and α-Tomatine Contents of Parts of the Tomato Plant (Adapted from References *1*–*3*)

plant part	mg/kg of fresh wt		% dehydrotomatine
	dehydrotomatine	α-tomatine	
large immature green fruit	14	144	8.8
roots	33	118	23
small immature green fruit	54	465	10
calyxes	62	795	7.3
leaves	71	975	6.9
small stems	138	896	13
large stems	142	465	25
flowers	190	1100	15
senescent leaves	330	4900	6.1

Table 4. Tomatine Content of Store-Bought Tomatoes (Adapted from References *1*–*3*)

tomato	mg/kg	
	dry wt	fresh wt
Beefsteak	15	0.9
Roma	7	0.4
standard tomato	4	0.3
cherry	42	2.8

ANALYSIS

Detection of tomato glycoalkaloids is of interest because they (a) are involved in host-plant resistance, (b) disrupt cell membranes, and (c) may have beneficial effects in the diet. The development of improved transgenic tomatoes has also stimulated interest in determining whether their changing glycoalkaloid content during different stages of fruit maturity differs significantly from that found in standard varieties.

Numerous methods have been proposed for the analysis of tomatine in different matrices. These include gravimetric, spectrophotometric, gas and liquid chromatographic, and immunoassay methods.

HPLC. HPLC is widely used to measure tomatine directly without the need to hydrolyze it to tomatidine or to subject it to other modifications (*1*, *2*, *61*−*65*). The following is an assessment of our results (**Tables 3**−**8**) on the analyses of tomatine and dehydrotomatine.

Because of difficulties in using refractive index and UV detection methods due to poor sensitivity and interferences, we developed an improved extraction HPLC-PAD method for the analysis of tomatine in different parts of the tomato plant, in commercial and field-grown (including transgenic) tomatoes,

Table 5. Tomatine Content per Fruit (Adapted from References *1–3*)

variety	fruit wt (g)	mg of tomatine/kg	mg of tomatine/fruit
ripe			
Sungold cherry	3.9	11	0.04
red pear cherry	6.5	1.3	0.01
yellow pear cherry	10.3	4.5	0.05
yellow cherry	11.4	9.7	0.11
tomatillos	34.0	0.5	0.02
green Zebra	66.9	0.6	0.04
standard	123	0.3	0.04
large yellow	227	1.1	0.24
unripe green			
small immature	3.4	548	1.86
medium immature	17.1	169	2.88
large immature	37.9	10	0.39
pickled	80.0	28	2.20
mature	127	16	2.04

Table 6. Tomatine Content of Processed Tomato Products (Adapted from References *1–3*)

	mg/kg	
sample	fresh	freeze-dried
stewed red	11	20
juice	28	49
red sauce	57	50
fried green	11	44
microwaved green	12	134
ketchup	25	
pickled green, brand X	28	353
pickled green, brand Y	72	989

Table 7. Tomatine Content of Freeze-Dried Tomatoes, Tomato Products, and Tomatillos Determined by HPLC and ELISA (Adapted from References *83* and *84*)

	mg/kg	
sample	HPLC	ELISA
Manteca red tomato	10	11.3
Manteca green tomato	308	312
breaker tomato	77	75
large immature tomato	397	386
mature green tomatoes	144	135
tomato roots	376	377
tomatillos	6	6.1
canned tomato sauce	64	57
pickled tomatoes	121	114

Table 8. Tomatine Content of Standard and Transgenic Tomatoes (Adapted from References *1–3*)

	mg/100 g of fresh wt			
variety	immature green	mature green	breaker	red
standard, parent	35	6.8	3.5	0.7
standard, transgenic	12	15	6.2	1.1
cherry, commercial	210	55	14	2.5
cherry, transgenic	280	52	27	3.9
cherry, transgenic	190	58	21	2.6

and in processed tomato products including juice, ketchup, salsa, sauce, and sun-dried tomatoes (*1*−*3*). Microwaving and frying did not affect tomatine levels of foods.

We found that tomatine consists of a mixture of the known tomato glycoalkaloid, α-tomatine, and a new glycoalkaloid, which we named dehydrotomatine. Dehydrotomatine differs from α-tomatine by having a double bond in ring B of the steroidal part of the molecule (**Figure 1**). We also found that an improved HPLC-PAD analytical method can be used to measure both the α-tomatine and dehydrotomatine contents of tomatoes and tomato plant parts and of a variety of processed tomato products sold commercially or prepared in the kitchen. Because the α-tomatine content of red tomatoes and most tomato products is quite low and the dehydrotomatine content in tomatoes appears to be an order of magnitude lower than that of α-tomatine, the highly sensitive HPLC-PAD method can be used to analyze dehydrotomatine in a variety of tomato substrates. The following results demonstrate this possibility.

Purified dehydrotomatine and α-tomatine were found to have the same concentration response by PAD detection and very different response by UV. Detection of dehydrotomatine is greater by a factor of ∼10 relative to α-tomatine (**Figure 3A**,**B**) (*1*, *41*). We found it difficult to use UV detection for low-tomatine red tomatoes and tomato products. In our hands, UV detection is not sufficiently sensitive for very low levels of dehydrotomatine. In contrast, PAD analysis is quite sensitive. Thus, the estimated lower detection limit by UV is 5 μg and that by PAD is 0.1 μg. Two commercial tomatine samples consisted of an 85:15 mixture of α-tomatine and dehydrotomatine.

The method was useful for the analysis of parts of the tomato plant including large immature green fruit (14 mg/kg of fresh weight), roots (34), small immature green fruit (54), calyxes (63), fresh leaves (71), small and large stems (140), and senescent leaves (315). The dehydrotomatine content of red tomatoes ranged from 0.05 to 0.42 mg/kg of fresh weight. The corresponding range for green tomatoes was from 1.7 to 45 mg/kg. The percent dehydrotomatine for the tomato plant parts was ∼7 for fresh and senescent leaves and calyxes, 10 for green fruit, 14 for small stems and flowers, and 23 for roots and large stems. The corresponding values for 15 different tomato varieties ranged from about 3 to 10%. A possible explanation for the variation in the dehydrotomatine content is offered below under Biosynthesis.

The observations with fresh and senescent leaves deserve further comment. Although the content of glycoalkaloids on fresh weight basis in brown, senescent leaves was much higher than in fresh leaves that came from the same plant, on a dry basis the values for both glycoalkaloids were similar (in mg/kg: dehydrotomatine, 465 dry weight for senescent leaves and 470 for fresh leaves; pure α-tomatine, 7300 for senescent leaves and 6400 for fresh leaves). These results demonstrate that drying of leaves on the plant does not degrade the tomato glycoalkaloids. We developed procedures for optimizing the analysis of glycoalkaloids in potato leaves that may be applicable to tomato leaves (*66*, *67*). Leonardi et al. (*20*) and Vaananen et al. (*68*) measured dehydrotomatine in tomatoes by HPLC, and Laurila et al. (*69*) measured dehydrotomatine by GC-MS as the aglycon tomatidenol.

Gas Chromatography—Mass Spectrometry. Gas chromatography alone or in combination with mass spectrometry for the analysis of tomatine has been widely studied (*70*−*73*). GC has an inherent disadvantage when applied to quantitative analysis of glycoalkaloids—it does not measure the glycoside directly. The glycoalkaloids have to be first hydrolyzed to the aglycons. These are then analyzed by GC without or with derivatization to more volatile derivatives. This multistep procedure does not give any information about the specific glycoside content when two or more glycosides are hydrolyzed to the same aglycon, as is the case with α-chaconine and α-solanine with their common aglycon solanidine and with solamargine and solasonine with their common aglycon sola-

sodine. However, GC-MS may be quite useful when information on the structure of the different aglycons is needed (*74*).

Mass Spectrometry. MS can be used to measure tomatine and other glycoalkaloids (*75*−*80*). The rapid technique requires limited sample pretreatment or purification and gives a highly specific ion signal for each glycoalkaloid. It was successfully applied to tomatine isolated from tomato fruit, which contains very low levels of tomatine. MS was also used to measure glycoalkaloids in Andean potatoes (*81*). Although MA can identify structures of glycoalkaloids **(Figure 3C)**, it is not always useful as a quantitative method.

Immunoassays. Analysis by immunoassays is based on the interaction between a monoclonal antibody and a corresponding antigen as analyte followed by detection of that interaction using enzymes. Generally, immunochemical assays are rapid and simple in design and do not require expensive instrumentation, the use of organic solvents, or highly purified plant extracts. Barbour et al. (*82*) developed an immunoassay for tomatine using polyclonal antibodies. However, the assay gave a nonlinear response to the glycoalkaloid. We developed a panel of monoclonal antibodies following immunization with a solanidine−BSA conjugate that binds the potato glycoalkaloids α-chaconine and α-solanine and their common aglycon, solanidine (*83*, *84*). One of the antibodies, Sol-129, was used in an immunoassay for tomatine in a variety of tomato matrices. The ELISA data correlated with HPLC analyses of the same samples (**Table 7**; **Figure 3D**). The ability to use the ELISA, especially an ELISA kit, merits further study because it would be useful for early rapid screening of newly developed tomato cultivars as well as for metabolic studies of α-tomatine and dehydrotomatine after oral consumption by animals and humans (*3*, *85*, *86*).

Spectrophotometry. So-called colorimetric methods for tomatine are simple to perform and are of special value to investigators with no access to modern instrumentation. However, impurities and other chromophores present in plant extracts may interfere with the analysis (*87*). These methods cannot be used with extracts containing more than one glycoalkaloid. Some of the reported colorimetric procedures for tomatine are outlined below.

Tomatine forms compounds of unknown structure that absorb in the visible region of the spectrum. Analytical methods based on such chromogens include those formed with sulfuric acid (λ_{max}, 620 nm) (*88*), anthrone (λ_{max}, 630 nm) (*89*−*91*), formaldehyde (λ_{max}, 575 nm) (*92*), paraformaldehyde (λ_{max}, 670 nm) (*93*), methyl orange (λ_{max}, 400 nm) (*94*), tropeollin (λ_{max}, 670 nm) (*95*), and bromthymol blue (λ_{max}, 620 nm) (*67*, *96*, *97*).

A bioassay based on the chemiluminescence of HepG 2 cells exposed to tomatine was used by Asano et al. (*98*) to measure the tomatine content of red tomatoes (5.42 mg/kg) and of tomatoes from the wild-type plants, *L. hirtsutum* and *L. peruvianum* (353 mg/kg). This fluorescent method yielded comparable values for tomatine and dehydrotomatine in red and green tomatoes and tomato leaves determined by HPLC. The value for tobacco mosaic virus-resistant transgenic tomatoes was the same as that of its host tomato. A rapid colorimetric assay based on recording the absorbance at 725 nm after injection into cultured cells of a green *leuco* base and tomatine was devised by Yamashoji (*99*). The decrease in absorbance after 5 min was proportional to the number of viable cells. The dose−response of tomatine in disrupting the integrity of cell membranes correlated with a colorimetric assay. A fluorescent method for tomatine, linear in the range of 1−10 μg, based on its ability to disrupt cholesterol-containing liposome cell membranes, was developed by Bacigalupo et al. (*100*).

Radioligand Assay. A radioligand assay for tomatine isolated from plant extracts is based on precipitating tomatine with [^{14}C]-cholesterol in ethanol and determining the radioactivity remaining in solution by scintillation counting (*5*). Unlike similar precipitation assays, the radioligand assay does not require purification of a precipitated complex. The assay was used to measure a 0.625% tomatine content of freeze-dried tomato leaves. An analogous assay for tomatine could probably be based on the use of [^{15}N]tomatine (*101*). This would entail measuring the ^{15}N of the tomatine−cholesterol complex.

Analysis of Glucose in Hydrolyzed Tomatine. A method for tomatine based on acid hydrolysis of the carbohydrate side chain (2 N H_2SO_4, 2 h, 90−100 °C) followed by enzymatic analysis of the liberated glucose with the aid of glucose oxidases and peroxidase is described by Ostrzycka (*102*).

BIOSYNTHESIS

α-Tomatine and Dehydrotomatine. High levels of fertilizer which provide compounds that provide sources of nitrogen such as ammonium sulfate, amino acids, and urea reduced both root weight and tomatine content in cultured tomato roots (*103*). The results suggest that tomatine production is related to weight increases in the root but that growing tissues are the principal sites of tomatine synthesis. In a related study, Hoffland et al. (*104*) found that the α-tomatine content of tomato leaves correlated with the C/N ratio of the leaves, suggesting that the glycoalkaloid is a C-based rather than an N-based compound and that the carbohydrate rather than the N concentration limits the biosynthetic rate of α-tomatine. Tomatine levels appear to be decreased by increasing N content in the soil. On the basis of observed patterns of glycoalkaloid biosynthesis in cell and shoot *teratoma* cultures of *Solanum dulcimara*, it appears that the biosynthesis of the aglycons occurs first, followed by glycosylation of the aglycon (*105*).

Both potato and tomato glycoalkaloids are formed in the plant by common biosynthetic pathways and are therefore interrelated, as discussed elsewhere (*106*). The starting point for the glycoalkaloids is cholesterol, although it is undetectable as formed by the mevalonic acid pathway responsible for the production of steroids in general. Cholesterol does not accumulate in plants but is immediately and completely converted to other substances. Acetate reacts with coenzyme A, forming the intermediates mevalonic acid, squalene, lanosterol, cycloartenol, and evanescent cholesterol. The exact pathway for the conversion of cholesterol to glycoalkaloids has not been fully elucidated. The N atom added in the formation of the F ring originates from an amino acid such as arginine.

Figure 4 illustrates possible biosynthetic and degradative pathways of tomato glycoalkaloids. The biosynthetic intermediate teneimine derived from cholesterol is partitioned; part of its double bond is hydrogenated by a hypothetical hydrogenase to tomatidine, and the remainder forms tomatidenol. Another possibility is that a part of the aglycon tomatidine is dehydrogenated to tomatidenol by a hypothetical dehydrogenase. This hypothesis, implying the existence of both types of aglycons in the same plant, is supported by investigations of Laurila et al. (*69*). Thus, although the leaves of the cultivated potato *Solanum tuberosum* contained α-solanine, the aglycon of which, solanidine, possesses a double bond, and those of the non-tuber-bearing wild-type *S. brevidens* contained α-tomatine, the aglycon of which, tomatidine, has no double bond, the somatic hybrids derived from the two cultivars contained the new

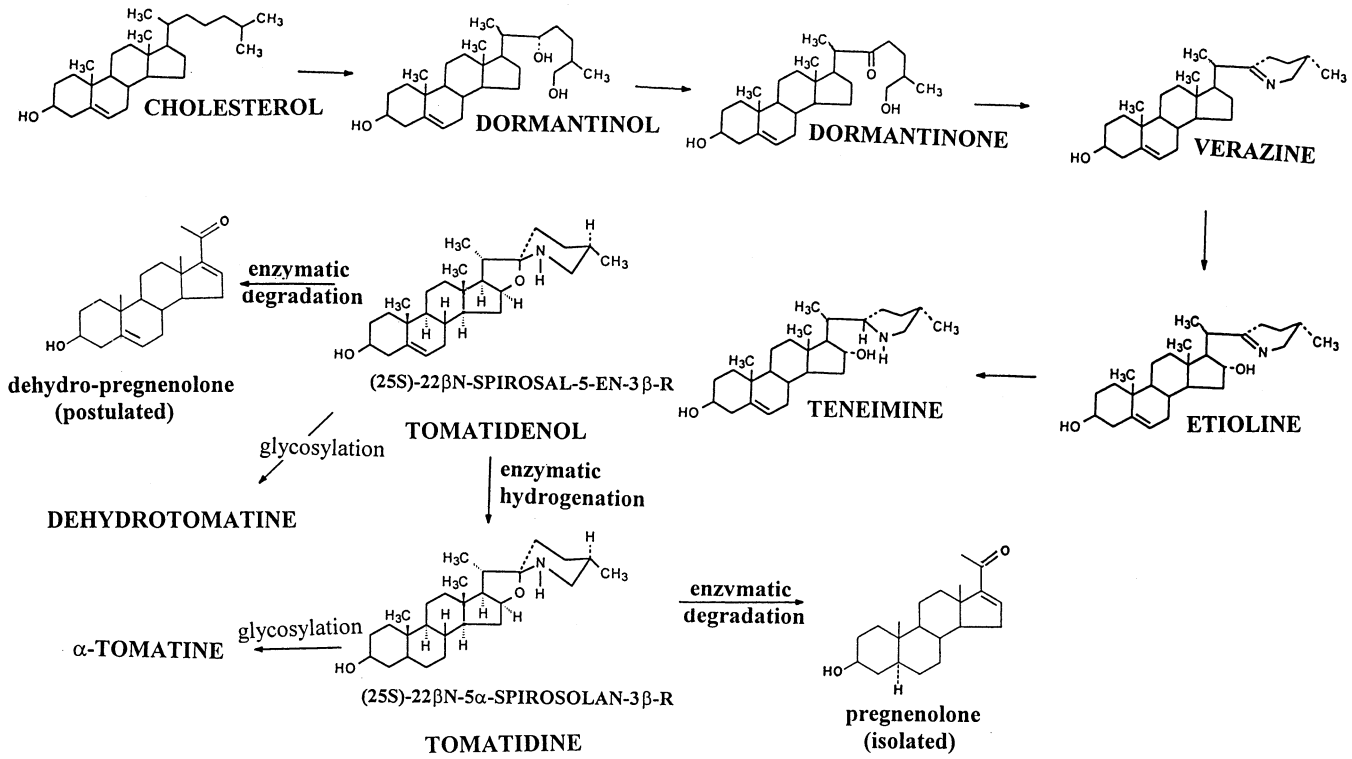

Figure 4. Postulated biosynthetic pathways from cholesterol to dehydrotomatine and α-tomatine. The degradation of α-tomatine to pregnenolone and the suggested analogous transformation of dehydrotomatine to dehydropregnenolone are also shown. Most of the enzymes involved are not known.

glycoalkaloid demissine, the aglycon of which, demissidine, has no double bond. The latter is probably derived by hydrogenation of the double bond of solanidine.

Indirect support for the hydrogenation hypothesis comes from our observation that different tomato varieties and different parts of the tomato plant contain different α-tomatine/dehydrotomatine ratios (*3*). Variation in the ratio may be ascribed to the action of a readily reversible NAD(P)-dependent reductase, acting to produce α-tomatine from dehydrotomatine or, in the opposite direction, to dehydrogenate α-tomatine to dehydrotomatine. The oxidation/reduction microenvironment could influence the balance of these reactions.

The two glycoalkaloids have the same carbohydrate side chain, so it is likely that tomatidine and tomatidenol are similarly glycosylated to the corresponding glycoalkaloids. However, the exact mechanism of glycosylation of the 3-OH group of the aglycons is unclear. Work with potatoes has shown that the plants contain enzymes capable of glycosylating solanidine in a stepwise manner, one sugar at a time. The enzymes must be able to synthesize UDP-glucose, UDP-galactose, and UDP-rhamnose and transfer the three sugars from the nucleoside to the acceptor molecule. Zimowski (*107*, *108*) isolated and characterized a galactosyltransferase from tomato leaves catalyzing the galactosylation of tomatidine by UDP-galactose to tomatidine monogalactoside. We found that solanidine UDP-glucosyltransferase from potatoes also glucosylated tomatidine to glucosyl-tomatidine (*109*−*111*). Evidently, the aglycons are rapidly glycosylated in a stepwise manner after being formed.

We also investigated the dynamics of incorporation of labeled [2-^{14}C]-DL-mevalonate into chlorophyll and the glycoalkaloids α-chaconine and α-solanine during their biosynthesis in potato sprouts in the light and in the dark (*112*). The data implicate a non-mevalonate pathway for the biosynthesis of both chlorophylls and the glycoalkaloids and are consistent with independent genetic control of the formation of the two classes of compounds during greening of potatoes.

Moehs et al. (*111*) have produced a cDNA encoding solanidine glucosyltransferase in yeast and transformed a library of cDNA into yeast. Certain cDNAs were found to encode an enzyme that glycosylated the aglycons, allowing the yeast to grow in the presence of normally inhibitory concentrations of solanidine, solasodine, or tomatidine. Suppression of the gene encoding this enzyme by an antisense-RNA approach resulted in reduced synthesis of the enzyme, a possible biomarker for glycoalkaloid synthesis.

The two potato glycoalkaloids, which share the common aglycon solanidine but not the same trisaccharide side chain, appear to be synthesized in discrete biosynthetic channels (*113*), so we do not know whether suppressing genes that govern the biosynthesis of α-chconine will influence the synthesis of α-solanine and vice versa. It is also not known whether the biosynthesis of the eggplant glycoalkaloids solamargine and solasonine, which share the same aglycon, solasodine, and of α-tomatine and dehydrotomatine, which share the same tetrasaccharide side chain but not the same aglycon, also occur in discrete channels. Moreover, safety considerations should guide efforts to alter the biosynthesis of glycoalkaloids. For example, we found that α-chaconine, α-solanine, solasonine, α-tomatine, and tomatidine were inactive and solanidine was active in an in vitro estrogen activity assay (*114*).

Evolutionary Aspects of the Biosynthesis of Glycoalkaloids. Until the recent discovery of dehydrotomatine, it was thought that tomatoes contain only one glycoalkaloid, usually called α-tomatine or tomatine. The question arises why each of the major *Solanum* plants produced two glycoalkaloids [potatoes, α-chaconine and α-solanine (*106*); eggplants, solamargine and solasonine (*115*); tomatoes, dehydrotomatine and α-tomatine]. One possibility is that nature initially created one glycoalkaloid. As phytopathogens became adapted over time to resist its effects, the plant created a second, biologically more potent one. An alternative possibility is that both glycoalkaloids developed at the same time during evolution in order to exert

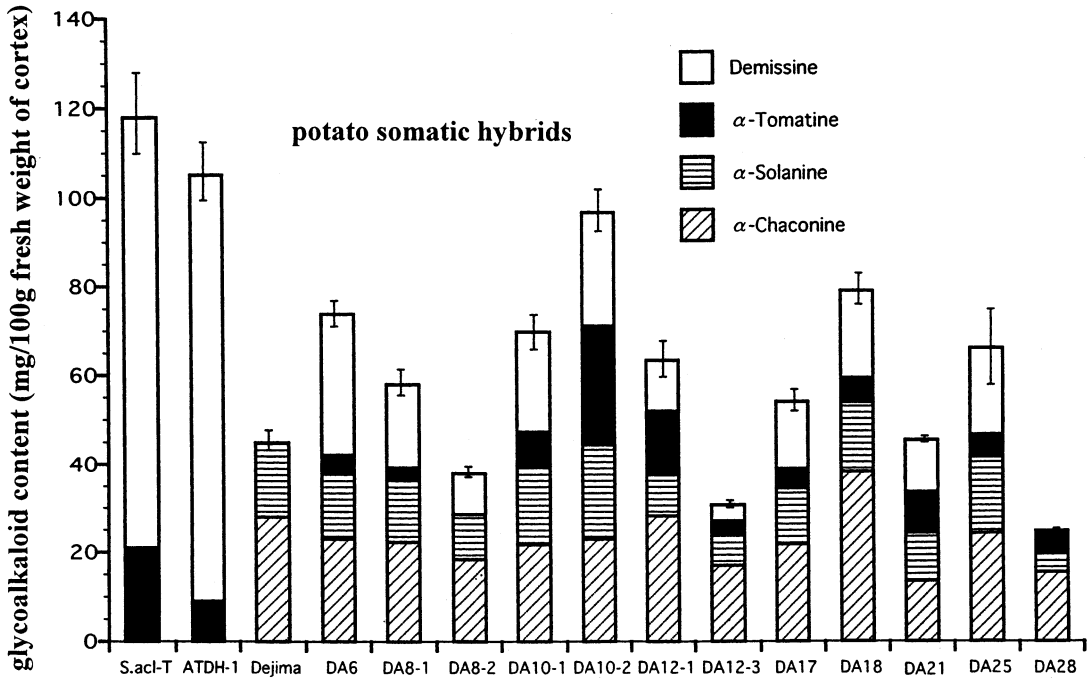

Figure 5. Constitutent glycoalkaloids in somatic hybrids of *S. acauele* and *S. tuberosum* (adapted from ref *120*).

the observed synergistic effect against phytopathogens. The second, evolutionary approach is more efficient because it allows the plant to produce a smaller total amount of the two glycoalkaloids while maintaining resistance. The biological potency of one of the two glycoalkaloids should be greater than of the other, and mixtures of the two glycoalkaloids may act synergistically both in the plant and in the diet. This is the case with potato glycoalkaloids α-chaconine and α-solanine (*116*). Whether this also true for tomato glycoalkaloids remains to be ascertained.

A referee pointed out other possible rationalizations for the dual glycoalkaloid hypothesis. It is possible that one compound might work better on one set of pests and the other for a different set. Differing interacting effects of the two glycoalkaloids may thus also guide the evolutionary issue on mammals, insects, and plant pathogens. Other factors that impact the evolution of a single versus a dual glycoalkaloid system include the difficulty in simultaneously developing pathways to two stable compounds and the potential effects of precursors on the synthesis of the final products. The driving forces for evolution of the dual glycoalkaloid model merit further study.

INHERITANCE OF TOMATINE IN SOMATIC HYBRIDS

Using the radioligand method of binding of the glycoalkaloids to ^{14}C-labeled cholesterol, precipitating the complex, and measuring the radioactivity remaining in solution or a spectrophotometric method based on chromogen formation with H_2SO_4 at 325 nm, Roddick and Melchers (*117*) analyzed the steroidal glycoalkaloid content of parents and somatic hybrids of *S. tuberosum* and *L. esculentum* var. *cerasiformae*. The total glycoalkaloid content of the hybrid tubers was found to be 5−15-fold greater than in the parents, whereas the foliar values were about the same. In addition, the α-tomatine content of leaf tissue of the hybrids ranged from 1.1 to 3.3% of total glycoalkaloid content, whereas the corresponding range for hybrid tubers was 61−71%. Thus, the leaf glycoalkaloids of the hybrids were largely of the potato type and those of the tubers of the tomato type. The results imply that the observed patterns are due to different biosynthetic potentials of the two plant parts.

To demonstrate the transfer of useful traits from wild species, Mattheij et al. (*118*) carried out somatic fusions between the cultivated potato *S. tuberosum* and the wild species *S. circaefolium*. Three of four hybrids were resistant to *Phytophthora infestans*, and all four hybrids were resistant to *Globdera pallida* pathotypes. With respect to glycoalkaloids, the hybrids contained solanidine, demissidine, solasodine, tomatidenol, and tomatidine glycosides, the total values of which ranged from 253 to 405 mg/kg. The tomatine values ranged from 50 to >200 mg/kg. The total value did not differ from the amounts present in the parent cultivars, suggesting that glycoalkaloids were inherited during hybridization.

Leaf and tuber glycoalkaloid patterns in somatic hybrids between *S. tuberosum* and *S. brevidens* were studied by Laurila et al. (*69*). The parental glycoalkaloid α-solanine in *S. tuberosum* and α-tomatine in *S. brevidens* were both present in the somatic hybrids. The hybrids also had the new glycoalkaloid, demissine. In somatic hybrids with a known genome composition, the proportion of α-tomatine correlated with the genome doses of *S. brevidens*. Evidently, transfer of glycoalkaloids from parents to somatic hybrid may be accompanied by the creation of new glycoalkaloids (*119*).

We also determined glycoalkaloid profiles and other characteristics of potato tubers of somatic hybrids the progenies of which were the cultivated potato *S. tuberosum*, containing α-chaconine and α-solanine, and the wild types *S. acuale*, containing α-tomatine and demissine (*120*). TLC, HPLC, and GC-MS studies revealed that all somatic hybrids, except one clone, contained all four glycoalkaloids derived from the fusion parents (**Figure 5**). The total glycoalkaloid levels of most hybrids were intermediate between those of their parents. The data show that glycoalkaloids, including α-tomatine, can be passed to progenies during breeding programs designed to develop improved potatoes as well as tomatoes (*121*).

Resistance to gray mold (*Botrytis cinera*) was successfully transmitted from the wild nightshade *Solanum lycopersicoides* Dun. into the cultivated tomato, *L. esculentum* (*122*, *123*). Such efforts should lead to further improvement in the quality of tomatoes. Changes in tomatine content were not reported in these studies. Backcrossing of the somatic hybrids with cultivated cultivars should reduce the glycoalkaloid content to safe levels. It should also make it possible to ascertain whether different types of glycoalkaloids act synergistically in the protection against phytopathogens.

METABOLISM OF TOMATINE DURING RIPENING OF TOMATOES

Effect of Fruit Maturation. As the tomato ripens and changes color from green to red, it produces enzymes, which metabolize the glycoalkaloids. The following describes some of the efforts designed to define the nature of the degradation products and the dynamics of these events (*73*).

The tomatine content of tomatoes decreases during maturation (*124*) as a result of enzymatic conversion to 3β-hydroxy-5d-pregn-16-en-20-one and possibly into 23-hydroxytomatidine (*125*). The enzymes responsible for this transformation are activated or synthesized during fruit maturation (*126*). Such enzyme activation probably does not occur in mature green fruits of wild lines with a high tomatine content. Artificially ripened fruit may have higher tomatine contents than those maturing on the plant.

Incubation of [^{14}C]tomatine into whole ripe tomatoes resulted in its rapid transformation to 3β-hydroxy-5α-pregne-16-en-20-one (allopregnenolone; **Figure 4**) (*6*). This study suggested that (a) cholesterol is a precursor of tomatine synthesis, (b) tomatoes contain tomatine-catabolizing enzymes; and (c) the enzymes are activated or synthesized during the maturation of the tomatoes because high-tomatine green tomatoes do not degrade tomatine.

In a series of studies Roddick and colleagues attempted to define the distribution in plant tissues and the dynamics of synthesis and degradation of tomatine during ripening. On the basis of the observation that tomatine was found in the supernatant of suspensions of pericarp tissue of green tomato fruit and in the expressed sap from intact tissues, Roddick (*127*) concluded that the glycoalkaloid is synthesized in microsomal organelles and accumulates in vacuoles and/or soluble phase of the cytoplasm. The tomatine content of sap was 0.4 mM. Using the radioligand assay, Eltayeb and Roddick (*128*) studied the pattern of tomatine accumulation and disappearance in several cultivars and mutants of tomato showing different fruit pigmentation and ripening characteristics. Normal ripening of red-, orange-, and yellow-fruited cultivars showed patterns of fruit growth and tomatine formation similar to those of nonripening mutants. The amount of tomatine per fruit showed a biphasic pattern of formation and decline. The relative contribution of growth and ripening to the decline in tomatine levels appears to vary with the cultivars.

Labeling studies on the incorporation of [2-^{14}C]mevalonic acid lactone into tomatine showed that (a) young fruits have the greatest tomatine-synthesizing ability, which decreases as the fruit matures; (b) tomatine is not transported into fruit from vegetative organs, that is, it is independently synthesized in the fruit; (c) excised fruits degraded tomatine at rates that were related to fruit age; (d) green fruit did not contain the aglycon tomatidine but did contain tomatine degradation products, Δ16-5α-pregnenolone-like compounds; (e) tomatine degradation products are channeled into carotenoids and chlorophyll; and (f) fruit ripening and tomatine degradation were accelerated by the treatment of different tomato cultivars with an insecticide (*128*, *129*).

These observations suggest that tomatine's disappearance from tomato fruits is governed by the physiological rather than chronological age of the fruits and that tomatine may have a dual role in the plant, that is, protection against phytopathogens and synthesis of pigments (carotene, lycopene, and chlorophyll), which can act as attractants for seed dispersal. Fortuitously, this may benefit human health.

Using a gravimetric procedure based on the formation of the tomatine−cholesterol complex, Courtney and Lambeth (*130*) compared the total glycoalkaloid content of in mature green fruit of seven lines of wild *Lycopersicon* species. The mean values ranged from about 7 to 114 mg/100 g of fresh weight. Significant differences were present among all lines except *L. esculentum* and among clusters within six lines. The second fruiting of clusters of six lines contained more glycoalkaloid than the first cluster. This study suggests that the high-glycoalkaloid *L. chmielewski* selection may be of value in studies of inheritance of tomato glycoalkaloids because it is compatible with *L. esculentum*.

Using a procedure based on acid hydrolysis of tomatine to tomatidine, followed by analysis the trimethylsilyl tomatidine derivative by GLC with anthracene as an internal standard, Juvik and Stevens (*72*) found that the foliar tomatine content (in milligrams per gram of dry weight) of several accessions of the species *Lycopersicon* ranged from about 2 to 23. For *L. esculentum*, the average value was ~4 mg/g and for *L. esculentum* var. *cerasiforme* and *L. pimpinellifolium*, 13 mg/g. A related study, based on an evaluation of progenies from crosses between tomato accessions with a high and low leaf tomatine contents, suggests that inheritance of tomatine from different accessions of *Lycopersicon* spp. is controlled by the segregation of two codominant alleles at a single locus (*71*). Although it remains to be shown to what extent the two alleles code for enzymes involved in tomatine synthesis or degradation, the results suggest that it should be possible to produce resistant tomato plants with increased tomatine content.

Leaves of the cold-tolerant and disease-resistant *Solanum lycopersicoides* Dunn. indigenous to Peru and known as "tomatillo", produced α-tomatine as the sole glycoalkaloid. The amount ranged from 3.2 to 3.5% of dry weight (*131*). The foliar content of *S. lycopersicoides* is much higher than that found in leaves of commercial tomato plants. This species could also be used in tomato breeding programs designed to enhance the tomatine content of the progenies.

Using an HPLC procedure, Voldrich et al. (*65*) determined the tomatine content in green tomatoes during postharvest ripening, processing, and storage. Green tomatoes contained 50−80 mg of α-tomatine/kg. Tomato salads and purees prepared from green tomatoes contained 10−30 mg/kg. Postharvest ripening of the green tomatoes and storage of the tomato products for several weeks resulted in decreases in the tomatine level, which occurred at a faster rate than changes in color.

High-Tomatine Red Tomatoes. A variant of *L. esculentum* var. *cerasiforme* indigenous to Peru produces tomato fruit with a very high α-tomatine content, in the range of 500−5000 mg/kg of dry weight (*132*). The high tomatine level correlated with the bitter flavor among 88 accessions of this variety. Peruvians seem to enjoy eating the bitter high-tomatine tomatoes. The high-tomatine tomatoes are consumed without apparent acute toxic effects; therefore, tomatine appears to be much safer for humans than are potato glycoalkaloids. This conclusion is

reinforced by the widespread consumption of high-tomatine "pickled green" and "fried-green tomatoes" without apparent ill effects.

The genetic determinant of high tomatine is recessive and monogenic and may be the result of a random mutation, possibly in response to stress due to low oxygen levels in the mountains of Peru. Because tomato plants produce tomatine-degrading enzyme(s) during ripening, the mutant producing high-tomatine tomatoes has a defect in its DNA, which prevents encoding the synthesis of tomatine-degrading enzymes during maturation. The authors suggest that the high-tomatine variety probably originated from a chance mutation of the normal allele coding for a tomatine-degrading enzyme. The high-tomatine plant offers a useful model to study tomatine catabolism.

Tomatine Content of Transgenic Tomatoes. Plant-engineering efforts designed to introduce desirable traits into tomatoes, such as tolerance to stress and pesticides and resistance to phytopathogens by gene manipulation (suppression, amplification, and introduction of new genes), raise questions about the tomatine content of the transgenic tomatoes. A priori, it cannot be predicted whether the genes encoding the formation of enzymes involved in tomatine synthesis are metabolically linked to the manipulated genes. We analyzed α-tomatine and dehydrotomatine in a variety of field-grown tomatoes supplied by several companies, including transgenic, slow-ripening tomatoes at different stages of maturity (**Table 8**). The tomatine level of the transgenic tomatoes was not different from that seen in the standard varieties grown under the same field conditions. Similar results were observed by other investigators (*98*, *133*−*135*). Plant genes encoding enzyme that govern tomatine synthesis appear not to be linked to the manipulated genes.

Tomatine—Carotene—Lycopene—Chlorophyll Biosynthetic Relationships. Studies on the relationship among fruit development, tomatine content, and tomatine chemistry (*124*, *126*, *136*, *137*) have indicated that chlorophyll and tomatine are degraded during fruit ripening, that the degradation of the tomatidine part of the molecule precedes that of the carbohydrate side chain, and that the degradation products appear to be preferentially incorporated into β-carotene and lycopene via biosynthesis.

Because tomatine is both synthesized and degraded as tomatoes mature, we investigated the tomatine content at different stages of maturity of widely consumed Japanese tomato varieties (*138*). As part of this effort, concurrent changes in content of three other tomato components, β-carotene, lycopene, and chlorophyll, were also measured. During the five maturity stages, artificially defined as starting 10 days after flowering (stage 1) followed by four more stages each 10 days apart, the average weight of the tomato increased 80-fold, from 1.7 to 136 g. The corresponding increase in the size of the tomatoes was from 15 to 66.3 mm along the major axis. Tomatoes harvested during the first stage contained 83 mg/kg of chlorophyll of fresh weight. The chlorophyll content then decreased by ∼25% during stage 2 and by ∼75% during stages 3 and 4. It then dropped precipitously to near zero during the final stage (50 days after flowering). The ratio of chlorophylls *a* to *b* remains at ∼2.5 during the first 30 days after harvest and then decreases to 1.88 on day 40 and to 0.66 on day 50. Not surprisingly, chlorophyll is evidently degraded during tomato ripening.

An inverse relationship between fruit weight and tomatine content of green tomatoes was noted. This decrease in tomatine does not appear to be influenced by tomato variety or location of the tomato bundles (trusses) on the vine. Tomatoes harvested during the first four stages of ripening contained undetectable quantities of β-carotene and lycopene. However, those harvested 50 days after flowering contained 12 mg/kg of fresh pericarp weight of β-carotene and 58 mg/kg of fresh pericarp weight of lycopene. These findings show that ripe red tomatoes contain high levels of lycopene. However, these levels for Momotaro tomatoes appear to be 3−4 times higher than those for tomatoes consumed in the United States.

These results show that chlorophyll and tomatine concentrations decrease rapidly during the growth of the tomatoes. Immature tomatoes contain no or low amounts of β-carotene and lycopene. There appears to be a sharp transition in the formation of the two compounds during maturation of tomatoes. The location of the tomato clusters and tomato variety did not significantly affect these results. It would benefit human health to (a) consume both green and red tomatoes and (b) create, through plant breeding and/or plant molecular biology techniques, improved tomatoes that contain the health-promoting ingredients β-carotene, chlorophyll, lycopene, and tomatine.

ANTIBIOTIC ACTIVITIES

Tomatoes are hosts for many pests that damage all parts of the tomato plant including pathogens, weeds, nematodes, and insects. Insects also vector tomato-damaging viruses. Such pests can reduce yield and quality (holes and spots on tomato surfaces) of the fruit (*139*). The fundamental molecular−cellular mechanisms by which glycoalkaloids resist pathogens—binding to cholesterol, disruption of cell membranes, inhibition of cholinesterases—may also be involved in the biological effects of glycoalkaloids in fungi, bacteria, protozoa, insects, worms, animals, and humans. To develop a better understanding of the role of tomatine in the plant and in nonvertebrate and vertebrate organisms, I shall first outline reported antibiotic activities of tomatine against a variety of plant pathogens.

Factors That Influence Antibiotic Activities of Tomatine. *pH Effects.* The p*K* value of the N-atom ($-NH + H^+ \rightleftarrows -NH_2^+$) of the steroidal part of α-tomatine is near 6.0 and that of tomatidine, near 6.4 (*140*). The N-atom therefore exists in the protonated (NH_2^+) form below pH 4 and in the unprotonated (NH) form above pH ∼7.5. Both forms are present at intermediate pH values. Because α-tomatine is inactive against fungi at pH ∼4 and is active at pH 8, only the ionized form appears to be the active fungitoxic species. A possible reason is that only this form binds to free cholesterol in vitro and to phytosterols present in membranes of fungi (*47*, *141*, *142*).

These considerations induced Schlosser (*143*) to test the hypothesis that the pH at the surface of tomatoes affects the antifungal activity of α-tomatine. The data show that tomatine in tomatine-rich green tomatoes did not inhibit the growth of the fungal species *Botrytis cinerea*, *Gleosporim fructigenum*, and *Monilia fructigena*. This unexpected result can be explained by the observation that the pH on the cell surfaces of the tomato pulp was found to be between 4.0 and 4.5. Evidently, pH can act as fungicidal barrier where fungal inhibition can occur at the inoculation site only when the pH is above ∼6.

Two possible reasons for the apparent inverse relationship between tomatine concentration and resistance are (a) the fungus lowers the pH on the surface of the leaves, reducing tomatine's effectiveness against cell membranes, and (b) α-tomatine induces fungal tomatinases, which transform the former to less active molecules, as discussed below.

Effect of Light. Leaves of tomato plants grown in sunlight were tougher, had higher concentrations of chlorogenic acid, rutin, and tomatine, and had less protein than leaves from plants

grown in the shade (*144*−*146*). Caterpillars (*Manducca sexa*) fed leaf diets from plants grown in sunlight consumed more but grew less than those consuming leaves from plants grown in the dark. From the viewpoint of the tomato plant, light appears to be a stress factor, which induces the synthesis of antifeeding compounds with resultant consequences for the behavior and growth of insect herbivores. The effect of UV light is mentioned below under Fungi.

Anticytokinin Activity. Tomatine inhibited fungal cytokinins (*147*). Whether such inhibition is operative in the phytopathogenicity of fungi is not known. Although tomatine inhibited hormonal activity in fungi, it did not bind to estrogen receptors of human breast cancer or invertebrate cells (*114*, *148*).

Tomatine−*Phytosterol Relationships*. Another factor, which affects the toxicity of tomatine to fungi and other pathogens, may be due to the presence of sterols in cell membranes. Glycoalkaloids complex to sterols such as ergosterol, which makes up ~80% of fungal membrane sterols. Such binding results in disruption of cell membranes followed by leakage of cell components and cell death (*115*, *149*−*152*).

To better assess the role of tomatine in host-plant resistance, Bloem et al. (*153*) compared the relative toxicity of tomatine to two herbivorous pests of tomatoes, *Heliotis zea* (the tomato fruitworm) and *Spodoptera exigua*. They also looked into the potential to alleviate tomatine toxicity by cholesterol in the wasp, using the model of Campbell and Duffey (*141*). They found that (a) tomatine toxicity to the larvae of *H. zea* was completely alleviated by the addition of equimolar concentrations of cholesterol to the diet and (b) the effect of cholesterol was less pronounced with *S. exigua*. These results and additional observations on the slopes of the dose−response growth curves suggest that there may be more than one mechanism of action by which tomatine acts against pests. They also suggest that the influence of tomato glycoalkaloids on plant−herbivore interrelationships is that, just like pathogens, they may be more active on one species than another. This in turn leaves opens the question of whether it is desirable to increase the tomatine content of plants to enhance disease resistance. The presence of sterols in the foliage and other parts of plants may counteract the adverse effects of tomatine to insects such as *S. exigua* and *H. zea*. To optimize disease resistance, breeding programs need to monitor both tomatine and sterol levels. To assess the potential value of tomatine in protecting plants against phytopathogens, plant cultivars should be developed with low phytosterol to α-tomatine ratios.

Tomatine and other stress factors acting on plasma membranes induce transcription of genes of the yeast *Saccharomyces cerevisiae* (*154*). The mechanism of the tomatine-induced transcription and the transduction pathway transmitting the signal at the plasma membrane to stress activators are not known.

Role of Other Antifeeding Compounds. In addition to tomatine, non-trichome-based antimicrobial defenses of the tomato plant against phytopathogens include a variety of other so-called antifeeding or deterrent compounds such as chlorogenic acid, rutin, and rishitin (**Figure 1**) (*155*−*158*). Tomato plants also accumulate chitinases and glucanases, proteinase inhibitors, sugar esters, 2-tridecanone, etc. Whether and how they impact antifeeding effects of tomatine is largely unknown.

Behavior of Insect Predators. Are insect predators deterred from eating allelochemical-fed prey? To answer this question, Traugott and Stamp (*159*) studied the feeding behaviors of the stinkbug predator (*Podisus maculiventris*) reared on control and chlorogenic acid- and tomatine-fed prey caterpillars (*Manduca sexta*). The results suggest that although the allelochemical-fed predators were easier to locate, they often deterred predation. A possible rationale for this behavior is that because the growth rate of predators consuming toxic prey will suffer, they adapt by consuming less. The results imply that feeding specialization by insect herbivores on plants containing toxic compounds could confer a measure of defense against generalist insect predators.

Glycolysis of α-Tomatine by Fungal Tomatinases. In healthy tomato plants the tomatine content is highest in the leaves, which suggests that the glycoalkaloid may inhibit tomato leaf spot caused by *Septoria lycopersici* (*160*). This leaf pathogen can detoxify tomatine by removing one glucose residue, forming the much less toxic β_2-tomatine. The enzyme did not remove the glucose from β_1-tomatine. This observation prompted several investigators to try to characterize the fungal tomatinase enzymes responsible for the detoxification of α-tomatine by removal of one or more sugar units from the glycoalkaloid. This is a very active area of research, and some of the interpretations are subject to change. **Figure 6** illustrates the large variation in the susceptibilities of different races of fungi to growth inhibition by tomatine (*161*).

The presence of high levels of tomatine in the outer layers of the tomato fruit inhibits the growth of *B. cinerea* in these fruits (*162*). An enzyme in this fungus hydrolyzed tomatine to the nontoxic tomatidine and the tetrasaccharide lycotetraose, which was split off as one unit. Renewed growth of the fungus does not occur in ripe fruit with a low tomatine content, presumably because of the increase in the amount of fungitoxic ferulic acid in the fruit during ripening. *Fusarium oxysporum* f. sp. *lycopersici* grown on tomato leaves produces a tomatine-induced extracellular enzyme, which cleaves tomatine to the non-fungitoxic tomatidine and the tetrasaccharide lycotetraose (*163*). This enzyme probably carries out the cleavage by a mechanism analogous to that produced by *B. cinerea*.

Tomatinase enzymes produced by *S. lycopersici* share physicochemical and immunochemical cross-reactivity but not substrate specificity properties with an enzyme isolated from fungal pathogens of oat roots (*164*, *165*). DNA probes for the two enzymes could be used to clone genes encoding enzymes required for saponin detoxification (*166*). Using a variety of analytical techniques (TLC and GC-MS), Osbourn et al. (*167a*) showed that the tomatinase present in *B. cinerea* does not remove the complete β-lycotetraose side chain but only the terminal xylose, forming β_1-tomatine as the final product. The β_1-tomatine was less toxic to fungi than is the parent compound.

A tomatinase from *S. lycopersici* cleaved the β-1,2-D-glucosyl bond of the lycotetraose side chains of α-tomatine to β_2-tomatine (*168*). The enzyme was substrate-inducible, because incubation of the fungus with α-tomatine resulted in accumulation of β_2-tomatine mRNA. Sandrock and VanEtten (*161*) then examined the susceptibility of 23 fungal strains to degrade α-tomatine. All tomato pathogens except *Phytophthora infestans* and *Pythium aphanidermatum* degraded α-tomatine (**Figure 6**). Pathogenicity of the fungi correlated with tolerance to α-tomatine and ability to degrade the glycoalkaloid. Although β_2-tomatine and tomatidine were generally less toxic to the fungi, they did inhibit some fungi. A surprising observation was that α-tomatine inhibited *Ph. infestans* and *Py. aphanidermatum*, which lacked sterols in their membranes, suggesting that tomatine can act by a mechanism other than binding to sterols.

Parallel studies report on the isolation and characterization of a tomatinase for *F. oxysporum* f. sp. *lycopersici*, which cleaved α-tomatine to tomatidine and the lycotetraose moiety (*169*−*172*). They showed that (a) tomatinase activity was

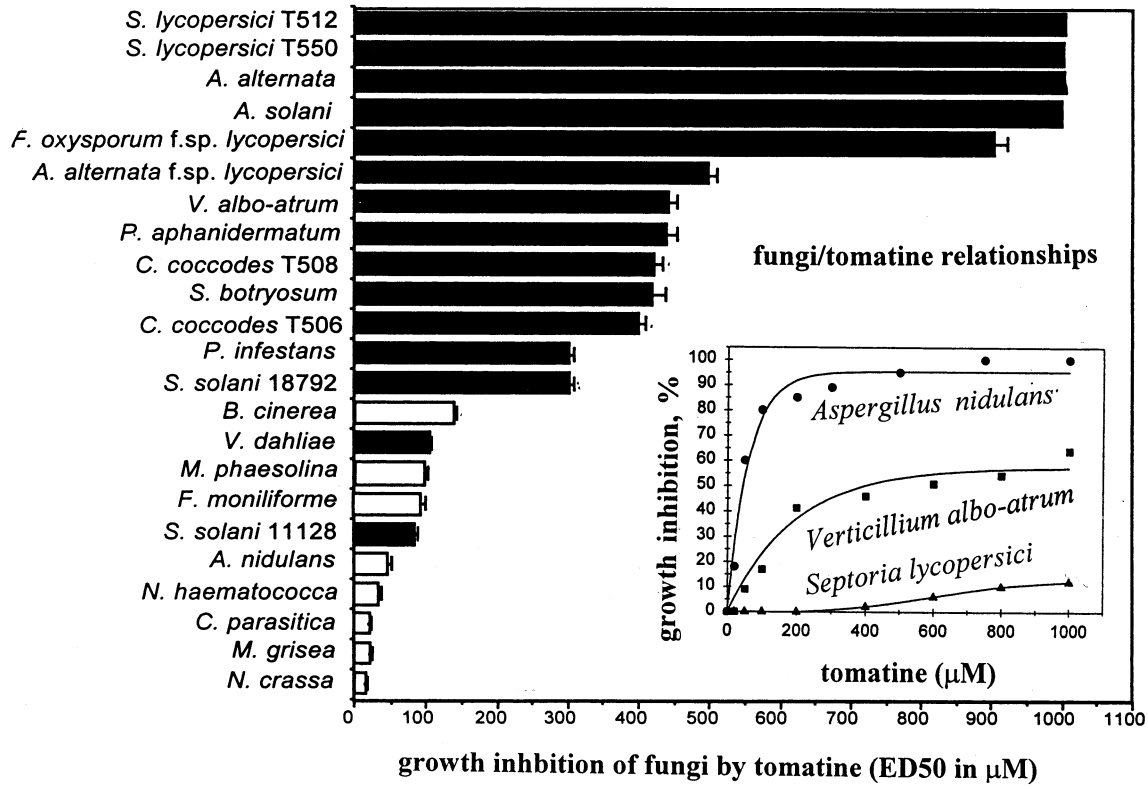

Figure 6. Growth inhibition of fungi by tomatine (adapted from ref *161*).

optimum at 45−50 °C and pH 5.5−7; (b) nonpathogenic fungi also had inducible tomatinase, which had the same molecular weight and acted mechanistically similar to the *Fusarium* enzyme; (c) the tomatinases from both sources were recognized by antibodies raised against the tomatinase from the *Fusarium* enzyme; (d) polyclonal anti-tomatinase antisera for *F. oxysporum* did not recognize a tomatinase induced by α-tomatine in *Fusarium solani*; and (e) *S. solani* isolates were less resistant to α-tomatine and had a lower tomatinase activity than did *F. oxysporum*. These observations suggest that *F. oxysporum* expresses tomatinase in vivo as a result of infection of the tomato plant and that the mechanism of resistance by *S. solani* differs from that by *F. oxysporum*.

The ability of fungi to degrade α-tomatine may be a pathogenicity factor of fungal tomato pathogens; that is, they evolved as a resistance mechanism against the toxicity of α-tomatine and other saponins. However, it is not clear why different classes of fungi have adapted to the toxicity of α-tomatine by producing enzymes that can remove either the β-1,3-linked xylose to form β_1-tomatine, the β-D-glucose to form β_2-tomatine, or the lycotetraose side chain to form tomatidine. Relative potencies of all possible α-tomatine and dehydrotomatine hydrolysis products against fungi merit study.

Induction of Panthotenate Synthetase. α-Tomatine also induces panthotenate synthetase in the fungus *F. oxysporum* f. sp. *lycopersici* (*173*). Southern blot analysis of the genomic DNA revealed the presence of a single copy of the panthotenate synthase gene in the fungus. If the induction of the gene turns out to be a response by the fungus to cellular stress induced by α-tomatine, this may be an additional biochemical mechanism by which phytopathogens can overcome resistance.

Induction of Steroid Hydroxylase Activity. The ascomycete *Gibberella pulicaris*, which can cause root and seedling rot in cereals and storage rot of fruits and vegetables, can metabolize tomatine within 2 h by first removing the lycotetraose side chain, forming tomatidine (*174*). The aglycon is then further transformed into 7α-hydroxytomatidine and 7α-hydroxytomatidenol. The hydroxylation may be carried out by a membrane-bound cytochrome P-450 oxygenase. The enzyme α-chaconinase was also isolated from *G. pulicaris*. It removed 1,2-bound L-rhamnose from the potato glycoalkaloid α-chaconine but was inactive against the potato glycoalkaloid α-solanine and against α-tomatine (*175*). It is also noteworthy that tomatidine can induce 11-α-hydroxylase activity in the fungus *Rhizopus nigricans* (*176*).

Does induction of steroid hydroxylase P450 enzymes of fungi by plant metabolites constitute a response to the toxic compounds? This seems to be the case, because α-tomatine and several other compounds induced the hydroxylation of progesterone by the fungus *Cochliobolus lunatus* (*177*). Fungi appear to possess a number of biochemical defenses against secondary metabolites.

Interaction of Tomatine with Specific Plant Pathogens ex Planta and in Planta. With the above description of parameters that affect the interaction of tomatine with phytopathogens as background, we will now examine specific plant pathogens discussed in alphabetical order.

Fungi. In what, in retrospect, has turned out be a classic study, Arneson and Durbin (*160*) determined the minimum concentration of α-tomatine that completely inhibited mycelial growth of 30 fungal species, 14 of which were tomato pathogens. The minimum concentration to kill 100% of the fungi (MC100) values ranged from 0.00013 for *Helminthisporium turcicum* to 0.85 for *S. lycopersici.* The tomato pathogens were less susceptible to α-tomatine than the nonpathogens. The MC100 values for the pathogens ranged from 0.0025 to 0.85. They also noted that, assuming a uniform distribution of α-tomatine in ground cytoplasm and vacuoles, the compound is present in tomato leaves at fungitoxic levels (0.003 M). Its concentration would be much higher and may approach that needed to inhibit

many other fungi if it is localized at specific intracellular sites. These considerations prompted the authors to suggest that tomatine should be evaluated as a possible resistance factor to different races of fungi. Subsequent studies outlined below indicate that this suggestion has been widely adopted.

(a) Beauveria brassiana. To find out whether tomatine and solanine are sufficiently toxic to a fungus such as *B. brassiana* to impede infection of tomato by an insect, Costa and Gaugler (*178*) evaluated the effects of these glycoalkaloids and the antibiotic nystatin on germination of the conidia and hyphal growth of this fungus. Nystatin was most inhibitory, followed by tomatine and then solanine. Colony formation and growth were inhibited by 100 mg/L of tomatine in unbuffered media. The sensitivity of *B. brassiana* to the test compounds appears to be in the middle range reported for other fungi. The data suggest that the toxic effect of tomatine on the fungus could retard insect infection.

(b) Botrytis cinerea. The necrotrophic fungus *B. cinerea* causes moldy diseases in fruits, vegetables, and flowers. Glazener and Wouters (*179*) studied the consequences of infection of tomato fruits with *B. cinerea*. The data suggest that tomatine may be partly responsible for protecting the fruit against the fungus. The fungus did not induce the formation of rishitin. Studies by Urbasch (*180*) showed that *B. cinerea* Pers. produces enzymes which can transform α-tomatine to two products: (a) the aglycon tomatidine and (b) a less polar unknown compound, which still contains the four sugar units.

(c) Cladosporium fulvum. Tomatine released from leaf cells following induction by the fungus appears to be involved in resistance of tomato cultivars to the tomato leaf mold pathogen *C. fulvum* (*181*). Tomatine was both fungistatic and fungicidal and caused an irreversible leakage of electrolytes from the hyphae, which resulted in inhibition of hyphal elongation of the fungi. The degree of resistance depended on the pH and nutrient status of the assay medium. The results imply that different assay techniques can give rise to differences in measured antifungal activity of tomatine.

(d) Fusarium caereleum. Factors that affect toxicities of glycoalkaloids to spores of *F. caereleum* were examined by McKee (*182*). Tomatine was more toxic than chaconine, demissine, or solanine. Solanidine was $^1/_5$ to $^1/_{10}$ times as active as the glycoalkaloids. Toxicity increased with increasing concentrations of sodium and potassium ions, decreased in the presence of calcium ions, was dependent on the sodium ion/calcium ion ratio, and correlated with the hemolytic activities of the test compounds. Toxicity increased with pH, suggesting that it is the nonprotonated forms of the compounds which disrupt cell membranes by penetrating the cholesterol monolayers of the membranes.

(e) Fusarium oxysporum f. sp. lycopersici. Infection of stems of both low- and high-resistant tomato plants with *F. oxypsorum* f. sp. *lysopersici* responsible for vascular wilt resulted in an increase in tomatine content (*183*). To obtain direct evidence for the involvement of tomatine in resistance of the tomato plant to *F. oxysporum* f. sp. *lycopersici*, Smith and MacHardy (*184*) studied its effect on both susceptible and resistant tomato lines. Tomatine did not appear to be the primary determinant of resistance, because the concentration of tomatine increased rapidly by the same amount 2 days after wounding or inoculation by both races. Tomatine may contribute to resistance by inhibiting spore production rather than spore germination or hyphal growth. Tomatine may act in a sequential two-component resistance process: vascular occlusion may restrict the upward distribution of the pathogen, thus impeding water movement and resulting in localized accumulation, which then prevents mycelial growth. The inhibition of germination of *F. solani* spores by extracts of dried tomato tops depended only on their tomatine content (*185*).

To find out whether tolerance of tomatine and rishitin correlates with virulence or pathogenicity of fungi, Suleman et al. (*186*) examined the effect of 17 *F. oxysporum* isolates on stems of the tomato plant. One group of four pathogenic isolates colonized tomato stems more aggressively than other isolates tested. Differences in virulence among pathogenic isolates correlated most strongly to tolerance of rishitin and more narrowly to that of tomatine. Because the formation of rishitin is induced and that of tomatine is not, these differences may be related to differences in timing of the availability of the two fungitoxic compounds.

(f) Fusarium solani. To overcome difficulties in correlating the sensitivity of a fungus to tomatine with its pathogenicity, Defago and Kern (*187*) infected green and red tomato fruits with five *F. solani* mutants that can grow in the presence of tomatine and wild strains that cannot. The mutants produced severe rot in the green tomato, whereas the wild strains did not. In contrast, both the mutants and wild types had the same effect on red tomatoes. The results imply that tomatine is liberated in the early stages of infection and inhibits further growth of the wild-type strains. A possible explanation for the insensitivity to tomatine of the mutants appears to be associated with their low sterol (ergosterol) content, which presumably prevents disruption of cell membranes. The reasons that mutants insensitive to tomatine are more pathogenic than the wild-type strains only for organs containing tomatine implies that tomatine may be the main resistance factor against *F. solani*.

(g) Paecilomyces fumosoreus. Because allelochemicals inhibit a wide variety of fungal plant pathogens, Lacey and Mercadier (*188*) investigated the effect of five such compounds, including tomatine, on the germination of conidia and plastophores and a mycelial growth of the fungus *P. fumosoreus*, a natural enemy of the whitefly (*Bemisia argentifolii*). The LC50% value for the inhibition germination by tomatine was 51.6 mg/L. This information should be useful in integrated pest control programs that concomitantly employ resistant plant species and fungi.

(h) Phytophthora infestans. Expectations are that α-tomatine should have no effect on *P. infestans*, a parasitic fungus that infects potatoes (*151*), because its cell membranes contain no sterols. However, α-tomatine stimulates the growth of the fungus but inhibits the germination of zoospores and the induction of rishitin in potato tubers (*189*). Reduced synthesis of rishitin in the presence of α-tomatine appears to be associated with the effect of the latter on spore germination.

(i) Phytophthora megasperma. To test the hypothesis that the susceptibility or resistance of fungi to α-tomatine may be due to differences in sterol content of the membranes, Steel and Drysdale (*190*) examined the effect of tomatine on two cultures of *P. megasperma*, which differed only in membrane sterol content. The one with a higher content was more susceptible to the antibiotic effect than the one with a lower content, suggesting that relative activities of tomatine in fungi are a function of the sterol content of their cell membranes.

(j) Rhizopus stolonifer. Treatment of tomato fruit with UV light retarded induction of rotting and loss of nutrients induced by the fungus *R. stolonifer* (*146*). UV light slowed the degradation of tomatine, because the tomatine content of tomatoes exposed to UV light was higher as compared to

untreated fruit. The resistance of the treated tomatoes artificially inoculated with the fungus correlated well with high tomatine content.

(k) Verticillium albo-atrum. An inducible extracellular β1,2-glucosidase (tomatinase) produced by the pathogenic fungus *V. albo-atrum* hydrolyzed α-tomatine to the weakly fungitoxic β_2-tomatine by removal of glucose (*191*). The enzyme was inactive with α-chaconine and α-solanine as substrate. Because colorimetric analytical methods for tomatine may not always be accurate, due to the errors associated with contaminating substances and in view of conflicting conclusions by previous investigators about the role of tomatine resistance to fungi that may be due to such errors, the authors measured tomatine by the sensitive analytical method based on complex formation with [^{14}C]cholesterol. They found the following concentrations (in micrograms per gram of fresh weight) of tomatine in the healthy susceptible *Craigella* cultivars: roots, 506; stems, 286; leaves, 1070. The corresponding values for an isogenic resistant line were 310, 170, and 591 μg/g. Because the concentration of tomatine in the resistant line was lower than in the susceptible one and on the basis of studies of resistance of the two lines to infection, indications are that α-tomatine was not involved in major gene resistance of tomato to *V. album-atrum*.

Viruses. Exposure of the tomato plant to the cucumber mosaic virus seems to affect tomatine synthesis (*192*). Tomatine also inhibited the growth of the tobacco mosaic virus in infected tomato plants. The in vitro inhibition of the virus occurred at a tomatine concentration of 0.005% (*193*). The compound was also active against the human herpes simplex virus (*194*) and reverse transcriptases produced by the human immunodeficiency viruses (*195*). A cream containing the glycoalkaloids solamargine and solasonine was highly effective against the herpes simplex virus in humans (*196*). It would be worthwhile to find out whether α-tomatine and dehydrotomatine can protect food and the consumer against viral infection.

Bacteria. (a) Phytopathogens. Bacteria can also infect tomato leaves. After invading wounded plant tissues, the bacteria pass into the xylem vessels and then into other plant tissues. Beimen et al. (*155*) describe an infection-induced response in leaves of tomato plants colonized with the bacterial pathogen *Clavibacter michiganense* susp. *michiganense* of the tomato. The response consisted of a time-dependent increase in the formation of chlorogenic acid, rutin, and tomatine as well as in cell wall-bound caffeic, coumaric, and ferulic acids (*197, 198*). Infection with the highly virulent strain resulted in the synthesis of 1600 nmol of tomatine/g of fresh weight compared to 775 nmol/g for the control leaves. The increase in tomatine content was lower with two less virulent strains. The authors suggest that bacteria-derived signals such as the exoenzymes cellulase, exopolysaccharide, or polygalactouronase, the expression of which is related to strain virulence, may initiate the defensive response in the tomato leaves.

Spraying of tomato seeds with 0.005% solutions of tomatine inhibited the development of bacterial black spot, macrosporiosis, and septoriosis in the tomato plants (*199*). Other beneficial effects were increased yield of tomatoes and higher vitamin C, sugar, and tomatine contents in the fruit. It would be of interest to find out whether glycoalkaloids could neutralize the effects *Bacillus thurigiensis*, a bacteria used for insect control (*200, 201*).

(b) Human Pathogens. The infection of fruits and vegetables with bacteria and viruses pathogenic to humans is a major public health problem (*202*). Such pathogens include *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enteritidis*, bacteriophages, and the polio virus (*203*−*205*). Tomatoes inhibit the growth of *Listeria* (*206*). The question of whether tomatine can protect against human pathogens as do potato glycoalkaloids (*207*) and whether human pathogens can induce the synthesis of antibiotic compounds by the tomato plant merits study (*208, 209*).

Tomatine also inhibited the growth of *Lactobacilli* spp. The inhibition was accompanied by the formation of tomatine−lactic acid conjugates (*210*−*212*). Because lactic acid is reported to help kill pathogens on tomatoes (*213*), the dietary significance of tomatine−lactic acid conjugates as well as the effectiveness of natural antimicrobial compounds (*214, 215*) against human pathogens on tomato surfaces and in tomato juice merit study.

Protozoa. (a) Flagellate Protozoan (Trypansoma cruzi). Infection of humans by the parasite *T. cruzi* causes chagas disease, a chronic illness widespread in Latin America. The glycoalkaloids α-chaconine, solamargine, and α-tomatine were found to inhibit the growth of the protozoa at micromolar concentrations (*216*). The antitrypanosomal activity may be associated with the abilities of the glycoalkaloids to disrupt cell membranes and inhibit cholesterol biosynthesis as a result of the interaction of the rhamnose moiety of the carbohydrate side chains with carbohydrate groups of lectins on the surface of the cell membranes and insertion of the steroid part of the molecule into the membrane.

(b) Ciliated Protozoan (Tetrahyhmena pyriformis). Biochemical pathways in the ciliated protozoan *T. pyriforamis* are similar to those of mammalian cells. α-Tomatine at 16.5 ppm inhibited cell growth of this protozoa by 50% (*217*). Cell lysis occurred at 37.5 ppm. The glycoalkaloid also inhibited the synthesis of DNA and RNA, stimulated the synthesis of proteins, and had variable effects on lipid and glycogen synthesis. Toxic manifestations are initiated by insertion of tomatine into the lipid bilayers of the cell membranes. This event initiates leakage of and changes in the biosynthesis of the cell components. *T. pyriformis* appears to be a good model for studying consequences of exposing cell membranes to tomatine. Tomatine formed stable complexes with cholesterol, β-amyrin, and tetrahymenol, a pentacyclic triterpenoid alcohol that functions as a cell membrane compound similar to cholesterol. However, the evidence is inconclusive as to whether the tomatine−tetrahymenol complex operates in the membrane disruption process.

It is also significant that tomatine is lethal to snails (*Lymnaea cubensis* and *Biophlaria glabratus*) that are responsible for the spread in tropical climates of animal and human protozoan infections such as schistosomiasis (*218, 219*). The molluscicidal effect of tomatine could be useful in controlling the spread of such major parasitic diseases.

Insect and Worm Pests. (a) Acridids (Locusta migratoria and Schistocerca gregaria). To answer the question of whether deterrence and toxicity are related, Cottee et al. (*220*) compared behavioral and toxic responses of two acridids, the graminivorous *L. migratoria* and the polyphagous *S. gregaria*, to eight secondary metabolites including tomatine. There was a correlation in both species between deterrence and toxicity of compounds injected into the hemolymph but not between deterrence and oral toxicity. The results imply that there is physiological basis for the behavioral response when the gut is bypassed and that avoidance responses may have evolved under selective pressures unrelated to oral toxicity.

(b) Army Worm (Spodoptera littoralis Boisd.). Dhillon (*221*) investigated how tomatine affects the development of the army worm, an insect pest of tomatoes. The results suggest that two

wild tomato species with a high tomatine content which resist this insect, *L. esculentum* var. *cerasiforme* and *L. pimpinellifolium*, provide potential sources of resistant germplasm that can be used to introgress genes coding for amplified tomatine content. The creation of cultivars with enhanced levels of the glycoalkaloid and increased host-plant resistance to the army worm is a desirable objective. As noted below, such tomatoes may also benefit nutrition.

(c) Colorado Potato Beetle (Leptinotarsa decemlineata Say). The damaging effect of the beetles results from the defoliation of potato plants. Feeding experiments by Barbour and Kennedy (*222*) did not reveal any differences in food consumption, growth rate, or survival of potato beetles fed foliage from susceptible tomato plants (*L. esculentum)* and resistant ones (*L. hirsutum).* Differences in dietetic indices did not correlate with foliar α-tomatine content of resistant or susceptible tomato cultivars. In a related study, Harrison and Mitchell (*223*) found that solanine and tomatine found in two regional host plants did not reduce leaf consumption or significantly alter behavior patterns of newly emerged beetles.

Effects of tomatine observed ex planta are not manifested in planta in view of the fact that studies linking tomatine to feeding deterrence against the beetles used mostly artificial diets supplemented with tomatine or tomato foliage coated or infiltrated with tomatine (*145*, *224*, *225*). Expression of α-tomatine-based resistance in tomatoes may depend on the presence of other factors such as phytosteroids and proteins. It is not known whether resistance factors such as tomatine and protease inhibitors act independently or synergistically.

On the basis of elegant studies on gustatory and sensory factors that influence host recognition and feeding behavior of the Colorado potato beetle, Zhang and Mitchell (*226*, *227*) conclude that in addition to tomatine other components of tomato leaves appear to influence chemosensory responses that allow the beetle to distinguish tomato from potato leaves. Tomatine may thus act by a dual mechanism consisting of both sensory suppression and physiological action on tissues of the beetles.

Rearing Colorado potato beetles on a synthetic diet supplemented with increasing concentrations of α-tomatine resulted in retarded growth and delayed development (*224*). High levels of tomatine in the diet induced agitated and restless behavior in the beetles. However, α-tomatine did not affect their survival. The mechanism could involve inhibition of acetylcholinesterase (*228*) and formation of a biologically inactive complex with sterols resulting in disruption (permeabilization or lysis) of the cell membranes and depolarization of neurons. Several factors could account for the variable results observed by different investigators. These include variability in interactions of different compounds in the plant and in the tomatine effect on different stages of insect development and feeding behavior (*229*).

Another complicating factor in defining the role of tomatine as a resistance factor to insects in tomato plants is the observation by Hare and Dodds (*230*) that infection of tomato plants with tobacco mosaic virus lowered the resistance of the plants to the beetles. The simultaneous increase in tomatine content had no effect on the survival. The viruses appear to facilitate adaptation of phytophagous insects such as beetles to host-plant species by altering nitrogen metabolism, which results in an increase in the nitrogen content of the plant. Virus infection thus changes the tomato plant into a better nutrient for the beetles. However, nitrogen availability to the plant was not related to the susceptibility of tomato plants to the fungus *B. cinerea* because the more susceptible leaves, with a high C/N ratio, contained more α-tomatine than the less susceptible ones (*104*).

(d) Diamondback Moth (Plutella xylostella L.). Tomatine was found to be ovicidal against the diamondback moth, which attacks cruciferous vegetables such as cabbage (*231*, *232*). Hatching of eggs decreased from 90 to 20% after treatment with a 0.1% tomatine solution. The lethal concentration that kills 50% of the eggs was estimated to be 0.33% of tomatine. It appears that tomatine is highly toxic to deposited eggs of the diamondback moth.

(e) Greenhouse Whitefly (Trialeuarodes vaporariorum Wetw.). Green-mature wild tomatoes (*L. hirsutum glabratum)* with a high foliar (3.39 g/kg of fresh weight) but normal tomatine content resisted infection by the greenhouse (glasshouse) whitefly, a major pest of tomatoes (*73*). In addition to tomatine, GC-MS data show the presence of unknown glycoalkaloids in the whitefly-resistant tomato variety.

(f) Green Peach Aphid (Myzus persicae Bejing). Junde and Lidao (*233*) studied the effects of adding 0.1−0.2% of secondary metabolites including tomatine to the diets of the aphids maintained on greenhouse tobacco plants. Tomatine was quite active, inducing a 89.4% mortality within 7 days of rearing after birth of the aphids. Although the polyphagous green peach aphid is only occasionally found on tomato plants, tomatine may contribute to the aversion toward the aphid. The deterrent effect of tomatine toward this aphid contrasts with the reported nondeterrent effect toward an aphid native to The Netherlands (*234*).

(g) Mediterranean Fruit Fly (Ceratitis capitata Wiedemann). Tomato fruit is a host for the Mediterranean fruit fly. Increased concentrations of α-tomatine in the range 25−200 ppm resulted in decreased larval survival, lower pupal weights, extended pupation period, and a longer period of adult emergence (*235*). The LC50 for α-tomatine fed to the larvae was 52.7 ppm. The apparent toxicity of α-tomatine to the fruit fly larvae in vitro could explain the low infestation rates in field-grown high-tomatine green tomatoes.

(h) Potato Aphid (Microsiphum euphorbiae Thomas). The potato aphid pest transmits potato leafroll virus and alfalfa mosaic virus. Gunther et al. (*236*) examined feeding, toxicity, and reproductive effects of potato and tomato glycoalkaloids and aglycons. Both α-chaconine and α-tomatine delayed the appearance and decreased the number of nymphs, and the aglycons tomatidine and solanidine were deterrent and lethal at high concentrations. Because the aglycons were quite active in this system, the mechanism of the described effects probably does not involve binding to sterols of cell membranes.

(i) Potato Leafhopper (Empoasca fabae Harris). Studies on the responses of individual, caged fifth-star nymphs of the potato leafhopper to several allelochemicals showed that tomatine was the most effective in restricting inhibition at 0.01−0.1 M concentrations (*237*). Tomatine adversely affected nymph survival, and the rate of mortality increased with tomatine concentration. The aglycon tomatidine was inactive.

(j) Red Flour Beetles (Tribolium castaneum) and Tobacco Hornworm (Manduca sexta). Weissenberg and colleagues (*238*) used larvae of the red flour beetles and tobacco hornworm as test organisms to evaluate the effect of structurally different glycoalkaloids and aglycons on the development of larval weight, pupation, and emergence. Beetle larval growth was inhibited on artificial diets containing solamargine, solasonine, and tomatine. The corresponding aglycons, solasodine, tomatidine, and tomatidenol, were inactive. Addition of cholesterol

or sitosterol to the diet abolished the inhibitory effect of solamargine and tomatine but not that of solasonine.

The test compounds affected the growth of the tobacco hornworm larvae differently. Tomatine had a marked inhibitory effect, whereas the other compounds did not affect larval development or sterol metabolism. The authors offer a detailed assessment of the relationship of the structures of the test compounds to the mechanisms of the observed effects involving complex formation with cholesterol, disruption and rearrangement of cell membranes, leakage of cell constituents, carbohydrate−carbohydrate interactions on cell surfaces, and inhibition of essential enzymes. Such an assessment facilitates the development of edible plants containing combinations of the potent but safe antifeeding phytochemicals.

(k) Root-Knot Nematodes (Meloidogyne incognita). Root-knot nematodes are obligate endoparasites of roots of tomatoes and other plants (*239*). They decrease plant yield by damaging roots, removing plant nutrients, and retarding root growth. There appears to be a direct correlation between the quantity of α-tomatine in the roots of tomato plants and their resistance to the root knot nematode (*240*, *241*). Inoculation with nematodes resulted in a greater decrease in tomatine of roots in nematode-resistant tomato isolines than in none-resistant ones. Tomatidine was more toxic to nematodes than tomatine.

(l) Soybean Looper (Pseudoplusia includens Walker). Soybean looper is an insect pest that defoliates many plants including soybeans and tomatoes. About 85% of collected larvae were found on soybean and 0.4% on tomato plants (*242*). The presence of tomatine in the diet lowered body weight, total weight gain, and larval survival, but not pupal weight. Larval growth followed a linear regression of tomatine concentration in the range of 0.01−0.1%. The effective dose of tomatine to reduce larval weight by 50% was 0.048% weight.

(m) Spiny Bollworm (Earia insulana). The spiny bollworm is of economic importance as a cotton pest of the Middle East. Weissenberg et al. (*243*) determined the effect of 23 secondary metabolites including tomatine and tomatidine on larval growth and pupation of the spiny bollworm. The results permitted the assessment of structure−biological activity relationships against this insect. The insecticide activity of the glycoalkaloids solasonine, solamargine, and tomatine was quite high, presumably because they can combine with cholesterol, which results in disruption of cell membranes and hemolysis of the larvae. The aglycons, solasodine and tomatidine, were markedly less potent against the larvae, presumably because they lack the sugar side chain and are therefore less hydrophilic and do not interact with cholesterol.

There appears to be a correlation between the hydrophilic/lipophilic nature of all the compounds tested and biological activity. The inhibiting activity of the steroidal glycosides did not depend on substitution at C-5, C-22, and C-25 atoms of the steroid part of the molecules or on the nature and number of the carbohydrate side chains associated with the glycoalkaloids. These findings make it possible to select plants for compounds possessing structural features that favor larval growth inhibition.

(n) Stinkbug (Podisuss maculiventris). Weiser and Stamp (*244*) evaluated the combined effect of the chlorogenic acid, rutin, and tomatine fed to prey with and without supplemental plant material, on the growth of the stinkbug, a generalist insect predator. They found that (a) prey scarcity depressed development, weight gain, and growth rate; (b) the chemicals in the diet negatively affected the predators when prey was scarce but not when predators were supplied with an excess of prey; and (c) feeding supplemental plant material did not affect the negative effects of the chemicals in the prey diet.

(o) Tomato Fruitworm (Heliothis zea). Tomatine, aldaric acid, chlorogenic acid, and rutin isolated from tomato leaves inhibited larval growth of the tomato fruitworm, an important tomato pest, and the wasp *Heliothis zea* (*53*). The antibiotic activity of tomatine, measured as the ED50 value, was 0.4% compared to 2.5% for the other compounds. The glycoalkaloid was therefore ∼6 times more active than the phenolic compounds. The authors suggest that growth inhibition of fruitworms fed on the leaves is probably derived from the combined effects of tomatine and the phenolic compounds.

To develop a better understanding of the mode of action of tomatine, Duffey and colleagues (*141*, *153*) studied the effect on larval growth of *H. zea* and the wasp *Hyposoter exigua*, a biological control agent of the former. The latter was 3 times more sensitive to tomatine than *H. zea*. Addition of equimolar cholesterol to the diet completely abolished tomatine toxicity to *H. zea* but incompletely to *H. exigua*. The species also differed in their abilities to consume and utilize food in the presence of tomatine. Although tomatine may exert its effect by forming complexes with insect sterols, the differences in toxicities of tomatine in the two species suggest more than one mode of action of tomatine. Such differential effects of herbivorous species to tomatine and other compounds should be taken into account when their impact on natural enemies is assessed.

The situation may even be complicated in view of the finding that the parasite *H. exiguae* (Viereck) acquires tomatine from its host *H. zea* after the host ingests the glycoalkaloid (*245*). The ingested tomatine was found to exert toxic effects in the parasite manifested in an extended larval period from 7 to 8.5 days, a significant reduction in the percent of pupal occlusion, and lower weight gain and smaller size. Although the parasite is not an efficient biocontrol agent in tomatoes, the apparent ingestion of tomatine by the parasite from a secondary source (its host) may complicate efforts of pest management based on breeding plants with a high content of tomatine.

Gallardo et al. (*246*) examined the consequences of exposing the corn earworm *H. zea* (Boddie) that was fed an artificial diet containing tomatine to the fungus *Nomuraea rileyi* (Farlow). Tomatine retained the antifungal properties in the second trophic level by inhibiting the development of *N. rileyi* in *H. zea*.

(p) Thrips palmi Karny. The polyphagous insect *T. palmi* attacks a wide range of vegetable plants but not tomatoes. Hirano et al. (*247*) demonstrated strong antifeeding activity of tomatine for this insect. Because arthropods go through a complicated process of host-plant selection that includes visual and olfactory cues before landing on plants, the thrips may or may not contact tomatine on tomato plants. Thus, although tomatine is repellent to *T. palmi*, it may not be important in resistance.

EFFECTS OF TOMATINE ON PLANT CELLS AND TISSUES

Tomatine is reported to inhibit the growth and development of plant-derived tissues, but not in tomato fruit or leaves. These include apple fruit, carrot root, celery seeds, oat, potato tuber, red beet, and wheat (*248*−*250*), tomato pollen (*251*), and corn seeds (*252*). Comparison of the relative effectiveness of several glycoalkaloids and aglycons on the inhibition of lettuce seed radicle elongation revealed that sugar side chains of the glycoalkaloids seems paramount in governing the extent of inhibition, which averaged ∼30% for all compounds tested compared to 50% for tomatine (*253*). The mechanism of the

inhibition involves disruptions of cell membranes followed by leakage of the cell contents from the plant tissues.

Studies on the membrane-disruptive properties of α-tomatine revealed that membrane leakage of liposome vesicles depended on the amount of 3β-hydroxysterols incorporated into the phosphatidylcholine liposomes and that leakage occurred with cucumber mesocarp and leaf disks of tobacco but not from potato and tomato leaf disks (*190*). The potato results can be explained by the absence of sterols in the cell membranes of this plant. The presence of sterol glycosides and acetylated sterol glycosides in tomato cell membranes, rather than sterols with a free 3β-OH group as in ergosterol and cholesterol, may explain why tomatine does not disrupt membranes of the tomato plant.

Spraying etiolated 4-day-old seedlings of mungbean, sicklepod, sesbania, sorghum, and wheat inhibited stem elongation by 7−13% (*254*). Tomatine was more effective than tomatidine in reducing the chlorophyll content in excised etiolated tissues of several plants. Inhibition by α-tomatine ranged from 16 to 89% and that by tomatidine from 0 to 30% of control values. Additional studies revealed increased electrolyte leakage of leaf disks of corn, kudzu, palmleaf morning glory, and wild senna plants at 24−72 h after exposure to 0.5 mM concentrations of the two compounds, with tomatidine being more effective than tomatine. Both α-tomatine and tomatidine therefore appear to exhibit a broad range of phytotoxic effects in edible plants and weeds.

The mechanism of action of tomatine on plant cells could also involve interaction with the plant hormone abscissic acid, in view of the reported induction of stomatal closure in epidermal peels of *Commelina communis* by both compounds (*255*). Tomatine was found to be more potent than abscissic acid and to reverse abscissic acid-induced stomatal closure.

PHARMACOLOGY IN VERTEBRATES

α-Tomatine is a biologically active molecule, as shown by its ability to disrupt cell membranes, bind cholesterol, inhibit acetylcholinesterase, and perturb acid−base equilibria in vivo. Some of the relevant observations with enzymes, cells, tissues, and animals are outlined below.

Intraperitoneal, Oral, and Subcutaneous Effects. Sackmann et al. (*256*) determined the following LD50 values (in milligrams per kilogram of body weight) of α-tomatine in mice: intraperitoneal (ip), 25; oral, 500; subcutaneous, >1000. Similar LD50 values (ip, 33.5; oral, 500) were obtained by Nishie et al. (*257*). The 15-fold lower oral compared to the ip toxicities appears to be due to poor absorption of the compound from the gastrointestinal tract, presumably as a result of formation of an insoluble complex of α-tomatine with cholesterol, which is then eliminated in the feces (see below). In terms of LD50 values, tomatine is ∼20 times less toxic than the corresponding values observed with the potato glycoalkaloids (*258*, *259*).

In contrast to the findings of Wilson et al. (*7*) that feeding of a single dose of 2% tomatine was toxic to the weanling rat, Cayen (*260*) found that rats fed 2% tomatine did not show any signs of toxicity. Body weight gain and liver weights were also normal. The difference could be due to differences in the age or sex of the rats in the two studies and/or the fact that tomatine was administered over a 24-h period rather than as a single dose.

Inhibition of Acetylcholinesterase. Tomatine was shown to be a competitive inhibitor of acetylcholinesterase following Lineweaver−Burk kinetics (**Figure 7A**) (*140*). Inhibitory activity of tomatine against bovine and human acetylcholinesterase was lower than that observed with the potato glycoalkaloids α-chaconine and α-solanine (*261*). Tomatine also inhibited acetylcholinesterase from Colorado potato beetles (*228*). Neurological manifestations associated with glycoalkaloid consumption may be due to inhibition of acetylcholinesterase.

Permeability and Viability of Intestinal Cells. In vitro studies showed that tomatine inhibited active transport by increasing the general permeability of membranes of the surface of averted rat jejunal sacs (*262*). The increase in the permeability of the small intestinal mucosal cells inhibits active nutrient transport and facilitates uptake of gut contents to which the gut would normally be impermeable. The results imply that the loss of affected cells from the intact mucus might facilitate fecal excretion of cholesterol from this source. Tomatine-induced reduction of cholesterol by this pathway may therefore complement excretion of dietary and endogenous cholesterol as the tomatine complex.

In a related study, Gee et al. (*263*) examined the effect of tomatine and other saponins on cultured cell lines of rat and human mucosal epithelium and on isolated rat jejunum. Changes in membrane integrity and increases in the permeability of brush border cells were reflected in leakage of lactate dehydrogenate and in the depolarization of the membranes. Because an increase in gut permeability may be associated with some forms of food allergy, sublethal concentrations of glycoalkaloids may be a factor in the hypersensitivity to some food ingredients.

Cardiotonic Effects. Oral administration of tomatine induces a cation effect on cardiac contractions in the frog, producing symptoms of tachycardia (*264*). To define the cellular basis for this effect, Berger and Alink (*265*) examined effects of solanine and tomatine in beating rat heart cells. The cells ceased beating within a few minutes after addition of 80 μg/mL solanine or 20 μg/mL tomatine. At lower concentrations, both compounds induced increases in the contraction frequencies of the cells. Because the nitrogen atom of tomatine has a p*K* of ∼6 and it can participate in acid−base equilibrium in the pH range of 4−8, the cited inhibition of beating of heart cells may arise from alteration of electric charge properties of heart cell membranes by positively charged tomatine ions.

Effects on Liver Size. We carried out studies to define possible effects of orally fed glycoalkaloids and aglycons on food consumption, body weight, and liver weight changes in mice (*266*). Orally consumed α-tomatine and tomatidine did not affect body and liver weights of the mice. Because solasodine induced significant hepatomegaly and tomatidine did not and because these two compounds are structurally similar, differing only by the presence of a 5,6-double bond in ring B and in the stereochemistry of ring F, it appears that the double bond is likely to be responsible for the observed difference in potency in inducing liver enlargement.

Effects on Growth of Hamsters. Figure 7D shows weight gains of hamsters fed green and red tomato diets and control diets fortified with 0.05−0.2% tomatine for 21 days (*24*, *267*). Green and red tomato feeding resulted in 12−20% lower gain and in lower food consumption compared to control diets. By contrast, added tomatine did not affect weight gain. Does eating of tomatoes by humans also result in lower weight gain?

Hemolysis of Red Blood Cells. α-Tomatine hemolyzes red blood cells both in vitro and in vivo (*7*, *268*). Injection of α-tomatine into mice caused a rapid drop in blood pressure, presumably due to hemolysis (*257*). Hemolysis results in formation of differently shaped erythrocyte ghosts and is presumably the result of tomatine-induced disruption of red blood cell membranes followed by leakage of the cell content. Cholesterol protected erythrocytes against hemolysis (*143*, *269*).

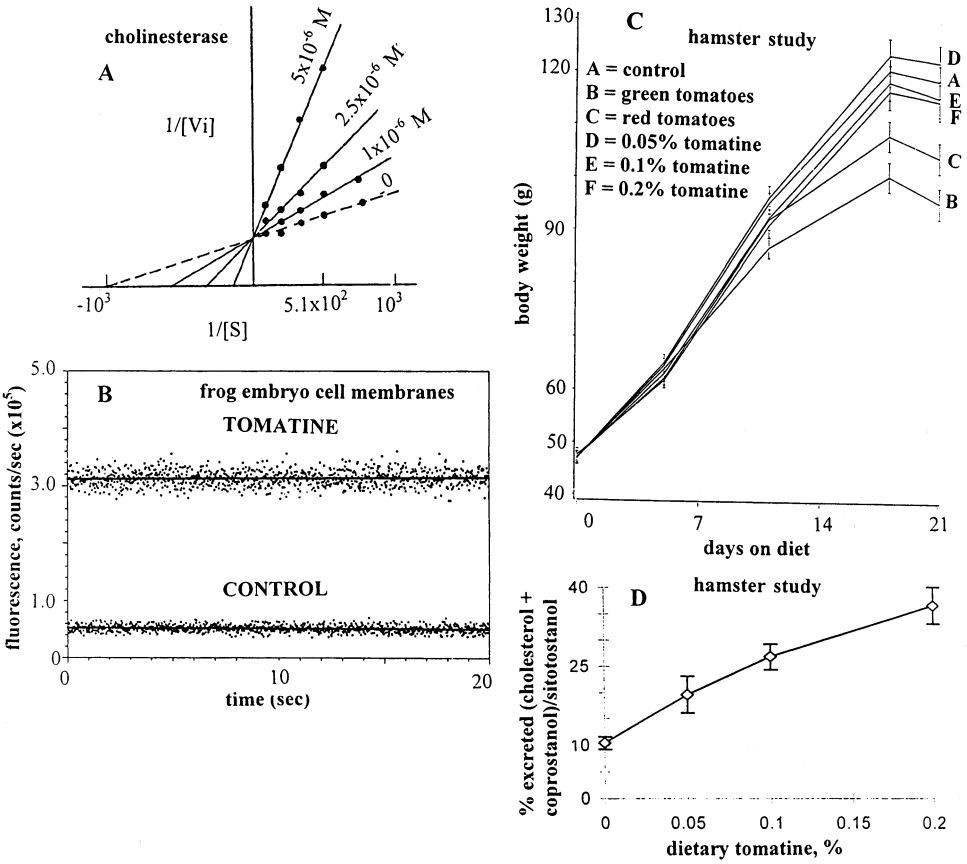

Figure 7. Biological data: (A) Lineweaver–Burk plot of the inhibition of cholinestarase by the indicated tomatine concentrations (adapted from ref *140*); (B) tomatine-induced disruption of frog embryo cell membranes measured by fluorescence (adapted from ref *152*); (C) growth of hamsters fed tomato- and tomatine-containing diets (adapted from refs *24* and *267*); (D) relationship between dietary tomatine and fecal levels of cholesterol and coprostanol (adapted from refs *24* and *267*).

There appears to be a lack of correlation between surface and interfacial activities of saponins including tomatine and their hemolytic properties (*270*). Because cytotoxic effects of pure α-tomatine differ from those of dehydrotomatine (*41*), it would be worthwhile to measure the respective abilities of the two glycoalkaloids to induce hemolysis.

Release of Calcium from Bone Tissue. Exposure of cultured mouse bone tissue to 1−10 μg/mL of either α-tomatine or solanine resulted in a significant increase in releases of calcium from treated tissues compared to controls (*271*). The aglycons tomatidine and solanidine showed no effect on calcium release from cultured bone. Because α-tomatine and solanine are reported to increase the calcium influx into cells (*272*), calcium release from bone tissue presumably arises from the cholesterol binding and membrane disruption of the glycoalkaloids.

Antidiuretic Effects. Intraperitoneally injected tomatine decreased diuresis in rats (*273*, *274*). The physiological effect was accompanied by increased corticosteroid and neutrophil levels and a decrease in the Na/K ratio in the serum.

Anti-inflammatory Effects. Tomatine administered intramuscularly to rats in the dose range of 1−10 mg/kg or orally in doses of 15−30 mg/kg induced a dose-dependent inhibition of induced edema. Similar anti-inflammatory effects were noted following subcutaneous administration of tomatine. Tomatidine was inactive (*275*). Injection of tomatine into guinea pigs prevented the effects of intradermally injected histamine and bradykinin against capillary permeability. The glycoalkaloid also offered some protection against effects of a lethal histamine aerosol and anaphylactic shock (*276*−*278*). Whether consumption of high-tomatine green tomatoes can protect against inflammation and allergies in humans merits study.

Potentiation of the Immune Response. Cytokines are mediators of lymphoid cell function during an immune response. T helper (Th) cells and macrophages are the major cytokine producers. Adjuvants combined with an antigen potentiate an immune response in an immunized species. Morrow et al. (*279*, *280*, *281a*) compared the immunopotentiating properties of novel aggregate formulations based on α-tomatine, a glycosylamide lipid, and a polymer. Of the three formulations, tomatine was found to induce cytokines at levels that were higher than those induced by reference control adjuvants.

Immunization of mice with a molecular aggregate prepared from tomatine and the 9-mer peptide from *Plasmodium berghei* sporozoite protein protected the mice against malarial infection (*281*). The stimulation of the immune system by tomatine could involve participation in a sequence of biochemical reactions known as the "oxidative burst", which entails cellular release of hydrogen peroxide (H_2O_2), a known immune modulator (*282*). This idea is supported by the observation that the saponin digitonin triggers a rapid and transient production of the superoxide anion (O^{2-}) in tomato cell cultures and that the tomato cells were able to discriminate H_2O_2 from O^{2-} (*283*). Will tomatine behave similarly to digitonin in influencing the redox system of tomato cells?

Cancer Chemotherapy. Tomatidine may benefit cancer chemotherapy by inhibiting multidrug resistance in human cancer cells (*284*). Although the mechanism of this beneficial effect is not known, it could involve disruption of cancer cell

membranes, thus facilitating the action of the chemotherapeutic agents (*285*−*289*).

MEMBRANE STRUCTURES

Structures of Cell Membranes. Tomatine, digitonin, and the polyene antibiotic filipin were used to study the planar distribution of cholesterol complexes in membranes (*290*). Ultrastructural studies showed that both tomatine and digitonin formed free complexes with 3β-hydroxysterols. The concentration of sterols in membrane bilayers must exceed a threshold level for complex formation to occur. In animal tissues (epidermis, leukocytes, liver, and sperm), tomatine does not distinguish among the common 3β-hydroxysterols such as cholesterol, cholestanol, 7-dehydrocholesterol, and lanosterol.

Related studies showed that tomatine facilitated studies of sterol-deficient domains in the green flagella *Chlamydomonas rheinhardi* (*291*), cholesterol distribution in cells of stria vascularis of guinea pigs and gebrils (*292*), glucose transport across plasma membranes (*293*), microfilaments and microtubules in fertilized eggs of mollusks (*294*), cytochemistry of hamster heart cell membranes (*295*), calcium influx into cells (*272*), and stress-induced changes in permeability of frog embryo (*152*) and plasma cell membranes (*154*). Tomatine−sterol interactions appear to be a useful cytochemical tool for freeze−fracture and related structural studies of cell membranes of animal tissues (*296*).

Effects on Embryos. Tomatine and extracts of tomatine-containing tomato pulp were found to be toxic to White Horn chicken embryos (*297*). To develop a better understanding of the biological effects of tomatine, we examined electrochemical effects of α-tomatine and tomatidine on cell membranes of frog embryos and frog skin (*152*). The fluorescence intensity of the membrane-potential-sensitive electrochemical dye increased by a factor of ∼6 when the dye-loaded embryos were exposed to 20 mg/L of α-tomatine **(Figure 7B)**. α-Tomatine also diminished sodium-active transport in frog skin by ∼16% compared to control values, as estimated from the change in the interstitial short-circuit current (ISC). The increase in fluorescence results from a depolarization of the membrane potential. Tomatidine had only a minor effect in both tests, so the carbohydrate moiety evidently binds to receptor sites of cell membranes, inducing the cited effects**.** Potato and eggplant glycoalkaloids behaved similarly in the frog embryo cell membrane assays (*115*, *149*).

In an in vitro test, we examined the developmental toxicities of tomatine to frog embryos (*298*, *299*). The in vitro results showed that tomatine caused malformations in frog embryos similar to those induced by potato glycoalkaloids. On the other hand, no conspicuous teratogenic effects were noted in the fetuses of pregnant female rats fed 250 mg/kg tomatine, whereas such effects were apparent in parallel studies with retinol palmitate, a known teratogen (*300*). Tomatine was also not teratogenic when fed to pregnant hamsters (*301*). It seems that although tomatine is toxic to cells, orally consumed tomatine does not induce in vivo teratogenicity in rodents, presumably because it forms an insoluble complex with cholesterol in the digestive tract, which is then eliminated in the feces, as discussed below.

Our findings raise several questions that merit further study. First, the "tomatine" used is a mixture of two glycoalkaloids differing only in a double bond in the second ring. Will various combinations of pure α-tomatine and dehydrotomatine present in tomato leaves and fruits harvested at various stages of maturity exert synergistic or antagonistic effects in the membrane assays? These techniques may be useful in defining relative potencies of beneficial effects of glycoalkaloids against phytopathogens, and adverse and beneficial effects in animal and human cells and tissues, without the use of live animals.

Table 9. Effect of Green and Red Tomato Diets on Plasma Cholesterol and Triglycerides in Hamsters Fed for 21 Days (Adapted from References *24* and *267*)[a]

	mg/dL					
diet	VLDL	LDL	HDL	total	triglycerides	LDL/HDL
control	139	52	130	313	627	0.41
green tomatoes	77	21	130	228	333	0.17
red tomatoes	98	29	112	240	432	0.26

[a] VLDL, very low density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Table 10. Ratios of Dietary Deuterated Sterols to the Internal Standard Sitostanol in the Feces of Hamsters (Adapted from References *24* and *267*)

diet	d-cholesterol/d-sitostanol	d-coprostanol d-sitostanol
control	0.107	0.71
green tomato	0.090	0.58
red tomato	0.083	0.55
tomatine, 0.05%	0.154	1.90
tomatine, 0.1%	0.215	2.97
tomatine, 0.2%	0.573	3.95

Effect on Cholesterol in the Small Intestine and in the Liver. In the course of studies on the mucosal cholesterol balance in the small intestine of the rat, it was noted that tomatine removed cholesterol from mucosal cells as well as the output of cholesterol into the lymph, suggesting that tomatine may have direct effects on mucosal cell function (*302*). Related studies showed tomatine precipitated cholesterol but not bile acids from micellar solutions in the lumen, affected cholesterol absorption and lipid metabolism, and induced sterol but not bile acid excretion in the rat (*260*, *303*).

A possible explanation for the tomatine-induced increased cholesterol synthesis in the liver is that cholesterol in the gastrointestinal tract forms a complex with tomatine, which is then excreted. The consequence is that a reduced amount of cholesterol is transported from the intestine to the liver via the enterohepatic circulation. The liver then compensates by synthesizing additional cholesterol. Evidently, increasing dietary cholesterol offsets the tomatine-induced hepatic cholesterol synthesis.

Lowering Plasma LDL Cholesterol and Triglycerides. The dynamics of cholesterol formation and metabolism in hamsters is similar to that in humans; therefore, we examined the effect of feeding tomatine as well as high-tomatine green and low-tomatine red tomatoes on hamster plasma lipoprotein and triglyceride levels, cholesterol distribution, fecal excretion of cholesterol, cholesterol metabolites, and bile acids (**Figure 7C**,**D**; **Tables 9** and **10**) (*24*, *267*). Two feeding studies were carried out: (1) hamsters were fed a 0.2 g of tomatine/100 g of a high saturated fat, high-cholesterol diet for 3 weeks and (2) hamsters were fed green (high-tomatine) or red (low-tomatine) freeze-dried tomato powders and diets containing three concentrations of tomatine. In the first study, plasma LDL cholesterol decreased by 41%. In the second, 59 and 44% reductions in LDL cholesterol were induced by the green and red tomatoes, respectively. The corresponding reductions in plasma triglyceride concentrations were 47 and 31%.

The fact that tomatine alone reduces both dietary cholesterol bioavailability and endogenous cholesterol and that our calcula-

tions show that the amount reduced is equivalent to the amount consumed suggests that tomatine forms an insoluble complex with cholesterol from both dietary cholesterol and from endogenous cholesterol produced by the liver. Liver cholesterol enters the digestive tract via the enterohepatic circulation. Although we do not know much about the dynamics of this event, it probably does not occur under the acid conditions of the stomach, because in vitro studies show that protonation of the ring nitrogen of tomatine prevents complex formation with cholesterol (*57*). Complex formation probably takes place in the alkaline environment of the duodenum. Whether acid or enzyme (glycosidase)-catalyzed hydrolysis of tomatine occurs in vivo is not known.

The fact that the high-tomatine green tomatoes are more effective in lowering plasma cholesterol and triglycerides than low-tomatine red tomatoes suggests that tomatine in green tomatoes contributes to the cholesterol-lowering effects. Red tomatoes are also a potent hypolipidemic food for hamsters, so obviously other components of tomatoes must be involved in inducing the observed hypolipodemias. These could include fiber, free amino acids, protein, sugars, and antioxidants. In fact, an additional study (*304*) showed that feeding hamsters l0% cellulose-containing diets supplemented with cholesterol levels of 0.025, 0.05, or 0.2% resulted in plasma LDL concentrations of 121, 175, and 326 mg/dL, respectively. The corresponding values with 10% red tomato diets were much lower: 64, 90, and 102 mg/dL. The tomato diets also reduced plasma triglyceride but not HDL (good) plasma cholesterol levels. The fecal content of cholesterol, coprostanol, and bile acids supports the hypothesis that the major pathway by which tomatine induces lowering of cholesterol is by complex formation described earlier. The mechanism of the triglyceride lowering effect is not known.

Tomatine was used to measure cholesterol esters in the plasma of 30 patients suffering from diabetes mellitus and in 10 patients with cirrhosis of the liver (*305*). Both diseases were associated with a decrease in plasma cholesterol. It was also used to measure the cholesterol content of plasma HDL and to separate mixtures of five oxidation products of cholesterol (*306*).

Removal of Cholesterol from Butteroil. Micich et al. (*307, 308*) covalently attached tomatine to a resin via acetal or ester linkages at an average value of 0.14 mM of tomatine/g of polymer. Passage of hexane solutions of cholesterol or cholesterol-containing butteroil through the tomatine-bound resin resulted in removal of cholesterol, which was left attached to the tomatine on the column. The resin could be regenerated by extraction of the bound cholesterol with acetone or ethyl acetate. This method has the potential for practical use to reduce the cholesterol content of foods.

CONCLUSIONS AND OUTLOOK

Glycoalkaloids may have evolved in nature to protect selected plants against bacteria, fungi, insects, and animals. It is striking that both green tomatoes and tomato leaves have a very high glycoalkaloid content, which makes them undesirable to eat because the green fruit and leaves not only taste bitter to animals but may not be safe to phytopathogens. An unanswered question involves the respective contributions of α-tomatine and dehydrotomatine to host-plant resistance of tomato plants and whether the two tomato glycoalkaloids act synergistically both in the plant and in the diet.

Stepwise removal of the carbohydrate groups by acid or enzyme hydrolysis can, in principle, generate a mixture of six compounds with four, three (two isomers), two, one, and zero sugar groups for each of the two glycoalkaloids. The isolation and characterization of all possible hydrolysis products and the assessment of their biological functions in the plant and in animals and humans are challenging problems.

Observations that the absence of a 5,6-double bond in the B-ring of tomatidine results in a much less toxic molecule in both pregnant and nonpregnant mice as compared to the structurally similar solasodine (which contains such a double bond) confirm related findings that glycoalkaloids without such a double bond such as tomatine are less toxic than those which have them such, as the potato glycoalkaloids α-chaconine and α-solanine. It may benefit food safety to create, through plant breeding and/or plant molecular biology methods, plant foods with modified glycoalkaloids that lack the double bond.

Plant scientists are challenged to (a) investigate the gene transcription mechanism and control of the synthesis and degradation of tomato glycoalkaloids in order to suppress genes that encode enzymes that degrade tomatine during ripening of tomatoes, allowing the production of cultivated red tomatoes with a higher tomatine content; (b) replace the glycoalkaloids α-chaconine and α-solanine in potatoes with tomatine, which is nontoxic to humans when consumed in amounts present in green tomatoes and which seems to be more effective as a protectant against phytopathogens; and (c) assess the tomatine levels of salt-tolerant (*309*), insect repellent-producing (*310*), and other genetically engineered tomatoes (*200*). Food and biomedical scientists, including nutritionists and microbiologists, are challenged to further define beneficial effects of α-tomatine and dehydrotomatine and hydrolysis products in the human diet in lowering cholesterol and triglycerides, in enhancing the immune system, in cancer chemotherapy, and in protecting against virulent fungi, bacteria, viruses, and protozoa.

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