differences in rates of growth and alkaloid synthesis. Considering that roots were not sterile, the extent of incorporation of mevalonic acid into tomatine in tomato roots (0.02%) is comparable with that previously reported for aseptically-cultured tomato roots (0.09%)[13]. Earlier grafting studies[4,14] using potato and/or tomato were concerned principally with establishing the organ of alkaloid synthesis and did not lead to definitive conclusions regarding transport of these compounds between stock and scion. However, taken together, the TLC analyses and radioactivity data reported here tend to rule out significant transport or interchange of glycoalkaloids between (or within) root and shoot in the potato and tomato plant (and possibly other related species). Low radioactivity in 'parent' alkaloids of nontreated regions might have resulted from transport of small quantities of the precursors. These findings are compatible with earlier work in which tomatine could not be detected in cultured root medium[4,7,8] or in bleeding sap from decapitated plants[4,6].

Plants producing steroidal glycoalkaloids of the 'Solanum type'[15] appear to differ from other solanaceous plants in which non-steroidal, non-glycosidal alkaloids are frequently synthesized in the root and transported to other non-synthesizing regions where they accumulate[16]. Assuming that alkaloid accumulation has some selective value, then long-distance alkaloid transport (e.g. root ⇆ shoot) is unlikely to be totally devoid of significance. Thus, in *Nicotiana* spp., transport of nicotine from roots to leaves probably compensates for the inability of the latter organ to synthesize this alkaloid on any scale[17]. Since both the root and the shoot are apparently capable of elaborating glycoalkaloids in potato and tomato[4], lack of alkaloid transport between these organs is perhaps not surprising.

The claim that tomatine is a 'water-soluble transport form'[9] should therefore be qualified to exclude long-distance transport between root and shoot, and probably also within these structures. However, transport of steroidal alkaloids at the subcellular level is a possibility, especially between the site of synthesis/glycosylation (not known, but probably particulate) and the site of accumulation (probably vacuolar[18]).

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Protective effects of vitamin E and dithiothreitol against the hemolysis of rat and goat erythrocytes induced by Tween 20 with or without ascorbic acid and azide

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Summary. Extensive in vitro hemolysis of erythrocytes, induced in vitamine E-deficient rats by 0.001% Tween 20 with ascorbic acid and azide, or in goats by 2.5% Tween 20, could be counteracted by either the inclusion of vitamin E in the cells or by the in vitro addition of 0.25–0.4 mM dithiothreitol.

We have reported[1] that vitamin E-deficient erythrocytes of milk-fed kids exhibit an extreme fragility with Tween 20, which can be protected against by either vitamin E or dithiothreitol (DTT), and that rat erythrocytes are hemolyzed with much-diluted Tween 20 irrespective of their vitamin E status. In the present work, we tried to discover a specific hemolytic condition for vitamin E-deficient rat erythrocytes. Our experimental idea was that at a low Tween 20 concentration which cannot induce any appreciable hemolysis, the addition of other chemicals might induce an extensive hemolysis only in the vitamin E-deficient erythrocytes. After several trials, it was found that vitamin E-specific hemolysis of rat erythrocytes was induced by Tween 20 with ascorbic acid and azide. We also examined the relationships between the hemolytic actions of various chemicals and the protective effects of vitamin E or DTT using the vitamin E-deficient erythrocytes of rats and goats.

Materials and methods. Mature male Wistar rats were fed either a vitamin E-deficient or a vitamin E-supplemented diet consisting of casein 15, sucrose 70.7, lard 5, cellulose 5.5 and vitamin-mineral mixture 3.8%. Contents of minerals and vitamins of these diets were the same as those recommended by the NRC feeding standard[2]. Only the vitamin E-supplemented diet contained 30 mg dl-α-tocopheryl acetate per kg. For more than 2 months 20 g of the diets were fed daily in the dark period of 18.00–6.00 h at about 23 °C.

The hemolytic procedure was as follows. 25 vol. of saline-phosphate buffer (pH 7.4)[3] was added to 1 vol. of heparinized rat blood taken by cardiac puncture and the mixture was centrifuged. The cell pellet was resuspended with the

Table 1. Percentage hemolysis induced by Tween 20, ascorbic acid and/or azide in rats fed vitamin E-deficient or vitamin E-supplemented diet after incubation for 30 min at 37 °C

Addition	Vitamin E-deficient diet	Vitamin E-supplemented diet	p
Tween 20	22±6	21±11	NS
Ascorbic acid	8±1	7± 2	NS
Azide	7±1	6± 2	NS
Tween 20+ascorbic acid	20±7	12± 5	NS
Tween 20+azide	9±2	12± 6	NS
Ascorbic acid+azide	12±1	10± 3	NS
Tween 20+ascorbic acid+azide	84±5	12± 2	0.01
Tween 20+ascorbic acid +azide+0.4 mM DTT	24±9	20± 1	NS
Tween 20 (×2 concentration)	72±5	72±11	NS

Data are shown as a mean for 4 and 3 animals fed vitamin E-deficient or vitamin E-supplemented diet, respectively, and its SD. p, Level of significance. Plasma vitamin E contents of rats fed vitamin E-deficient or vitamin E-supplemented diet were 0.82±0.18 (SD) and 10.11±0.48 μg · ml^{-1}, respectively.

same volume of saline-phosphate buffer. Tween 20 solution contained 0.02% Tween 20 in 0.9% NaCl. Ascorbic acid solution, azide solution or ascorbic acid and azide solution contained either 2.2 mM ascorbic acid neutralized with dilute NaOH or 10 mM Na azide, and were made isotonic with NaCl. 0.3 ml of the cell suspension containing about 5 μl of washed erythrocytes, 0.5 ml of saline-phosphate buffer, 1.0 ml of either ascorbic acid solution, azide solution, ascorbic acid and azide solution or 0.9% NaCl and 0.1 ml of Tween 20 solution or 0.9% NaCl were mixed and incubated at 37 °C for 30 min. Final concentrations of Tween 20, ascorbic acid and azide of the incubation mixture were 0.001%, 1.2 and 5.3 mM, respectively. Immediately after the incubation, the mixture was cooled by ice and 2.0 ml of cold saline-phosphate buffer was added. Percentage hemolysis was calculated by dividing the OD at 540 nm of the supernatant of the above solution by the OD of the completely hemolyzed sample and multiplying by 100.

Japanese meat-type male goats were used for the experiment at about 3 months of age. From 1 week to 2 months of age they were fed either vitamin E-deficient or vitamin E-supplemented milk[1] and thereafter weaned and fed about 150 g of either vitamin E-deficient or vitamin E-supplemented purified ration[4] with 15 g of rice straw daily. Only the vitamin E-supplemented ration contained 200 mg dl-α-tocopheryl acetate per kg. The hemolytic procedure was the same as shown previously[1]. The mixture of cell suspension and Tween 20 solution was incubated at 37 °C for 15 min. Vitamin E contents of rat and goat plasma were measured by the fluorometric method[5].

Results. As shown in table 1, in the presence of 1 or 2 of the substances Tween 20, ascorbic acid and azide, no significant differences in the percentages hemolysis were detected between vitamin E-deficient and vitamin E-supplemented rats. However, in the presence of all 3 chemicals, the percentage hemolysis of the vitamin E-deficient group became significantly higher than that of vitamin E-supplemented group (84 vs 12%). The percentages hemolysis of the vitamin E-deficient erythrocytes increased rapidly from 20 min after the start of incubation. When DTT was added to the above medium, the hemolysis of vitamin E-deficient erythrocytes was prevented and there was no significant difference between the percentages of hemolysis of the 2 groups. In the presence of twice the concentration of Tween 20, the erythrocytes of both groups were extensively hemolyzed, and the addition of DTT could not prevent this hemolysis. DTT exerts its protective effect only in the vitamin E-specific hemolysis.

Table 2. Percentage hemolysis induced by Tween 20 in goats fed vitamin E-deficient or vitamin E-supplemented diet, after incubation for 15 min at 37 °C

Concentration of Tween 20 (%)	Vitamin E-deficient diet	Vitamin E-supplemented diet	p
0	4± 3	5± 2	NS
0.5	21±14	3± 1	NS
1.0	71±11	3± 1	0.01
1.5	77±10	4± 3	0.01
2.0	86± 3	11±11	0.01
2.5	87± 1	28±19	0.01
2.5+0.25 mM DTT	9± 2		
2.5* (vitamin E-fortified cells)	15± 5	4± 1	0.05

Data are shown as mean of 3 animals and its SD. p, Level of significance. * Heparinized blood was incubated with 250 μg of dl-α-tocopheryl acetate and 25 μg of penicillin G potassium per ml for 18 h at 37 °C. Plasma vitamin E contents of goats fed vitamin E-deficient or vitamin E-supplemented diet were 0.89±0.15 (SD) and 5.39±1.20 μg · ml^{-1}, respectively.

As shown in table 2, the percentages of hemolysis of vitamin E-deficient erythrocytes of goats were significantly higher than those of vitamin E-supplemented ones at 1.0–2.5% Tween 20, and either uptake of vitamin E by the cells or in vitro addition of DTT could prevent the hemolysis of vitamin E-deficient erythrocytes. These facts are similar to our previous observation[1] on the pre-ruminant kid.

Discussion. Only vitamin E-deficient goat erythrocytes were hemolyzed with 1.0–2.5% Tween 20 during an incubation of 15 min at 37 °C. However, rat erythrocytes were hemolyzed with 0.002% Tween 20 during an incubation of 30 min at 37 °C irrespective of their vitamin E status. The increased fragility of rat erythrocytes in Tween 20 might be related to their membrane lipid composition. About half of the phospholipid of rat erythrocytes is lecithin, whereas that of goat erythrocytes is sphingomyelin and their lecithin content is negligible[6,7]. The vitamin E-specific hemolysis of rat erythrocytes was induced by the addition of ascorbic acid and azide to 0.001% Tween 20, and DTT could prevent this hemolysis. It has been proposed that peroxides and free radicals are mainly responsible for the hemolysis induced by polyoxyethylene derived surfactants[8]. Tween 20 is polyoxyethylene sorbitan monolaurate, and aqueous solu-

tions of Tween 20 undergo autoxidation[9]. Ascorbic acid itself can autoxidize and generate H_2O_2 at an obviously slow rate[10], but in the presence of other autoxidizable substances ascorbic acid can stimulate both O_2 consumption and H_2O_2 production[11]. Ascorbic acid can react with superoxide anion to generate H_2O_2[12]. In the presence of traces of metal ions, superoxide anion and H_2O_2 might generate the highly reactive hydroxy radical. If catalase is inhibited by azide, the membrane lipid peroxidation[13,14] may progress due to the increased generation of H_2O_2 and other oxidants. The depletion of cellular reduced glutathione and the oxidation of protein thiol groups may cause cell lysis. We have a hypothesis that the oxidative damage of membrane ATPase is crucial for this, since according to our unpublished result, the potassium loss preceded the cell lysis, and the inactivation of ATPase occurring during the treatment could be prevented by the addition of DTT. Vitamin E is a radical scavenger[15,16] and may play a structural role in protecting membrane lipid-protein complexes against oxidative damage.

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A queen bee extract (*Apis mellifera* L.) (Hymenoptera) reduces the fecundity of *Tenebrio molitor* L. (Coleoptera)

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Summary. 3 different doses of crude queen bee extract were administered to pairs of adult *Tenebrio molitor* L.; the extract exerted an inhibitory action on fecundity, but did not induce complete sterility of the females.

The queen of *Apis mellifera* is known to hinder the development of the worker bees' ovaries by means of a pheromone[1-3] which has been isolated and identified as trans-9-keto-2-decenoic acid[4,5]. Both the pure substance (natural or synthetic) and crude extracts of queen bee are active in other insects as well: in *Kalotermes flavicollis* (Isoptera) they influence caste differentiation[6-8], in *Aedes aegypti* (Diptera) they cause death and delay moulting and metamorphosis[9,10]; but more frequently they exert an inhibitory action on fecundity, as they do in bees. This is so in *Formica fusca* (Hymenoptera)[11], *Musca domestica* (Diptera)[12] and *K. flavicollis*[8], and even in a crustacean, *Leander serratus*[11].

The experiments reported here show that crude extracts of queen bee, administered orally, also reduce fecundity in *Tenebrio molitor* L. (Coleoptera).

Queen bees at least 1-year-old were homogenized in alcohol. The lipoproteins were precipitated from the solute with acetone[13]; the solute was then concentrated in a vacuum at 30 °C, dissolved in alcohol, and spread on wheat wafers which were fed to pairs of *Tenebrio molitor* as their only food. 3 doses were used: 1:100, 2:100 and 4:100 (ratios by weight of crude extract to wafer at room conditions). The controls were fed wafers treated with alcohol alone. Every 4 days the pairs were given drinking water on dampened filter paper.

Single pairs of *T. molitor* which had moulted less than 24 h earlier were placed in plastic boxes with fine wood sawdust, where they laid their eggs. On the 5th, 9th, 13th and 17th days after metamorphosis each pair was moved to another box and the eggs counted. Thus 4 lots of eggs, laid successively, were counted per couple.

Since the treatment with the 3 different doses of extract was done at different times a separate series of control pairs was set up in each case.

Table 1 shows the average number of eggs per lot laid by

Table 1. Average number of eggs (by lot) laid by treated and control pairs

	Treated insects			Controls			
Dose	No. pairs	No. lots	No. eggs per lot	No. pairs	No. lots	No. eggs per lot	t
1:100	27	108	25.56 ± 1.32	20	80	27.46 ± 1.73	0.89
2:100	23	92	23.64 ± 1.57	23	92	28.68 ± 1.64	2.22*
4:100	24	96	22.08 ± 1.52	22	88	28.72 ± 1.67	2.94*
Total	74	296	23.82 ± 0.85	65	260	28.32 ± 1.00	3.51*

* $p < 0.05$.