domestic animals. The compound proved promising against different helminths of fowl, cat and dog. The high order of anthelmintic activity coupled with a wide margin of safety "acute toxicity and general pharmacology" calls for detailed investigations.

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Distribution of steroidal glycoalkaloids in reciprocal grafts of *Solanum tuberosum* L. and *Lycopersicon esculentum* Mill.

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Summary. TLC analyses of steroidal glycoalkaloids of the scion and stock of reciprocally-grafted potato and tomato plants and tracer studies involving administration of [^{14}C]-labeled alkaloid precursors to scion and stock suggest that alkaloid transport between root and shoot does not take place in these species.

Steroidal glycoalkaloids are particularly prominent in members of the Solanaceae but their function in the plant is still uncertain[2]. Because of their toxicity, particularly to fungi and insects, it is generally thought that they may play a protective role in plants. Their sites of biosynthesis and accumulation are reasonably well understood[3,4], but their mobility in the plant, knowledge of which could help clarify their function, has long been a subject of debate. Kern[5] found measurable quantities of alkaloid in sap exuded from decapitated tomato plants but later work[4,6] failed to confirm this finding and alkaloids have not been detected in liquid culture medium supporting the aseptic growth of excised roots of *Lycopersicon* spp.[4,7,8]. However, the extent to which excision and cultural conditions could have influenced results is not known. Nor would either of these methods provide information on transport from the shoot (which is the main region of alkaloid synthesis) to the root. More recently, Segal and Schlösser[9] proposed that the aglycone of steroidal glycoalkaloids is the physiologically-active moiety with the glycoside being the 'water-soluble transport form'.

In this study, root/shoot transport of the steroidal glycoalkaloids of potato (α-solanine [**I**] and α-chaconine [**II**]) and tomato (α-tomatine [**IV**]) has been investigated using reciprocal grafts between these plants. Phylogenetically-related species were chosen to help ensure biochemical compatability (the alkaloids are chemically similar, but chromatographically distinct) and graft formation. Use of grafted plants also overcomes a problem associated with normal plants viz. radioactivity appearing in metabolites of non-treated parts due to transport of a radioactive precursor, followed by synthesis.

Materials and methods. Tomato (cv. Suttons Potentate Best of All) and potato (cv. Majestic) plants were grown in John Innes No. 2 compost for approximately 8 weeks and 4 weeks respectively. Glasshouse temperature was 20–22 °C. 'Whip and tongue' grafts were made by cutting obliquely into the stem of intact plants 10 cm above soil level, upwards in the scion species and downwards in the stock species, and inserting the scion 'lip' into the stock groove. The graft region was then bound with aluminium foil and sellotape. Plants were grown in this condition for approximately 4 weeks after which stems were cut, 5 cm above the graft union in the stock and 5 cm below the union in the scion. Grafted plants were grown on for between 2 weeks and 3 months. Plants grown for 3 months on a tomato stock were re-potted after 4 weeks; those on a potato stock, after 4 and then 8 weeks.

2-week-old grafted plants (approximately 25–30 cm) were removed from their pots, their roots cleaned of compost and washed, and divided into stock and scion by cutting 2 cm above and below the graft union. The union itself was discarded. 3-month-old plants (approximately 60–70 cm with tomato as stock and approximately 130 cm with potato as stock) were cleaned, as above, but divided into a greater number of parts viz. a) leaf + petiole b) stem of scion excluding the 3 cm zone directly above the graft union c) the 3 cm of scion stem above the graft union d) the 3 cm of stock stem directly below the union e) remainder of the stock stem f) root system g) tubers (where appropriate). For each of the 4 groups of plants used (potato on tomato, 2 weeks and 3 months; tomato on potato, 2 weeks and 3 months) 3 individual plants were analyzed.

In radiotracer studies, 4-week-old grafted plants were used, with all side shoots removed. To 1 batch of plants, 37kBq of [4-^{14}C]-cholesterol (in 0.1 cm^3 acetone) were applied to each of 5 selected young leaves every second day for 10 days (i.e. a total application of 925kBq). In another batch, root systems were thoroughly washed then placed in aerated liquid nutrient medium[10] containing 925kBq of DL-[2-^{14}C]-mevalonic acid lactone in a darkened glass container. After 7 days, root solutions were renewed and left for a

further 7 days. All plants were grown in a constant environment chamber (25 °C day, 15 °C night; 65% relative humidity). Plants were harvested 2 weeks after the 1st application and treated roots washed for 24 h. Plants were divided into leaf, stem, fruit, tuber and root as above.

Tissues were homogenized in 96% (v/v) methanol containing 2% (v/v) acetic acid and extracted for 24 h. After filtering, residues were re-extracted in 64% (v/v) methanol for 24 h. Extracts were again filtered and filtrates combined and evaporated to dryness under vacuum at 45 °C. Flask contents were taken up in 2% (v/v) acetic acid, adjusted to pH 10.0 with concentrated ammonia, heated in a water bath at 80 °C for 30 min and placed in a refrigerator for at least 16 h. Precipitated alkaloids were separated by centrifugation at 27,000 × g for 20 min, washed with 1% (v/v) ammonia and centrifuged as previously. Precipitates were dried in a $CaCl_2$ desiccator followed by extraction 3 times into hot methanol, with centrifuging, as above, between each extraction. Methanol extracts were combined and reduced to approximately 1 ml by rotary evaporation at 35 °C.

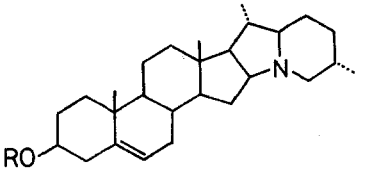
Aliquots of extracts (approximately 25 µl) were applied to TLC plates coated with Kieselgel G (0.25 mm) as were also authentic, α-tomatine, tomatidine (**V**), α-solanine, α-chaconine (Sigma Chemical Company, Poole, U.K.) and solanidine (**III**) (Koch-Light Laboratories, Colnbrook, U.K.). TLC plates were developed in 95% (v/v) methanol which clearly separated potato (R_f 0.21) and tomato (R_f 0.65) alkaloids and/or in 95% ethanol which separated α-solanine (R_f 0.18) and α-chaconine (R_f 0.39). The locating reagent was modified Dragendorff's reagent[11] (sensitivity approximately 0.3 µg) and identity of alkaloids was confirmed by co-chromatography with authentic compounds and chromogen formation on spraying with 50% (v/v) sulphuric acid and heating (100 °C, 30 min). Different alkaloids produce different colors and characteristic color changes[12]. Radioactivity of alkaloids was determined by elution from TLC plates and liquid-scintillation spectrometry.

Results. In the 2-week-old grafted plants, alkaloid patterns were very similar in both types of graft (i.e. potato on tomato and tomato on potato). In potato, whether scion or stock, solanine was readily identifiable, but confirmation of chaconine required use of a 2nd solvent system and chromogen characteristics. Tomatine was not detected. In tomato, whether stock or scion, similar methods confirmed the presence of tomatine only. Further TLC using ethyl acetate:methanol:water (80:5:5) and the aglycones tomatidine and solanidine as standards indicated that traces of Dragendorff-positive compounds of low R_f observed in both solvents were not aglycones. This was subsequently confirmed by chromogen formation.

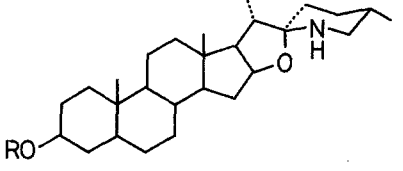
In the 3-month-old grafts, extracts of stocks (both potato and tomato) showed only the 'parent' alkaloids with no indications of alkaloids of the scions. In scions of both species, all parts again contained the 'parent' alkaloids along with other Dragendorff-positive material. Further TLC and chromogen analyses verified that this was not the 'stock' alkaloid. No compounds resembling aglycones in R_f or color were observed.

Data from radiotracer experiments are shown in the table. Precursor applied to leaves was significantly incorporated into leaf alkaloids in both species. In tomato, 0.55% of the applied label found its way into tomatine whereas the figure for potato alkaloids was 0.13%. Root-administered alkaloid was also incorporated into root alkaloids of both species although at a lower level than in leaves (0.02% in tomato, 0.07% in potato). Locating reagents did not reveal tomato alkaloids in potato or vice versa but radioactivity was present in the corresponding R_f zones although levels were very low (0.05%–6.5% of the label in alkaloid in the organ of application). Low radioactivity (not exceeding 0.003% of applied label) was observed in 'parent' alkaloids of all nontreated scions and stocks.

Discussion. Although the possibilities of biochemical modification/degradation of transported alkaloids, and failing to allow sufficient time for alkaloid transport and accumulation to occur cannot be wholly discounted, the use of taxonomically-similar species and grafted plants as young as 2 weeks and as old as 3 months render these unlikely. Differential incorporation of label into tomato and potato tissues, and also leaves and roots, is probably due mainly to



I R = Rham-glu-gal-, α-solanine
II R = 2Rham-glu-, α-chaconine
III R = H, solanidine



IV R = Xyl-2glu-gal-, α-tomatine
V R = H, tomatidine

Distribution of radioactivity in alkaloids and corresponding R_f zones of scions and stocks of reciprocally-grafted tomato and potato plants

		Radioactivity (dpm) in tomato (T) and potato (P) alkaloids and corresponding R_f zones								Percent of incorporated label in scion/stock 'parent' alkaloid and corresponding stock/scion R_f zone			
		A (Tom/Pot)		B (Pot/Tom)		C (Tom/Pot)		D (Pot/Tom)		A (Tom/Pot)	B (Pot/Tom)	C (Tom/Pot)	D (Pot/Tom)
		T	P	P	T	P	T	T	P				
Scion	Leaf	305470	–	72946		151[b]	3038	280[b]	701	99.49	99.17	0.19[b]	1.18[b]
	Stem	167	–	ND[a]	–	1023[b]	342	1557[b]	529	0.05	ND[a]	1.31[b]	6.54[b]
	Flower	313	–	394	–	226[b]	823	–	–	0.10	0.54	0.29[b]	–
	Fruit	–	–	–	–	43[b]	216	–	–	–	–	0.06[b]	–
Stock	Root	470[b]	374	210[b]	457	76723	–	21976	–	0.15[b]	0.29[b]	98.15	92.29
	Tuber	616[b]	626	–	–	–	–	–	–	0.20[b]	–	–	–

[a]Data not available; [b]corresponding R_f zone; alkaloid not detectable by TLC. [4-^{14}C] - cholesterol was applied in acetone solution to selected leaves of plants A and B; [2-^{14}C] - mevalonic acid lactone in nutrient solution to the roots of plants C and D.

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