rough handling of the specimens during experiments easily destroyed their photoresponsivity.

Recent investigations have indicated that the electrophysiological characteristics of *Paramecium* membranes are essentially similar to those of metazoan excitable cells[10]. Thus *Paramecium* is potentially a valuable organism for the study of mechanisms underlying light-excitation processes in the membrane.

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## The effect of chronic marginal vitamin C deficiency on the α-tocopherol content of the organs and plasma of guinea-pigs

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*Summary.* Chronic marginal vitamin C deficiency lasting 270 days induced hypertriglyceridaemia, an abrupt fall of ascorbic acid in all organs and a significant decrease of α-tocopherol in the liver and the lungs in guinea-pigs.

Experiments in which a deficiency of vitamin C was ameliorated by the administration of vitamin E, and vice versa, were suggestive of a synergic effect of the 2 vitamins[1-3]. The plasma level of vitamin E is known to be affected by changes in triglyceridaemia and cholesterolaemia[4-7]. We undertook this study to ascertain whether the assumed synergism between vitamins C and E and changes in the lipid metabolism caused by a prolonged latent vitamin C deficiency[8] induce changes in α-tocopherol levels in the blood and organs of guinea-pigs exposed to marginal vitamin C deficiency over a long period.

*Material and methods.* We induced a vitamin C deficiency lasting 270 days in 18 growing male guinea-pigs[9]; 20 animals served as controls. Both groups were fed a scorbutogenic diet[10] containing an adequate amount of α-tocopherol (47 mg/kg diet). The deficient group received 0.5 mg oral ascorbic acid/animal/day vs 5 g ascorbic acid/kg diet received by controls. After 16 h of fasting the animals were anesthetized with ether and killed. Blood was collected by cardiac puncture. Blood samples were assessed for concentrations of vitamin C[11], α-tocopherol[12] and triglycerides[13]. The amount of vitamin C[14], α-tocopherol[15] and total fats[16] was determined in organs. The results were evaluated by Student's t-test.

*Results.* Food intake and weight curves were virtually the same in both groups (body weight at the end of experiment: controls 866±31 g, deficiency 836±28 g). Deficient animals showed a significantly increased weight of the liver (controls 28.6±1.7 g, deficiency 35.5±2.5 g; $p < 0.05$) and a decreased weight of the testes (controls 4.7±0.2 g, deficiency 3.6±0.3 g; $p < 0.01$). The average content of fat in organs was not significantly changed except for a distinct fat accumulation in the liver of experimental animals. Chronic vitamin C deficiency elicited a rapid rise of triglyceridaemia, exceeding almost 4-fold the values of controls (controls 1.7±0.3, deficiency 6.4±0.7 mmoles/l; $p < 0.001$).

Vitamin C concentration in the plasma and organs of the deficient group decreased significantly to 4-10% of the values found in controls (table).

Prolonged vitamin C deficiency resulted in a decrease of α-tocopherol concentration in the liver, lungs and kidneys to approximately a half of the control values; its concentration in testes, epididymal fat and blood plasma did not change significantly (table).

*Discussion.* The marked decrease of α-tocopherol in some organs of the guinea-pigs exposed to prolonged marginal vitamin C deficiency can be attributed only to the interaction of ascorbic acid with α-tocopherol as both groups of guinea-pigs consumed the same amount of food and hence also the same amount of α-tocopherol. A direct interaction of free ascorbate and tocopherol radicals was observed in

The effect of chronic marginal vitamin C deficiency on the content of vitamin C and α-tocopherol in the organs and plasma of guinea-pigs

| | Vitamin C (μmoles/kg) | | α-Tocopherol (μmoles/kg) | | | |
|---|---|---|---|---|---|---|
| | Control, n = 20 | Deficiency, n = 18 | Control | n | Deficiency | n |
| Liver | 1561.1±41.4 | 68.3±4.1 | 26.7±3.3[a] | 12 | 14.2±3.9[a] | 11 |
| Lung | 1768.3±69.1 | 99.3±7.8 | 20.9±4.3[b] | 13 | 10.2±2.3[b] | 11 |
| Kidney | 524.0±13.3 | 36.5±2.7 | 3.6[c] | | 2.1[c] | |
| Testes | 1229.3±31.6 | 120.1±6.5 | 8.7±2.3 | 12 | 10.8±1.9 | 10 |
| Plasma (μmoles/l) | 129.7±15.7 | 11.4±1.1 | 25.0±3.5 | 13 | 28.9±3.5 | 14 |

Mean values ± SEM; vitamin C content in all tissues of deficient guinea-pigs is significantly lower than in controls, $p < 0.001$; [a,b] Significantly different from controls, $p < 0.05$; [c] α-Tocopherol in the kidneys was determined in a pooled sample.

vitro[17]. There is also growing evidence of the interaction of the 2 vitamins in animal tissues. In some conditions, vitamin C can improve $\alpha$-tocopherol metabolism; in others it may increase the demand of the body for vitamin E[18-20]. Decreased $\alpha$-tocopherol content in the liver, lungs and kidneys of guinea-pigs with chronic marginal vitamin C deficiency suggests that in these organs $\alpha$-tocopherol replaced the missing ascorbic acid in some redox-processes. There is also a possibility that in conditions of long-lasting low ascorbate levels in the above organs $\alpha$-tocopherol is more susceptible to oxidation to quinons.

In the light of known relations between the plasma levels of lipids and vitamin E[4-6], the absence of changes in the plasma level of vitamin E in vitamin C-deficient guinea-pigs, which showed a nearly 4-fold increase in triglyceridaemia, is surprising.

The results suggest that chronic marginal deficiency of vitamin C can lead also to relative deficiency of vitamin E.


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## Biosynthetic relationships among daunorubicin, doxorubicin and 13-dihydrodaunorubicin in *Streptomyces peucetius*


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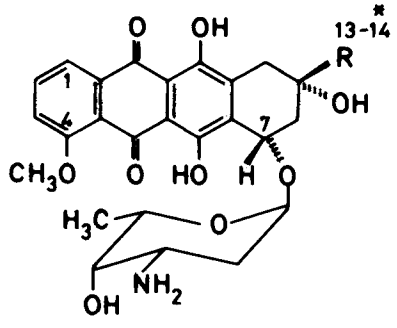
*Summary.* By feeding $^{14}$C-daunorubicin to a doxorubicin-producing mutant of *Streptomyces peucetius,* labelled doxorubicin and 13-dihydrodaunorubicin have been obtained; this indicates that the former compound is a precursor of both the latter ones in the fermentation process.


Doxorubicin (DX), one of the most useful drugs in antitumor chemotherapy together with the related compounds daunorubicin (DA) and 13-dihydrodaunorubicin (DDA), is a fermentation derived anthracycline antibiotic[1,2]. Several groups have studied the biosynthesis of this class of antibiotic[3-6]. Very recently Oki and coworkers[7] demonstrated that a mutant of *S. peucetius* subsp. *caesius* ATCC 27959, unable to produce DX, could efficiently convert DA to DX and DDA.

The present study, in which a DX-producing mutant of *S. peucetius* fed with $^{14}$C-DA was used, unambigously confirms that in the fermentation process DA is a precursor of DDA and of DX, and that the latter compound is formed by oxidation at the carbon atom in position 14 of DA itself.

*Feeding experiments.* Strain M 76 F.I., used in this study, derives from *S. peucetius* var. *caesius* ATCC 27952. The maintenance, seed and production media have been described elsewhere[8]. Fermentations were carried out at 28 °C in 100-ml Erlenmeyer flasks with rotary shaking for 6 days in 20 ml of production medium. One of the cultures grown for 3 days was supplemented with 100 µg of DA-[14-$^{14}$C] · HCl (prepared by G.P. Vicario, Laboratorio Radionuclidi, Farmitalia Carlo Erba, Nerviano) with sp. act. 100.8 µCi/mg and incubated for 3 more days. At the end of the fermentation, hydrolysis with oxalic acid was performed according to McGuire et al.[6], in order to release the individual glycosides, which were then quantitatively determined by HPLC. The following values were obtained: DX, 35 µg/ml: DDA, 45 µg/ml and DA, 165 µg/ml.

*DX and DDA purification.* The contents of the fermentation flask supplemented with labelled DA were divided into 2 samples of 10 ml each and separately processed as follows. Each sample was sonicated and adjusted to pH 8.6, and the antibiotics were extracted 5 times with 40 ml of a chloroform/methanol mixture (4:1, v/v). The extracts were




R = $COCH_3$ Daunorubicin (DA)

R = $COCH_2OH$ Doxorubicin (DX)

R = $CHOHCH_3$ 13-Dihydrodaunorubicin (DDA)