vitro[17]. There is also growing evidence of the interaction of the 2 vitamins in animal tissues. In some conditions, vitamin C can improve α-tocopherol metabolism; in others it may increase the demand of the body for vitamin E[18-20]. Decreased α-tocopherol content in the liver, lungs and kidneys of guinea-pigs with chronic marginal vitamin C deficiency suggests that in these organs α-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 α-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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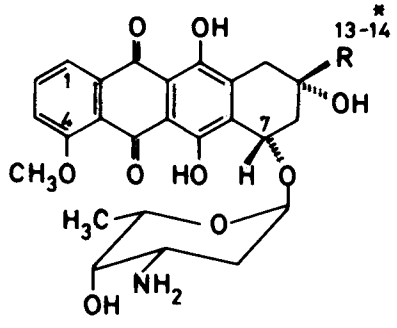
Summary. By feeding ^{14}C-daunorubicin to a doxorubicin-producing mutant of *Streptomyces peucetius,* labelled doxorubicin and 13-dihydrodaunorubicin have been obtained; this indicates that the former compound is a precursor of both the latter ones in the fermentation process.

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)

pooled and evaporated to dryness. The aqueous phase was centrifuged and the mycelium resuspended in 20 ml of water for radiochemical determination. The radioactivity administered was totally recovered (≥ 98%) in the 3 following fractions: chloroform extract 47%, aqueous phase 13% and mycelial cake 40%. The total recovery of the administered radioactivity demonstrates that in the side chain of DA no metabolic degradation occurs up to CO_2 formation. The chloroform extract was dissolved in methanol and chromatographed on several sheets of Whatman paper 3MM buffered with phosphate M/15 pH 5.4. The solvent system used was n-propanol/ethylacetate/water (7:1:2, v/v/v) (system A). A strip from the sheets was scanned for radioactivity and the 2 main peaks detected were at R_f 0.2 and 0.4 corresponding to DX and DA + DDA respectively. The red bands, wich corresponded to the radioactive peaks, were cut and eluted exhaustively with methanol/HCl 0.01 N (4:1, v/v). The neutralized eluates were concentrated in vacuo to about $^1/_{10}$ of the volume, diluted with H_2O, adjusted to pH 8.6 with Tris·HCl buffer 1 M and extracted with chloroform/methanol (4:1, v/v) (acid-base step). The extract containing DA + DDA was chromatographed on analytical silica gel plates in the solvent system chloroform/methanol/acetic acid/water (80:20:7:3, v/v/v/v) (system B) in order to separate DA (R_f 0.2) from DDA (R_f 0.3). Both the products were found to be labeled when scanned for their radioactivity. Also, DX was submitted to chromatography in the solvent system B for further purification. Spots or bands were scraped off and eluted as above for radiochemical analyses. DX, DA and DDA were subsequently chromatographed in TLC on analytical silica gel plates with solvent system chloroform/acetone (4:1, v/v) (system C) in order to reach a constant specific activity. The chemical purity of DX, DA and DDA was checked by HPLC; the radiochemical purity was controlled by scanning the plates run in system C.

Results and discussion. The specific activities of the antibiotics considered are reported in the table, from which it appears that both DX and DDA are significantly labeled. These data confirm that DA is a precursor of DX and DDA. The different values of specific activity of DX and DDA found at the end of the fermentation are the consequence of the different moments at which their production occurs, as the specific activity of DA gradually decreases throughout the fermentation owing to its constant dilution with the unlabeled biosynthesized DA.

Among the schemes proposed for the biosynthesis of antracycline antibiotics, one has been reported[9] for which 10-decarbo-methoxy-ε-rhodomicinone glycoside is considered to be the precursor of DDA by the following 2 different pathways; via DA and via 13-deoxydaunorubicin. Our data confirm the former hypothesis even though the latter cannot be completely ruled out. If this 2nd pathway had been operating in this strain, the specific activity of DDA could also have been lower than the specific activity of DA itself. The value of the specific activity of DDA, which is higher than that of DA, and the lack of evidence for production of 13-deoxydaunorubicin in detectable amounts, makes it unlikely that this 2nd biosynthetic pathway is operating, at least under the conditions and with the strain employed in our study.

Specific activities of doxorubicin (DX), daunorubicin (DA) and 13-dihydrodaunorubicin (DDA), after feeding of daunorubicin-[14-^{14}C] to cultures of *Streptomyces peucetius* strain M76 F.I.

Sample	Purification steps	Specific activities (nCi/μg) DX	DA + DDA	DA	DDA
1	Paper chromatography in system A	1.360	0.680	-	-
	Acid-base step	1.488	0.707	-	-
	TLC in system B	1.494	-	0.637	0.784
	TLC in system C	1.492	-	0.639	0.792
2	Paper chromatography in system A	1.376	0.680	-	-
	Acid-base step	1.490	0.702	-	-
	TLC in system B	1.501	-	0.632	0.778
	TLC in system C	1.504	-	0.636	0.786
	Mean values of final specific activities	1.498	-	0.638	0.789

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Nucleating agents in the haemolymph of an intertidal mollusc tolerant to freezing

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Summary. Nucleating agents are found to be present in the haemolymph of the common mussel *Mytilus edulis*. Such substances are found only during winter, and they cause the haemolymph to freeze at temperatures around −6 °C. In summer supercooling points are around −15 °C.

The mussel *Mytilus edulis* is commonly found in the intertidal zone in arctic and temperate regions. In winter, such sessile invertebrates are exposed to temperatures down to −30 °C for a period of 1-6 h twice daily[2]. *Mytilus edulis* is reported to be freeze-tolerant[3], and ice-formation is shown to take place in the extracellular compartments[4,5]. Insects tolerant to freezing are shown to have nucleating agents in the haemolymph[6,7]. The nucleating agents are