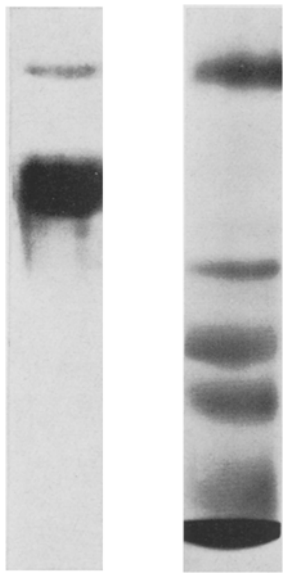


Fig. 1. Disc acrylamide gel electrophoresis patterns of control, and granuloma collagen lysate with several new degradation products. 14-day-old granuloma, 3-day incubation of tissue on collagen gel.

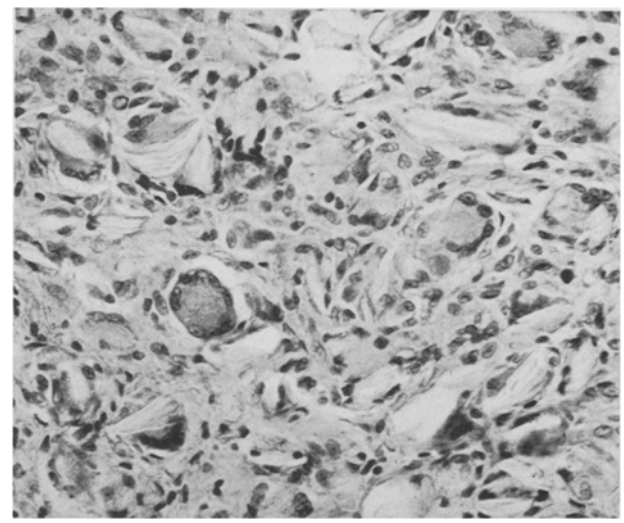

Fig. 2. Granuloma, photomicrograph of central area showing talc, macrophages and giant cells. H.E. ×205.

to be associated with fibroblast proliferation. Periods of higher collagen lysis occurred with increased fibroblast populations. However, in our studies maximum lysis occurred at 14 days, when histological examination showed a nearly exclusive macrophage and giant cell population (Figure 2), with only occasional fibroblasts at the periphery of the lesion. Enzyme histochemical examination of cryostat sections of tissue (macrophages and giant cells) surrounding the explants demonstrated intense acid phosphatase (pH 5.2) and aminopeptidase (pH 5.5 and 6.8) activity[6]. From these observations, it is believed that a collagenase was associated with the highly lytic enzyme-active macrophage and giant cell population in the central areas of the talc granulomas.

This preliminary observation is part of a continuing study which will be reported more extensively at a later date.

*Zusammenfassung.* Ein kollagenolytisches Enzym wurde in Talkgranulomen und im Zusammenhang mit Makrophagen sowie Riesenzellen gefunden und elektrophoretisch charakterisiert.

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## Interaction of Daunomycin with Nucleic Acids: Effect of Photoirradiation of the Complex

The biological activity of daunomycin is believed to be related to its ability to interact with the 'primer' DNA[1,2], thus inhibiting not only DNA-dependent RNA synthesis[3,4], but also DNA duplication[4]. Photodynamic activity of daunomycin against some DNA and RNA viruses has been described[5,6].

Daunomycin, a fluorescent glycosidic antibiotic of the anthracycline group, could sensitize photodynamic effects[7]. Preliminary irradiation (UV and visible) experiments of its DNA complex indicated that a stable combination of daunomycin with DNA took place. Various methods were used to test the stability of the photoirradiated complex: dialysis, gel filtration, solvent extraction and enzymatic digestion. We report here studies on the effects of UV-irradiation of daunomycin-nucleic acid complexes.

*Materials and methods.* In a typical experiment, a solution of calf thymus DNA ($5 \times 10^{-3} M$ of DNA phosphorus)

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and daunomycin ($5 \times 10^{-4} M$) in $0.01 M$ Na phosphate buffer (pH 6.8) was irradiated with UV-light (Westinghouse sterilamp, 15 W) in contact with air at room temperature. The distance between the light source and the sample was 10 cm.

For the binding study, after photoirradiation daunomycin was extracted (5 times) by water-saturated phenol at room temperature. The optical density of DNA solution was measured at 475 nm after exhaustive dialysis against water or after ether extraction.

*Results and discussion.* As shown in Table I, calf thymus DNA irradiated in presence of daunomycin, extracted with phenol and dialyzed, showed a gradual increase of optical density at 475 nm with increasing length of irradiation. Optical density in the visible region was not observed without irradiation. Similarly, the control solution of irradiated DNA alone showed no absorption in the visible region.

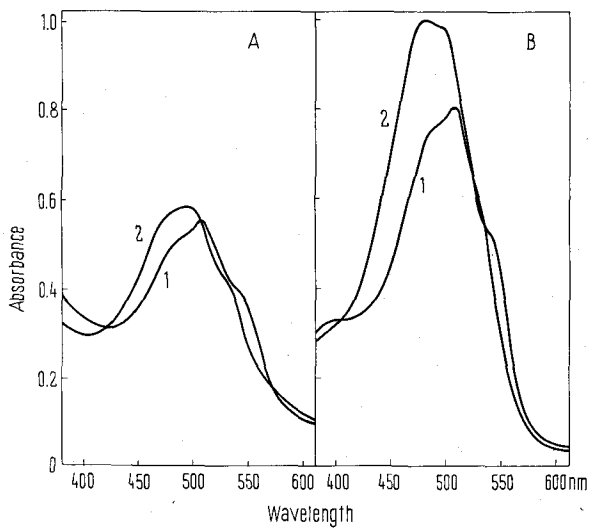


Fig. 1. Spectral changes of daunomycin-DNA physical and photoirradiated complex upon exposure to DNase. A) Daunomycin-DNA irradiated (for 18 h) complex (cf. legend to Table I). 1. Before DNase digestion. 2. After digestion by DNase (100 μg/ml) for 12–24 h at 37 °C, in $0.015 M$ of Mg-Ac. B) Daunomycin-DNA (1/10) physical complex in $0.01 M$ sodium phosphate; pH 6.8. 1. Before DNase digestion. 2. After digestion by DNase.

The visible absorption spectrum of DNA irradiated in the presence of daunomycin, after phenol extraction (Figure 1 A, curve 1), exhibits a form very close to that of DNA-daunomycin physical complex (non irradiated) (Figure 1 B, curve 1). When DNA-daunomycin photoirradiated complex is hydrolized by pancreatic DNase, the absorption maximum shifts to shorter wavelength (Figure 1 A, curve 2), but the spectra characteristic of the pure daunomycin was not restored, as we found after digestion of DNA-Daunomycin physical complex (Figure 1 B, curve 2).

Digestion products of $^3$H-Daunomycin-DNA irradiated complex (Figure 2 B) were separated by thin-layer chromatography on silica gel. The enzymatic hydrolysate has 2 strongly red-colored and radioactive spots showing the fluorescence characteristic of daunomycin; one spot corresponds to free daunomycin in Rf value, the other UV absorbing spot remains at the origin. As a control, $^3$H-daunomycin-DNA complex without exposure to photoirradiation was digested by DNase. Elution of the control (Figure 2 A) resulted in removing over 99% of the radioactivity from the origin. UV-absorbing material (digestion products of DNA) was found to be retained at the origin. Repeated experiments of this nature, showing association of radioactivity and fluorescence characteristic of daunomycin with DNA components, clearly indicated that a firm combination had been generated between daunomycin and DNA, as an effect of irradiation.

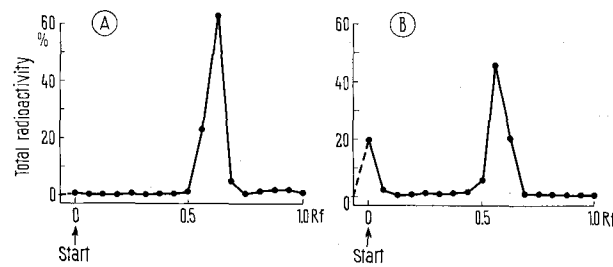


Fig. 2. Silica gel thin-layer chromatogram of A) non-irradiated $^3$H-daunomycin-DNA complex, B) irradiated $^3$H-daunomycin-DNA complex after DNase digestion. In B) a solution of DNA ($6 \times 10^{-3} M$ of DNA phosphorus) and of Daunomycin ($6 \times 10^{-4} M$, specific activity 20 μC/mg) in $0.01 M$ sodium phosphate was irradiated for 24 h. After phenol extraction and dialysis, the DNA was treated by pancreatic DNase (100 μg/cc) for 12 h at 37 °C. Solvent system: Benzyl alcohol-Formic Acid-Ethyl Formate-$H_2O$ (4:1:4:5, v/v).

Table I. Effect of irradiation on solvent extraction of daunomycin from its native DNA complex

| Irradiation (h) | O.D. 475 nm |
|---|---|
| 0 | 0.010 |
| 14 | 0.443 |
| 18 | 0.553 |
| 22 | 0.636 |
| 30 | 0.737 |

In these experiments 16.5 ml of aqueous solution ($1 \times 10^{-2} M$ Na phosphate buffer, pH 6.8) of DNA ($5 \times 10^{-3} M$ of DNA phosphorus) and of daunomycin ($5 \times 10^{-4}$) were irradiated in dish (9.5 cm of diameter) at 10 cm from a light source. Daunomycin was extracted ( × 5) by phenol. Phenol was removed by exhaustive dialysis against water in a darkened room at about 4 °C. Phenol in external solution was assayed by UV absorbance.

Table II. Effect of irradiation on solvent extraction of daunomycin from its denatured DNA complex

| Irradiation (h) | O.D. 475 nm |
|---|---|
| 0 | (colourless) |
| 22 | 0.159 |
| 30 | 0.280 |
| 41 | 0.323 |

DNA was denatured in phosphate buffer by heating in boiling water for 10 min, after which the solution was quickly cooled in ice for 30 min. Conditions are the same as those described in legend to Table I. Phenol was removed by ether extraction. The OD 475 nm was read after evaporation of each sample to 10 ml.

Also heat-denatured DNA irradiated in presence of daunomycin and worked out in the same experimental conditions described for native DNA, formed a stable combination with daunomycin (Table II).

The observations reported in this communication are only qualitative; and at this stage in the investigation no attempt was made to characterize the complex that is formed by photoirradiation and no conclusion can be drawn with respect to the mechanism of the reaction. Studies are in progress to elucidate whether daunomycin reacts with free purine and pyrimidine nucleosides or nucleotides. Preliminary experiments with apurinic and apyrimidinic DNA (Figure 3) show that only with apyrimidinic DNA it is possible to obtain after photoirradiation a stable combination with daunomycin showing a spectrum similar (curve 1) to that of daunomycin-DNA irradiated complex. This suggests that purinic bases may be reactive sites of DNA.

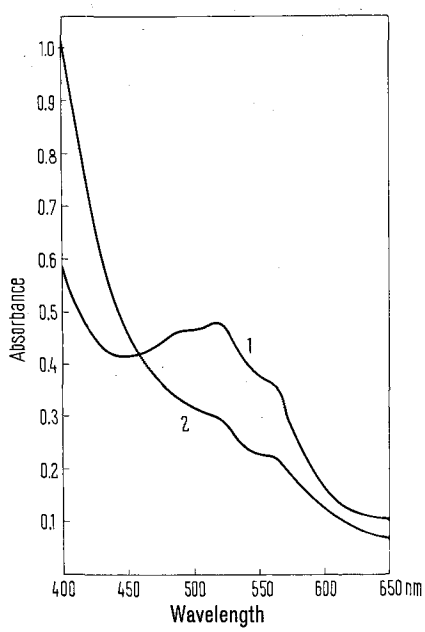


Fig. 3. Spectra of apyrimidinic DNA-Daunomycin 1) and apurinic DNA-Daunomycin irradiated complexes 2). Apurinic and apyrimidinic DNA's were obtained according to TAMM[8] and SHAPIRO[9] respectively. In this experiment a PCQ-XI photochemical lamp (Ultraviolet Products) was used. A solution of DNA (about $8 \times 10^{-3} M$ of nucleotide phosphorus) and of Daunomycin ($2 \times 10^{-4}$) in $0.01 M$ Na phosphate (pH 6.8) was irradiated for 15 min. The spectrum was recorded after phenol extraction.

Daunomycin-DNA complexes are irradiated in the UV region so that both bases and daunomycin are excited. The obvious possibility of direct damage to DNA has as yet to be established. Using visible light so that only daunomycin is excited, preliminary experiments indicated that, besides direct damage to DNA, a stable combination of daunomycin with native DNA took place. The possibility may exist that, in the case of photoinactivation of viruses[5,6], reaction mechanisms similar to photochemical binding may be involved. In conclusion, the unusual strength of the photochemical binding of daunomycin to DNA raises the possibility of the formation of a covalent bond between daunomycin and nucleic acids, as a consequence of irradiation. The results observed could have interesting implications for the explanation of the biological effects of daunomycin.

*Riassunto.* Quale effetto dell'irradiazione UV è stato osservato un legame insolitamente forte tra daunomicina ed acidi nucleici, che suggerisce la possibilità della formazione di un legame covalente.

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## Morphogenic Effects of Halogenated Thymidine Analogs on *Drosophila* III. 5-Iododeoxyuridine[1]

When *Drosophila* larvae are fed a mixture of 5-bromodeoxyuridine (BUdR) and 5-fluorouracil (FU), the hatching adult flies show a variety of developmental lesions including instances of supernumerary tissue growth as well as bristles in place of hairs[2,3]. Under similar conditions of treatment, FU is ineffective in stimulating growth while BUdR administered alone induces a low frequency of developmental modifications. These observations suggest that treatment with BUdR is the prime factor in upsetting normal growth processes in *Drosophila* while FU amplifies this effect.

BUdR, a thymidine analog, is incorporated into DNA, and the level of its incorporation can be increased by inhibiting de novo synthesis of thymidine monophosphate. 5-Fluorodeoxyuridine is a potent inhibitor of thymidylate synthetase[4], and has been used to create thymidine deficient conditions in a number of biological systems[5-7]. Since the presence of FU increased the amount of BUdR incorporated into *Drosophila* DNA[8], presumably one of the roles of FU following ingestion by *Drosophila* larvae is the inhibition of thymidylate synthetase. In order to

[1] This research was supported by a grant No. DRG-1113 from the Damon Runyon Memorial Fund.

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