value of the enzymatic activities with acetylcholine and quinolinium compound were 68 and 67 sec respectively, the denaturation curves were identical.

The localizations of the particle bound esterase activity over a continuous (0·8–1·7 M) sucrose gradient were very similar for both substrates.

8-Bu-Q was not hydrolysed faster in normal than in the eserinized rat brain homogenates. Apparently 8-Bu-Q is not detectably hydrolysed by AChE. In rat plasma, 8-Bu-Q is hydrolysed very quickly. This is an indication that it is possible to differentiate with this substrate between AChE and cholinesterase (EC 3.2.1.8) activity.

Acknowledgement—The synthesis of the 1-methyl-acylquinolinium derivatives was performed by Mr. G. P. Overbeek.

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Interaction of some daunomycin derivatives with deoxyribonucleic acid and their biological activity

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THE BIOLOGICAL activity of daunomycin is dependent on the ability to interact with primer DNA.[1]

In order to obtain further information about the mode of action of daunomycin, we studied some physicochemical properties of the complex which DNA forms with some daunomycin derivatives and their biological activity.

In the interaction of a chemical compound with biological material, the observed effects are in some cases highly specific, so that small changes in the molecule structure lead to marked changes in the biological activity.

We tested two groups of daunomycin derivatives. The derivatives of the first group have changes in acetyl side chain (R) in 9-position of the saturated ring of daunomycinone.

Among these the following were studied:

14-hydroxy-daunomycin (R = —CO.CH_2OH),
13-dihydro-daunomycin (R = —CHOH.CH_3),
daunomycin oxime (R = —CNOH.CH_3),
daunomycin semicarbazone (R = —CNNHCONH_2.CH_3),
daunomycin thiosemicarbazone (R = —CNNHCSNH_2.CH_3).

The derivatives of the second group have alterations in the amino-sugar (daunosamine). Among these are the following: *N*-acetyl-daunomycin, daunomycin-*N*-guanidine-acetamide, 2-amino-2-α-D-deoxy glucosyl-daunomycinone. A natural derivative of daunomycin with 2 moles of daunosamine

per mole of daunomycinone differs from these. The exact position of the second amino-sugar is not known.

The *in vitro* interaction of daunomycin and its derivatives with calf thymus DNA has been examined by the effects of DNA on absorption intensity of the chromophore at 480 mμ, by the effects of antibiotics on viscosity of DNA and by the displacement of methyl green from its DNA complex.

The reaction of daunomycin with DNA in solution is accompanied by a change in the spectral properties of the chromophore.[2]

The variation of absorbance at 480 mμ can be taken as an indication of extent of binding.[3] Like daunomycin, in presence of DNA, the visible absorption spectra of the derivatives studied were depressed and red shifted.

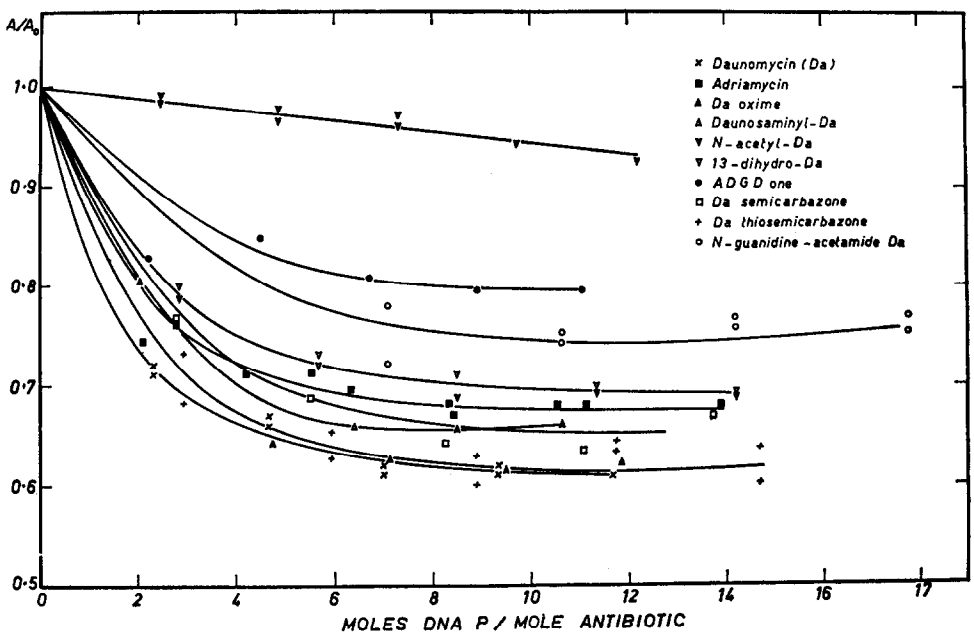


FIG. 1. Effect of calf thymus DNA on absorption intensity of daunomycin and its derivatives at 480 mμ. The antibiotic concentration was $0{\cdot}5 \times 10^{-4}$ M in pH 7·0 tris buffer of $1{\cdot}25 \times 10^{-2}$ M. DNA-antibiotic complexes were prepared by mixing 2 ml of antibiotic solution with 2 ml of DNA solution at various concentrations. The optical density at 480 mμ was read after storage over night. ADGDone: 2-amino-2-α-D-deoxy-glucosyl-daunomycinone.

Figure 1 shows the decrease of absorption intensity at 480 mμ of these substances on adding DNA. The hypochromic effect of DNA on the absorption of chromophore at 480 mμ, although indicating for all the substances an interaction with DNA did not reveal marked difference for most compounds.

In contrast DNA had a weak effect on the absorption intensity of *N*-guanidine-acetamide-daunomycin and especially of 2-amino-2-deoxy-glucosyl-daunomycinone and, as has already been observed,[2] for *N*-acetyl-derivative.

These spectrophotometric titrations cannot be used to construct absorption isotherms, because our conditions of measurement required a high DNA concentration.

At this DNA concentration the free antibiotic concentration was too small to be quantitated. In attempting to obtain further indications on the affinity of tested compounds for DNA, we studied the effect of daunomycin and its derivatives on viscosity of DNA.

According to Lerman's hypothesis[4] on the interaction of amino acridines with DNA, an increase in intrinsic viscosity in the complex is one of criteria for intercalation of ring systems between base pairs of double-helical DNA.

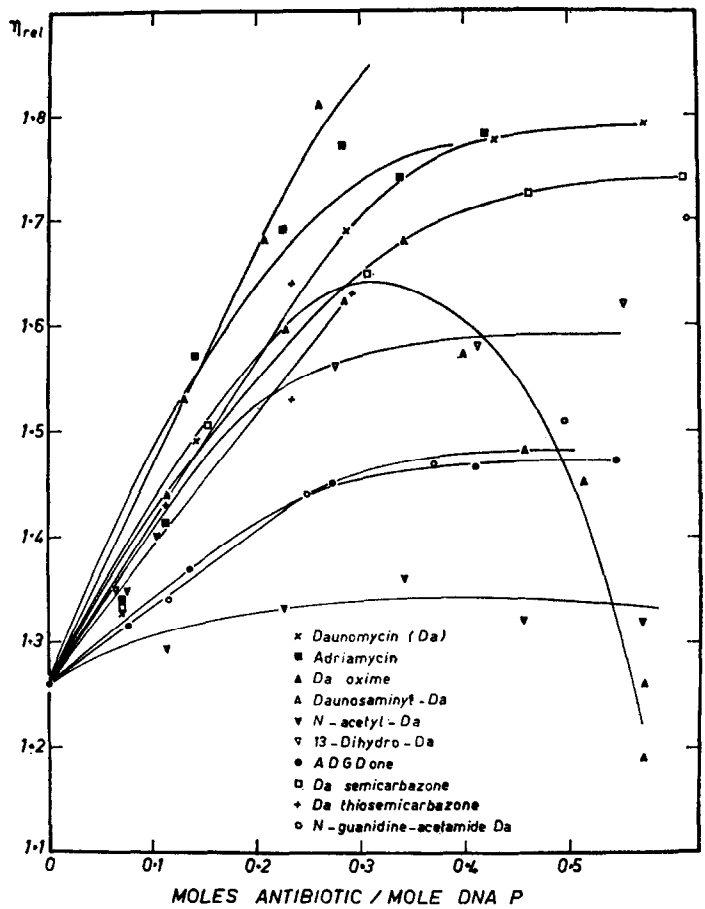


FIG. 2. Effect of daunomycin and its derivatives on viscosity of DNA. All solutions were made in 0·1 M NaCl. The DNA concentration was 50 μg/ml in all cases.

Figure 2 shows the effect of the antibiotics on relative viscosity of DNA by increasing antibiotic–DNA ratios. The increase in relative viscosity up to a molar antibiotic–DNA ratio of 0·2 is similar for most substances tested.

On the contrary a very moderate increase in relative viscosity was observed for *N*-acetyl-derivative, daunomycin-*N*-guanidine-acetamide and 2-amino-2-deoxy-glucosyl-daunomycinone, i.e. for the compounds with alterated amino-sugar.

The intrinsic viscosity increase of the rod-like DNA-drugs complex has been correlated with an increase in the molecule length of rod-like DNA.[5, 6] The measurements in this study report only the relative viscosity of native DNA of high molecular weight. A theoretical investigation and a quantitative analysis was beyond the scope of the present study. We may outline that viscosity enhancement could be interpreted as an effect similar to intrinsic viscosity increase.

The ability of daunomycin and its derivatives to bind to DNA was analysed further by the displacement of methyl green from its DNA complex (Fig. 3). The ability of the competing compound to displace methyl green can be taken as an indication of affinity for DNA.[7] From the results obtained (Fig. 3), the compounds are arranged in order of decreasing affinity for DNA (Table 1). Only the compounds with alterated amino-sugar (daunomycin-*N*-guanidine-acetamide, 2-amino-2-deoxy-glucosyl daunomycinone, and *N*-acetyl-daunomycin) behaved differently from the other compounds.

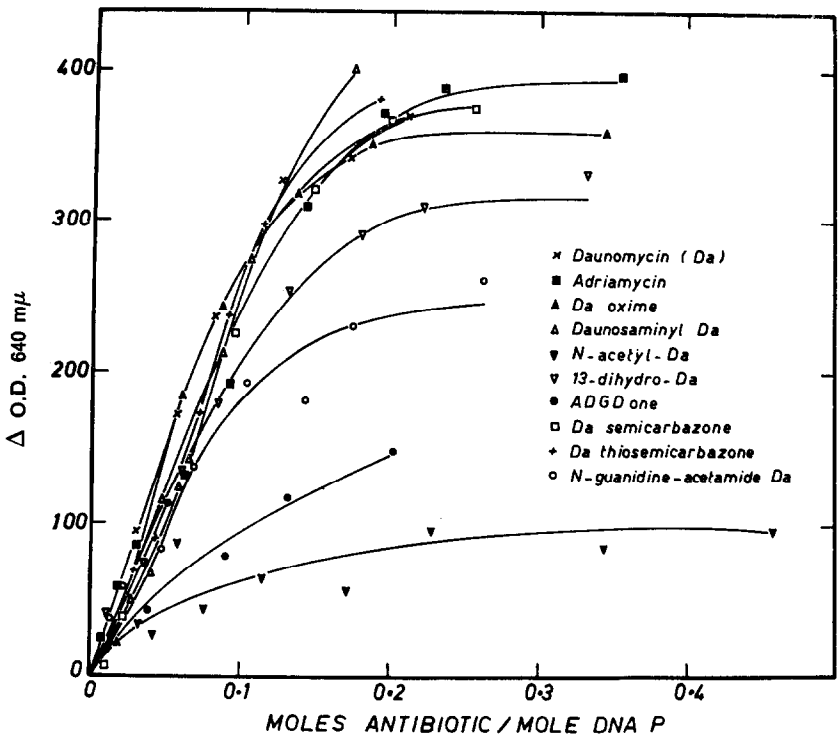


FIG. 3. Displacement of methyl green from its complex with calf thymus DNA by daunomycin and its derivatives. 0·5 ml of antibiotics solution at various concentrations were added to 2 ml MG-DNA complex (substrate for alkaline DNase[8] in 0·025 M tris, pH 7·5). The final concentrations of DNA is 0·12 mg/ml. The O.D. 640 mμ was read after storage in the dark overnight. The results are expressed as the difference at O.D. 640 with and without antibiotic.

Thus, although none of these methods affords an exact estimate of the affinities of the tested compounds for DNA, they can give some indication of their ability to bind to DNA.

It appeared significant the observed correspondence between the decrease in extinction of the compounds caused by DNA, the drug-induced increase in relative viscosity of DNA and their ability to displace methyl green from its DNA complex.

Thus, the tested compounds can be divided into two groups, one which showed an affinity for DNA similar to that of daunomycin, including the derivatives with alterated acetyl side chain and 4′-daunosaminyl-daunomycin, and the other which showed a reduced ability to bind to DNA, including the derivatives with alterated amino-sugar.

Likewise when these compounds were tested for the effects on mitotic activity (Table 1), it was possible to divide them into two groups, i.e. one which presented a more or less elevated action which increased with the increase in dose, and the other which did not cause mitotic inhibition.

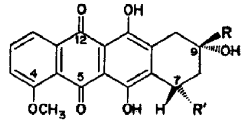
The inhibiting activity on DNA synthesis appeared restricted to a more limited number of substances. A 50 per cent inhibition of DNA synthesis was obtained only with daunomycin at a concentration of 1 μg/ml, with adriamycin and 4′-daunosaminyl-daunomycin at a concentration of 2 μg/ml, and with 13-dihydro-daunomycin and 2-amino-2-deoxy-glucosyl-daunomycinone at a concentration of 5 μg/ml.

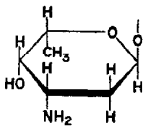
Likewise the proliferative activity appeared markedly reduced in cultures treated with daunomycin, adriamycin and more moderately with daunosaminyl-daunomycin and 13-dihydro-daunomycin.

In conclusion, the examination of the interaction of daunomycin and its derivatives with DNA *in vitro* and of their biological activity reveals three different groups of compounds (Table 1).

Whenever the amino hydrogens of daunosamine are not substituted and CO group of acetyl side chain is not alterated, the compounds are found to complex strongly to DNA and to inhibit mitotic

TABLE 1. BIOLOGICAL ACTIVITY OF DAUNOMYCIN AND ITS DERIVATIVES

General structure 

Structure of daunosamine 

N	Compound	R	R′	Antibiotic/DNAP*	50% Inhibiting doses: Mitotic Index 2 hr† (M × 10⁶)	Mitotic Index 8 hr† (M × 10⁶)	DNA Synthesis‡ (M × 10⁶)	Cell proliferation§ (M × 10⁶)
1	Daunomycin.HCl	—CO.CH₃	Daunosamine	0·076	0·44	0·18	1·6	0·89
2	Adriamycin.HCl	—CO.CH₂OH	Daunosamine	0·090	0·43	0·09	3·4	1·9
3	4′-Daunosaminyl-Daunomycin.2HCl	—CO.CH₃	4′-Daunosamiyl-Daunosamine	0·090	0·96	0·27	2·7	5·5
4	Daunomycin-thiosemicarbazone	CH₃C=NNHCSNH₂	(?)	0·090	1·60	1·20	>8·3	>8·3
5	Daunomycin.oxime.HCl	CH₃C=NOH	Daunosamine	0·078	4·30	4·70	>8·6	>8·6
6	Daunomycin semicarbazone	CH₃C=NNHCONH₂	Daunosamine	0·090	5·10	2·60	>8·5	>8·5
7	13-Dihydrodaunomycin.HCl	—CHOH.CH₃	Daunosamine	0·114	3·50	1·20	8·8	7·9
8	*N*-guanidine-acetamide-daunomycin.HCl	—CO.CH₃	*N*-guanidine-acetamide-daunosamine	0·150	>7·60	>7·60	>7·6	>7·6
9	2-Amino-2-deoxy-glucosyl-daunomycinone.HCl	—CO.CH₃	2-Amino-2-α-D-deoxy-glucose	>0·200	>8·80	8·80	8·8	>8·8
10	*N*-acetyl-daunomycin.HCl	—CO.CH₃	*N*-acetyl-daunosamine	>0·200	>8·30	>8·30	>8·3	>8·3

* Molar ratio antibiotic: DNA P for 50 per cent displacement of methyl green from its complex with calf thymus DNA by Daunomycin and its derivatives. The displacement of methyl green was measured as described in legend to Fig. 3.

† 24 hr after the transplant HeLa cells were treated with the antibiotic at different concentrations (from 0·05 to 5 μg/ml). Part of the cultures were fixed after 2 hr and the other part after 8 hr from the beginning of the treatment.

‡ DNA synthesis was studied by incorporation of [³H]thymidine, using the autoradiographic method of stripping film.

The radioactive precursor (2 μg/ml, s.a. 16 mc/mM) was introduced into the medium after 1 hr of antibiotic treatment; HeLa cells were fixed after 7 hr from this treatment.

§ 16 hr following the transplant, the cultures of HeLa cells (in 6 cm Falcon plates) were treated for 8 hr with the antibiotic, and 50 hr after the beginning of treatment the cultures were trypsinized.

activity, DNA synthesis and proliferation of HeLa cells. This includes daunomycin, adriamycin and daunosaminyl-daunomycin. When CO group of acetyl side chain is alterated, the derivatives preserve the ability to bind to DNA and a reduced antimitotic activity, but are inactive or only slightly active in inhibiting DNA synthesis and cellular proliferation. Examples are daunomycin-thiosemicarbazone, daunomycin-oxime, daunomycin-semicarbazone and 13-dihydro-daunomycin.

When daunosamine is exchanged for α-D-glucosamine or if amino group is masked, the derivatives are found to bind to DNA very weakly and to lack biological activity.

The results once again suggest that the ability of these compounds to bind to DNA is closely connected with the special chemical structure of the amino sugar.[2]

Work is proceeding in this laboratory to elucidate further possible correlations between the chemicophysical properties of the complex formed by daunomycin derivatives and their biological activity.

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Effect of phenobarbital treatment on lysosomal enzyme activity in rat liver

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Phenobarbital is known to stimulate the formation of smooth-surfaced endoplasmic reticulum in the liver cell[1] and to induce a number of microsomal drug metabolizing enzyme systems.[2] In the course of studies on the effect of phenobarbital on the submicrosomal distribution of the enzyme UDP glucuronyltransferase from rat liver[3] a reduced acid phosphatase (EC 3.1.3) activity per gram of liver was found after this pretreatment during 4 days. Acid phosphatase was used as marker enzyme for the lysosomal fraction in which it is known to be located.[4] A decrease in the activity of four lysosomal enzyme activities is reported in the present study.

Materials and methods

Male rats (TNO, Zeist, The Netherlands) weighing 190–250 g who had free access to food and water were used. The animals received intraperitoneal injections of phenobarbital (90 mg/kg) at 4 p.m. on each of 4 consecutive days. About 9 a.m. on the day after the last injection the rats were decapitated, the livers were excised and removed into ice-cold 0·154 M KCl. A 20% (w/v) homogenate in 0·154 M KCl was made with a Potter–Elvehjem homogenizer with teflon pestle at 0–4°.

All enzyme activities were measured in the diluted homogenates during incubations at 37°. UDP glucuronyltransferase was assayed as described before[3] with *p*-nitrophenol as substrate; the enzyme preparations were activated by the detergent Triton X-100 *in vitro*.[3,5] Cathepsin B and C were determined by the microdiffusion method of Conway as described by Bouma.[6] Glucose-6-phosphatase