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Degradation of doxorubicin and daunorubicin in human and rabbit biological fluids

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Abstract: The stability of doxorubicin (DOX) and daunorubicin (DNR) in rabbit and human plasma, bile and urine and in rabbit faeces was studied in the presence or absence of light, and at body, room and cold room temperatures. Fluorescence was determined by spectrofluorimetry after normal and reversed phase HPLC. Under each set of conditions, DOX and DNR fluorescence decreased with time; the decrease was more rapid with DOX. As the parent drugs were degraded, apolar compounds were formed which behaved like 7-deoxyaglycones and generally did not compensate for the loss in fluorescence of the parent drug. The degradation of anthracyclines occurred even in the absence of light, was not due to bacterial contamination and was faster at higher pH or temperature. The rapid degradation of DOX and DNR in biological fluids at body temperature may have implications on the disposition of anthracyclines *in vivo*. Prior to analysis, biological fluids and solutions containing anthracyclines should be processed quickly at 4°C, in the absence of light, and at a pH no greater than 6 to avoid degradation.

Keywords: *Doxorubicin; daunorubicin; anthracyclines; normal-phase HPLC; reversed-phase HPLC; degradation* in vitro*; biological fluids.*

Introduction

The anthracycline antibiotics doxorubicin (Adriamycin) (DOX) and daunorubicin (DNR) are widely used in the treatment of neoplastic diseases [1]. The stability of DOX and DNR has been studied in different infusion fluids [2–5]; however, only a minimal amount of information is available on the stability of anthracycline antitumour agents in biological fluids. In human whole blood samples, Eksborg *et al.* [6] observed rapid degradation of DOX and DNR. In human plasma samples, these authors reported a higher rate of degradation with increasing temperature and with repeated freezing and thawing of samples. The instability of other anthracyclines in human plasma has also been observed [7, 8]. Assaying total fluorescence, Tavoloni *et al.* [9] reported no loss of fluorescence over 144 h when DOX was dissolved in fresh rat bile and exposed to high-intensity light at room temperature.

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When studying the pharmacokinetics of DOX and DNR, it is important to take precautions in the handling of biological samples to avoid artifacts prior to the quantitative determination of the drugs and their metabolites. The purpose of the present study was to ascertain the stability of DOX and DNR in solutions at pH 4.0–10.0 and in rabbit and human plasma, bile and urine as well as in rabbit faeces. The influence of temperature, exposure to light, microbial contamination and pH on the degradation of DOX and DNR was also considered.

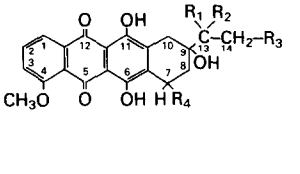
Materials and Methods

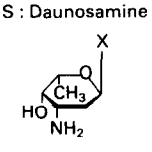
Chemicals

DOX was obtained from Roger Bellon (Paris, France). DNR, daunorubicinol (DOL) and 7-hydroxydaunorubicin aglycone (DNRONE) were provided by Rhone-Poulenc (Vitry-sur-Seine, France). Doxorubicinol (DOXOL) was prepared by reduction of DOX using $NaBH_3CN$ [5]. 7-Hydroxydoxorubicin aglycone (DOXONE), 7-hydroxydoxorubicinol aglycone (DOXOLONE) and 7-hydroxydaunorubicinol aglycone (DOLONE) were prepared by mild acid hydrolysis of DOX, DOXOL and DOL, respectively [10]. DNR, DOX, DOL and DOXOL were converted to their 7-deoxyaglycones: 7-deoxydaunorubicin aglycone (7-d DNRONE), 7-deoxydoxorubicin aglycone (7-d DOXONE), 7-deoxydaunorubicinol aglycone (7-d DOLONE) and 7-deoxydoxorubicinol aglycone (7-d DOXOLONE) by reductive hydrolysis using $Na_2S_2O_4$ as described by Smith *et al.* [11]. Figure 1 illustrates the structures of the different anthracyclines and their aglycones. All other chemicals and solvents used for analysis were of analytical grade.

Rabbit sample collection

New Zealand White rabbits were obtained from Animalabo (Brussels, Belgium). For collection of faeces and urine, the rabbits were placed in metabolism cages. Arterial blood samples were withdrawn from the midline ear artery into heparinized tubes and immediately centrifuged to separate the plasma used for the assay. To collect rabbit bile,





COMPOUND	R_1	R_2	R_3	R_4
DNR	=O		–H	–O–S
7–hydroxy DNR–ONE (DNR–ONE)	=O		–H	–OH
7–deoxy DNR–ONE	=O		–H	–H
DNR–OL (DOL)	–OH	–H	–H	–O–S
7–hydroxy DNR–OL–ONE (DOL–ONE)	–OH	–H	–H	–OH
7–deoxy DNR-OL-ONE	–OH	–H	–H	–H
DOX	=O		–OH	–O–S
7–hydroxy DOX–ONE (DOX–ONE)	=O		–OH	–OH
7–deoxy DOX–ONE	=O		–OH	–H
DOX–OL	–OH	–H	–OH	–O–S
7–hydroxy DOX–OL–ONE (DOXOL–ONE)	–OH	–H	–OH	–OH
7–deoxy DOX–OL–ONE	–OH	–H	–OH	–H

Figure 1
Chemical structures of daunorubicin (DNR) and doxorubicin (DOX) and their metabolites.

cannulation of the common bile duct was performed under general anaesthesia. With the rabbit in the dorsal recumbent position, the common bile duct was isolated through a ventral midline incision. A silicone catheter (0.8 mm i.d., Vygon, Ecouen, France) was threaded through a small incision in the common bile duct and secured with a 3–0 silk suture. The bile was collected in glass tubes and assayed as such.

Human sample collection

Fresh human plasma and urine were obtained from a volunteer. Human common duct bile was collected by means of a choledochal T tube inserted into a patient for the treatment of obstructive jaundice.

Incubation of samples

The influence of different pH levels (pH 4–10) on the stability of DNR and DOX was studied. A stock solution of DOX or DNR was prepared and diluted with buffers or biological fluids to obtain a final anthracycline concentration of 10 $\mu g\ ml^{-1}$. DOX or DNR was stored at 37°C in the following 0.1 M buffers: borate buffers pH 9.0 and 10.0; phosphate buffers pH 6.0, 7.0 and 8.0; and acetate buffers pH 4.0 and 5.0. Aliquots were removed for analysis every 5 min when degradation was rapid and otherwise at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72 and 96 h.

To study the stability of anthracyclines in biological fluids DOX or DNR was added to human and rabbit plasma, bile and urine. Human samples were stored at 37°, 23° and 4°C, rabbit samples at 39° (rabbit body temperature), 23° and 4°C, and exposed to normal room lighting (TL fluorescent lamps 65 W, Philips, Eindhoven, The Netherlands). Aliquots of biological fluids were removed as described for buffers.

To evaluate degradation due to bacterial contamination, human plasma and urine containing 10 $\mu g\ ml^{-1}$ of DOX or DNR were incubated at 37°C after sterile filtration through a 0.22 μm filter (Millipore, Bedford, Mass.).

To study the influence of light on anthracycline degradation in biological fluids, plasma, bile and urine containing 10 $\mu g\ ml^{-1}$ DOX or DNR were protected from light and incubated at 37° and 4°C. Aliquots of each biological fluid were removed for analysis as described above.

Rabbit faeces were homogenized and DOX or DNR (2 $\mu g\ ml^{-1}$) was added. The mixture was protected from light and incubated at 39°C. An aliquot was sampled for analysis at 0, 15, 30, 45, 60, 90 and 120 min.

Chromatography

Normal-phase (NP) HPLC. DNR (for DOX analysis) and DOX (for DNR analysis) were used as internal standards. Anthracyclines were extracted at alkaline pH with chloroform–methanol (6:1, v/v). An aliquot of the organic layer was injected into the chromatograph using a Lichrosorb Si-60 column and an isocratic eluting mixture of chloroform–methanol–acetic acid–0.3 mM $MgCl_2$ in water (720:210:40:30, v/v) at a flow rate of 1.5 ml min^{-1}. Detection was performed by spectrofluorimetry at 480 nm (excitation) and 560 nm (emission) as described previously [12]. The same intrinsic fluorescence was assumed to determine the concentration of each compound.

Reversed-phase (RP) HPLC. The biological samples were mixed with an equal volume of acetonitrile and centrifuged. The aliquots of buffers were mixed with borate buffer (0.5 M pH 9.2) and acetonitrile (1:1:2, v/v) and centrifuged. The supernatants were

chromatographed isocratically on a μBondapak-phenyl column (Waters, Milford, Mass.) using an eluting mixture of formate buffer (16 mM pH 4)–acetonitrile (35:65, v/v) at a flow rate of 2 ml min^{-1} and as described by Robert [13]. Detection was performed by spectrofluorimetry [12].

All samples were assayed by NP HPLC to quantify parent drug and metabolites. Rabbit urine and light-protected human plasma were assayed by RP HPLC and rabbit urine was also assayed by direct fluorimetry using interference filters at 480 nm for excitation and 560 nm for emission. RP HPLC was used to identify DOX and DNR as well as their degradation products in all biological fluids and buffers.

Data analysis

The time needed for 50% loss in the initial fluorescence of the drug ($t_{50\%}$ value) was determined from the plot of percentage of original drug fluorescence against time.

Results

Chromatography

More than 99% of DOX and DNR were extracted from plasma and phosphate-buffered saline pH 7.4 (NaCl 140 mM, KCl 3 mM, KH_2PO_4 8 mM in distilled water) [12, 14]. Similar results were obtained from bile and urine. Figure 2 illustrates the separation of DOX and DNR and some of their metabolites. Table 1 shows the retention times

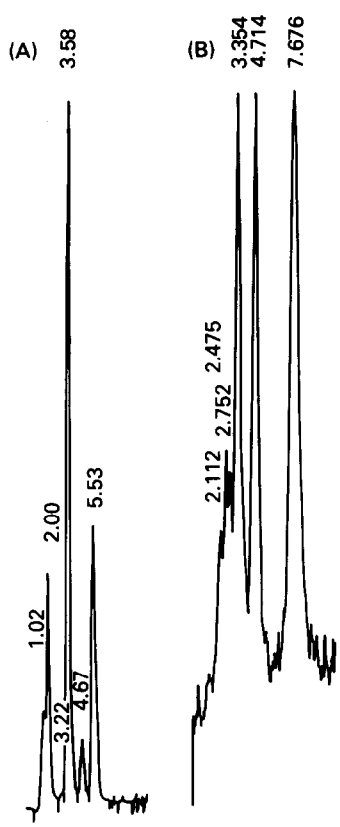


Figure 2
Separation of several anthracyclines and their aglycones by HPLC. Chromatogram A (normal-phase HPLC). Retention times were: 1.8 min (DNRONE), 2.0 min (DOXONE), 3.6 min (DNR), 4.7 min (DOL), 5.5 min (DOX). Chromatogram B (reversed-phase HPLC). Retention times were: 2.5 min (DOXOL), 3.4 min (DOX), 4.7 min (DOXONE), 7.7 min (7-d DOXONE).

Table 1
Relative retention times of various anthracyclines by reversed-phase HPLC

Compound	Retention time ratio*	Compound	Retention time ratio*
DOXOL	0.46	DOL	0.70
DOXOLONE	0.54	DOLONE	0.84
DOX	0.61	DNR	1.00
DOXONE	0.77	7-d DOLONE	1.26
7-d DOXOLONE	0.82	DNRONE	1.39
7-d DOXONE	1.23	7-d DNRONE	2.31

* Retention time ratio = retention time of compound/retention time of DNR.

relative to that of DNR for the various standards analysed by RP HPLC. The NP method gave similar results to the RP method for the quantitation of DOX and DNR. In the RP method the different aglycones of DOX or DNR were separated from each other, from parent drugs and from endogenous fluorescent constituents of biological fluids.

Degradation in buffers

The degradation of DOX and DNR in 0.1 M buffers (pH 4.0–10.0) at 37°C and the formation of apolar compounds are shown in Fig. 3. At pH 9.0 and 10.0, the degradation of DOX and DNR was very rapid. Decomposition was indicated by an immediate colour change of the solutions from red to blue–purple and finally complete discoloration. No apolar compound formation was observed. However, the traces of DOXONE, DNRONE and DOL contaminating DOX and DNR were degraded like the parent drug into non-fluorescent compounds. At pH 4.0–8.0, DOX was more labile than DNR. Both anthracycline degradation and apolar compound formation decreased with pH and at pH 4.0–5.0 there was almost no formation of apolar compound. At pH 7.0 and 8.0, when DOX became exhausted, the concentration of apolar compound, which never exceeded

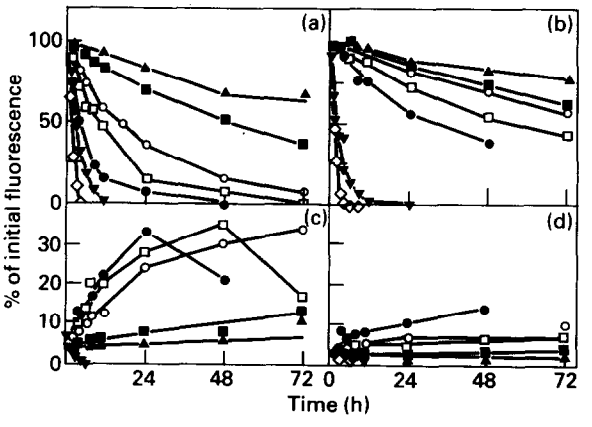


Figure 3
Degradation of parent drug and aglycone formation after incubation of DOX and DNR at 10 μg ml^{-1} in 0.1 M buffers at 37° C. Degradation of DOX (a) and DOX-ONE formation (c) after incubation of DOX. Degradation of DNR (b) and DNR-ONE formation (d) after incubation of DNR. (▲) pH 4.0, (■) pH 5.0, (○) pH 6.0, (□) pH 7.0, (●) pH 8.0, (▼) pH 9.0, (◇) pH 10.0.

35% of the total fluorescence, decreased (Fig. 3,C). For DNR, the appearance of fluorescent apolar compounds never exceeded 10% of the total fluorescence. With both drugs, the loss in fluorescence of the parent drug was never compensated by the formation of fluorescent apolar compounds. DOX and DNR were thus degraded mainly into non-fluorescent compounds. The apolar compounds of DOX and DNR formed in buffers were co-chromatographed with DOXONE and DNRONE, respectively. None of the other compounds presented in Table 1 were observed in buffers.

Degradation in rabbit samples

Plasma. The variations in initial DOX and DNR fluorescence, determined by NP HPLC, when incubated in rabbit plasma at 39°, 23° and 4°C, are shown in Fig. 4. The degradation of DNR was less rapid than that of DOX (Table 2). At 39°C, DOX and DNR were rapidly degraded while there was an increase in formation of a fluorescent unidentified product. On a NP column, this degradation product was eluted as a highly apolar compound and had an intrinsic fluorescence higher than that of DOX or DNR. Most of the fluorescence attributed to this compound was retained on a RP column. The retention of red degradation products of anthracyclines was also visualized by passing concentrated solutions of these drugs through either C_{18} Sep-pak mini-columns (Waters, Millford, Mass.) or glass columns filled with C_{18} (reversed-phase). However, in RP HPLC, in addition to DOX and DNR, two apolar compounds were observed; these were eluted with the same retention time as those of 7-d DOXONE and 7-d DOXOLONE for DOX and those of 7-d DNRONE and 7-d DOLONE for DNR. As the fluorescence due

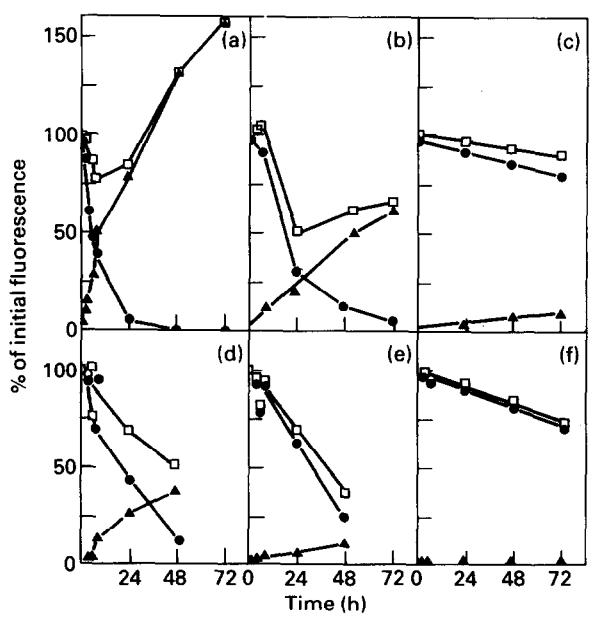


Figure 4
Variation of sample fluorescence after incubation of DOX and DNR at 10 μg ml^{-1} in rabbit plasma. Decrease of DOX initial fluorescence (●), formation of apolar compound (▲) and variation of total fluorescence (□) after incubation of DOX in rabbit plasma at 39° C (a), 23° C (n) and 4° C (c). Decrease of DNR initial fluorescence (●), formation of apolar compound (▲) and variation of total fluorescence (□) after incubation of DNR in rabbit plasma at 39° C (d), 23° C (e) and 4° C (f).

Table 2
Degradation of DOX and DNR in rabbit and human biological fluids

Conditions		Light exposure*	DOX		$t_{50\%}$† (h)	DNR		
Rabbit			39° C	23° C	4° C	39° C	23° C	4° C
Plasma	(pH 7.6)	+	5.6 ± 0.3‡	16.2 ± 0.9‡	>96 (74.0)§	20.7 ± 0.6‡	26.1	>96 (66.8)§
Bile	(pH 9.1)	+	3.4 ± 0.4‡	11.9	93.0	29.6 ± 10.6‡	46.1	>96 (72.8)§
Urine	(pH 9.3)	+	0.2			2.2		
	(pH 8.6)	+	0.3			27.1		
	(pH 8.5)	+	2.0	4.0	24.4	36.1	87.4	>96 (86.9)§
Faeces		−	0.7			1.2		
Human			37° C	23° C	4° C	37° C	23° C	4° C
Plasma	(pH 7.4)	+	4.1 ± 0.7‡	8.1	>96 (67.1)§	9.3 ± 3.1‡	25.6	82.5
		−	4.6 ± 0.1‡		96.0 (75.4)‡	18.1 ± 0.1‡		>96 (100)§
Bile	(pH 8.8)	+	3.3 ± 0.6‡	27.4	>96 (67.4)§	7.6 ± 1.6‡	36.0	>96 (52.4)§
		−	3.7		>96 (65.1)§	12.6		>96 (91.8)§
Urine	(pH 7.3)	+	13.0			11.6		
	(pH 6.9)	+	13.8			23.2		
	(pH 6.2)	+	46.5	>96 (82.7)§	>96 (100)§	49.3	>96 (80.0)§	>96 (99.2)§
	(pH 5.3)	−	50.6		>96 (98.9)§	>96 (68.8)§		>96 (100)§

*Incubation under room light (+) or in the dark (−).
†$t_{50\%}$ is the time for 50% loss of the initial fluorescence of the drug.
‡Mean ± standard deviation of three separate determinations.
§Values in parentheses are percentages of the initial DOX or DNR concentration at the end of the 96-h incubation period.

to the parent drugs decreased, that due to apolar compounds increased. The amount of apolar compounds was thus greater in case of the more labile anthracycline DOX. Even at 4°C, when almost no apolar compound was formed, the fluorescence of DOX and DNR decreased.

Bile. When DOX and DNR were incubated in rabbit bile at 39°, 23° and 4°C, the fluorescence of both substances decreased with time, degradation being greater at higher temperatures (Fig. 5, Table 2). Apolar compounds which never compensated for the loss in DOX and DNR fluorescence were found in smaller amounts than in plasma. By RP HPLC, in addition to the parent drugs, only one apolar compound was observed, which eluted with a retention time identical to those of 7-d DOXONE and 7-d DNRONE, respectively for DOX and DNR.

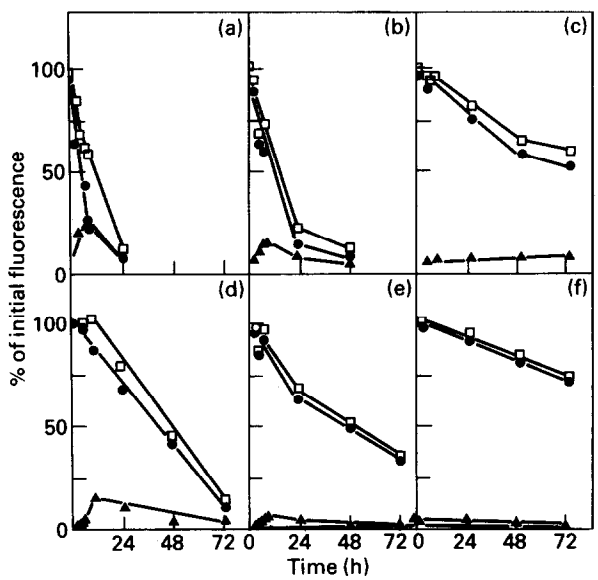


Figure 5
Variation of sample fluorescence after incubation of DOX and DNR at 10 μg ml^{-1} in rabbit bile. Conditions and symbols are identical to those described in the legend of Fig. 4.

Urine. In rabbit urine at 39°C, DOX was more rapidly degraded than was DNR (Fig. 6, Table 2). However, since urinary pH was variable from one set of experiments to the other, degradation rates of DOX and DNR varied accordingly. When the urinary pH fell below 8.7, there was a marked increase in stability that was greater for DNR than DOX in accordance with the results shown in Fig. 3. The variations of sample fluorescence in rabbit urine samples were confirmed by RP HPLC and direct fluorimetric measurement of a ten-fold urine dilution. Degradation of DOX and DNR was accompanied by the rapid appearance of apolar compounds. By RP HPLC, significant amounts of two apolar compounds were detected; those eluted were as 7-d DOXONE and 7-d DOXOLONE for DOX and 7-d DNRONE and 7-d DOLONE for DNR. The concentrations of 7-d DOXONE and 7-d DNRONE increased before those of 7-d DOXOLONE and 7-d DOLONE and thereafter all aglycones concentrations decreased without the appearance of other fluorescent compounds.

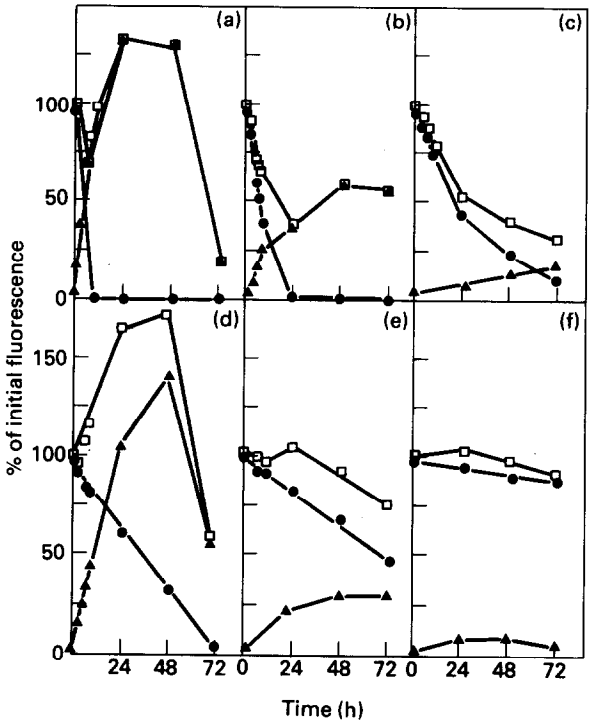


Figure 6
Variation of sample fluorescence after incubation of DOX and DNR at 10 μg ml^{-1} in rabbit urine. Conditions and symbols are identical to those described in the legend of Fig. 4.

Faeces. Both DOX and DNR were rapidly degraded at 39°C in rabbit faeces with a $t_{50\%}$ about 1 h (Table 2).

Degradation in human samples

Plasma. When DOX was incubated at 37°C in human plasma, the fluorescence disappeared completely after 24 h without being compensated entirely by the appearance of apolar compounds. As described for rabbit plasma, DNR was more stable than DOX (Table 2) and the rate of appearance and amount of apolar compounds were higher in case of DOX. These apolar compounds were those found in rabbit plasma.

Bile. In human bile at 37°C, both DOX and DNR were completely degraded within 24 h and were more stable at lower temperatures (Table 2). The amounts of apolar compounds formed in bile never accounted for more than one-third of the initial drug fluorescence. These compounds were identical to those found in rabbit bile.

Urine. There was almost no difference between the degradation rates of DOX and DNR in human urine (Table 2). As described for rabbit urine, the rate of DOX and DNR degradation varied according to the urinary pH. In agreement with the pH effect on drug stability (Fig. 1), the higher the urinary pH the faster the degradation. The loss of parent drug fluorescence was compensated by an increase in apolar compounds which, as in rabbit urine, had higher intrinsic fluorescences and were eluted in RP HPLC like

the standards of 7-d DOXONE and 7-d DOXOLONE for DOX and 7-d DNRONE and 7-d DOLONE for DNR. At 37°C, the graphs of concentration against time pattern of these aglycones were similar to that described for rabbit urine, but these aglycones were more stable in human urine which has a lower pH than rabbit urine.

Degradation rates at 37°C were not different in sterile and non-sterile plasma and urine (results not shown). When biological samples were protected from light, degradation at 37° and 4°C was slowed much more for DNR than for DOX (Table 2). Nevertheless, degradation still occurred in the absence of light, especially in human plasma and bile at 37°C.

Both in rabbit and human biological fluids, DNR was less labile than DOX. In both, the degradation of DOX and DNR and the formation of apolar compounds was decreased when the temperature of incubation was reduced. However, even at 4°C when the formation of apolar compounds was minimal, the fluorescence of the parent drug decreased.

Discussion

In this study rapid and sensitive HPLC methods of analysis were used; these offered excellent recovery of anthracyclines with direct extraction and allowed for qualitative and quantitative analysis of aglycones, parent anthracyclines and metabolites [12–14]. The effectiveness of separation of the isocratic RP method was comparable with that achieved by other authors [15] and with that of the gradient elution method proposed by Andrews *et al.* [16]. The lack of additional reference compounds prevented a complete comparison of methods.

It has been shown that in the presence of light, even room light, degradation of DOX and DNR occurred, as measured by the loss in fluorescence [9, 17–19], visible absorbance [3, 20] or UV [4] absorbance. This degradation was inversely proportional to the drug concentration [9] and was thus more rapid in dilute samples than in concentrated solutions [2, 9, 21]. Since in the present work, incubation of DOX or DNR in various solutions (in the dark or in normal room light) resulted in a loss in fluorescence, of the parent drug, that was not entirely compensated by the formation of fluorescent apolar compounds, it is possible that degradation products are formed that have a shifted or weaker fluorescence [5, 22].

As previously reported [4], DOX was more labile than DNR and the degradation of both anthracyclines increased with pH [4, 10, 23]. In addition to light, temperature and pH, anthracycline stability may be also influenced by factors such as the concentration of the buffer [10] and the buffering effect of the excipient [4] present in formulated products of anthracyclines [2–4, 21].

When incubated in rabbit or human biological fluids, both DOX and DNR were rapidly degraded at body temperature but more slowly at room and cold room temperatures. As for DOX and DNR in buffers, the degradation of DOX and DNR in biological fluids was more rapid than that observed from the variation of total fluorescence, due to the formation of fluorescent, apolar degradation products. Degradation compounds produced in buffers and biological fluids are qualitatively and quantitatively different.

In accordance with studies *in vivo* [24, 25], the degradations *in vitro* of DOX and DNR in rabbit and human biological fluids were qualitatively identical, though differing quantitatively.

As reported by others for human plasma [15], apolar compounds behaved in RP HPLC like 7-deoxyaglycones, which have at least 2.5 times higher intrinsic fluorescence than those of the parent drugs [25]. The formation of highly apolar compounds has also been observed when anthracyclines were incubated in acellular culture media [26, 27]. These compounds may correspond to the aglycone reported by Gray and Phillips [18]; the structural elucidation of these compounds is now under study. Owing to their higher intrinsic fluorescences, the true concentrations of apolar compounds reported in Figs 4–6 were probably overestimated. This could explain why the total fluorescence due to the parent drug and to these degradation products sometimes exceeded 100%. Thus methods measuring the total fluorescence decrease [9] are inadequate and underestimate the degradation of parent drugs. Rapid extraction and HPLC separation are preferable.

In bile, the degradation rates of DOX and DNR can be explained almost entirely by the high biliary pH [4, 7, 8, 10, 23].

The formation *in vitro* of 7-deoxyaglycones has been reported in rat [24, 25], hamster [24] and rabbit [28] liver and kidney homogenates as well as their excretion in rat [23], rabbit [29] and human [30] bile and in human urine [5, 22]. The transformation of DNR into 7-d DNRONE and then into 7-d DOLONE were described as being sequential events [24, 25, 31]; this explains the pattern of 7-deoxyaglycones observed in urine in the present work. This pattern also applied to DOX, but since DOX is a weaker substrate for glycosidic cleavage, lower amounts of 7-deoxyaglycones are formed than with DNR [31].

The rate of degradation of parent drugs was drastically influenced by the urine pH, and since in the experiments the pH varied from 5.3 to 7.3 for human urine and from 8.5 to 9.3 for rabbit urine, the degradation rates showed greatest variations in those biological fluids (Table 2). However, in rabbit urine, DOX and DNR degradation rates were lower than those that would be expected from the pH and therefore, as previously suggested [17, 20], stabilizing factors could exist in urine. Degradation could not be attributed to bacterial contamination since DOX and DNR were labile in fresh sterilized urine and was not due to photolysis only as it still occurred in light-protected samples.

In all fluids examined in the present work, anthracycline degradation and, consequently, apolar compound formation increased with temperature; similar observations for human plasma have been made by others [6–8].

As suggested previously [22], aglycones reported in several pharmacokinetic studies may have been produced, at least in part, artifactually during sample processing. Aglycones are probably devoid of antitumour activity [31]. Therefore a high level of apolar compounds together with a low concentration of parent drug would only have a pharmacological meaning if degradation did not occur after sampling. The degradation of anthracyclines in biological fluids has thus important implications for pharmacokinetic studies. Due to degradation *in vitro* after sampling, the true concentration of DOX and DNR could have been underestimated in plasma, bile and urine samples. Since human urinary pH often varies with diet and ranges from pH 4.6 to 8.0 [32], inter- and intra-individual variations in degradation rates of DOX and DNR may lead to variable estimation of urinary drug excretion. Since anthracyclines are extensively excreted in rabbit [29] and human [30] bile, the biliary instability of those drugs may have led to underestimated biliary levels. Although it is not possible to extrapolate these results *in vitro* to situations *in vivo*, the instability of anthracyclines in biological fluids at body temperature may be a major factor influencing the pharmacokinetics and thereby the therapeutic and toxic effects of these drugs *in vivo*.

In conclusion, the implications of the findings are important for the handling of

biological samples and solutions of anthracyclines inasmuch as the experimental conditions are similar to those in which they are handled in clinical and laboratory use. Precautions should be taken when degradation is rapid in the particular sample and especially for DOX. In any case, samples should be protected from light and should be immediately cooled. Solutions should be kept at highest possible concentrations and at a pH no greater than 6, and should be protected from light. These precautions should be taken into account when preparing intravenous solutions of anthracyclines. If these storage precautions cannot be applied in certain situations, intravenous solutions of anthracyclines should not be prepared in advance but should be prepared extemporaneously. Nevertheless these precautions may not suffice as in some instances degradation of DOX and DNR was observed even at 4°C. To avoid any artifacts it is recommended that drugs be extracted immediately from any sample.

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References

[1] R. C. Young, R. F. Ozols and C. E. Myers, *N. Engl. J. Med.* **305**, 139–153 (1981).
[2] D. M. Hoffman, D. D. Grossano, L. Damin and T. W. Woodcock, *Am. J. Hosp. Pharm.* **36**, 1536–1538 (1979).
[3] D. Ketchum, E. Wolf and G. P. Sesin, *Am. J. Intrav. Ther. Clin. Nutr.* **8**, 15–18 (1981).
[4] G. K. Poochikian, I. C. Cradock and K. P. Flora, *Am. J. Hosp. Pharm.* **38**, 483–486 (1981).
[5] S. Takanashi and N. R. Bachur, *Drug Metab. Disp.* **4**, 79–87 (1976).
[6] S. Eksborg, H. Ehrsson, I. Wallin and A. Lindfurs, *Acta Pharm. Suec.* **18**, 215–220 (1981).
[7] Y. M. Peng, D. Ormberg, D. S. Alberts and T. P. Davis, *J. Chromatogr.* **233**, 235–247 (1982).
[8] D. L. Reynolds, L. A. Sternson and A. J. Reptal, *J. Chromatogr.* **222**, 225–240 (1981).
[9] N. Tavoloni, A. M. Guarino and P. D. Berk, *J. Pharm. Pharmacol.* **32**, 860–862 (1980).
[10] F. Arcamone, G. Cassinelli, G. Franceschi, S. Penco, C. Pol, S. Redaelli and A. Selva, in *Adriamycin* (S. K. Carter, Ed.), pp. 9–22. Springer, New York (1972).
[11] T. H. Smith, A. N. Fujiwara, W. W. Lee, H. Y. Wu and D. W. Henry, *J. Org. Chem.* **43**, 3653–3660 (1977).
[12] R. Baurain, D. Deprez-De Campeneere and A. Trouet, *Cancer Chemother. Pharmacol.* **2**, 11–14 (1979).
[13] J. Robert, *J. Liquid Chromatogr.* **3**, 1561–1572 (1980).
[14] R. Baurain, M. Masquelier, C. Peterson, D. Deprez-De Campeneere and A. Trouet, *Curr. Chemother.* **2**, 1130–1132 (1972).
[15] W. Bolanowska, T. Gessner and H. Preisler, *Cancer Chemother. Pharmacol.* **10**, 187–191 (1983).
[16] P. A. Andrews, D. E. Brenner, F.-T. E. Chou, H. Kubo and N. R. Bachur, *Drug Metab. Dispos.* **8**, 152–156 (1980).
[17] J. P. Daugherty and S. C. Hixon, *J. Pharm. Pharmacol.* **33**, 556 (1981).
[18] P. J. Gray and D. R. Phillips, *Photochem. Photobiol.* **33**, 297–303 (1981).
[19] B. A. Williams and T. R. Tritton, *Photochem. Photobiol.* **34**, 131–134 (1981).
[20] J. P. Daugherty, S. C. Hixon and K. L. Yielding, *Biochim. Biophys. Acta* **565**, 13–21 (1979).
[21] M. B. Garnick, G. R. Weiss, G. D. Steele, M. Israel, D. Schade, M. J. Sack and E. Frei, *Cancer Treat. Rep.* **67**, 133–142 (1983).
[22] S. Takanashi and N. R. Bachur, *J. Pharmacol. Exp. Ther.* **195**, 41–49 (1975).
[23] N. Tavoloni and A. M. Guarino, *Pharmacology* **21**, 244–255 (1980).
[24] M. A. Asbell, E. Schwartzbach, F. J. Bullock and D. W. Yesair, *J. Pharmacol. Exp. Ther.* **182**, 63–69 (1972).
[25] H. S. Schwartz and N. B. Parker, *Cancer Res.* **41**, 2343–2348 (1981).
[26] J. T. Hjelle, R. Baurain, M. Masquelier and A. Trouet, *J. Pharmacol. Exp. Ther.* (in press, 1984).
[27] D. Londos-Gagliardi, R. Baurain, J. Robert and G. Aubel-Sadron, *Cancer Chemother. Pharmacol.* **9**, 45–48 (1982).
[28] H. Loveless, E. Arena, R. L. Felsted and N. R. Bachur, *Cancer Res.* **38**, 593–598 (1978).
[29] N. R. Bachur, R. C. Hildebrand and R. S. Jaenke, *J. Pharmacol. Exp. Ther.* **191**, 331–340 (1974).

[30] C. E. Riggs, R. S. Benjamin, A. A. Serpick and N. R. Bachur, *Clin. Pharmacol. Ther.* **22**, 234–241 (1977).
[31] N. R. Bachur and M. Gee, *J. Pharmacol. Exp. Ther.* **197**, 681–686 (1976).
[32] J. Wallach, in *Interpretation of Diagnostic Tests*, p. 18. Little Brown, Boston (1978).

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