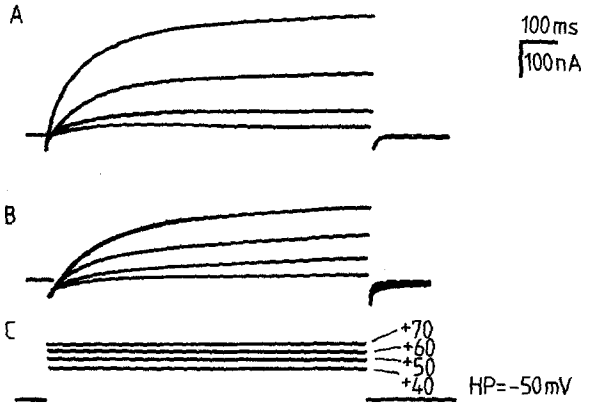

Figure 3. Comparison of Ca-dependent K-currents in control saline (A) and in the presence of 10 nM CARP (B) at different voltages. Numbers on the right to the voltage curves show values by which the neurone was depolarized from the holding potential (HP = −50 mV). D-cluster neurone.

Since both Ca-inward and Ca-activated K-currents decreased simultaneously in the presence of CARP, so that the relationship between them was linear, it is suggested that depression of K(Ca) current is merely the consequence of Ca-inward current inhibition. Recently it has became evident that neuronal membranes possess two types of voltage-sensitive calcium channels: a transient, and a slowly inactivating one. In conclusion, the data presented above suggest a specific blocking action of the CARP on slowly inactivating Ca-current component. It is proposed therefore that CARP could be a selective Ca-channel blocker.

Our experiments revealed that CARP strongly depresses the Ca-dependent K-current and the slowly inactivating Ca-inward current of snail neurones. In both cases the $(Ca)_i$ may decrease, which is consistent with the idea proposed by Twarog[4,5] for the relaxation mechanism of catch.

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1 Hirata, T., Kawahara, A., and Muneoka, Y., Hiroshima J. med. Sci. *35* (1986) 397.
2 Hirata, T., Kubota, I., Takabatake, I., Kawahara, A., Shimamoto, N., and Muneoka, Y., Brain Res. *422* (1987) 374.
3 Twarog, B. M., J. cell. comp. Physiol. *44* (1954) 141.
4 Twarog, B. M., Life Sci. *5* (1966) 1201.
5 Twarog, B. M., J. gen. Physiol. *50* (1967) 157.
6 Sakharov, D. A., and Salánki, J., Acta physiol. hung. *35* (1969) 19.
7 Gola, M., Hussy, N., Crest, M., and Ducreux, C., Neurosci. Lett. *70* (1986) 354.
8 Lux, D. H., and Hofmeier, G., Pflügers Arch. *394* (1982) 70.
9 Nowycky, M. C., Fox, P. A., and Tsien, R. W., Nature *316* (1985) 440.

0014-4754/88/11-120998-03$1.50 + 0.20/0

Fructose-1,6-diphosphate reduces acute ECG changes due to doxorubicin in isolated rat heart

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Summary. Doxorubicin (DXR) (0.17×10^{-4} M) induces an acute cardiotoxicity in isolated rat heart; there is a progressive widening of the SαT segment, with a decrease in force derivatives and in the coronary flow. Concurrent perfusion with fructose-1,6-diphosphate (FDP) (10^{-5}–10^{-4} M) dose-dependently reduces the SαT enlargement but fails to affect the reduction in force derivatives and coronary flow. The target of cardiac protection by FDP might be the ionic mechanisms underlying the action potential configuration.
Key words. Isolated rat heart; doxorubicin; acute cardiotoxicity; fructose-1,6-diphosphate.

Doxorubicin (DXR) produces both acute and chronic cardiotoxic effects which are associated with marked ECG and hemodynamic changes in animals and in humans[1]. Various pathogenetic mechanisms have been proposed to explain the acute cardiac damage, such as free radical production[2], membrane phospholipid peroxidation[3], intracellular ATP decrease[4] and histamine release[5]. Fructose-1,6-diphosphate (FDP) is a metabolic regulator which has been successfully employed in acute myocardial ischemia[6] and in hemorrhagic shock[7], which are both characterized by a significant decrease in energy supply. Under these conditions, FDP causes a regression of electrocardiographic (ECG) ischemic changes and prevents arrhythmias in acute myocardial infarction[6,7]. In the present study, the effects of FDP on isolated rat hearts perfused with DXR are investigated. It appears from these experiments that FDP is able to reduce ECG alterations but not the contractile force changes induced by DXR.

Materials and methods. Isolated hearts. Female Sprague-Dawley rats (250–300 g) were injected with heparin 500 IU/kg i.p. and then killed by cervical dislocation. Hearts were rapidly removed and placed in a cold physiological solution; then the aorta was cannulated to allow retrograde coronary perfusion with Locke solution at 37 °C, aerated with 100% O_2, pH 7.4, in a Langendorff apparatus (perfusion pressure 60 mm Hg). Locke solution had the following composition (mM/l): NaCl 153.97, KCl 5.63, $CaCl_2$ 2.18, $NaHCO_3$ 1.78, glucose 5.09. The hearts were perfused for an initial 30-min stabilization period.

Cardiac parameters. The cardiac electric activity was recorded by a computerized on-line evaluation system to allow the measurement of various ECG parameters[8]. ECG monitoring was performed before and during perfusion with the drugs (1 h), by means of 2 atraumatic electrodes[9], one recording from the right atrium and the other from the heart apex. This system was used to measure heart rate (beats/min), the SαT segment duration (ms) and T-wave voltage (mV). The contractile function was recorded by an isometric transducer (Basile DY2) directly connected to an ADCI/T channel of a Battaglia Rangoni ESO 600 polygraph. The first derivative of the contraction or relaxation over the time ($\pm dF/dt$) was determined starting from the force signal elaborated by an AO/DP/NS operational channel (Battaglia-

Effects of DXR (0.17×10^{-4} M), FDP ($10^{-5} - 5 \times 10^{-5} - 10^{-4}$ M) and DXR + FDP on SαT segment (SαT) (ms), T-wave (Tw) (mV), heart rate (HR) (beats/min), contractile force (F) (g), ±dF/dt (g/s), and coronary flow (CF) (ml/min) in isolated perfused rat heart. C, hearts perfused with drug-free Locke solution.

Time (min)	Basal	5	15	30	45	60
SαT						
C	13.1 ± 0.4	0.15 ± 0.01	−0.10 ± 0.08	0.20 ± 0.09	−0.15 ± 0.08	0.08 ± 0.05
FDP 10^{-5}	12.2 ± 0.25	0.08 ± 0.03	0.12 ± 0.08	0.10 ± 0.04	−0.06 ± 0.01	0.05 ± 0.10
FDP 5×10^{-5}	12.5 ± 0.25	0 ± 0	0.25 ± 0.08	−0.10 ± 0.03	−0.25 ± 0.01	0.03 ± 0.02
FDP 10^{-4}	14.7 ± 3.7	0 ± 0	−0.25 ± 0.25	0.25 ± 0.25	−0.08 ± 0.04	−0.08 ± 0.08
DXR	12.8 ± 1.1	0.80 ± 0.30	1.3 ± 0.33*	2.1 ± 0.43*	2.7 ± 0.51*	3.3 ± 0.54*
DXR + FDP 10^{-5}	15.5 ± 0.67	0.75 ± 0.25	1.4 ± 0.47*	1.9 ± 0.47*	2.2 ± 0.47*	2.7 ± 0.59*
DXR + FDP 5×10^{-5}	11.4 ± 1.4	0.62 ± 0.31	1.0 ± 0.20*	1.2 ± 0.14*	1.6 ± 0.31*	1.9 ± 0.51*
DXR + FDP 10^{-4}	13.5 ± 0.66	0.65 ± 0.15	0.95 ± 0.18*	1.1 ± 0.21*	1.4 ± 0.31*	1.6 ± 0.33*
Tw						
C	1.9 ± 0.12	−0.05 ± 0.01	−0.05 ± 0.01	−0.12 ± 0.06	−0.20 ± 0.05	−0.32 ± 0.04
FDP 10^{-5}	1.1 ± 0.14	0.07 ± 0.02	0.07 ± 0.02	−0.06 ± 0.01	−0.15 ± 0.05	−0.15 ± 0.04
FDP 5×10^{-5}	2.7 ± 1.2	−0.05 ± 0.05	−0.05 ± 0.05	−0.25 ± 0.05	−0.55 ± 0.25	−0.55 ± 0.25
FDP 10^{-4}	2.0 ± 0.40	0.50 ± 0.08	−0.05 ± 0.05	−0.10 ± 0.03	−0.15 ± 0.05	−0.25 ± 0.05
DXR	1.9 ± 0.19	−0.06 ± 0.06	−0.07 ± 0.06	−0.18 ± 0.07	−0.26 ± 0.09	−0.34 ± 0.11
DXR + FDP 10^{-5}	2.0 ± 0.26	−0.02 ± 0.05	−0.10 ± 0.04	−0.17 ± 0.06	−0.27 ± 0.02	−0.35 ± 0.06
DXR + FDP 5×10^{-5}	1.6 ± 0.09	−0.05 ± 0.09	−0.05 ± 0.01	−0.15 ± 0.01	−0.20 ± 0.01	−0.20 ± 0.02
DXR + FDP 10^{-4}	2.0 ± 0.15	0.05 ± 0.04	−0.03 ± 0.02	−0.10 ± 0.03	−0.18 ± 0.06	−0.25 ± 0.07
HR						
C	222.0 ± 21.3	−4.8 ± 4.4	−6.2 ± 2.8	−11.5 ± 4.9	−20.7 ± 3.0	−20.2 ± 6.8
FDP 10^{-5}	207.0 ± 2.3	−1.9 ± 0.70	−18.0 ± 2.0	−35.5 ± 10.1	−35.5 ± 13.9	−40.5 ± 26.5
FDP 5×10^{-5}	204.5 ± 17.5	−26.0 ± 11.0	−33.0 ± 18.1	−37.0 ± 22.1	−37.0 ± 22.1	−42.0 ± 20.1
FDP 10^{-4}	253.0 ± 43.2	0 ± 0	−12.6 ± 8.2	−24.5 ± 10.5	−25.7 ± 18.7	−35.5 ± 17.7
DXR	225.8 ± 15.8	−12.8 ± 4.8	−22.8 ± 3.5	−35.0 ± 8.1	−49.4 ± 11.2	−66.6 ± 16.1*
DXR + FDP 10^{-5}	237.0 ± 28.3	−43.5 ± 9.7*	−65.2 ± 15.3*	−73.7 ± 15.8*	−91.2 ± 21.3*	−100.2 ± 25.6*
DXR + FDP 5×10^{-5}	221.5 ± 13.3	−28.0 ± 5.4*	−46.0 ± 8.6*	−42.0 ± 8.6*	−63.0 ± 13.7*	−79.2 ± 16.3*
DXR + FDP 10	242.2 ± 12.4	−20.6 + 5.3	−39.0 ± 6.2*	−61.5 ± 13.7*	−81.0 ± 16.3*	−103.0 ± 21.5*
F						
C	1.5 ± 0.16	−0.01 ± 0.04	−0.08 ± 0.06	−0.07 ± 0.08	−0.07 ± 0.11	−0.13 ± 0.10
FDP 10^{-5}	1.9 ± 0.11	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.05 ± 0.02	−0.05 ± 0.01
FDP 5×10^{-5}	1.3 ± 0.25	−0.12 ± 0.05	0.04 ± 0.01	−0.15 ± 0.10	−0.20 ± 0.18	−0.20 ± 0.19
FDP 10^{-4}	1.4 ± 0.10	0.05 ± 0.01	0.10 ± 0.01	0.10 ± 0.02	0.15 ± 0.05	0.12 ± 0.02
DXR	1.3 ± 0.09	−0.12 ± 0.05	−0.18 ± 0.11	−0.30 ± 0.15	−0.47 ± 0.18	−0.63 ± 0.19
DXR + FDP 10^{-5}	1.8 ± 0.30	−0.30 ± 0.06	−0.22 ± 0.06	−0.22 ± 0.07	−0.32 ± 0.14	−0.55 ± 0.23
DXR + FDP 5×10^{-5}	1.7 ± 0.28	−0.32 ± 0.11	−0.28 ± 0.09	−0.29 ± 0.09	−0.39 ± 0.18	−0.50 ± 0.14
DXR + FDP 10^{-4}	1.6 ± 0.06	−0.26 ± 0.08	−0.30 ± 0.06	−0.41 ± 0.12	−0.46 ± 0.14	−0.52 ± 0.12
+dF/dt						
C	63.9 ± 4.1	−1.8 ± 1.1	−6.1 ± 1.7	−5.5 ± 2.6	−7.4 ± 2.9	−7.4 ± 3.3
FDP 10^{-5}	40.3 ± 0.85	0 ± 0	1.3 ± 0.50	1.1 ± 0.08	−0.80 ± 0.05	−1.5 ± 0.90
FDP 5×10^{-5}	31.2 ± 2.9	−2.3 ± 0.90	1.1 ± 0.02	−1.0 ± 0.05	−2.5 ± 0.50	−4.6 ± 0.51
FDP 10^{-4}	61.1 ± 6.1	2.5 ± 0.91	3.5 ± 0.80	4.6 ± 0.35	3.4 ± 0.91	3.4 ± 0.92
DXR	53.3 ± 3.5	−6.9 ± 5.4	−11.4 ± 4.9	−16.2 ± 6.5	−23.5 ± 7.3*	−27.6 ± 6.5*
DXR + FDP 10^{-5}	61.3 ± 12.3	−7.4 ± 4.1	−14.7 ± 4.3	−16.9 ± 4.9	−23.4 ± 8.7*	−23.2 ± 8.4*
DXR + FDP 5×10^{-5}	39.1 ± 8.0	−5.3 ± 1.3	−7.8 ± 2.3	−9.1 ± 2.8	−13.1 ± 4.4	−15.8 ± 3.9
DXR + FDP 10^{-4}	66.3 ± 3.6	−11.3 ± 4.5	−16.1 ± 2.7	−21.1 ± 5.1	−19.2 ± 5.9	−25.7 ± 5.6*
−dF/dt						
C	40.1 ± 2.5	−0.32 ± 2.2	−2.5 ± 1.5	−4.2 ± 3.4	−4.5 ± 3.5	−7.5 ± 2.5
FDP 10^{-5}	47.2 ± 0.22	−1.4 ± 0.21	−2.8 ± 0.10	5.5 ± 0.25	−7.0 ± 1.1	−8.5 ± 0.50
FDP 5×10^{-5}	29.1 ± 7.3	−5.1 ± 1.0	−2.3 ± 0.05	−4.9 ± 0.80	−8.0 ± 0.90	−9.1 ± 0.31
FDP 10^{-4}	34.8 ± 0.15	0 ± 0	1.2 ± 0.05	−1.4 ± 0.01	−0.8 ± 0.02	−1.5 ± 0.9
DXR	38.6 ± 4.5	−4.4 ± 2.1	−5.7 ± 1.0	−10.7 ± 1.8	−18.9 ± 3.9*	−24.5 ± 5.3*
DXR + FDP 10^{-5}	43.6 ± 9.3	−9.1 ± 2.6	−10.8 ± 3.2	−12.5 ± 4.4	−18.9 ± 6.8*	−22.4 ± 8.0*
DXR + FDP 5×10^{-5}	39.5 ± 6.9	−7.2 ± 2.5	−9.1 ± 2.4	−9.6 ± 2.1	−13.0 ± 3.2	−17.3 ± 3.1*
DXR + FDP 10^{-4}	44.7 ± 3.1	−11.2 ± 3.5	−14.9 ± 3.7*	−17.4 ± 5.3*	−16.9 ± 4.7*	−22.5 ± 4.6*
CF						
C	5.2 ± 0.48	−0.05 ± 0.05	−0.17 ± 0.10	−0.32 ± 0.13	−0.45 ± 0.16	−0.60 ± 0.13
FDP 10^{-5}	5.7 ± 0.25	−0.10 ± 0.01	−0.17 ± 0.02	−0.15 ± 0.03	−0.45 ± 0.09	−0.50 ± 0.13
FDP 5×10^{-5}	5.3 ± 0.15	−0.16 ± 0.01	−0.10 ± 0.01	−0.15 ± 0.03	−0.48 ± 0.02	−0.60 ± 0.07
FDP 10^{-4}	5.2 ± 0.75	0 ± 0	−0.15 ± 0.01	−0.15 ± 0.02	−0.30 ± 0.06	−0.45 ± 0.05
DXR	5.6 ± 0.50	−0.76 ± 0.18	−0.74 ± 0.24	−1.1 ± 0.33	−1.5 ± 0.21*	−2.2 ± 0.17*
DXR + FDP 10^{-5}	5.4 ± 0.56	−1.7 ± 0.35*	−1.8 ± 0.38*	−1.9 ± 0.43*	−2.2 ± 0.52*	−2.5 ± 0.53*
DXR + FDP 5×10^{-5}	4.9 ± 0.50	−0.72 ± 0.28	−0.92 ± 0.37	−0.87 ± 0.40	−1.6 ± 0.72*	−1.8 ± 0.70*
DXR + FDP 10^{-4}	5.6 ± 0.20	−0.96 ± 0.26*	−1.5 ± 0.24*	−1.9 ± 0.17*	−2.5 ± 0.32*	−2.5 ± 0.43*

Data are mean ± SE changes from basal values.
Each value represents the mean of n = 5 (control and FDP) and n = 10 (DXR and DXR + FDP) experiments.
Student's t-test for grouped data: * $p < 0.05$ vs control values.

Rangoni, Bologna, Italy). The coronary flow was measured by determining the volume of the solution spontaneously drained by the heart in 1 min.

Drugs. DXR-HCl (Farmitalia-Carlo Erba, Milano, Italy) and FDP (Biomedica Foscama, Ferentino, Italy) were dissolved in distilled water and then added to Locke solution. The final concentrations were respectively 0.17×10^{-4} M for DXR-HCl and $10^{-5} - 5 \times 10^{-5} - 10^{-4}$ M for FDP. The concentration of DXR was selected on the basis of dose-response curves which indicated that this dose was associated with significant changes of ECG and contractile force during the whole of the experimental procedure. Moreover the dose of DXR used in our study is in the range employed by various authors in experiments on anthracycline cardiotoxicity in vitro [10, 11]. Higher doses produced excessive cardiotoxicity signs. The addition of FDP and/or DXR did not appreciably change the pH and the osmolality of the Locke solution. All other chemicals were of analytical grade.

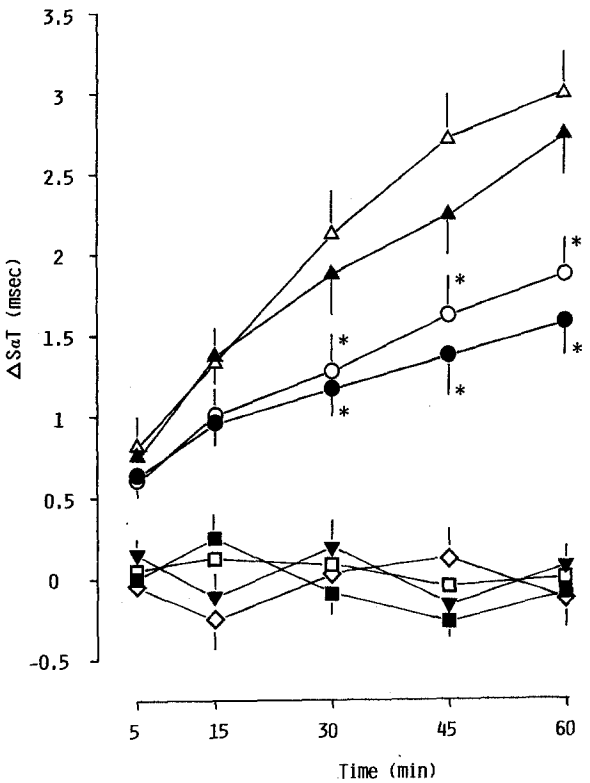
Statistical analysis. The data are presented as means ±SE. Student's t-test for paired or grouped data was used to determine the significance of differences and p values less than 0.05 were considered to be significant; n indicates the number of the experiments.

Results. No changes were observed in any of the parameters examined, either in controls or in hearts perfused with FDP. Although DXR did not significantly modify the contractile force and the T-wave, it induced a progressive widening of the SαT segment which became significant 15 min after the beginning of the perfusion (table). This effect was dose-dependently reduced by FDP (fig.), whereas DXR-induced decrease in the heart rate, +dF/dt, −dF/dt and in the coronary flow was not prevented by FDP (table). The same degree of protection was obtained with FDP given before or in combination with DXR.

Discussion. The present study shows that FDP reduces the SαT widening induced by DXR, but fails to affect the other parameters recorded. The enlargement of the SαT segment might depend on the altered repolarization phase of cardiac cells [13, 14] due to the inhibition of Ca^{2+} turnover into the sarcoplasm caused by DXR [12]. In addition to this, the inhibition of Ca^{2+} transport across sarcolemmal membranes [15] and the impairment of energy production by rat heart slices exposed to DXR [4] could participate in the SαT segment prolongation. The activity of FDP in improving the ATP production by the glycolytic pathway may explain the protective effect of the drug on the SαT segment, since it has been shown that ATP production by glycolysis is effective in preserving membrane function, which could include the maintenance of Ca^{2+} ion homeostasis [16]. An additional protective mechanism of FDP is likely to accord with the stimulation of Mg^{2+}-dependent Ca^{2+}-ATPase activity in cell membranes [17].

The failure of FDP to prevent the reduction of cardiac contractile force derivatives induced by DXR might depend on the multifactorial pathogenesis of cardiac damage including the formation of semiquinone and oxygen radicals [2] and lipid peroxidation [3].

As the pathogenetic mechanisms of acute cardiotoxicity by DXR may be involved in DXR chronic effects [18], the protective action of FDP against DXR acute alterations might be useful in limiting chronic cardiac damage.



Changes in the SαT interval from basal value in hearts perfused with Locke solution (control) (▼), FDP 10^{-5} M (□), FDP 5×10^{-5} M (■), FDP 10^{-4} M (◇), DXR 0.17×10^{-4} M (△), DXR + FDP 10^{-5} M (▲), DXR + FDP 5×10^{-5} M (○), and DXR + FDP 10^{-4} M (●). Each point represents the mean of n = 5 (control and FDP) and n = 10 (DXR and DXR + FDP) experiments ±SE (vertical bars).
Student's t-test for grouped data: * $p < 0.05$ vs DXR values.

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1 Young, R. C., Ozols, R. F., and Myers, C. E., N. Engl. J. Med. *305* (1981) 139.
2 Doroshow, J. H., Cancer Res. *43* (1983) 460.
3 Myers, C. E., McGuire, W. P., Liss, R. H., Ifrim, I., Grotzinger, K., and Young, R. C., Science *197* (1977) 165.
4 Neri, B., Cini-Neri, G., and D'Alterio, M., Biochem. biophys. Res. Commun. *125* (1984) 954.
5 Klugmann, F. B., Decorti, G., Candussio, L., Grill, V., Mallardi F., and Baldini, L., Br. J. Cancer *54* (1986) 743.
6 Markow, A. K., Oglethorpe, N. C., Blake, M. I., Lehan, P. H., and Hellems, H. K., Am. Heart J. *100* (1980) 639.
7 Markow, A. K., Oglethorpe, N., Young, B. Y., and Hellems, K. H., Circ. Shock *8* (1981) 9.
8 Danesi, R., Del Tacca, M., and Soldani, G., J. pharmac. Meth. *16* (1986) 251.
9 Curtis, M. J., Macleod, B. A., Tabrizchi, R., and Walker, M. J. A., J. pharmac. Meth. *15* (1985) 87.
10 Okano, C., Kwok, O., Hokama, Y., and Chou, S. C., Res. Commun. chem. Path. Pharmac. *45* (1984) 279.
11 Saman, S., Jacobs, P., and Opie, L. H., Cancer Res. *44* (1984) 1316.
12 Monti, E., Piccinini, F., Favalli, L., and Villani, F., Biochem. Pharmac. *32* (1983) 3303.
13 Jensen, R. A., Acton, E. M., and Peters, J. H., J. cardiovasc. Pharmac. *6* (1984) 186.
14 Bernardini, C., Del Tacca, M., Danesi, R., and Della Torre, P., Archs int. Pharmacodyn. *283* (1986) 243.
15 Caroni, P., Villani, F., and Carafoli, E., FEBS Lett. *130* (1981) 184.
16 Goto, M.,Yatani, A., and Tsuda, Y., Jap. J. Physiol. *27* (1977) 81.
17 Galzigna, L., and Rigobello, M. P., Experientia *42* (1986) 138.
18 Doroshow, J. H., Locker, G. Y., and Myers, C. E., Cancer Treat. Rep. *63* (1979) 855.

0014-4754/88/11-12/1000-03$1.50 + 0.20/0