Eur J Cancer Clin Oncol, Vol. 20, No. 1, pp. 129–135, 1984.
Printed in Great Britain.

0277-5379/84$3.00+0.00
Pergamon Press Ltd.

Dose–Response Relationships of Chronic Adriamycin Toxicity in Rabbits*†

H. J. LONG,‡ S. S. DIAMOND, C. P. RAFLO and R. A. BURNINGHAM
Clinical Investigation Center, Naval Regional Medical Center, San Diego, CA 92134, U.S.A.

Abstract—*Doxorubicin was administered chronically to 55 female New Zealand White rabbits in order to determine a chronic dose–response relationship for doxorubicin-induced cardiomyopathy, skeletal myopathy and nephropathy. Systolic time interval recording as a measure of cardiac function in doxorubicin-induced cardiomyopathy in the rabbit is compared to histologic grading of the cardiomyopathy. Histologic evidence of cardiomyopathy was not seen at cumulative doses less than* 100 mg/m^2 *but incidence and severity of cardiomyopathy increased with increasing doxorubicin dosage. The most severe lesions were seen at cumulative doses in excess of* 400 mg/m^2. *The dose–response of skeletal myopathy paralleled that of cardiomyopathy but the severity of histologic abnormalities and the incidence of skeletal myopathy was less than half that of cardiomyopathy at all dose levels. Nephropathy was a consistent finding at cumulative doses of doxorubicin in excess of* 100 mg/m^2.

INTRODUCTION

CHRONIC intravenous administration of the anthracycline drugs, doxorubicin and daunomycin, in the rabbit has been reported to result in a characteristic cardiomyopathy manifested by focal to diffuse myofiber degeneration with vacuolation followed by necrosis and interstitial fibrosis identical to that seen in humans [1–4]. This cardiomyopathy results in irreversible congestive heart failure with resultant dependent tissue edema, hydrothorax, hydropericardium, ascites and chronic passive congestion of lungs and liver [3]. In humans, these changes are seen with increasing frequency and severity at cumulative doses of doxorubicin exceeding 500 mg/m^2 [4]. The rabbit appears to be more sensitive than humans with symptomatic cardiomyopathy consistently seen at cumulative doses in excess of 250 mg/m^2 and early histologic changes at cumulative doses in excess of 120 mg/m^2 [1]. Complex histologic grading systems were developed in an effort to quantitate the degree of cardiomyopathy [1, 3] so that dose–response relationships could be determined, and a suitable model system could be developed for comparative testing of new anthracycline compounds, identification of cardioprotective agents and determination of synergistic cardiotoxicity, when administered with other antineoplastic agents or cardiac irradiation.

A number of non-invasive cardiac function studies, including systolic time interval recording, have been shown to correlate with the development of symptomatic cardiomyopathy in humans, but they have not been able to predict the development of mild, asymptomatic cardiomyopathy, [5, 6] and they have not been significantly correlated with the severity of cardiomyopathy as measured by endomyocardial biopsy [7, 8]. Systolic time interval recording has been demonstrated to be a simple, accurate and reproducible technique for measurement of cardiac function in the rabbit [9]. Although a pre-ejection period (PEP)/left ventricular ejection time (LVET) ratio in excess of 0.466 is diagnostic

Accepted 1 June 1983.
*Supported in part by the Bureau of Medicine and Surgery Clinical Investigation Program Grants 6-16-696, 7-16-1042 and 7-16-1043.
†The opinions or assertions expressed herein are those of the authors and are not to be construed as official or as necessarily reflecting the views of the Department of the Navy or the naval services at large.
‡To whom requests for reprints should be addressed.

of left ventricular dysfunction [10] at the 95% confidence level, and an elevated PEP/LVET ratio has been consistently seen in rabbits receiving doxorubicin in excess of 200 mg/m^2 [11], there have been no attempts to correlate the absolute level of PEP/LVET with histologic degree of cardiomyopathy in the rabbit. Similar comparisons in humans have reported no correlation between the level of PEP/LVET and histologic degree of cardiomyopathy [7].

Investigators have described a skeletal myopathy after chronic administration of doxorubicin in rabbits with resultant focal myofiber degeneration, vacuolation, necrosis and fibrosis similar to the changes in the myocardium [3]. A cumulative dose–response relationship has not been described, nor has there been any attempt to correlate skeletal muscle changes with severity of myocardial abnormalities, although such a correlation may permit serial skeletal muscle biopsy as a monitor of anthracycline cardiac toxicity without the risk or expense of endomyocardial biopsy.

A cumulative dose-related anthracycline-induced nephropathy has been well described in rabbits with glomerulonephrosis manifested by vacuolation of podocytes and parietal epithelial cells lining the urinary space affected glomeruli, mesangial thickening and glomerulosclerosis, dilated proximal tubules containing protein and cellular casts, and interstitial nephritis with stromal fibrosis [3, 12, 13]. Similar renal abnormalities have been described in only one human case with interstitial nephritis after a cumulative doxorubicin dose of 180 mg/m^2 [14]. In one study over 300 rabbits received various cumulative dose levels of doxorubicin and various dose levels of cardiac irradiation. The dose–response relationship was described for doxorubicin-induced nephropathy with increased frequency and severity of renal lesions in 71% of rabbits as the total cumulative doses increased to a level of 202 mg/m^2 [13]. Although the incidence of renal lesions was said to significantly parallel the incidence of the myocardial lesions ($P < 0.0001$), there was no attempt to correlate the degree of nephropathy with degree of cardiomyopathy, and there was no correlation between congestive heart failure and nephropathy.

This study describes cumulative dose–response data regarding the development of doxorubicin-induced nephropathy, skeletal myopathy and cardiomyopathy in rabbits treated with various cumulative doses of doxorubicin. Correlation is demonstrated between histologic degree of cardiomyopathy and severity of cardiac dysfunction as measured by increases in PEP/LVET ratio.

MATERIALS AND METHODS

Fifty-five female New Zealand White rabbits, weighing approximately 2 kg each, were housed singly in stainless steel cages and provided with a diet of commercial rabbit pellets* and water *ad libitum* while under experimentation. Five rabbits served as untreated controls and received no therapy during the study. Fifty rabbits received once weekly intravenous bolus injections of doxorubicin† at various dose levels and schedules (Table 1) via the marginal ear vein. Body surface area was calculated according to the formula: BSA = 56.33 cm^2; weight = 0.436 g [15]. All rabbits were repeatedly treated until the specified cumulative dose (160 mg/m^2: 5 rabbits; 200 mg/m^2: 5 rabbits; 240 mg/m^2: 4 rabbits; 280 mg/m^2: 4 rabbits) or until death (Table 2). *In vivo* cardiac function was measured by performing serial systolic time interval recordings with calculation of PEP/LVET ratio once weekly on anesthetized, supine rabbits immediately prior to each doxorubicin administration as previously described [9, 10]. The final PEP/LVET ratio that was recorded prior to animal death or termination was used to reflect cardiac function immediately prior to death and was correlated with histologic grading of the severity of cardiomyopathy. PEP/LVET $\geqslant$0.466 was considered to be indicative of significant cardiac dysfunction.

Table 1. *Treatment schedules*

Dose	No. of rabbits
Control	5
10 mg/m^2/week	5
20 mg/m^2/week	5
30 mg/m^2/week	5
40 mg/m^2/week	25
40 mg/m^2/q^3 week	5
60 mg/m^2/q^3 week	5

Table 2. *Total cumulative doxorubicin dosage*

Cumulative dosage (mg/m^2)	No. of rabbits
0–100	6
101–200	6
201–300	21
301–400	12
401–500	4
501–600	1
601–700	3
701–800	1

*Standard Rabbit Ration, Star Milling Co., Perris, CA, U.S.A.
†Adriamycin, Adria Laboratories, Inc., Columbus, OH, U.S.A.

Immediately after death or euthanasia, each rabbit was necropsied. Representative samples of left ventricular free wall and skeletal muscle from the thigh and kidney were fixed in 10% neutral-buffered formalin. Samples were paraffin-embedded, sectioned and stained with hematoxylin–eosin and Masson-Trichrome stains. All histologic sections were reviewed by a veterinary pathologist (S.S.D.) without knowledge as to cumulative dosage of adriamycin. Severity of cardiomyopathy and nephropathy was determined according to modification of the grading systems proposed by Van Vleet [3] (Tables 3 and 4), which are quantitative, easily performed and appear comparable to more complex histologic grading systems [1, 13, 16]. Severity of skeletal myopathy was determined using the same criteria as for cardiomyopathy.

Table 3. Histologic grade of doxorubicin-induced cardiac and skeletal myopathy

Histologic grade	Characteristics
0	normal
1	focal vacuolation, myofiber degeneration, fibrosis
2	mild, diffuse vacuolation, myofiber degeneration, fibrosis
3	moderate diffuse vacuolation, myofiber degeneration, fibrosis
4	severe diffuse vacuolation, myofiber degeneration, fibrosis

RESULTS

Histologic evidence of anthracycline-induced cardiomyopathy was demonstrated in increasing numbers of rabbits who received cumulative doses of doxorubicin in excess of 100 mg/m^2. Between 100 and 199 mg/m^2, 1 of 6 rabbits demonstrated only grade 1 cardiomyopathy and cardiac dysfunction with a PEP/LVET ratio of 0.500, although clinical signs of congestive heart failure were not evident. Only 6 of 21 rabbits who received 200–299 mg/m^2 developed an abnormal PEP/LVET ratio, although 75% had histologic evidence of cardiomyopathy. Of the 12 rabbits who received 300–399 mg/m^2, 46% had PEP/LVET >0.466 and demonstrated cardiomyopathy. Cardiomyopathy and abnormal PEP/LVET were seen in 8 of 9 rabbits who received in excess of 400 mg/m^2.

Table 4. Histologic grade of doxorubicin-induced nephropathy

Histologic grade	Characteristics
0	normal
1	focal glomerulonephrosis, tubular dilation with casts; mild fibrosis
2	mild diffuse glomerulonephrosis, tubular dilation with casts; moderate fibrosis
3	severe diffuse glomerulonephrosis, tubular dilation with casts; severe fibrosis

The PEP/LVET ratio increased progressively with increased severity of histologic grade of cardiomyopathy (Table 6). The mean PEP/LVET ratio increased from 0.337 ± 0.066 for normal controls and pre-doxorubicin measurements to >0.500 for rabbits with grade 2, 3 or 4 cardiomyopathy. The mean PEP/LVET ratio for 11 rabbits with grade 1 cardiomyopathy was 0.451 ± 0.076, a value that is at the upper limit of normal for rabbits. Rabbits who received some doxorubicin but who did not manifest histologic evidence of cardiomyopathy (grade 0) had a mean PEP/LVET ratio of 0.411 ± 0.097, a value that was

Table 5. No. of rabbits with cardiomyopathy vs cumulative doxorubicin dose

Cumulative dose mg/m^2)	Histologic grade*						PEP/LVET ratio†		
	0	1	2	3	4	Mean ± S.D.	<0.466	>0.466	Mean ± S.D.
0–99	6					0	6		0.355 ± 0.043
100–199	5	1				0.167 ± 0.408	5	1	0.426 ± 0.078
200–299	12	4	1	4		0.857 ± 1.195	15	6	0.435 ± 0.111
300–399	6	3	2	1		0.833 ± 1.030	7	6	0.44 ± 0.077
400+	1	3	1	3	1	2.000 ± 1.323	1	8	0.563 ± 0.142

*Histologic grade of cardiomyopathy = 0.0813 + 0.00308 (dose), $r = 0.362$, $P < 0.01$.
†PEP/LVET = 0.3634 + 0.000299 (dose), $r = 0.430$, $P < 0.001$.

Table 6. Histologic grade of cardiomyopathy vs PEP/LVET ratio

	Normal control	Histologic grade				
		0	1	2	3	4
No. of rabbits	51	32	11	2	9	1
Mean PEP/LVET ± S.D.	0.337 ± 0.066	0.411 ± 0.097	0.451 ± 0.076	0.600 ± 0.141	0.517 ± 0.141	0.500

distinctly higher than our previously reported mean for normal rabbits of 0.337 ± 0.066. This elevated PEP/LVET ratio is still within the normal range but may reflect mild cardiac dysfunction even before there is histologic evidence of cardiomyopathy.

Skeletal myopathy

Representative sections of thigh muscle were reviewed for histologic changes similar to those seen in cardiac muscle after various cumulative doxorubicin doses (Table 7). The lesions or skeletal myopathy were less pronounced than the cardiac lesions but the number of rabbits demonstrating skeletal myopathy increased at cumulative doxorubicin doses in excess of 200 mg/m^2. Only 1 of 6 rabbits that received between 100 and 199 mg/m^2 had skeletal myopathy of grade 1. Five of 21 rabbits that received 200–299 mg/m^2 demonstrated skeletal myopathy. Between 300 and 399 mg/m^2, one-third of the rabbits had skeletal myopathy, as did two-thirds of the rabbits that received in excess of 400 mg/m^2. Only 3 rabbits had grade 3 or greater lesions, and the mean histologic score was only 0.78 ± 0.67 at the highest dose level.

Nephropathy

Doxorubicin nephropathy was seen more frequently than cardiomyopathy and was seen with increasing frequency and severity at cumulative doses exceeding 100 mg/m^2 (Table 8).

Table 7. No. of rabbits with skeletal myopathy vs cumulative doxorubicin dose

Cumulative dose (mg/m^2)	Histologic grade* 0	1	2	3	4	Mean ± S.D.
0–99	6					0
100–199	5	1				0.17 ± 0.41
200–299	16	1	1	1	2	0.67 ± 1.35
300–399	8	4				0.33 ± 0.49
400+	3	5	1			0.78 ± 0.67

*Histologic grade skeletal myopathy = 0.1621 + 0.00115 (dose), $r = 0.198$, $P > 0.1$.

Table 8. No. of rabbits with nephropathy vs cumulative doxorubicin dose

Cumulative dose (m/m^2)	Histologic grade* 0	1	2	3	Mean ± S.D.
0–99	6				0
100–199	3	3			0.50 ± 0.55
200–299	3	9	5	4	1.48 ± 0.98
200–399	2	2	6	2	1.67 ± 0.98
400+		3		6	2.33 ± 1.00

*Histologic grade of nephropathy = 0.4348 + 0.003616 (dose), $r = 0.479$, $P < 0.001$.

Grade 2 or 3 nephropathy was seen in 42.9% of rabbits treated to cumulative doses of 200–299 mg/m^2 and in 67% of rabbits who received in excess of 300 mg/m^2. The mean histologic score increased from 0.50 ± 0.55 for rabbits receiving between 100 and 199 mg/m^2 to 2.33 ± 1.00 for those receiving in excess of 400 mg/m^2.

DISCUSSION

Doxorubicin has been one of the most extensively used antineoplastic agents since its commercial release in the United States in 1974 [17]. It is a highly active agent in all hematologic malignancies and has high degrees of activity in most solid tumors [18]. Its clinical use has been limited by a cumulative dose-related cardiac toxicity manifested by a characteristic histologic picture of focal to diffuse myofibrillar degeneration, necrosis and myocardial fibrosis that is recognized in endomyocardial biopsy tissue at cumulative doses as low as 200 mg/m^2, and culminates in congestive heart failure with increasing frequency at cumulative doses in excess of 500 mg/m^2 [4]. Numerous anecdotal reports of synergy between doxorubicin and other antineoplastic agents and mediastinal irradiation have been published [19–21]. Because of the ethical difficulties encountered when using humans for toxicity studies, a suitable animal model was needed. Numerous animals have demonstrated acute cardiac toxicity following single injections of doxorubicin [12]. The rabbit is the only animal to consistently develop a cumulative dose-related cardiomyopathy after repeated chronic doxorubicin administration. With the use of serial systolic time interval measurement, each rabbit can serve as its own control and small groups of animals can be treated to the point of cardiac dysfunction rather than empirically treating multiple groups of animals to various dose levels. Euthanasia of the animals and histologic study has been required for the few studies [1–3, 16] using the rabbit model. Histologic studies require large numbers of rabbits and large quantities of study drug, thus making the rabbit a prohibitively expensive model for study of anthracycline cardiac toxicity. The smaller number of rabbits needed for studies using serial systolic time interval recording of cardiac function in the rabbit makes a more attractive model for a comparative study of new anthracycline compounds and a search for cardioprotective agents and synergistic cardiotoxic drugs.

This study demonstrates that prolongation of the PEP/LVET ratio correlates well with development of doxorubicin cardiomyopathy,

and that as the histologic severity of cardiomyopathy increases the PEP/LVET ratio also increases. Previous reports [9–11] have documented the reproducibility of systolic time intervals in the rabbit and confirmed its accuracy in documenting cardiac dysfunction in rabbits with congestive heart failure. Using the PEP/LVET ratio and histologic grading, it has been demonstrated that the rabbit develops cardiac dysfunction and histologic lesions identical to those seen in humans, with the same increasing frequency and severity as cumulative doxorubicin dosage increases beyond 200 mg/m^2. This was found in nearly 100% of the rabbits that received greater than 400 mg/m^2.

The results of this study indicate that the use of the PEP/LVET ratio as a measure of the presence of anthracycline-induced cardiomyopathy is useful only at the higher cumulative dosage levels, where the correlation with histologic presence of cardiomyopathy is high. As in previously reported human studies [7], there is poor correlation at lower cumulative dose levels and at the milder grades of histologic damage. There is a significant correlation between cumulative doxorubicin dosage and level of PEP/LVET ($r = 0.430$, $P < 0.001$) and histologic grade of cardiomyopathy ($r = 0.362$, $P < 0.01$). There is also a significant correlation between histologic grade of cardiomyopathy and level of PEP/LVET ($r = 0.394$, $P < 0.01$). When the PEP/LVET ratio is greater than 0.466 and it is used as the definition of cardiac dysfunction, the specificity is high (0.75) but the sensitivity is low (0.57). Of the 23 rabbits with grade 1 or greater cardiomyopathy on histologic review, there were 10 false negatives using PEP/LVET $\geqslant$0.466 as the definition of cardiac dysfunction. By this criterion, there were only 8 false positives of 32 rabbits without histologic evidence of anthracycline-induced cardiomyopathy. By changing the definition of significant cardiac dysfunction to PEP/LVET $\geqslant$0.450, then the sensitivity is increased to 0.70 and specificity is decreased to 0.71.

The rabbit develops a cumulative dose-related nephrotoxicity at doses in excess of 100 mg/m^2. The frequency and severity of the nephropathy increases with increasing cumulative dosage. There has been speculation that this nephrotoxicity may contribute to cardiac dysfunction seen in the rabbit, and it may limit usefulness of this animal as a model for anthracycline-induced cardiac toxicity. The current study did not include tests of renal function and does not address the possibility that renal failure, altered fluid and electrolyte excretion may contribute to the degree of cardiac dysfunction seen in the rabbit. There does not appear to be a direct correlation ($r = 0.198$, $P > 0.1$) between severity of histologic abnormalities in the heart and the kidney (Table 9), although the nephropathy appears to be more severe at all dose levels of doxorubicin than the cardiomyopathy. Although nephropathy was demonstrated in 20 of the 23 rabbits with histologic evidence of cardiomyopathy, there were 19 rabbits with nephropathy in the absence of histologic evidence of cardiomyopathy. These results confirm the observation of others [13] that the incidence of nephropathy is high at all dose levels above 100 mg/m^2 but suggests no direct relationship to the presence or severity of cardiomyopathy.

Nephropathy has not been widely demonstrated in humans after chronic treatment with doxorubicin, daunomycin or rubidazone, but it is a potential toxicity of new anthracycline drugs and should be looked for in preclinical and clinical studies of newer anthracycline drugs. Further investigation of the role of nephropathy in the development of cardiac dysfunction in the rabbit is needed before this animal can be adopted as a suitable model for anthracycline cardiotoxicity.

To our knowledge skeletal myopathy has not been described in humans following chronic doxorubicin administration but has been recognized as a toxicity in the rabbit. This study confirms a cumulative dose–response relationship for skeletal myopathy in the rabbit after chronic administration of cumulative doses of doxorubicin in excess of 200 mg/m^2. However, only 13/24 rabbits exhibiting grade 1 or greater cardiomyopathy also demonstrated skeletal myopathy and 4 rabbits exhibited skeletal myopathy in the absence of cardiomyopathy. At all dose levels, skeletal myopathy was not as frequent a finding nor were the lesions as severe as cardiac lesions at the same dose level. The skeletal muscle lesions were focal and mild even in rabbits with severe cardiomyopathy, thus the severity of skeletal myopathy did not appear to be dose-

Table 9. Comparison of histologic grade of nephropathy and cardiomyopathy in doxorubicin-treated rabbits

		Cardiomyopathy histologic grade*				
		0	1	2	3	4
Nephropathy histologic grade	0	12			2	
	1	10	2	2	3	
	2	4	3		3	
	3	5	5		1	1

*Histologic grade of cardiomyopathy = 0.5445 + 0.2136 (histologic grade of nephropathy), $r = 0.198$, $P > 0.1$.

related. There was only a weak correlation ($r = 0.276$, $P < 0.05$) between the presence or severity of skeletal myopathy when compared to the presence and severity of cardiomyopathy (Table 10).

Table 10. Comparison of histologic grade of skeletal myopathy and cardiomyopathy in doxorubicin-treated rabbits

		Cardiomyopathy histologic grade*				
		0	1	2	3	4
Skeletal myopathy histologic grade	0	26	6	3	3	0
	1	1	5	1	4	0
	2	1	0	0	0	1
	3	1	0	0	0	0
	4	1	0	1	1	0

*Grade of cardiomyopathy = 0.704 + 0.345 (grade of skeletal myopathy), $r = 0.276$, $P < 0.05$.

A cumulative dose–response relationship has been confirmed for the development of doxorubicin-induced cardiomyopathy, nephropathy and skeletal myopathy. Systolic time interval measurement has been demonstrated to confirm cardiac dysfunction in a high percentage of rabbits with histologic evidence of doxorubicin-induced cardiomyopathy of grade 2 or greater. In the rabbit a PEP/LVET ratio >0.450 is both sensitive and specific for cardiac dysfunction. Due to the focal nature of anthracycline-induced skeletal myopathy and the poor correlation to degree of cardiomyopathy, serial skeletal muscle biopsy would not appear to be a reasonable substitute for serial systolic time interval recording or histologic examination of the myocardium in future rabbit studies of anthracycline drugs.

REFERENCES

1. JAENKE RS. Delayed and progressive myocardial lesions after adriamycin administration in the rabbit. *Cancer Res* 1976, **36**, 2958–2966.
2. JAENKE RS. An anthracycline antibiotic-induced cardiomyopathy in rabbits. *Lab Invest* 1974, **30**, 292–304.
3. VAN VLEET JF, FERRANS VJ. Clinical and pathologic features of chronic adriamycin toxicosis in the rabbit. *Am J Vet Res* 1980, **41**, 1462–1469.
4. LENAZ L, PAGE JA. Cardiotoxicity of adriamycin and related anthracyclines. *Cancer Treat Rev* 1976, **3**, 111–120.
5. BALCERZAK SP, CHRISTAKIS J, LEWIS RP, OLSON HM, MALSPEIS L. Systolic time intervals in monitoring adriamycin-induced cardiotoxicity. *Cancer Treat Rep* 1978, **62**, 893–899.
6. EWY GA, JONES SE, FRIEDMAN MJ, GAINES J, CRUZE D. Noninvasive cardiac evaluation of patients receiving adriamycin. *Cancer Treat Rep* 1978, **62**, 915–922.
7. MASON JW, BRISTOW MR, BILLINGHAM ME, DANIELS JR. Invasive and noninvasive methods of assessing adriamycin cardiotoxic effects in man: superiority of histopathologic assessment using endomyocardial biopsy. *Cancer Treat Rep* 1978, **62**, 857–864.
8. BRISTOW M. In: BRISTOW M, ed. *Drug-induced Heart Disease*. Amsterdam, Elsevier, 1980, Ch. 10.
9. LONG HJ, DIAMOND SS, BURNINGHAM RA, RAFLO CP. Systolic time interval recordings as a measure of cardiac function in the healthy rabbit: reference values. *Am J Vet Res* 1982, **43**, 1497–1499.
10. LONG HJ, DIAMOND SS, BURNINGHAM RA, RAFLO CP. Systolic time interval recordings in rabbits with congestive cardiomyopathy induced by chronic anthracycline administration. *Am J Vet Res* In press.
11. LONG HJ. Systolic time intervals: an effective measure of cardiac function in anthracycline-induced cardiomyopathy of rabbits. *Cancer Treat Rep* 1978, **62**, 911–914.
12. YOUNG DM. Pathologic effects of adriamycin (NSC-123127) in experimental systems. *Cancer Chemother Rep* 1975, **6**, 159–175.
13. FAJARDO LF, ELTRINGHAM JR, STEWART JR, KLAUBER MR. Adriamycin nephrotoxicity. *Lab Invest* 1980, **43**, 242–253.
14. BURKE JF JR, LAUCIUS JF, BRODOVSKY HS, SORIANO RZ. Doxorubicin hydrochloride-associated renal failure. *Arch Intern Med* 1977, **137**, 385–386.
15. SPECTOR WS, ed. *Handbook of Biological Data*. Philadelphia, PA, Philadephia, W. B. Saunders, 1956, 258.
16. ELTRINGHAM JR, FAJARDO LF, STEWART JR, KLAUBER MR. Investigation of cardiotoxicity in rabbits from adriamycin and fractionated cardiac irradiation: Preliminary results. *Front Radiat Ther Oncol* 1979, **13**, 21–35.
17. RDA lists 42 approved NDA's for oncologic drugs. *Clin Cancer Lett* August 1979, 6.

18. DORR RT, FRITZ WL. *Cancer Chemotherapy Handbook*. Amsterdam, Elsevier 1980, 388.
19. BILLINGHAM ME, MASON JW, BRISTOW MR, DANIELS JR. Anthracycline cardiomyopathy monitored by morphologic changes. *Cancer Treat Rep* 1978, **62**, 865–872.
20. MINOW RA, BENJAMIN RS, GOTTLIEB JA. Adriamycin (NSC-123127) cardiomyopathy: an overview with determination of risk factors. *Cancer Chemother Rep* 1975, **5**, 195–201.
21. PRAGA C, BERETTA G, VIGO PL *et al.* Adriamycin cardiotoxicity: a survey of 1273 patients. *Cancer Treat Rep* 1979, **63**, 827.