Cancer Chemother. Pharmacol. 4, 95–101 (1980)

Cancer Chemotherapy and Pharmacology

Evaluation of Antitumor Drug Side Effects in Small Animals

W. T. Bradner, J. E. Schurig, J. B. Huftalen, and G. J. Doyle

Bristol Laboratories Division, Bristol-Myers Company,
P.O. Box 657, Syracuse, NY 13201, USA

Summary. *This is an initial report on the development of screening tests for side effects of antitumor drugs, with small amounts of compound and short time intervals. These tests are based on acute dosing of mice and various blood or serum measurements: (a) total white blood cell count for leukopenia; (b) BUN for kidney toxicity; (c) SGPT for liver toxicity; and (d) creatine phosphokinase MB isozyme (CPK-MB) for cardiotoxicity. A correlation with the toxicity observed in other species is developed by establishing the effect of a prototype compound for each toxicity and tests of one or more compounds expected to lack such toxicity. On the basis of the limited number of compounds studied all four tests, although varying in sensitivity, seem to correlate with the results of tests in other species and with known effects in man. Final validation of these acute tests, especially the CPK-MB, will require both further study of histopathologic effects and correlation with results from clinical trials of an extended list of agents.*

Introduction

The selection of new antineoplastic agents for clinical trial is based on tests in animal tumor systems to determine efficacy, and on toxicologic studies in large animals to characterize toxic effects and determine safe dose levels for human use. More recent emphasis on the development of improved forms of existing drugs has led to a plethora of analogs of comparable effectiveness but varying broadly in potency. Since a major goal of analog programs is the identification of active compounds with reduced life-threatening side effects, a severe testing bottleneck has developed because of lack of efficient methods for evaluating such effects. Subjection of every new tumor-active agent to full-scale preclinical toxicology in large animals (dogs and primates) would be too slow and of such overwhelming cost as to exceed existing resources. Furthermore, many analogs tend to be available only in small quantities. In cases where synthesis is low-yielding or with natural products where isolation is difficult, there is reluctance to invest in the preparation of large quantities of a drug for testing in a critical screen that may have a high rejection rate. To circumvent these problems, we are developing screening tests applicable to small animals, primarily mice, which give a reasonably accurate indication of organ-specific toxicities. Mice have been under study for quantitative toxicity and prediction of starting dose in human trials for many years [4–6].

In this report we present data relative to hematologic, cardiac, renal, and hepatic toxicity of anticancer drugs and discuss the application and limitations in developing rapid side-effects screens in small animals.

Reprint requests should be addressed to: W. T. Bradner

Materials and Methods

Dose Selection

The doses of test drugs studied were based on the acute LD_{50} in heavy ($\geq$ 25 g) BDF_1 [(C57 BL/6 × DBA/2)F_1] male mice. In each experiment the animals were distributed uniformly within $\pm$ 2 g of the mean weight. Dose-response titrations were calculated in either 0.75 or 0.5 increments, depending upon the drug under study. Mitomycin C was dissolved in water; sterigmatocystin was suspended in hydroxypropylcellulose solutions; and the remaining drugs were dissolved in saline. The compounds were injected IP in a constant volume 0.5 ml per dose. Control animals received vehicle without drug.

Acute LD_{50} Determination

Sets of ten animals per dose were treated with single IP injections of the test drug and observed for 60 days. The LD_{50} based on deaths up to 14 days was calculated by the method of Weil [18]. The animals were observed for later deaths but these were usually not included in the calculations, because in most cases the delayed onset and gross pathology suggested the likelihood of local chemical irritation rather than general pharmacologic effect as described by Avery et al. [2].

0344-5704/80/0004/0095/$ 01.40

Leukopenic Tests

Typically, a group of 40 mice were bled from the retro-orbital plexus [13] and total white blood cell (WBC) counts were performed on the Coulter Counter Model S (Coulter Electronics Inc., Hialeah, Florida). One or two days later, sets of ten mice each were injected with three different dose levels of drug and one set with vehicle as a procedural control. Serial leukocyte counts were performed on each mouse on Days 3, 5, and 7 after dosing and the results of each test were compared with the appropriate pretreatment value. Statistical analyses were performed by means of a Digital PDP 11/34 (Digital Equipment Corp., Maynard, MA) programmed for a paired *t*-test on individual differences [15]. The mean change from pretreatment values was calculated at each time point after dosing for tabulating the data.

Serum Chemistries

Serial serum chemistries were performed in a similar manner. Blood was collected in Microtainers (Becton-Dickenson Co., Rutherford, NJ) and allowed to clot for serum separation. Appropriate dilutions, mixing with reagents, and spectrophotometric measurements for individual clinical chemistry tests were performed in the Centrifichem System 400 (Union Carbide Co., Rye, NY). The tests studied were: L-alanine amino-transaminase (SGPT), blood urea nitrogen (BUN), and creatine phosphokinase MB isozyme (CPK-MB). Measurements of CPK were made after activation with reagents to give total or MM isozyme levels based on the procedure of Rao [12]. The difference between total CPK and CPK-MM gives an estimate of the level of CPK-MB isozyme, which has been associated specifically with damage to heart muscle.

Results

Control Values

Table 1 lists control values for the measurements performed in mice prior to dosing. These values were computer-calculated and based on the number of animals listed. They are in general agreement with those reported by Harrison et al. [7], who used a different automated analyzer. Although pilot studies were performed with alkaline phosphatase (alk phos) and aspartate aminotransferase (SGOT) for liver function, and serum hydroxybutyric acid dehydrogenase (HBD) for cardiac effects, these measurements appeared less predictive in the mouse than SGPT and CPK-MB, respectively, for these purposes, and thus were not utilized further. Additional clinical tests, such as differential leukocyte counts, platelet counts, and serum creatinine are being explored.

Table 1. Clinical chemistry and hematology values for male BDF_1 mice

Test	*n*	Mean[a]	SD	95% confidence limits
BUN	996	18	4	10 – 26
SGPT	336	20	8	4 – 36
SGOT	246	46	17	12 – 80
Alk P	361	68	12.5	43 – 93
CPK-Total	583	270	193	0 –656
CPK-MM	583	252	188	0 –628
CPK-MB	583	17	15	0 – 47
HBD	213	134	56	41 –246
WBC	1450	10.2	2.4	5.3– 15.1

[a] Average of all measurements

Leukopenia

The following agents were tested for leukopenic effects after a single dose: adriamycin, mitomycin C, and *cis*-dichlorodiamminoplatinum II (*cis*-DDP) and bleomycin. Representative results are shown in Table 2. All doses of adriamycin and mitomycin C were significantly leukopenic on at least one of the measurement days. *cis*-DDP was significantly leukopenic at the three lowest doses on Day 3, but not at the LD_{50}. This could possibly be due to hemoconcentration associated with renal effects of the drug. Bleomycin gave variable results with a significant ($P \leq 0.01$) decrease only at one low dose time point.

Renal Toxicity

Two drugs known to be highly nephrotoxic, *cis*-DDP [8] and phleomycin [11] and two drugs with minimal nephrotoxicity, adriamycin and mitomycin C, were studied with BUN as a marker for nephrotoxicity. The results are shown in Table 3. With the nephrotoxic drugs severe rises in BUN were noted, occasionally as high as 427 mg/100 ml. In the experiment with *cis*-DDP the very low variability in the control group BUN values led to calculated significance for a postdose change that was well within normal limits for the BDF_1 mouse. In contrast, changes among treated animals that were highly significant clinically were not significant statistically, due to the high variability. An alternative parameter for assessing renal toxicity is the incidence of animals that manifested a BUN rise above normal limits (> 30 mg/100 ml). A column showing this incidence is therefore included for each day of measurement. The fact that a dose-response is observed among surviving animals suggests that the incidence may be a more clinically meaningful method of evaluating the data.

Hepatic Toxicity

The liver carcinogen sterigmatocystin was used as an example of a highly hepatotoxic chemical. In a kinetic study a sharp rise in SGPT was observed within 24 h, the

Table 2. Effect of antitumor agents on total white blood cell count

Drug	Dose mg/kg IP	Predose WBC[a]	Day 3		Day 5		Day 7	
			WBC	%Δ	WBC	%Δ	WBC	%Δ
Adriamycin	14.1	10 ± 0.5[b]	6 ± 0.5** (9/10–10)[c]	−43	5 ± 0.8** (7/10–1)[c]	− 4	9 ± 2 (6/10–10)[c]	−16
	10.6	9 ± 0.4	6 ± 0.6	−37	5 ± 0.5**	−40	8 ± 1	− 7
	8	10 ± 0.6	6 ± 0.5**	−36	7 ± 0.6** (8/10–9)[c]	−30	10 ± 1	7
Vehicle	–	10 ± 0.5	11 ± 0.9	8	9 ± 0.7	− 8	12 ± 1	21
Mitomycin C	7.5	11 ± 0.5	6 ± 1**	−42	5 ± 1** (9/10–11)[c]	−51	9 ± 0.9**	−22
	5.6	12 ± 0.4	6 ± 0.5**	−47	6 ± 0.5**	−52	10 ± 0.8	−15
	4.2	11 ± 0.5	7 ± 0.3**	−38	7 ± 0.8**	−35	14 ± 0.6*	26
Vehicle	–	11 ± 0.7	9 ± 0.7**	−19	12 ± 0.8	2	9 ± 0.9*	−22
cis-DDP	17.8	11 ± 0.6	10 ± 2	− 7	7 ± 0.8** (7/10–12)[c]	−38	[d]	
	13.4	12 ± 0.6	5 ± 0.4**	−53	8 ± 0.9*	−31	7 ± 1 (7/10–11)[c]	−34
	10.0	10 ± 0.7	5 ± 0.6**	−47	9 ± 0.5	−16	13 ± 4 (9/10–10)[c]	29
Vehicle	–	10 ± 0.8	11 ± 0.9	5	10 ± 1	− 4	8 ± 0.8	−19
Bleomycin	160	10 ± 0.6	9 ± 0.6	−13	8 ± 0.3* (9/10–10)[c]	−22	9 ± 0.9 (8/10–10)[c]	−16
	120	9 ± 0.8	7 ± 0.6	−15	9 ± 0.8 (9/10–9)[c]	− 1	8 ± 0.6 (9/10–9)[c]	−14
	90	9 ± 0.6	6 ± 0.4**	−32	8 ± 0.6	−17	7 ± 0.4	−20
Vehicle	–	11 ± 0.4	10 ± 0.5	− 8	12 ± 1	12	11 ± 0.5	− 1

[a] × 10^3 cmm
[b] Values are mean ± SEM
[c] Survivors/treated with adjusted predose WBC
[d] Less than three survivors
* $P \leq 0.05$, ** $P \leq 0.01$, i.e., significant differences from predose values

level returning to normal by Day 3 (Table 4). Bleomycin appeared to cause a delayed rise in SGPT, whereas adriamycin and mitomycin C had minimal effects on this enzyme (Table 4).

Cardiac Toxicity

Pilot studies on measurement of HBD, CPK, and CPK-MB revealed high variability with all these tests when performed with mice. The CPK-MB seemed most promising, because of a better clinical correlation for the drugs tested and its known association with cardiac damage. Because day-to-day variation was much wider than was seen with other types of tests, the vehicle-treated control mice were used as a comparative base rather than the pretreatment values. In a kinetics study of adriamycin a significant increase in CPK-MB was observed on Day 3 at 10.6 and 8 mg/kg (Table 5). In similar experiments with bleomycin and mitomycin C, a group of animals given 10.6 mg/kg of adriamycin was included, to serve as a positive control on the procedure. Bleomycin caused a significant rise in CPK-MB, whereas mitomycin C was without effect even at lethal doses.

Discussion

We have studied a number of approaches to screening side effects of antitumor drugs in small animals, emphasizing tests that could be performed with acute dosing and a short observation interval. This approach to side effects screening might be questioned because acute dosing may or may not produce the same toxicities as develop on chronic treatment. Whether or not such toxicities do in fact develop is not critical if a clear correlation can be established between acute effects observed with a series of various drugs and the side effects seen in large-animal toxicology studies and in clinical trials. This correlative evidence should serve to validate each test and the data we

Table 3. Effect of anticancer drugs on blood urea nitrogen

Drug	Dose mg/kg IP	Predose BUN[a]	Day 4			Day 7		
			BUN	%Δ	BUN ≥ 30	BUN	%Δ	BUN≥30
cis-DDP	17.8	15 ± 0.7[b]	427 ± 162	2747	4/5	[d]		
	13.4	16 ± 0.6	257 ± 119	1555	5/9	28 ± 8	82	2/5
	10.0	16 ± 0.9	43 ± 26	171	1/10	19 ± 0.9*	17	0/8
Vehicle	–	15 ± 0.4	20 ± 0.7**	31	0/10	19 ± 0.6**	21	0/10
Phleomycin	13.2	18 ± 0.7	86 ± 24* (8/10–18)[c]	370	6/8	92 ± 31 (7/10–18)[c]	405	5/7
	9.9	18 ± 0.9	41 ± 7**	129	6/10	43 ± 9*	144	6/10
	7.4	20 ± 0.6	23 ± 4	18	2/10	24 ± 3	19	2/10
Vehicle	–	17 ± 0.7	16 ± 0.5*	− 9	0/10	18 ± 0.7	4	0/10
Adriamycin	14.1	14 ± 0.7	19 ± 7	37	1/10	14 ± 0.5 (8/10–13)[c]	8	0/8
	10.6	14 ± 0.6	15 ± 0.9	9	0/10	17 ± 0.7* (9/10–14)[c]	20	0/9
	8	15 ± 0.7	14 ± 0.6	− 5	0 10	15 ± 0.4	− 1	0/10
Vehicle	–	14 ± 0.8	18 ± 1*	29	0/10	17 ± 0.8	24	0/10
Mitomycin C	7.5	18 ± 0.7	17 ± 0.9	− 4	0/10	[d]		
	5.6	17 ± 0.5	13 ± 0.8**	−20	0/10	15 ± 0.8*	−12	0/9
	4.2	17 ± 0.6	13 ± 0.8**	−22	0/9	16 ± 0.6	− 4	0/9
Vehicle	–	16 ± 0.5	18 ± 0.7**	18	0/10	19 ± 0.5**	23	0/10

[a] mg/100 ml
[b] Values are mean ± SEM
[c] Survivors/treated with adjusted predose BUN
[d] < 3 survivors
* $P \leq 0.05$, ** $P \leq 0.01$, i.e., significant differences from predose values

have obtained so far seem to meet this standard as follows:

Myelosuppression

Three widely differing drugs known to be clinically myelosuppressive caused significant leukopenia in the BDF_1 mouse, whereas the nonmyelosuppressive drug bleomycin did not. Data accumulating from the testing of other drugs in clinical trial and preclinical toxicology continue to support the reliability of the mouse leukopenic test [1, 3].

Renal Toxicity

Measurements of azotemia are considered somewhat insensitive for assessing toxicity to the kidney, since histologic damage can be detected in animals maintaining normal serum values. Nevertheless, for screening purposes, BUN appears to be a reliable test with good correlation. Sharp rises in BUN could be demonstrated with drugs known to be nephrotoxic, *cis*-DDP and phleomycin, and not with others known to have minimal or no nephrotoxicity. The practice of reporting BUN results as means of experimental groups alone is open to question, since we observed that fewer of the animals given lower doses respond. Thus we believe the morbidity incidence should be included.

Hepatic Toxicity

Of the various liver function tests explored, SGPT seemed suitable to use alone and rose quickly following a toxic dose of sterigmatocystin. A delayed rise observed after bleomycin treatment could be secondary to other effects. Bleomycin hepatic toxicity in the dog has been described as minor and reversible [17]. Still less effect was seen, as expected, with adriamycin and mitomycin C.

Cardiac Toxicity

A considerable amount of research has been performed in the search for animal models for anthracycline cardiotoxicity. Studies of EKG and histopathological effects of

Table 4. Effect of antitumor drugs on SGPT

Drug	Dose mg/kg IP	Predose SGPT[a]	Day 1		Day 3		Day 5	
			SGPT	%Δ	SGPT	%Δ	SGPT	%Δ
Sterigmatocystin	283	15 ± 2[b]	135 ± 16**	788	25 ± 2**	63	16 ± 2 (9/10–16)[c]	1
	141.5	13 ± 1	72 ± 6**	442	18 ± 2**	36	10 ± 1* (9/10–13)[c]	−27
	70.8	12 ± 1	24 ± 3**	97	13 ± 1	2	13 ± 2	6
Vehicle	–	16 ± 1	18 ± 6	15	19 ± 3	22	19 ± 6	23
Bleomycin	160	15 ± 1	43 ± 7**	192	57 ± 9**	286	183 ± 39* (5/10–14)[c]	1247
	80	16 ± 2	24 ± 3*	48	21 ± 3	27	59 ± 15 (4/10–15)[c]	293
	40	13 ± 1	17 ± 5	38	17 ± 2*	33	66 ± 10** (8/10–13)[c]	424
Vehicle	–	14 ± 0.9	18 ± 4	29	11 ± 1	− 19	12 ± 1	−16
Adriamycin	14.1	15 ± 2	34 ± 6**	125	18 ± 3 (9/10–15)[c]	18	18 ± 3 (7/10–14)[c]	28
	7.0	16 ± 1	22 ± 2**	44	13 ± 0.8	− 15	14 ± 1	−12
	3.5	14 ± 1	15 ± 0.9	6	11 ± 1	− 24	14 ± 1	0
Vehicle	–	14 ± 1	21 ± 4	45	11 ± 1	− 20	13 ± 2	− 8
Mitomycin C	7.5	20 ± 3	16 ± 3	− 20	36 ± 10 (9/10–22)[c]	66	20 ± 2 (6/10–21)[c]	− 5
	3.8	29 ± 4	16 ± 1**	− 43	17 ± 3*	− 39	19 ± 4	−34
	1.9	24 ± 2	16 ± 2**	− 33	16 ± 2*	− 34	15 ± 2**	−39
Vehicle	–	26 ± 4	19 ± 2*	− 26	18 ± 2	− 29	18 ± 1	−29

[a] IU
[b] Values ar mean ± SEM
[c] Survivors/treated with adjusted predose SGPT
* $P \leq 0.05$; ** $P \leq 0.01$, i.e., significant differences from predose values

Table 5. Effect of antitumor drugs on CPK-MB

Drug	Dose mg/kg IP	Predose CPK-MB (IU)	*n*	CPK-MB					
				Day 3[b]	*n*	Day 5[b]	*n*	Day 10[b]	*n*
Vehicle	–	17 ± 4[a]	10	14 ± 3	10	24 ± 5	10	16 ± 4	9
Adriamycin	14.1	10 ± 4	10	24 ± 4	9	22 ± 4	9	4 ± 2	7
	10.6	9 ± 2*	20	44 ± 6*	19	22 ± 2	19	17 ± 3	19
	8	4 ± 2*	10	36 ± 4*	10	27 ± 5	9	27 ± 5	9
Vehicle	–	22 ± 3	10	22 ± 2	10	12 ± 2	10	11 ± 3	9
Bleomycin	160	22 ± 4	10	63 ± 19*	5	[c]			
	120	28 ± 4	10	165 ± 33*	7	47 ± 10*	3	[c]	
	90	20 ± 3	10	59 ± 16*	9	81 ± 18*	6	[c]	
Adriamycin	10.6	16 ± 3	10	92 ± 21*	9	42 ± 13*	7	26 ± 6*	8
Vehicle	–	15 ± 4	10	22 ± 3	10	33 ± 11	10	22 ± 6	10
Mitomycin C	7.5	16 ± 3	10	6 ± 2*	10	37 ± 6	6	–	0
	5.6	13 ± 4	10	30 ± 5	10	32 ± 6	10	11 ± 4	3
	4.2	10 ± 2	10	35 ± 6	9	29 ± 6	10	15 ± 2	9
Adriamycin	10.6	25 ± 3	10	59 ± 8*	7	86 ± 20*	5	8 ± 2*	3

[a] n = Number of evaluable mice
[b] Values are mean ± SEM
[c] < 3 survivors
* $P \leq 0.05$, i.e., significant difference from corresponding value obtained with vehicle alone

anthracyclines in rat, rabbit, and hamster have all been reported. Serum enzyme changes have not been considered good predictors of anthracycline cardiomyopathy in either animals or man during chronic dosing with these drugs at therapeutic levels [19]. However, Olson and Capon [10] noted that acute single doses of adriamycin in rats caused serum CPK and LDH changes, which were followed by evidence of fine-structure damage to myofibrils. Rosenoff et al. [14] have reported light microscopic cardiomyopathic changes in BDF_1 mice as early as 4 days after an acute single dose of adriamycin. Induction of cardiac lesions in mice resembling those seen in patients following adriamycin treatment has been observed by Lenaz et al. [9]. This information supports the concept that the cardiac enzyme changes observed following acute dosing may be associated with insult to cardiac tissue, leading to more permanent damage. Our limited data tend to support this concept, since adriamycin caused a rise in CPK-MB and mitomycin C did not. Bleomycin might appear to be a 'false-positive', because of the rise it caused in CPK-MB. However, bleomycin *is* cardiotoxic at extremely high doses. Myocarditis has been observed in monkeys following high doses of bleomycin [16], but this has never been a clinical problem because pulmonary toxicity has limited human doses to much lower levels. Preliminary data we have obtained with other anthracycline antibiotics (to be reported in the future) suggest a reasonably good rank order in comparison of acute CPK-MB effects with reports of cardiotoxicity in the literature. The biggest difficulty with this test has been one of reproducibility. Occasional experiments fail to show differential effects between treated animals and controls. For this reason, a dose of adriamycin known to cause a rise in CPK-MB is included in each experiment. If this rise fails to occur the experiment is considered invalid and is repeated.

These studies of side effects in mice have as their objective the development of screening tests requiring small amounts of compound, which can assist in selecting analogs for further development. As such, these tests, together with antitumor tests, contribute to comparative efficacy studies. They are *not* preclinical toxicology studies. Use of the IP route of injection would be inappropriate for preclinical toxicology but is the most convenient and widely used route for antitumor screening, thus permitting some degree of direct relationship between tumor tests performed and side-effects data we are collecting in the same species.

Although this initial work has been limited to acute single doses developed from a prototype compound for each type of toxicity, it will be important in the future to study (a) early and later time points for measurements; (b) lower doses of drug to reach end-points; (c) other routes of administration; (d) multiple dose schedules; (e) other related clinical tests; and (e) early and late histopathological effects. The impetus for developing these screens was primarily to evaluate large numbers of chemotherapeutic analogs; however, they may also be useful in (a) testing various regimens for control of side effects; (b) testing the safety of agents used in combination; (c) profile testing of novel chemotherapeutic drugs; and (d) the study of side-effect blocking agents or treatments.

References

1. Anderson T, McMenamin MB, Schein PS (1975) Chlorozotocin, 2-[3-(2-chloroethyl)-3-nitrosouriedo]-D-glucopyranose, an antitumor agent with modified bone marrow toxicity. Cancer Res 35:761
2. Avery TL, Roberts D, Price RA (1973) Delayed toxicity of 4′Demethylepipodophyllotoxin 9-(4,6-0-2-thenylidine-β-D-glycopyranoside) (NSC 122819; VM-26) in mice. Cancer Chemother Rep [1] 57:165
3. Bradner WT, Huftalen JB (1978) Marcellomycin: An anthracycline without leukopenic effect in BDF_1 mice. Proc Am Assoc Cancer Res 19:46
4. Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper HE (1966) Quantitative comparison of toxicity of anti cancer agents in mouse, rat, hamster, dog, monkey, and man. Cancer Chemother Rep 50:219
5. Goldsmith MA, Slavik M, Carter SK (1975) Quantitative prediction of drug toxicity in humans from toxicology in small and large animals. Cancer Res 35:1354
6. Guarino AM, Rozencweig M, Kline I, Penta JS, Venditti JM, Lloyd HH, Holzworth DA, Muggia FM (1979) Adequacies and inadequacies in assessing murine toxicity data with antineoplastic agents. Cancer Res 39:2204
7. Harrison SD, Jr, Burdeshaw JA, Crosby RG, Cusic AM, Denine EP (1978) Hematology and clinical chemistry reference values for C57BL/6 X DBA/2 F_1 mice. Cancer Res 38:2636
8. Kociba RJ, Sleight SD (1977) Acute toxicology and pathologic effects of *cis*-diamminedichloroplatinum (NSC 119875) in the male rat. Cancer Chemother Rep [1] 55:1
9. Lenaz L, Sternberg SS, DeHarven E, Vidal PM, Philips FA (1978) Cardiac lesions in adriamycin treated mice. Proc Am Assoc Cancer Res 19:213
10. Olson HM, Capen CC (1977) Subacute cardiotoxicity of adriamycin in the rat: Biochemical and ultrastructural investigations. Lab Invest 37:386
11. Rakieten N, Nadkarni MV, Rakieten ML, Gordon BS (1969) Toxicologic and pharmacologic evaluation of phleomycin including special studies on its nephrotoxicity. Toxicol Appl Pharmacol 14:590
12. Rao PS, Lukes JJ, Ayers SM, Mueller H (1975) New manual and automated method for determining activity of creatine kinase isoenzymes MB by use of dithiothreitol: Clinical applications. Clin Chem 21:1612
13. Riley V (1960) Adaptation of orbital bleeding technic to rapid serial blood studies. Proc Soc Exp Biol Med 104:751
14. Rosenoff SH, Olson HM, Young DM, Bostick F, Young RC (1975) Adriamycin-induced cardiac damage in the mouse: A small-animal model of cardiotoxicity. J Natl Cancer Inst 55:191
15. Steel R, Torrie J (1960) Principles and procedures of statistics. McGraw-Hill, New York, p 72

16. Thompson G, Baker J, Fleischman R, Schaeppi U, Cooney D, Davis R (1970) Toxicity of bleomycin (NSC 125066), a new carcinostatic antibiotic, in dogs and monkeys. Pharmacologist 12:241
17. Thompson GR, Baker JR, Fleischman RW, Rosenkrantz H, Schaeppi UH, Cooney DA, Davis RD (1972) Preclinical toxicological evaluation of bleomycin (NSC-125066), a new antitumor antibiotic. Toxicol Appl Pharmacol 23:544
18. Weil CA (1952) Tables for convenient calculation of median effective dose (LD_{50} or ED_{50}) and instructions for their use. Biometrics 8:249
19. Young DM (1975) Pathologic effects of adriamycin (NSC-123127) in experimental systems. Cancer Chemother Rep [3] 6:159

Received June 25, 1979/Accepted October 30, 1979