*Europ. J. Cancer* Vol. 16, pp. 1433–1440
© Pergamon Press Ltd. 1980. Printed in Great Britain

0014-2964/80/1101-1433 $02.00/0

# *In Vivo* Tumor Response to Single and Multiple Exposures of Adriamycin*

D. W. SIEMANN and R. M. SUTHERLAND

*Multimodality Research Section, Cancer Center, Box* 704, *University of Rochester Medical Center*, 601 *Elmwood Avenue, Rochester, N. Y.* 14642, *U.S.A.*

**Abstract**—*The response of murine EMT6/Ro tumors and KHT sarcomas to adriamycin (ADR) given either as a single dose, 2 equal doses or multiple dose increments (all to the same total dose) was determined using a tumor growth delay assay. The total ADR uptake in the tumors was similar for the various drug schedules. An* $LD_{10/60}$ *dose of ADR (lethal to* 10% *of the mice in* 60 days) *led to a growth delay equivalent to one doubling time in these established tumor lines as well as a first generation transplanted mammary tumor. Two doses, each equal to half the* $LD_{10/60}$ *dose, separated by* 1–7 days *or* 1–2 days, *were as effective against the EMT6/Ro tumor and KHT sarcoma respectively as a single* $LD_{10/60}$ *dose. These findings suggest a lack of repair of ADR damage in these tumors. However, such a total dose* ($LD_{10/60}$), *given as multiple daily increments, resulted in a growth delay less than that following a single* $LD_{10/60}$ *exposure likely due to cell repopulation.*

## INTRODUCTION

THE CYTOTOXIC antibiotic adriamycin (ADR) has been shown to be an effective antineoplastic drug against a wide spectrum of solid tumors and hematologic diseases [1, 2]. ADR also is presently being used in combined modality trials [2, 3] because of its oncoloytic activity in both clinical studies [1, 2] and animal model systems [4–7]. However, only a few attempts have been made in animal models to correlate tumor response and drug concentration within the tumor [8, 9]. Further, most *in vivo* studies of tumor response to ADR have concerned single drug exposures [4, 5, 9], and those investigations dealing with repeated drug doses usually were limited to treatments given soon after tumor cell inoculation [5, 7]. Little information on the simultaneous comparison of single and multiple drug exposures against palpable solid tumors is available. Since clinically ADR may be administered by a variety of dose regimen [10], the present study was undertaken to determine the ADR level in mouse tumors following single, split and multiple drug exposures and to evaluate and compare the response to such treatments in tumors of different growth properties such as doubling time and metastatic potential. These experiments were carried out primarily using the EMT6 tumor and KHT sarcoma, two *in vivo* mouse tumor models commonly employed for radiobiological and chemotherapeutic studies [4, 11–14]. However, for comparison with these established tumor lines selected experiments employing a first generation spontaneous mammary tumor also were performed.

Accepted 10 April 1980.
*This work was presented in part at the 6th International Congress of Radiation Research, Tokyo, Japan, May 13–19, 1979, and was supported by NIH grants CA-20329, CA-11198 and CA-11051.

## MATERIALS AND METHODS

### *Animals and tumor systems*

The EMT6/Ro tumor is derived from the original tumor characterized by Rockwell, Kallman and Fajardo [15]. This subline is grown as an exponential culture *in vitro* and harvested for tumor injections by incubation in 0.05% trypsin. KHT sarcoma cells [16] are maintained *in vivo* and prepared from solid tumors by a mechanical transfer procedure [17]. For experiments, $2 \times 10^5$ EMT6/Ro or KHT sarcoma cells were injected i.m. into the hind limb of 8–14-week-old female BALB/cKa mice (Bio Breeding Labs, Ottawa, Canada) or C3H/HeJ mice (Jackson Labs, Bar Harbor, ME), respectively. The first generation transplanted mammary tumors were obtained by preparing a cell suspension from

a single spontaneous C3H/HeJ mouse mammary tumor and injecting a large number of cells into the calves of C3H/HeJ female mice. After the tumors had grown to a weight of ~0.2 g, the animals were allocated to a number of groups and treated or were kept as controls.

*Adriamycin treatments and fluorescence measurements*

ADR was dissolved in 15 min in physiological saline and given as a single or multiple i.p. injection to a total dose which results in the death of 10% of the animals in 60 days ($LD_{10/60}$). This dose was 11.0 mg/kg and 13.0 mg/kg for non-tumor-bearing BALB/cKa and C3H/HeJ mice respectively.

The tumor ADR equivalents at various times after drug injection were determined as has been previously described [13]. Briefly, after the animals were killed, a tumor homogenate was prepared and analyzed immediately for total drug fluorescence using the technique for extracting and quantifying ADR equivalents described by Schwartz [18]. However, although the measurement of ADR equivalents may be representative of the active drug level in the tumor, it should be recognized that this technique does not allow for a separation of the ADR products comprising the total drug fluorescence [19].

*Measurement of tumor response*

*Tumor growth delay studies.* Following the injection of tumor cells, each animal's tumor growth was determined by passing the tumor bearing leg through a series of increasing diameter holes in a plastic rod [14]. This measurement was converted to a tumour weight using a calibration curve (inset, Fig. 2) which was obtained by weighing tumors excised from tumor bearing legs of varing diameters. When the tumors reached a weight of ~0.2 g, the mice were allocated to various groups and each group was given its assigned treatment. Following treatment, the tumor size was measured 3–5 times a week and the growth delay resulting from the treatment then was determined by measuring the delay in the time between the control and treated tumors to grow to a weight of 0.8 g (4 times the starting weight).

*Survival studies with EMT6/Ro tumor and KHT sarcoma.* To determine the number of clonogenic cells per EMT6/Ro tumor, the animal was killed, the tumor aseptically removed and using scalpel and iris scissors minced until a fine paste was obtained. A single cell suspension was prepared by incubating the cells for 30 min in an enzyme "cocktail" modified from the one previously described by Brown *et al.* [11] containing pronase (0.025%), collagenase (0.025%) and DNAse (0.02%). After counting in a hemocytometer, the cells were plated in plastic dishes containing basal minimum essential medium with 15% fetal calf serum (FCS). The dishes were incubated for 12 days at 37°C, harvested, and stained with methylene blue and colonies of over 50 cells counted.

For clonogenic studies using the KHT sarcoma, surviving tumor cells were determined using an *in vitro* agar colony assay [17]. Following tumor excision, a suspension of single tumor cells was prepared by a combined mechanical and trypsinization procedure [12]. The cells were plated with $10^4$ heavily irradiated tumor cells in 0.2% agar containing alpha-minimum essential medium supplemented with 10% FCS. In about 2 weeks, the surviving cells formed colonies which were counted with the aid of a dissection microscope.

## RESULTS

*Adriamycin pharmacokinetics in tumors*

The ADR pharmacokinetics in EMT6/Ro tumors following single doses of 11.0 mg/kg and 5.5 mg/kg are shown in the upper panel of Fig. 1. A simple one component open model with first order decay for the terminal disposition phase was applied. For both doses, the ADR equivalents reach a maximum at 1–3 hr after injection. The half-life ($t_{\frac{1}{2}}$) (±S.E.) of the terminal phase following a dose of 11.0 or 5.5 mg/kg was 67 ± 10 and 58 ± 19 hr, respectively. These $t_{\frac{1}{2}}$s were found to be not significantly different when analyzed by covariance.

The ADR equivalents measured in the tumor following 2 doses of 5.5 mg/kg separated by 1 day and 5 daily doses of 2.2 mg/kg are the data points shown in the middle and lower panels of Fig. 1, respectively. The dashed lines in these 2 panels have not been fitted to the data points but rather are the expected results based on the curves shown in the upper panel and were calculated using the peak height and rate of elimination constant. In the middle panel, the initial portion of the dashed line (first 24 hr) is the solid curve of the upper panel (for an ADR dose of 5.5 mg/kg). For times greater than 24 hr, the same curve from the upper panel has been redrawn but now using as the origin the ADR

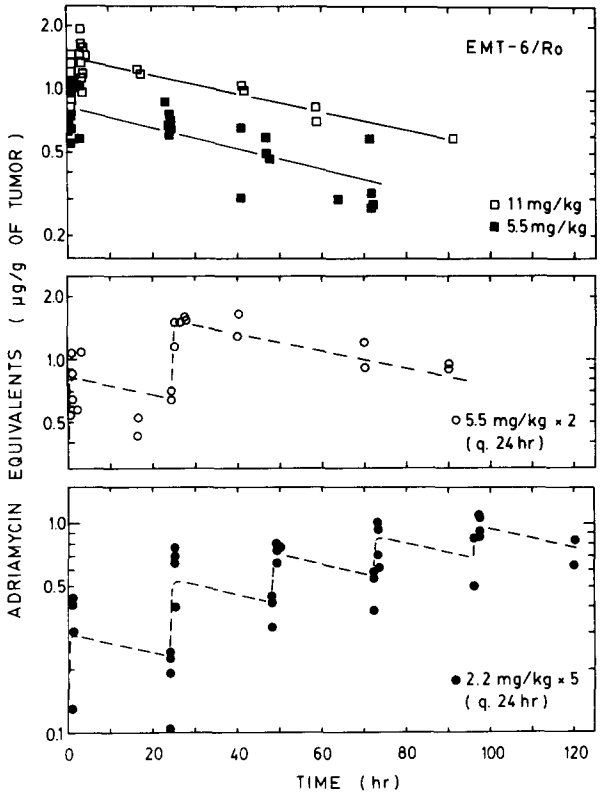


*Fig. 1. ADR equivalents as a function of time after a single i.p. injection of* 11.0 *or* 5.5 mg/kg (*upper panel*), *two doses of* 5.5 mg/kg *separated by* 24 hr (*middle panel*), *or* 5 *daily doses of* 2.2 mg/kg (*lower panel*). *Each data point represents one animal. The terminal slopes in the upper panel have been fitted by the method of least squares. The dashed lines in the middle and lower panels have been drawn using the curves from the upper panel* (*see text*).

equivalents in the tumor 24 hr post-ADR treatment. The dashed line in the lower panel was drawn assuming that the ADR equivalents remaining 24 hr after the completion of each 2.2 mg/kg dose were the result of the accumulation of drug from daily increases (of an amount equivalent to 1/5 the maximum achieved after a dose of 11.0 mg/kg) and subsequent decays with a $t_{\frac{1}{2}}$ of 67 hr. The results show that for these single, split, and multiple regimes the maximum ADR level obtained is linearly related to dose, but the tumor ADR $t_{\frac{1}{2}}$ appears unaltered. The data imply that with a constant $t_{\frac{1}{2}}$ and dose-dependent maxima, the total area under the ADR concentration versus time curves for various ADR dose schedules given to the same total dose, is equivalent. These findings are in agreement with and extend the results of a previous investigation which indicated that repeated drug exposures did not alter the ADR pharmacokinetics in normal or neoplastic tissues [13].

### *Tumor response studies—single doses of adriamycin*

Initially, the response of EMT6/Ro tumors and KHT sarcomas to single doses of ADR was studied as a function of time after tumor cell inoculation. At days 3–13 after tumor cell injection, the mice were treated with an $LD_{10/60}$ dose of ADR (13.0 mg/kg for C3H/HeJ mice; 11.0 mg/kg for BALB/cKa mice). In both tumor systems, ADR treatment given soon after tumor cell inoculation led to greater tumor growth delays than observed for treatments given at later times (Table 1). However, it should be noted that because of the different tumor doubling times, EMT6/Ro tumors and KHT sarcomas treated on the same day were not equally sized.

In studies concerning the response of established macroscopic tumors to ADR, mice with equally sized tumors were selected from a large number of mice all injected on the same day with the same number of cells from the same cell suspension. The animals were randomized into groups and kept as controls or treated with ADR. The mean tumor weight for each group as a function of time after

*Table 1. Tumour growth delay for ADR treated KHT sarcomas* (13.0 mg/kg) *and EMT6/Ro tumours* (11.0 mg/kg) *as a function of the day of drug treatment*

| Day of ADR treatment | Tumor growth delay at 0.8 g (days ± S.E.*) KHT sarcoma | EMT6/Ro tumor |
|---|---|---|
| 3 | 4.4 ± 0.7 | 10.5 ± 2.0† |
| 5 | 4.3 ± 0.7 | 10.6 ± 6.9‡ |
| 7 | 2.7 ± 0.6 | 6.4 ± 1.0 |
| 9 | 2.0 ± 0.5 | 8.3 ± 1.5 |
| 11 | 1.5 ± 0.6 | 4.6 ± 0.6 |
| 13 | — | 3.8 ± 1.7 |

*All data are the mean ± S.E. of 9–15 mice.
†Not including 3 mice whose tumors did not regrow in 60 days.
‡Not including 2 mice whose tumors did not regrow in 60 days.

treatment then was calculated. Figure 2 illustrates the response of EMT6/Ro tumors (upper panel) and KHT sarcomas (lower panel) to the various protocols. These data indicate that a 0.5 $LD_{10/60}$ and $LD_{10/60}$ dose of ADR lead to a tumor growth delay of 2 and 4.5 days in the EMT6/Ro tumor and 1 and 2 days in the KHT sarcoma respectively.

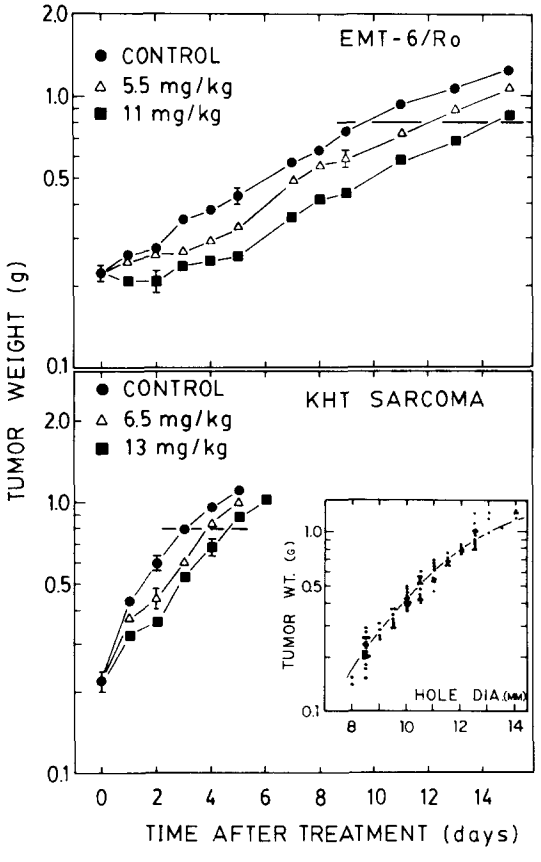


*Fig. 2. EMT6/Ro (upper panel) and KHT sarcoma (lower panel) tumor weight as a function of time after a 0.5 $LD_{10/60}$ (△) and $LD_{10/60}$ (■) dose of ADR. Tumor response to a given treatment was determined by converting from a hole diameter measurement (Methods) to tumor weight using a calibration curve shown in the inset. The data shown represent the mean tumor weight ±S.E. of a group of 8–10 mice.*

For comparison, with these well established tumor lines, the response of a first generation C3H/HeJ mammary tumor to single doses of 6.5 and 13.0 mg/kg was evaluated. The results (Fig. 3) show that increasing the ADR dose from a dose equivalent to 0.5 $LD_{10/60}$ to that of an $LD_{10/60}$ increases the tumor growth delay from 2 to 4.5 days.

The effect of ADR exposure on tumor cell survival in the EMT6/Ro tumor and KHT sarcoma is illustrated in Fig. 4. In these experiments, mice with equally sized tumors (0.2 g) were treated with saline or an $LD_{10/60}$ dose of ADR. At various times after the treatment, two tumors from each group were pooled, dissociated, and the cells immediately plated. By multiplying the tumor cell recovery times the *in vitro* plating efficiency, the clonogenic cells per tumor then were calculated. The results demonstrate that by 48–72 hr following the ADR exposure, the clonogenic cells per tumor in the treated groups are approximately a factor of 2 lower than corresponding values obtained from saline treated animals.

*Tumor response studies—split and multiple doses of ADR*

Since ADR may be given in the clinic using a variety of schedules, the response of tumors to two doses separated by various time intervals or multiple daily doses also was investigated. In the split dose studies, mice were given a 0.5 $LD_{10/60}$ dose of ADR followed immediately or from 1–7 days later by a second 0.5 $LD_{10/60}$ dose. The results, shown in Fig. 5, indicate that two ADR doses of 5.5 mg/kg separated by as much as 7 days are as effective against the EMT6/Ro tumor as a single dose of 11.0 mg/kg of ADR given on day zero. Similarly, for the KHT sarcoma, two doses of 6.5 mg/kg separated by 1 or 2 days lead to the same tumor growth delay as a dose of 13.0 mg/kg given on day zero. However, when a comparison was made between the tumor responses following an $LD_{10/60}$ dose of ADR administered either as 1, 2, 5 or 10 dose increments given at 24 hr intervals (D = 1 × 11, 2 × 5.5, 5 × 2.2, or 10 × 1.1 mg/kg), tumor growth delay was found to decrease with increasing fraction number (Fig. 6).

## DISCUSSION

In this study, the effects on tumor response of single and multiple doses of ADR, given to a total dose equivalent to the single $LD_{10/60}$ value, were investigated. The single $LD_{10/60}$ values for the two strains of mice employed were obtained from complete lethality dose response curves, but such curves were not determined for the various split and multiple dose regimen also investigated. Nevertheless, little change in animal lethality was observed for these treatment protocols in this study. Others [5] also have reported little difference between the $LD_{10}$ values determined for single doses of ADR and those obtained using a variety of multi-fraction exposures.

The results of Table 1, indicating that ADR is more effective if given soon after tumor cell inoculation than if used to treat

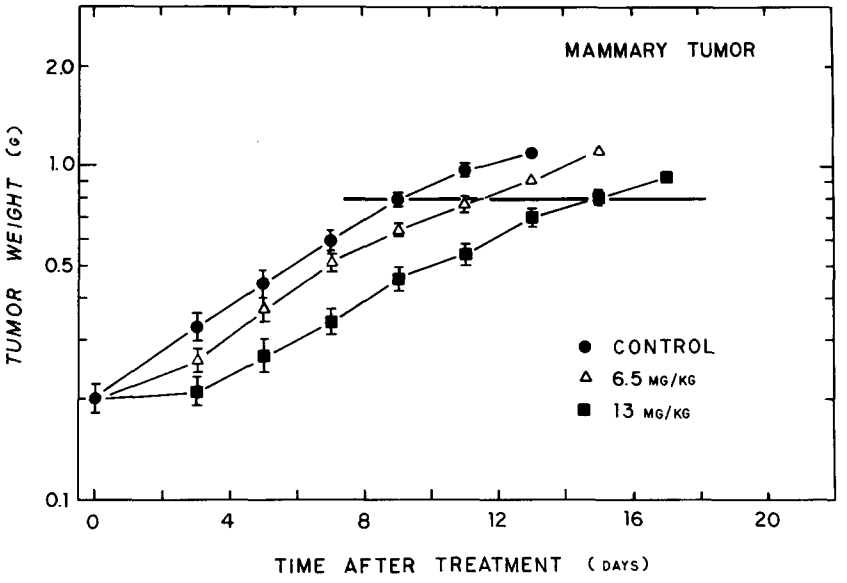


*Fig. 3. Mammary tumor weight as a function of time after a* 0.5 $LD_{10/60}$ (△) *and* $LD_{10/60}$ (■) *dose of ADR. Tumor response was determined as described in Fig. 2. Each data point represents the mean* ±S.E. of 9 animals.

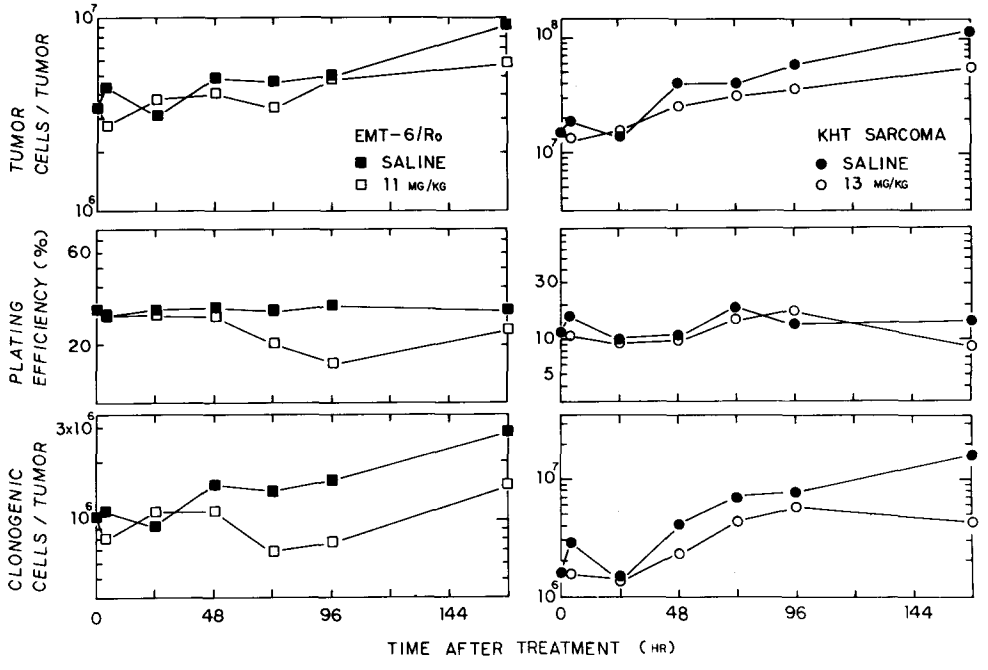


*Fig. 4. Tumor cell survival as a function of time after an* $LD_{10/60}$ *dose of ADR in the EMT6/Ro tumor* (□, ■) *and KHT sarcoma* (○, ●). *The data points shown are the mean of two separate studies; in each study and at each time point, two tumors from the control and ADR treated groups were pooled. Error bars were not included in the figure but ranged from* 5 *to* 20% *of values shown.*

advanced disease or established macroscopic tumors, are in agreement with previous findings, having also been observed in the Lewis lung carcinoma, P388 leukemia and Ridgeway osteogenic sarcoma [5]. The longer tumor growth delays seen at times shortly after tumor implantation likely are related to an increased uptake of the chemotherapeutic agent in the smaller tumors such as has been found for micrometastases compared to primary tumors in the Lewis lung carcinoma [8], and in hamster cervical carcinomas, varying by a factor of 10 in weight [20]. Alternatively, a drug exposure soon after tumor cell in-

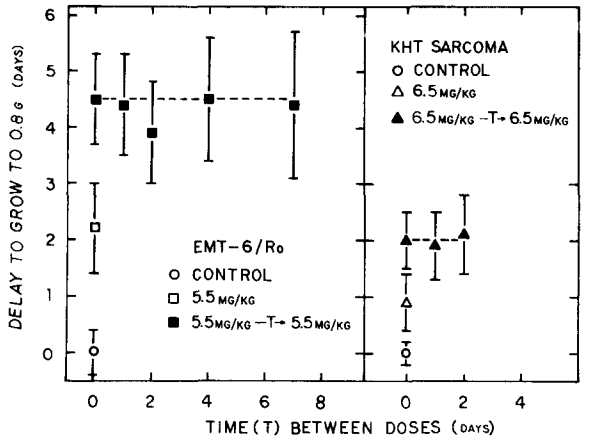


*Fig. 5. Tumor growth delay in the EMT6/Ro tumor (□, ■) and KHT sarcoma (△, ▲) as a function of time between two ADR doses of* 5.5 mg/kg *and* 6.5 mg/kg, *respectively. Each data point represents the mean difference in time* (±S.E.) *for the tumors of* 8–10 *control and ADR treated mice to grow from* 0.2 *to* 0.8 g.

jection might interfere with the ability of a tumor to become established and hence lead to an improved tumor response.

When macroscopic KHT, EMT6/Ro or mammary tumors (~0.2 g) were treated with ADR, increasing the dose by a factor of 2 also increased the tumor response to a similar degree (Figs. 2 and 3). Thus, over this ADR dose range, tumor growth delay appears to be directly related to the exposure dose, i.e., the area under the ADR concentration vs time curve (Fig. 1). Although an $LD_{10/60}$ dose of ADR leads to a greater tumor growth delay in the EMT6/Ro tumor than in the KHT sarcoma (4.5 vs 2 days), it should be noted that the tumor doubling time of the former tumor is 4–5 days and that of the latter is 2 days such that in both cases an $LD_{10/60}$ dose yields a tumor growth delay equivalent to one doubling time (Fig. 2). A similar result also is evident in the first generation transplanted mammary tumor (Fig. 3). Such a tumor growth delay of one doubling time should be approximately equivalent to a change in tumor cell survival by a factor of 2 and this appears to be the case for both the EMT6/Ro tumor and KHT sarcoma (Fig. 4).

When two ADR doses of 5.5 mg/kg, separated by up to 7 days, are administered to EMT6/Ro tumor bearing animals, the tumor growth curve initially follows that observed for a single dose of 5.5 mg/kg until the point at which the second dose of 5.5 mg/kg is given. At that time, there is a further tumor growth delay such that the tumor growth curve for the two doses of 5.5 mg/kg now matches that obtained following a single dose of 11.0 mg/kg. Hence, both treatments lead to the same tumor growth delay (Fig. 5). Similar results are observed for the KHT sarcoma for separations of up to 2 days between doses of 6.5 mg/kg (Fig. 5). Longer time separations between the two doses were not evaluated using this tumor since KHT sarcomas doubled so rapidly that it was not possible to employ tumor growth delay at 0.8 g as the endpoint.

The present results (Fig. 5) suggest that ADR damage is not repaired in these mouse tumors. This interpretation is supported by the small size or complete lack of an initial shoulder on most [6, 21–24] although not all [25, 26] ADR cell survival curves. In addition others have indicated that there is little evidence for *in vitro* repair of split-dose damage [21, 25] or for *in vitro* or *in vivo* recovery of cell survival [6, 27] after exposure to ADR. Alternatively, the present observations may result from long cell cycle delays observed following similar doses of ADR [28, 29] or simply reflect an accumulation and addition of damage due to the long ADR $t_{\frac{1}{2}}$ in the tumors (Fig. 1). Clearly, further studies to resolve these possibilities are needed.

*In vitro* studies had suggested that multiple small fractions might be more effective than a high dose pulsed exposure of ADR [21]. However, in the present study, when the same total dose is given as a series of smaller fractions rather than as a single dose or split doses, tumor growth delay decreases with increasing fraction number (Fig. 6). Yet it appears that the total drug exposure is similar for the various protocols since tissue ADR pharmacokinetics are unaltered during a fractionation regimen [13] and the tumor ADR level after each dose increment is dependent only on the dose of ADR given and the ADR equivalents remaining from the previous dose (Fig. 1). Conceivably, the time required for the longer fractionated regimen to be given would allow for tumor growth during the treatment interval such that the later dose fractions are delivered to larger tumors. Because the ADR uptake may be reduced in larger tumors [8, 20], the later fractions would hence be less effective. Also, the initial doses of a multi-dose regimen may lead to little cell cycle delay and an accumulation of ADR to a sufficiently high tumor level may be required for the drug to become effective. Both these factors would tend to decrease tumor growth delay with increasing fractionation as is seen in Fig. 6.

In summary, this study was carried out in three different tumor lines; two well established transplanted tumors and one first gene-

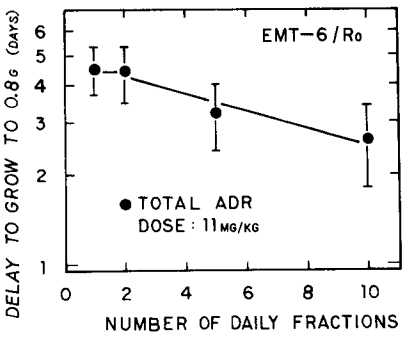


*Fig. 6. The relationship between tumor growth delay and fraction number for a fixed total dose of ADR of* 11.0 mg/kg *with fractions separated by* 24 hr. *Each data point represents the mean difference in time* (±S.E.) *for the tumors of* 8–10 *control and ADR treated animals to grow from* 0.2 *to* 0.8 g.

ration spontaneous tumor. The findings show that an $LD_{10/60}$ dose of ADR leads to a tumor growth delay equivalent to one doubling time in all three tumor models. For the split dose regimen studied, the results are suggestive of an absence of *in vivo* repair of ADR damage in the EMT6/Ro tumor and KHT sarcoma. However, with increasing number and decreasing size of daily fractions, the drug treatments become less effective likely due to tumor cell proliferation during the multidose schedules. These results form the basis of studies aimed at elucidating the interaction of single and multiple doses of ADR and single and fractioned doses of radiation.

**Acknowledgements**—The authors thank Mr. Dan Kirkpatrick for his skillful technical assistance and Drs. E. M. Lord and K. T. Wheeler for their constructive criticism of the manuscript. The adriamycin was provided by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland.

## REFERENCES

1. R. H. Blum, An overview of studies with adriamycin (NSC-123127) in the United States. *Cancer Chemother. Rep.* **6,** 247 (1975).
2. R. H. Blum and S. K. Carter, Adriamycin, a new anticancer drug with significant clinical activity. *Ann. intern. Med.* **80,** 249 (1974).
3. S. K. Carter, Adriamycin—a review. *J. nat. Cancer Inst.* **55,** 1265 (1975).
4. K. K. Fu, A. C. Begg, L. J. Kane and T. L. Phillips, Interaction of radiation and adriamycin (ADR) on the EMT6 tumor as a function of tumor size and assay method. *Int. J. Radiat. Oncol. biol. Phys.* **5,** 1249 (1979).
5. A. Goldin and K. Johnson, Experimental tumor activity of adriamycin (NSC-123127). *Cancer Chemother. Rep.* **6,** 137 (1975).
6. G. M. Hahn, J. Braun and I. Har-Kedar, Thermochemotherapy: synergism between hyperthermia (42–43°) and adriamycin (or bleomycin) in mammalian cell inactivation. *Proc. nat. Acad. Sci. (Wash.)* **72,** 937 (1975).
7. H. S. Schwartz and G. B. Grindey, Adriamycin and daunomycin: a comparison of antitumor activities and tissue uptake in mice following immunosuppression. *Cancer Res.* **33,** 1837 (1973).
8. M. G. Donelli and S. Garattini, Differential accumulation of anticancer agents in metastases compared with primary tumors in experimental models. In *Recent Advances in Cancer Treatments.* (Edited by H. J. Tagnon and M. J. Staquet) p. 177. Raven Press, New York (1977).
9. A. Martini, M. G. Donelli, A. Mantovani, M. A. Pacciarini, E. Fogar-Ottaviano, L. Morasca, S. Garattini and F. Spreafico, Antineoplastic activity and pharmacokinetics of adriamycin and daunomycin in tumor bearing mice. *Oncology* **34,** 173 (1977).
10. W. A. Creasey, L. S. McIntosh, T. Brescia, O. Odujinrin, G. T. Aspens, E. Murray and J. C. Marsh, Clinical effects and pharmacokinetics of different dosage schedules of adriamycin. *Cancer Res.* **36,** 216 (1976).
11. J. M. Brown, N. Y. Yu and P. Workman, Pharmacokinetic considerations in testing hypoxic cell radiosensitizers in mouse tumours. *Brit. J. Cancer* **39,** 310 (1979).
12. R. P. Hill and R. S. Bush, A lung colony assay to determine the radiosensitivity of the cells of a solid tumour. *Int. J. Radiat. Biol.* **15,** 435 (1969).
13. D. W. Siemann and R. M. Sutherland, A comparison of the pharmacokinetics of multiple and single dose administrations of adriamycin. *Int. J. Radiat. Oncol. biol. Phys.* **5,** 1271 (1979).

14. D. W. Siemann, R. P. Hill and R. S. Bush, Optimum pre-irradiation breathing times of 100% $O_2$ and 5% $CO_2$: 95% $O_2$ for a murine sarcoma. *Int. J. Radiat. Oncol. biol. Phys.* **2,** 903 (1977).
15. S. C. Rockwell, R. F. Kallman and L. F. Fajardo, Characteristics of a serially transplanted mouse mammary tumor and its tissue-culture-adapted derivative. *J. nat. Cancer Inst.* **49,** 735 (1972).
16. R. F. Kallman, G. Silini and L. M. Van Putten, Factors influencing the quantitative estimation of the *in vivo* survival of cells from solid tumors. *J. nat. Cancer Inst.* **39,** 539 (1967).
17. J. E. Thomson and A. M. Rauth, An *in vitro* assay to measure the viability of KHT tumor cells not previously exposed to culture conditions. *Radiat. Res.* **58,** 262 (1974).
18. H. S. Schwartz, A fluorometric assay for daunomycin and adriamycin in animal tissues. *Biochem. Med.* **7,** 396 (1973).
19. N. R. Bachur, Adriamycin (NSC-123127) pharmacology. *Cancer Chemother. Rep.* **6,** 153 (1975).
20. H. A. Eddy, Adriamycin uptake in the Syrian hamster cervical carcinoma following ionizing radiation. *Radiat. Res.* **74,** 579 (1978).
21. S. C. Barranco, Review of the survival and cell kinetics of adriamycin (NSC-123127) on mammalian cells. *Cancer Chemother. Rep.* **6,** 147 (1975).
22. B. Drewinko, P. R. Roper and B. Barlognie, Patterns of cell survival following treatment with anti-tumor agents *in vitro*. *Europ. J. Cancer* **15,** 93 (1979).
23. G. M. Hahn and D. P. Strande, Cytotoxic effects of hyperthermia and adriamycin on Chinese hamster cells. *J. nat. Cancer Inst.* **57,** 1063 (1976).
24. P. R. Twentyman and N. M. Bleehen, Changes in sensitivity to cytotoxic agents occurring during the life history of monolayer cultures of a mouse tumour cell line. *Brit. J. Cancer* **31,** 417 (1975).
25. J. E. Byfield, M. Lynch, F. Kulhanian and P. Y. M. Chan, Cellular effects of combined adriamycin and X-irradiation in human tumor cells. *Int. J. Cancer* **19,** 194 (1977).
26. S. H. Kim and J. H. Kim, Lethal effect of adriamycin on the division cycle of HeLa cells. *Cancer Res.* **32,** 323 (1972).
27. G. M. Hahn, Radiation and chemically induced potentially lethal lesions in non-cycling mammalian cells: recovery analysis in terms of X-ray- and ultraviolet-like-systems. *Radiat. Res.* **64,** 533 (1975).
28. L. A. Dethlefsen, R. M. Riley and J. L. Roti Roti, Flow cytometric (FCM) analysis of adriamycin-perturbed mouse mammary tumors. *J. Histochem. Cytochem.* **27,** 463 (1979).
29. E. Esser, J. Haut, J. Schumann, M. Wannenmacher and U. Wingenfeld, Experimentelle Untersuchungen zur Proliferationskinetik und Strahlenbehandlung nach Vincristin und Adriamycin. *Strahlentherapie* **163,** 682 (1977).