Eur J Cancer Clin Oncol, Vol. 22, No. 5, pp. 577–582, 1986.
Printed in Great Britain.

0277–5379/86$3.00+0.00
© 1986 Pergamon Journals Ltd.

Actinomycin D and Radiation: Effects on Mouse Lung

SALLY A. HILL,* ELIZABETH L. TRAVIS† and JULIANA DENEKAMP*

**Gray Laboratory of the Cancer Research Campaign, Mount Vernon Hospital, Northwood, Middlesex HA6 2RN, U.K. and †Experimental Radiotherapy Department, M.D. Anderson Hospital, Houston, TX 77030, U.S.A.*

Abstract—*The effect of actinomycin D* (0.4 mg/kg) *on radiation-induced lung damage in the mouse was investigated. The drug was administered either* 4 weeks *before, immediately after, or* 16 weeks *after single doses of* 240 kV *X-rays applied to the thorax of CBA mice. Lung damage was assessed by measuring respiration rate, with a whole body plethysmograph. Dose–response curves were obtained at* 2-week *intervals from* 12 *to* 40 *weeks after irradiation. Actinomycin D had no significant effect on respiration rate in this study. A summary of other experimental studies is included which shows conflicting results.*

INTRODUCTION

THE CLINICAL use of combined radiation therapy and adjunctive chemotherapy has increased in recent years, often with relatively little information available about optimum dosage or scheduling of the drug relative to radiotherapy. The earliest studies indicated that a conventional full dose of chemotherapy could not be added to a conventional full dose of radiotherapy without increasing the probability of excessive normal tissue injury in the irradiated field. Thus it soon became apparent that, when the two agents were used in concert, the dose of one or both must be decreased. Empirical schedules were developed for clinical treatments, but the experimental data for optimum doses and scheduling have generally lagged behind. At a conference concerned with drug–radiation interactions it was generally shown that the greatest risk of normal tissue morbidity occurs when the two agents are used in close sequence [1]. Unfortunately this is also the time at which the maximum effect on the primary tumour is achieved. However, for spatial cooperation (i.e. radiotherapy for the local tumour and chemotherapy for the systemic disease) it may be more important to find the interval that allows the maximum dose of each agent to be used, and avoid any interaction or synergism on the tumour or the normal tissue. In this way a normal full course of radiotherapy could perhaps be given, together with an effective course of chemotherapy.

Actinomycin D was the first cytotoxic drug that was recognised as enhancing normal tissue injury in irradiated fields. D'Angio *et al.* [2] reported an earlier appearance and enhanced level of radiation reaction in the skin and mucous membranes of patients and confirmed these observations in mouse studies. Furthermore they demonstrated that a drug dose given some time after irradiation could re-evoke a response in the irradiated field, the so-called 'recall' phenomenon.

Many normal tissue assays now exist which allow the degree of injury after irradiation to be quantified so that dose–response curves can be plotted. Field and Michalowski [3] showed that the time course and sensitivity of normal tissue injury is broadly similar in a range of species (including mouse and man) and it therefore seems reasonable to study the time course in rodents, with some expectation that it will be relevant to man.

In the present study we have chosen to investigate the radiosensitizing action of actinomycin D, by combining it with graded doses of radiation to the thorax. Functional lung damage can be assessed at frequent intervals in each group of mice by a non invasive measurement of the respiration rate [4]. Rapid shallow breathing has been shown to be an indication of acute pneumonitis and of later pulmonary fibrosis [5].

MATERIALS AND METHODS

Eight to twelve-week-old, male CBA mice were treated with single doses of 8–20 Gy 240kV X-rays (HVL 1.3mm Cu; dose rate 2.5 Gy-min). Full

Accepted 27 September 1985.
Financed entirely by The Cancer Research Campaign, 2 Carlton House Terrace, London, SW1Y 5AR, U.K.
Correspondence and reprint requests to: Dr S.A. Hill, Gray Laboratory of the Cancer Research Campaign, Mount Vernon Hospital, Northwood, Middlesex HA6 2RN, U.K.

details of the irradiation procedure have been described in detail previously [4]. Briefly, four mice were irradiated simultaneously in specially constructed jigs, which fixed the position of the thorax in relation to the X-ray beam. Two apertures allowed both lungs to be irradiated, while the rest of the body, including the oesophagus in the thoracic mid-line, was protected by 3.0 mm of lead. Approximately 10 min before irradiation the mice were anaesthetised with 60 mg/kg sodium pentobarbital.

Actinomycin D was dissolved in sterile saline and injected intraperitoneally at a dose of 0.4 mg/kg, either 4 weeks before, immediately after, or 16 weeks after the same range of X-ray doses. Following treatment, lung damage was assessed functionally by measuring the respiration rate of the mice at 2-week intervals using a whole body plethysmograph [4]. A mean breathing rate could then be calculated for each treated group and plotted as a function of either dose or time. Two sets of experiments were performed one year apart.

RESULTS

Figure 1 shows breathing rates plotted as a function of time after treatment. There was no significant variation with age in unirradiated controls; the mean frequency was 340 breaths per minute, with a range from 330 to 350. Low doses of radiation (8 and 11 Gy) produced no significant elevation of breathing rate over the whole period of the study. Higher doses, however, did produce an increased breathing frequency, with the extent of the increase and the time of onset being dose-dependent. Thirteen grays produced a persistent elevation from 15 to 35 weeks, without any of the animals dying of pulmonary insufficiency. Sixteen grays resulted in a progressive increase of breathing rate from 12 to 18 weeks, with all of the mice dying by 20 weeks. The highest dose group showed a significant elevation at 10 weeks, with breathing rates rising to > 450 breaths per minute at 16 weeks, followed rapidly by death from pulmonary insufficiency.

A dose of 0.4 mg/kg actinomycin D was chosen for the combination study because it had been shown in pilot studies to be just below the toxic limit for our CBA mice. However, approx. 15% of the experimental animals in this study died or were sacrificed within 3 weeks of drug injection, irrespective of the radiation treatment. On post-mortem examination, they were found to have grossly distended stomachs and the entire gastro-intestinal tract was full and rigid. Actinomycin D is known to induce degeneration and necrosis of the epithelial cells of the intestine [6], and Yatvin [7] has reported delayed stomach emptying in rats treated with this drug.

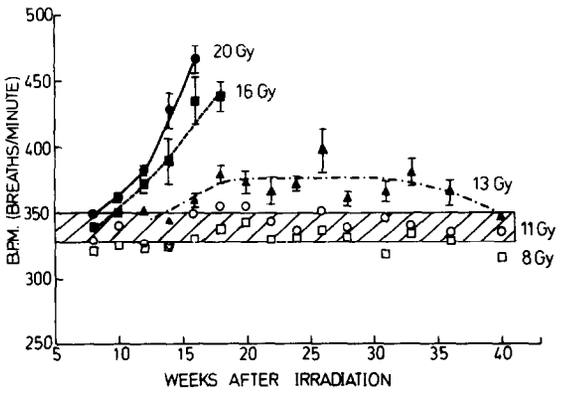

Fig. 1. Mean breathing frequency (± 1 S.E.M.) as a function of time for groups of eight mice treated with single doses of X-rays to both lungs. Hatched area represents range of values (± 1 S.E.M.) for sham-irradiated controls.

The influence of actinomycin D on the response to one of the radiation doses (13 Gy) is shown in Figs. 2 and 3 for the two separate experiments. Three panels are shown in each, representing the different sequences of drug and radiation. The upper pannel shows actinomycin D 4 weeks before

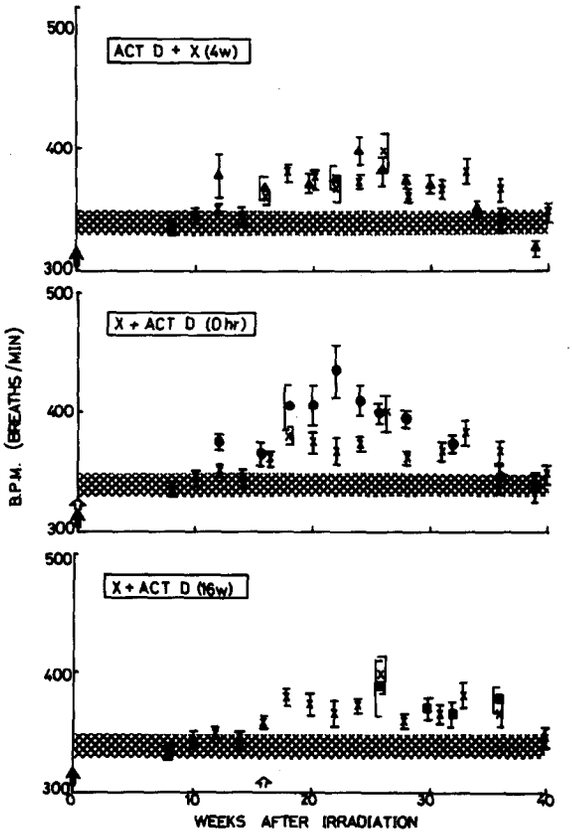

Fig. 2. Breathing rate in breaths per minute (± 1 S.E.M.) plotted as a function of time after a single dose of 13 Gy X-rays, given either alone (X) or in combination with 0.4 mg/kg actinomycin D (▲, ●, ■). First Experiment. Arrows show time of administration of radiation (closed arrow) and drug (open arrow). Range of values for sham-irradiated controls are indicated by hatched area.

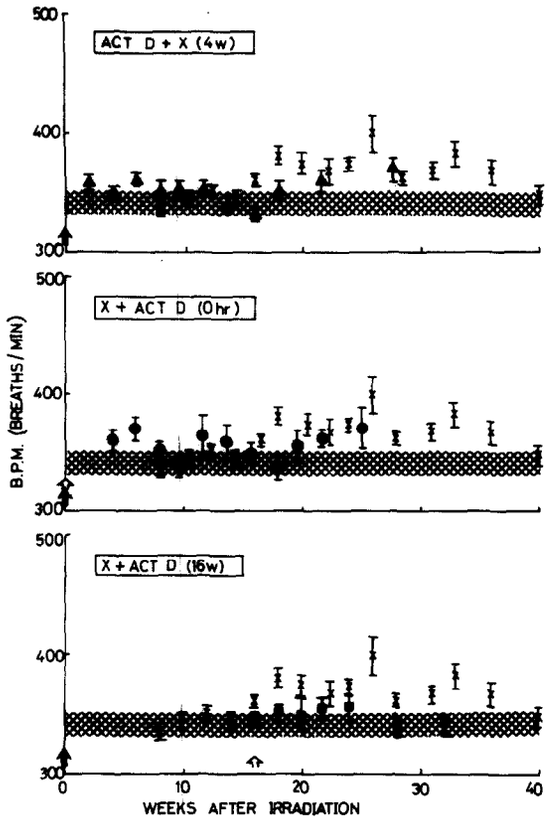

Fig. 3. As for Fig. 2, repeat experiment. Breathing rate for groups of animals treated with 13 Gy *X-rays alone* (X) *or with actinomycin D administered before, concurrently, or after* 13 Gy *X-rays* (▲, ●, ■).

X-rays. The middle panel shows the two agents given in close sequence. The lower panel shows the drug given 16 weeks after irradiation. In each panel the response to 13 Gy X-rays alone or X-rays plus drug are compared, with the hatched area indicating the control range. In the first experiment (Fig. 2) the functional tests were started 12 weeks after irradiation (panels A and B) or 10 weeks after the drug treatment (panel C). The two schedules with a long interval between the two agents produced a very similar response to that seen with X-rays alone (panels A and C). The groups receiving concurrent treatments showed a somewhat higher reaction, particularly at 18–28 weeks. The damage subsided however by 36–40 weeks and no deaths occurred. A comparison with Fig. 1 indicates that the combined treatment was less effective than 16 Gy, i.e. the enhancement was ≤ 1.2.

Figure 3 shows the same data for the repeat experiment. All of the values are lower than in the first study. A slightly elevated breathing rate was seen at 2–8 weeks in the combined treatment groups (panels A and B) but during the normal response time (15–35 weeks) the mice receiving the combination showed *less* functional damage than those given X-rays alone in all three panels. In this experiment therefore there was definitely *no* indication of an enhanced effect.

Dose–response curves were generated for every assay time from 12 to 36 weeks. Two examples of these are shown in Figs. 4 and 5, assessed at 16 and 26 weeks respectively. At the earlier time a full dose–response curve is obtained, and no animals have succumbed to lethal lung damage. Panels A, B and C show the three schedules that were tested, and open and closed symbols are used to indicate the two experiments. None of the six panels shows a significant enhancement of the damage compared with that from radiation alone, and the treatment 4 months post irradiation does not appear to produce a 'recall' phenomenon.

DISCUSSION

These results indicate that with large single doses of drug and radiation there is no significant enhancement of the radiation damage to the lung for any of the three schedules tested. The time of onset of measurable changes may be slightly earlier after the combined treatment but there is no significant increase in the severity in most groups.

The published experimental studies of actinomycin

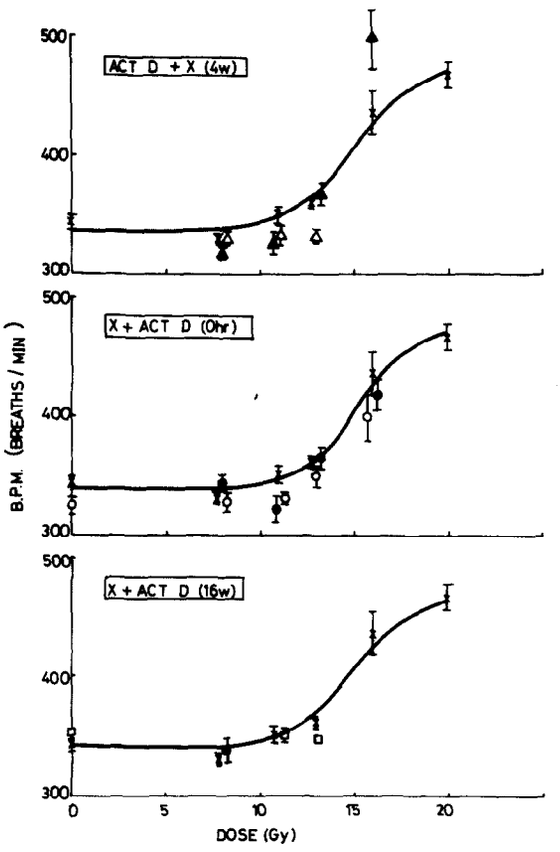

Fig. 4. Mean breathing rate as a function of X-ray dose, 16 weeks *after irradiation.* X, *X-rays alone;* ▲, ●, ■, *X-rays plus actinomycin D, first experiment;* △, ○, □, *X-rays plus actinomycin D, second experiment.*

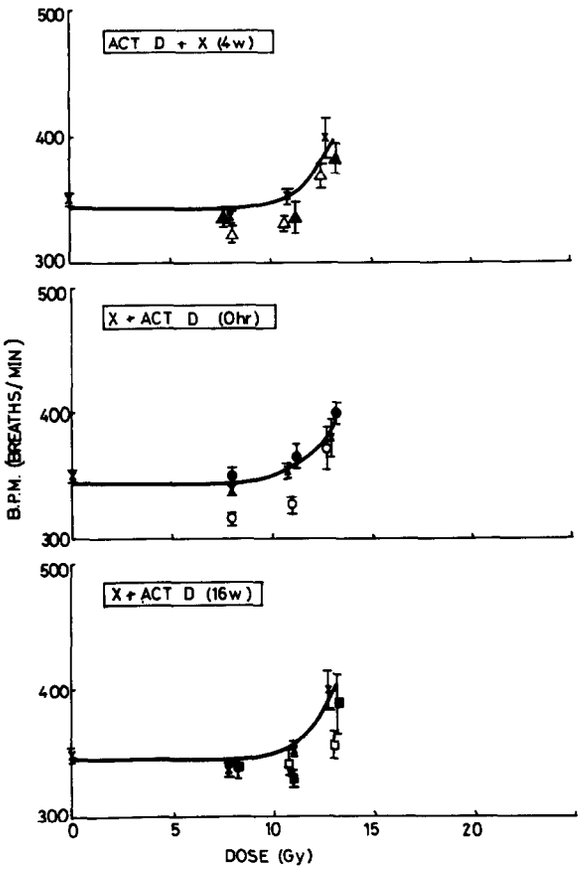

Fig. 5. Dose–response curves for breathing rate at 26 weeks *after irradiation.* X, *X-rays alone;* ▲, ●, ■, *X-rays plus actinomycin D, first experiment;* △, ○, □, *X-rays plus actinomycin D, second experiment.*

D plus radiation are summarised in Table 1 for both lung and other normal tissues. Mouse lung has previously been shown to be sensitized by actinomycin D to radiation injury (lethality) if a high dose (0.75 mg/kg) actinomycin D was given 2 hr before thoracic irradiation [8]. A lower dose of 0.15 mg/kg gave no enhancement when used as a single treatment, and only produced a marginal effect when given repeatedly 2 hr before each of 10 radiation treatments. Surprisingly this low dose did enhance lung damage if given 1 month before 10 fractions of radiation, although it had no effect in the reverse sequence [8]. Neither Steel *et al.* [9] nor Collis [10] saw enhancement of pulmonary dysfunction when actinomycin D was given before irradiation, although a small effect was seen at one radiation dose level with concurrent treatment [10].

Several other experimental studies have indicated that actinomycin D can enhance radiation damage in a variety of normal tissues. D'Angio *et al.* [2] originally reported increased epilation when low doses of actinomycin D were given within 6 hr of radiation. More recently however, Redpath and Colman [12] were unable to reproduce these findings. Even with high drug doses, they observed enhanced skin damage only when drug treatment was combined with fractionated radiation therapy. Single dose studies have also been reported for intestine, oesophagus and testis, each of which has shown increased damage following the combined treatment, particularly when drug and radiation have been given concurrently (see Table 1).

In general the clinical data are sparse and inconclusive, particularly since other chemotherapeutic agents are usually included in the protocol. At least four studies have shown enhanced pulmonary toxicity when actinomycin D is given *during* the course of radiotherapy [20–23]. Reports of enhancement with longer intervals are more rare. One case was reported with actinomycin D given 12 months before radiation [24] and one case when the drug was given 3 months post-irradiation [25] or when a second course of chemotherapy was given [23]. Several studies have failed to produce any evidence of enhanced radiation toxicity to the lung when chemotherapy including actinomycin D is combined with thoracic irradiation [26, 27]. Wara *et al.* [28] reviewed the clinical data and attempted to construct dose–response curves from which to estimate a dose enhancement factor. They found a DEF* of 1.3 for concurrent therapies and recommended that radiation doses be reduced whenever the drug was used.

The present data, together with the sometimes conflicting data in Table 1, show that actinomycin D is not a potent enhancer of radiation injury. Although several studies have reported a DEF of 1.2–1.3 for concurrent treatments, such an enhancement was not seen in these experiments. Had it been present, it would have been readily detected since the resolution of the system would allow a factor of 1.1 to be quantified with 95% confidence. The drug dose that would be used clinically would be much lower than that used here, although it is difficult to make direct comparisons of doses because of the marked difference in half life and therefore drug exposure in mouse and man. In the clinic the drug is likely to be combined with other drugs in a 'cocktail' schedule, and would also be combined with fractionated radiotherapy. It is possible that drug interactions may be important in patients, or that an effect on the repair capacity of the target cells might magnify the influence of actinomycin D in a fractionated schedule. This was suggested by Redpath and Colman [12], to explain their observations of enhanced skin reactions in mouse feet when actinomycin D was combined with radiation in a 5-fraction regime; no enhancement was seen in single dose studies. Further

*DEF = $\frac{\text{Radiation dose to give effect without drug.}}{\text{Radiation dose to give effect with drug.}}$

Table 1. *Summary of experimental studies of actinomycin D. Enhancement of normal tissue injury**

Endpoint	Drug dose (mg/kg)	Drug before	Concurrent	Drug after	Number of fractions	Reference
Lung						
Lethality	0.15	–	1.0 (2 hr)	–	SD	
$LD_{50/160}$	0.75	–	1.6 (2 hr)	–	SD	(8)
	0.15	1.2 (4 weeks)	1.1 (2 hr)	1.0 (4 weeks)	10F	
Median survival time	0.75	none (2 weeks)	–	–	SD	(9)
Breathing rate	0.2	–	none (1 hr)	–	SD	
	0.3	none (3 weeks)	± (1 hr)	–	SD	(10)
Breathing rate	0.4	1.0 (4 weeks)	1.0 (0 hr)	1.0 (16 weeks)	SD	This study
Hair follicles						
Epilation	0.15	+ (6 hr)	+ (½ hr)	+ (6 hr)	SD	(2)
Dysplasia	0.1	+ (4 hr–2 days)	+ (0 hr)	–	SD	
	0.15					(11)
Skin						
Peak skin reaction	0.7	1.0 (2 weeks)	1.0 (2 hr)	1.0 (1 day)	SD	
		–	1.1 (2 hr)	–	2F	
	0.15	–	none (2 hr)	–	SD	(12)
	0.3	–	+ (2 hr)	–	5F	
	0.6	–	+ (2 hr)	–	5F	
Mortality + crypt survival (dog)	0.004	none (16 hr)	–	–	12F	
	0.008	+ (16 hr)	–	–		(13, 14)
	0.01	+ (16 hr)	–	–		
$LD_{50/6}$	0.8	± (4 hr–4 days)	± (0–2 hr)	+ (4 hr–2 days)	SD	
	1.2	± (4 hr–4 days)	+ (0–2 hr)	+ (4 hr–2 days)	SD	(15)
	1.7	+ (4 hr–4 days)	+ (0–2 hr)	+ (4 hr–2 days)	SD	
$LD_{50/28}$	0.3	–	1.2 (1 hr)	–	SD	
	0.5	+ (6 hr–2 days) none (3,5,7days)	1.8 (1 hr)	+ (3 hr–2 days) none (3,5,7 days)	SD	(16)
	0.65	–	2 (1 hr)	–	SD	
Crypt survival	0.5	+ (1–5 days)	1.25 (0 hr)	–	SD	
	1.0	–	1.6 (0 hr)	–	SD	(17)
Crypt survival	0.75	1.0 (7 days)	1.2–1.3 (2 hr)	1.2 (2 days)	SD	(8, 18)
Oesophagus						
$LD_{50/28}$	0.75	–	1.6 (2 hr)	–	SD	(8)
Testis						
Histology	0.075	–	+ (½ hr)	–	SD	(19)

* All experiments performed on mice unless otherwise stated.

experiments would be needed to investigate these aspects in the lung.

Acknowledgements — We are grateful to Mrs. Lynda Hall and the animal house staff for care of the mice, to Dr. Boris Vojnovic and Mr. Brian Bloomfield for construction of the plethysmograph and Mrs. Jean Wilson and Mrs. Eileen Marriott for secretarial assistance.

REFERENCES

1. Phillips TL. Proceedings of the Conference on combined modalities chemotherapy/radiotherapy. *Int J Radiat Oncol Biol Phys* 1979, **5**, 1721–1723.
2. D'Angio GJ, Farber S, Maddock CL. Potentiation of X-ray effects by actinomycin D. *Radiol* 1959, **73**, 175–177.
3. Field SB, Michalowski A. Endpoints for damage to normal tissues. *Int J Rad Oncol Biol Phys* 1979, **5**, 1185–1196.
4. Travis EL, Vojnovic B, Davies EE, Hirst DG. A plethysmographic method for measuring function in locally irradiated mouse lung. *Br J Radiol 1979*, **52**, 67–74.
5. Travis EL, Down JD, Holmes SJ, Hobson B. Radiation pneumonitis and fibrosis in mouse lung assayed by respiratory frequency and histology. *Rad Res* 1980, **84**, 133–143.
6. Schwartz HS, Sternberg SS, Philips FS. Pharmacology of Mitomycin C. IV Effects in vivo on nucleic acid synthesis; comparison with actinomycin D. *Can Res* 1963, **23**, 1125–1136.
7. Yatvin MB. Effect of actinomycin D on the distribution of injected tryptophan in the rat. *Arch Int Physiol Biochem* 1970, **78**, 608–610.
8. Phillips TL, Wharam MD, Margolis LW. Modification of radiation injury to normal tissues by chemotherapeutic agents. *Cancer* 1975, **35**, 1678–1684.
9. Steel GG, Adams K, Peckham MJ. Lung damage in C57Bl mice following thoracic irradiation: enhancement by chemotherapy. *Br J Radiol* 1979, **52**, 741–747.
10. Collis CH. The response of the lung to ionising radiation and cytotoxic drugs. MD Thesis, University of Cambridge 1981.
11. Griem ML, Malkinson FD. Some effects of radiations and radiation modifiers on growing hair: Progress Report. *Front Radn Ther Onc* 1969, **4**, 24–45.
12. Redpath JL, Colman M. The effect of adriamycin and actinomycin D on radiation-induced skin reactions in mouse feet. *Int J Radiat Oncol Biol Phy* 1979, **5**, 483–486.
13. Concannon JP, Summers RE, King J, Tcherkow G, Cole C, Rogow E. Enhancement of X-ray effects on the small intestinal epithelium of dogs by actinomycin D. *Am J Roentgenol* 1969, **105**, 126–136.
14. Concannon JP, Summers RE, Cole C, Weil C, Kopp U, Sigdestad CP. Effects of X-irradiation and actinomycin D on the intestinal epithelium of dogs. *Radiol* 1970, **97**, 157–164.
15. Concannon JP, Dalbow MH, Hagemann RF, Frich JC, Hodgson SE, Weil CS, Martinelli R. Radiation and actinomycin D mortality studies in C57B1/6 mice. *Cancer* 1973, **32**, 553–561.
16. Smith WW, Carter SK, Wilson SM, Newman JW, Cornfield J. Joint lethal effects of actinomycin D and radiation in mice. *Can Res* 1970, **30**, 51–57.
17. Hagemann RF, Concannon JP. Mechanism of intestinal radiosensitization by actinomycin D. *Br J Cancer* 1973, **46**, 302–308.
18. Phillips TL, Fu KK. The interaction of drug and radiation effects on normal tissues. *Int J Radiat Oncol Biol Phys* 1978, **4**, 59–64.
19. Maddock CL, Rev-Kury L, Brown BL. Testicular damage resulting from actinomycin D X-irradiation and their combination in CAF mice. *Rad Res* 1965; **26**, 584–592.
20. Cassady JR, Tefft M, Filler RM, Jaffe N, Hellman S. Considerations in the radiation therapy of Wilms' tumour. *Cancer* 1973, **32**, 598–608.
21. Jenkins RDT, Sonley MJ. Wilms' tumour — treatment results 1969–73. Implications for the future. *Proc Am Assoc Cancer Res* 1975, **16**, 239.
22. Baeza MR, Barkley HT, Fernandez CH. Total-lung irradiation in the treatment of pulmonary metastases. *Radiol* 1975, **116**, 151–154.
23. Pearson D, Deakin DP, Hendry JH, Moore JV. The interaction of actinomycin D and radiation. *Int J Radiat Oncol Biol Phys* 1978, **4**, 71–73.
24. Braun SR, Do Pico GA, Olson CE, Caldwell W. Low-dose radiation pneumonitis. *Cancer* 1975, **35**, 1322–1324.
25. McInerny DP, Bullimore J. Reactivation of radiation pneumonitis by adriamycin. *Br J Radiol* 1977, **50**, 224–227.
26. Cox JD, Gingerelli F, Ream NW, Maier JG. Total pulmonary irradiation for metastases from testicular carcinoma. *Radiology* 1972, **105**, 163–167.
27. Rab GT, Ivins JC, Childs DS, Cupps RE, Pritchard DJ. Elective whole lung irradiation in the treatment of osteogenic sarcoma. *Cancer* 1976, **38**, 939–942.
28. Wara WM, Phillips TL, Margolis LW, Smith V. Radiation pneumonitis: a new approach to the derivation of time-dose factors. *Cancer* 1973, **32**, 547–552.