RESULTS

Animal Survival. All animals which had not been previously irradiated tolerated a dose of 8 mg/kg c-DDP, with a maximum transient weight loss of 2.5 g during the first week. Some deaths (4 of 32) occurred after 10 mg/kg and the LD_{50} was about 14 mg/kg. Acute drug tolerance was reduced in animals which had previously received bilateral renal irradiation. When 8 mg/kg c-DDP was given 1 or 3 months after X-rays there were only a few deaths (2 of 12), but when the same drug dose was given after 6 months most animals died. Death generally occurred at 1–2 weeks, but some animals died as late as 1–2 months after c-DDP. Animal survival data are summarized in Table 2.

Renal Function. The time course for renal damage after 4–10 mg/kg c-DDP alone is shown in Fig. 1. A drug dose of 4 mg/kg did not cause significant damage, but the first test was at 4 weeks after injection, and it is possible that at earlier times there would have been measurable renal impairment. Maximum damage after 6–10 mg/kg occurred within 1 week, followed by recovery of function until 17 weeks, with the suggestion of a second wave of damage from 17–23 weeks. From 23–35 weeks renal function remained unchanged, with persistent damage after doses of 6–10 mg/kg. These results were consistent with those from an earlier experiment (14).

c-DDP given at 1–6 months after renal irradiation caused more damage than either drug or X-rays alone. This is illustrated in Fig. 2, which shows the time course for renal damage when a single drug dose (6 mg/kg) was given 6 months after 8–12 Gy. c-DDP caused greater functional impairment in the preirradiated animals at all testing times and after all radiation doses, although the effect was greatest from 15 weeks onwards (*i.e.*, after the initial damage caused by c-DDP alone had partially resolved). Increased renal damage was also observed with drug doses of 4 and 8 mg/kg given after irradiation (data not shown in Fig. 2).

In order to compare the effects of administering c-DDP at different times after irradiation, renal function after a single drug dose (6 mg/kg) given at 1, 3, or 6 months after 12 Gy is shown in Fig. 3. When kidney damage was considered as a function of time after c-DDP injection (3*A*) it appeared that drug given at 6 months caused more damage than at 1 or 3 months. The development of renal injury after X-rays is, however, progressive, and the component of damage due to irradiation alone was consequently larger when c-DDP was given at 6 months than at earlier times (see —— in Fig. 3*A*). If the same data were replotted as a function of time after irradiation (Fig. 3*B*), then the extent and progression of damage after X-rays plus drug appeared to be very similar, whether c-DDP was given after 1, 3, or 6 months. Fig. 3 also clearly illustrates that the

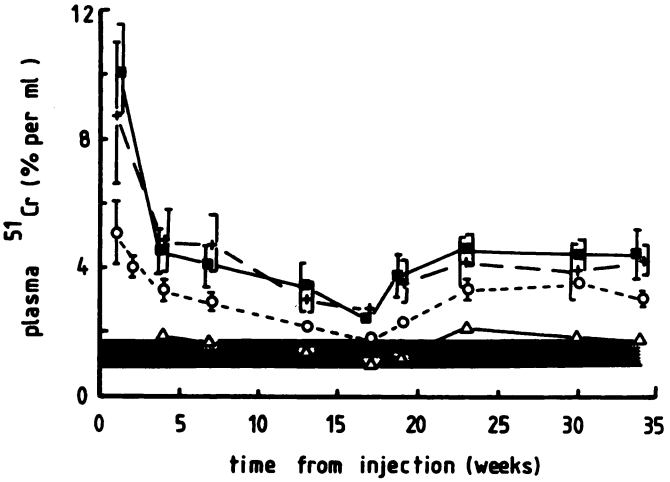

Fig. 1. Time course for changes in renal function after c-DDP alone. Results are expressed as residual ^{51}Cr-labeled EDTA in plasma at 30 min after giving the tracer. *Points*, mean ± 1 SE (*bars*) of a group of 4–6 mice; ■, control response of untreated mice throughout the testing period (mean ± 1 SD). △, 4 mg/kg; +, 8 mg/kg; ○, 6 mg/kg; ■, 10 mg/kg.

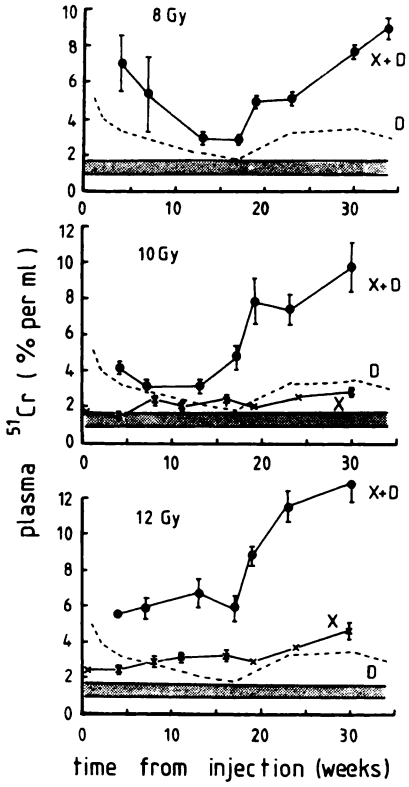

Fig. 2. Kidney damage from 4–30 weeks after 6 mg/kg c-DDP given at 6 months after renal irradiation (●). ——, Response to drug alone (*D*), and development of damage after 10 and 12 Gy X-rays alone (*X*) are shown for an equivalent testing period, *i.e.*, from 6 months after irradiation for a period of 30 weeks *Points*, mean of a group of 5–6 mice (± 1 SE) (*bars*).

Table 1 *Treatment schedule*

Bilateral kidney irradiation (1, 3, or 6 months) → c-DDP injections → test (4–30 weeks)

X-ray dose (Gy)	X-rays alone	c-DDP (4–8 mg/kg) after X-rays at		
		1 month	3 months	6 months
0	+[a]			+
8	NT[b]	NT	NT	+
10	+	+	+	+
12	+	+	+	+
14	+	+	+	NT
15	+	NT	NT	NT
16	+	NT	NT	NT
17	+	NT	NT	NT

[a] +, results presented in this paper.
[b] NT, not tested.

Table 2 *Animal survival at 2 months after c-DDP injection*

c-DDP dose (mg/kg)	% survival with no X-ray pretreatment	% survival after pretreatment with 10–12 Gy at		
		1 month	3 months	6 months
0	100 (34/34)[a, b]	97 (29/30)[b]		
4	100 (22/22)[b]	100 (12/12)	100 (12/12)	100 (12/12)
6	100 (22/22)[b]	100 (12/12)	100 (12/12)	100 (11/11)
8	100 (22/22)[b]	83 (10/12)	92 (11/12)	17 (2/12)
10	88 (28/32)[b]			
12	100 (10/10)			
14	40 (4/10)			
16	30 (3/10)			

[a] Numbers in parentheses, number surviving per number treated.
[b] Includes toxicity data from previously published experiments (14).

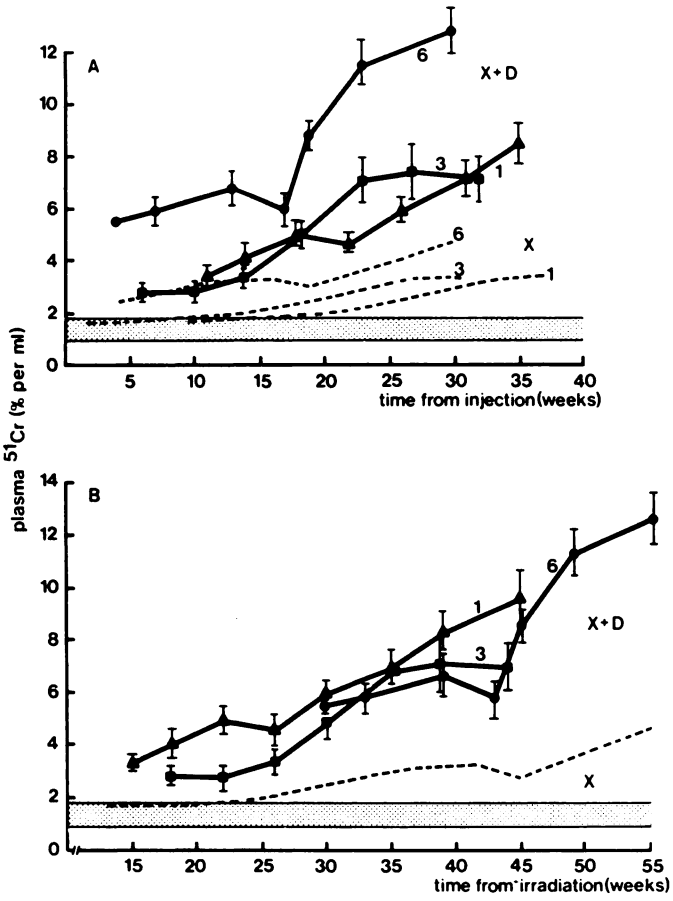

Fig. 3. Kidney damage when 6 mg/kg c-DDP (*D*) was given 1 (▲), 3 (■), or 6 (●) months after 12 Gy X-rays (*X*). *A*, damage as a function of time after c-DDP injection. -----, Response to 12 Gy X-rays only for the appropriate testing times (*i.e.*, from 1, 3, or 6 months after irradiation for a period of 30 weeks). *B*, damage as a function of time after irradiation. -----, Response to 12 Gy X-rays alone.

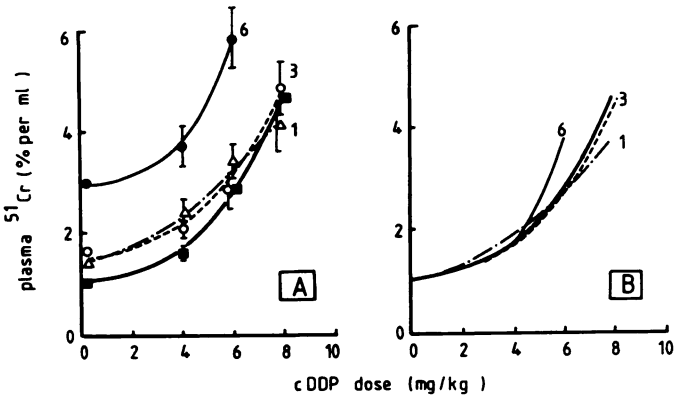

Fig. 4. *A*, c-DDP induced renal damage during the early period (6–11 weeks) after drug as a function of c-DDP dose. ■, c-DDP alone; △, c-DDP given 1 month after 12 Gy, tested 11 weeks after drug and 15 weeks after X-rays; ○, c-DDP given 3 months after 12 Gy, tested 6 weeks after drug and 18 weeks after X-rays; ●, c-DDP given 6 months after 12 Gy, tested 7 weeks after drug and 33 weeks after X-rays. *Bars*, SE. *B*, curves from *A* corrected for damage due to 12 Gy X-rays alone by normalizing against the control value.

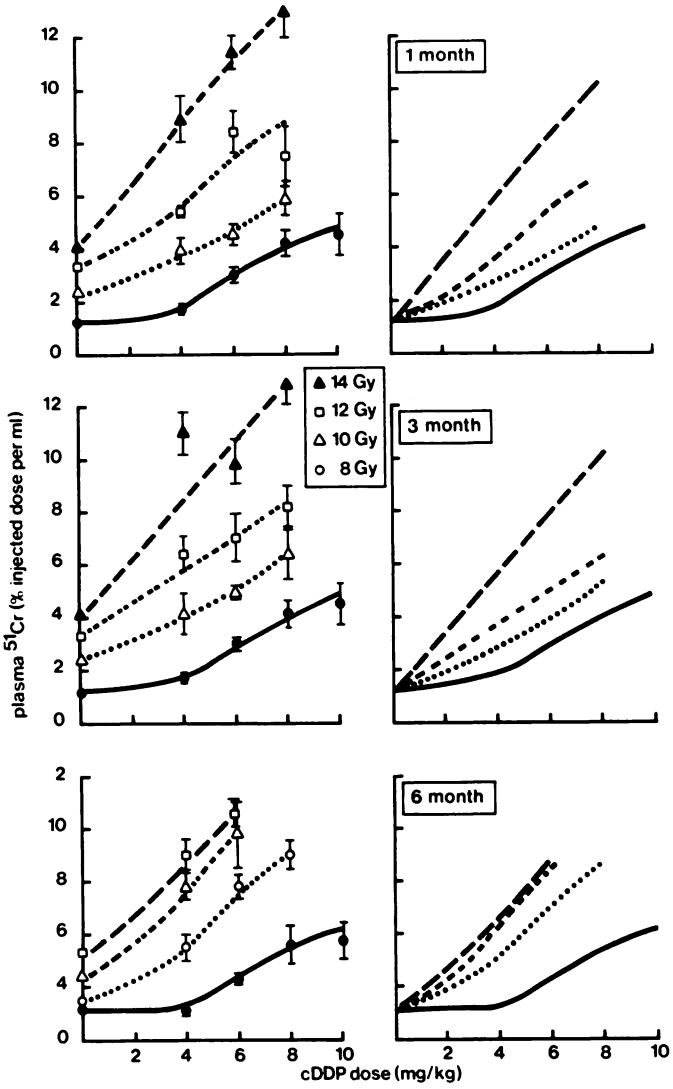

Fig. 5. Drug dose effect curves for late kidney damage when c-DDP was given 1, 3, or 6 months after 8–14 Gy, compared with the response to drug alone (●). The data show renal function at 40–45 weeks after irradiation and 35, 32, and 19 weeks after c-DDP for the 1-, 3-, and 6-month intervals, respectively. *Right*, curves corrected for damage due to X-rays alone by downward shifting of all curves to begin at the control level. *Bars*, SE.

rate of development of damage was faster after the combined treatments than after X-rays alone.

In order to determine the extent to which c-DDP toxicity was increased by prior irradiation, renal damage has been plotted as a function of drug dose for both irradiated and nonirradiated mice. Damage during 2 separate time periods is considered; firstly an early period of 6–11 weeks after drug injection (Fig. 4) and secondly a much later period of 19–35 weeks after injection which corresponds to 40–45 weeks after irradiation (Fig. 5). Early c-DDP induced renal damage was not increased by irradiation either 1 or 3 months previously (Fig. 4). c-DDP given 6 months after irradiation caused more early damage than drug alone, but after a simple linear correction for radiation damage (by a vertical downward shifting of all the combined treatment curves to begin at a level equivalent to zero X-ray dose) the curves for preirradiated and control animals were superimposable (Fig. 4*B*).

Drug dose response curves for long term c-DDP induced kidney damage are shown in Fig. 5. The data are from measurements at 19–35 weeks after c-DDP injection and 40–45 weeks after irradiation, *i.e.*, well into the period where radiation injury is expressed. On the *left* of Fig. 5 are shown the amount of renal damage caused by c-DDP given at 1, 3, and 6 months after irradiation, and on the *right* these dose response curves have been reproduced after correcting for damage due to X-rays alone (by a downward shift to normalize the curves against the control level). These data demonstrate that when c-DDP was given at 1–6 months after irradiation there was a marked increase in late renal damage which could not be explained by simple, linearly additive toxicities. The increase

Table 3 *DEF_d and DEF_x*

A. DEF_d				
Interval between x and d (mo)	X-ray preirradiation dose (Gy)			
	8	10	12	14
1		1.22 ± 0.25[a]	1.80 ± 0.17	ND[b]
3		1.31 ± 0.28	1.89 ± 0.44	ND
6	1.78 ± 0.33	2.35 ± 0.52	2.67 ± 0.52	ND

B. DEF_x			
Interval between x and d (*t*)	c-DDP dose (mg/kg)		
	4	6	8
0.5 h[c]		1.19 ± 0.09[d]	
1 wk[c]		1.19 ± 0.07	
1 mo	1.27 ± 0.13	1.27 ± 0.07	1.27 ± 0.10
3 mo	1.35 ± 0.21	1.47 ± 0.17	ND
6 mo	1.65 ± 0.12	1.69 ± 0.11	2.08 ± 0.32

[a] Calculated at residual plasma activity level of 3.5% ± integrated square error (from fractional dose errors using envelopes through error bar on the dose response curves).
[b] ND, not determined, since the measured response was too high for direct comparison with control data.
[c] From previously published data (14).
[d] Calculated at residual plasma activity level of 4% ± integrated square error.

in damage was dependent on both the initial X-ray dose and the interval between irradiation and drug, with the largest DEF_d[4] occurring after the highest radiation doses and for the longest intervals between treatments. Changes in the DEF_d with increasing interval may, however, be a consequence of changes with time in the response to drug alone (see "Discussion"). Drug dose effect factors for all schedules are given in Table 3.

To assess the extent to which X-ray damage was increased when c-DDP was given 1–6 months after irradiation, the data were replotted as a function of X-ray dose (Fig. 6). There was clearly more renal damage after the combination of X-rays plus c-DDP than after irradiation alone, whether drug was given after 1, 3, or 6 months. Some of this effect was due to direct drug toxicity and on the *right* of Fig. 6 the dose response curves for all the combined treatments have been corrected for drug toxicity by linear downward adjustment of the curves (see above). Even after this correction there was a large enhancement of X-ray damage, whether drug was given at 1, 3, or 6 months. The extent to which X-ray damage was increased was not markedly dependent on drug dose, but there was a gradual increase in the DEF_x[5] with increasing interval between irradiation and drug (see Table 3).

The dose response curves for both drug and X-rays alone (see Figs. 4–6) were nonlinear, making interpretations concerning additivity *versus* nonadditivity difficult. A simple, linear downward shifting of the curves to normalize against control values (as has been adopted here) may not adequately account for additive killing of the 2 agents. However, for such highly nonlinear curves, with threshold responses, an isobologram analysis was not useful and is probably invalid. In the absence of further knowledge of the precise relationship between ^{51}Cr plasma levels and cell killing in the kidney, the simplest procedure for correcting for drug or X-ray alone damage was adopted for these analyses.

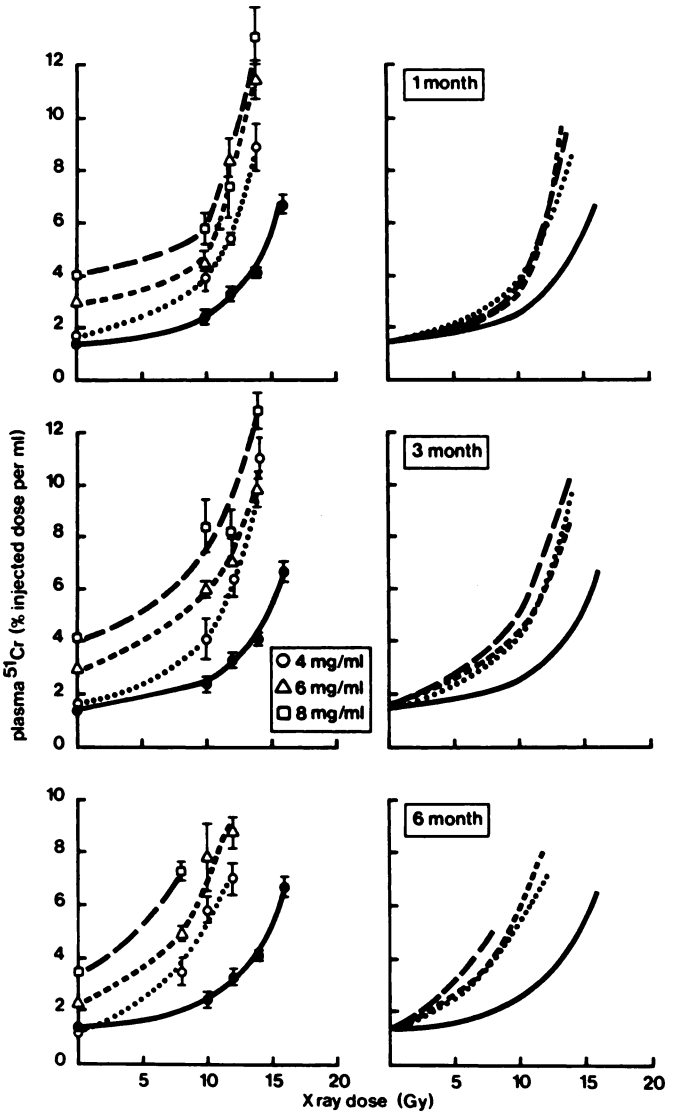

Fig. 6. X-ray dose effect curves for late kidney damage after X-rays alone (●), or when 4–8 mg/kg c-DDP was given 1, 3, or 6 months after irradiation. The data are the same as in Fig. 5, *i.e.*, tested 40–45 weeks after irradiation and 19–35 weeks after drug. *Right*, curves corrected for drug alone damage by downward shifting of all curves to begin at the control value. *Bars*, SE.

DISCUSSION

The results presented in this paper demonstrate that mice with previously irradiated kidneys were more susceptible to damage by subsequent c-DDP injection. There was a more rapid onset and development of damage when c-DDP was given 6 months after irradiation than when it was given at earlier times. During the first 11 weeks after drug administration the extent of renal damage could be explained in terms of linearly additive toxicities from radiation and drug. However, with longer follow up there was a more severe late damage (19–35 weeks after drug; 40–45 weeks after irradiation), which may represent greater than additive toxicities of the 2 agents. There are a number of possible explanations for these observations: (*a*) changes in the response to drug alone with time, and the influence of the choice of assay time; (*b*) progressive alterations in drug pharmacokinetics in mice with irradiated kidneys, and (*c*) alterations in the time of expression of radiation injury by c-DDP. These possibilities will be discussed in turn.

Choice of Assay Time. The results in Fig. 5 and Table 3 show that the amount of renal damage was greater when c-DDP was given after a 6-month interval than after 1 or 3 months. Inspection of the curves in Fig. 1 (response after drug alone), however, suggests that this could be partly a consequence of the choice of assay time. Due to the experimental design, it was not

[4] $$DEF_d = \frac{\text{c-DDP dose with no preirradiation}}{\text{c-DDP dose with preirradiation}}$$
for a given level of damage, after correction for X-ray alone damage.

[5] $$DEF_x = \frac{\text{Dose X-rays alone}}{\text{Dose X-rays + drug}}$$
for a given level of damage, after correction for drug alone damage.

possible to analyze all data at constant times from both irradiation and drug administration. The 6-month data were analyzed at 45 weeks after irradiation and thus 19 weeks after drug. At this time there was a dip in the measured drug damage after all doses (Fig. 1) which flattened the drug dose response curve and, consequently, the DEF values were larger. If the drug response was constant with time or if it had been possible to analyze all data at 50 weeks after irradiation, the DEF_d for the 6-month interval would probably be close to that for the shorter intervals.

Support for this comes from the curves in Fig. 3*B* showing that for a given drug and X-ray dose the progression of damage as a function of time after irradiation was very similar whether drug was given at 1, 3, or 6 months. Thus, analysis of damage at a constant time after X-rays should lead to equivalent DEFs for all intervals between irradiation and c-DDP, providing that the drug dose response curve does not change with time.

It is important to note, however, that a constant DEF_d for increasing interval between irradiation and drug does not necessarily imply that the extent of drug induced damage was the same at all intervals. This can be seen from Fig. 7 which schematically illustrates the progression of damage when drug is given at increasing intervals after irradiation. If the 1-, 3-, and 6-month curves for a given drug and X-ray dose are superimposed when plotted as a function of time after X-rays, as occurred in practice, then the drug killing (Fig. 7, ↑) must increase with interval between treatments. This is due to the progressive nature of the radiation damage, whether linear (Fig. 7*A*) or curved (Fig. 7*B*), and the increased rate of progression of damage after combined treatment compared with after irradiation alone. If drug killing was the same at all intervals after irradiation, then the 1-, 3-, and 6-month curves would appear progressively later in time (Fig. 7*C*), and the DEF_d would decrease with interval between irradiation and drug. In practice the time course for development of damage was the same whether drug was given at 1, 3, or 6 months, and there was certainly no reduction in DEF with increasing interval. The DEF_d was, in fact, larger for the 6-month interval although, as explained above, this may be an artifact due to the different testing times after drug administration.

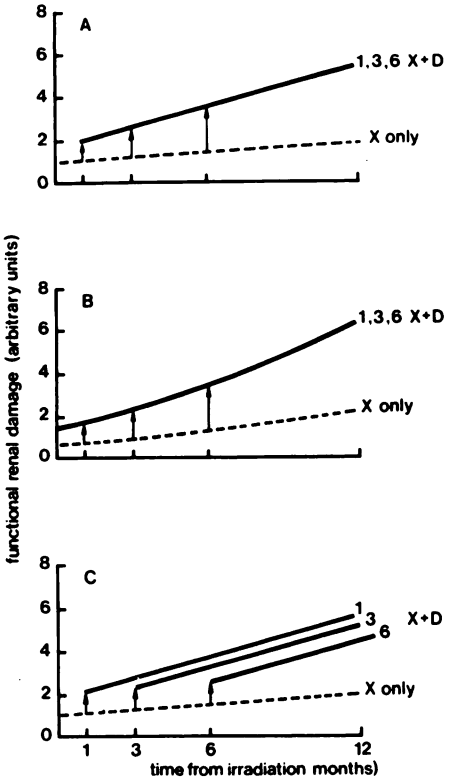

Fig. 7. Models for the rate of expression of functional kidney damage after X-rays (*X*) alone (——) or for c-DDP (*D*) given 1, 3, or 6 months after X-rays (——). ↑, amount of damage induced by a constant drug dose given at the various intervals. If the 1, 3, and 6 months combined treatment curves are all superimposed then the drug must cause greater damage (*larger* ↑) at the later intervals, whether damage progression is linear (*A*) or quadratic (*B*). If drug killing is independent of interval (*C*), damage expression is earlier for the shorter intervals.

The model shown in Fig. 7 assumes that residual plasma activity at 30 min after ^{51}Cr-labeled EDTA injection is linearly related to cell kill in the kidney, and the conclusion that there is greater drug killing with longer intervals between irradiation and drug depends on this assumption. It is, in fact, not known precisely how the measured renal function relates to cell killing and this assumption may not be valid. The increased lethality which occurred when c-DDP was given 6 months after irradiation (Table 2) and the reports of altered c-DDP pharmacokinetics after renal irradiation (see below) do, however, support the conclusion of increased drug cell killing with increasing time after irradiation. It appears, therefore, that c-DDP given 6 months (or longer) after renal irradiation may be more hazardous than when the same drug dose is given before or 1–3 months after irradiation.

Altered c-DDP Pharmacokinetics. As has previously been discussed by Brown (18), treatment schedules involving more than one drug dose or the administration of drug after irradiation or during a fractionated course of radiotherapy may result in altered pharmacokinetics, thus affecting the total drug exposure of a tissue.

In addition to the present data there is one other report of a reduced tolerance to c-DDP in animals with previously irradiated kidneys.[6] In those experiments rat kidneys were bilaterally irradiated with 20 Gy, given as 9 fractions in 11 days, and subsequently given i.v. injections of c-DDP. The LD_{50}/10 days (acute drug toxicity) was reduced from 10–7 mg/kg when the drug was given 9 months after irradiation. There was no reduction in the LD_{50}/10 days when c-DDP was given after 3 months, although animals which survived the acute effects did develop more severe late radiation nephritis. These results agree well with the present observations. Moulder *et al.*[6] also measured the 24-h excretion of total platinum in both control and preirradiated rats and found that drug clearance was reduced by approximately 50% at 6–9 months after renal irradiation.

Increased c-DDP toxicity after previous treatment with nonlethal doses of c-DDP has also been reported (19). In these experiments, guinea pigs were pretreated with 1, 2, or 3 doses of c-DDP 1 day before i.v. injections of 10 mg/kg [^{195m}Pt]c-DDP. Urinary excretion of the labeled platinum was significantly reduced in pretreated animals. Renal clearance of platinum in patients has also been shown to decrease with successive c-DDP treatments (20), although this was only associated with reduced creatine clearance in those patients who had previously received high dose platinum (>100 mg/m^2).

Increases in c-DDP toxicity after prior exposure to the drug could simply be the result of the increased total drug dose and accumulative toxicity. In both the clinical and animal studies, however, the urinary and plasma levels of platinum demonstrated a significant decrease in platinum clearance after prior drug exposure (19, 20).

The above studies demonstrate that renal damage caused

[6] J. E. Moulder, J. S. Holcenberg, B. A. Kamen, M. Cheng, and B. L. Fish. Renal irradiation and the pharmacology and toxicity of methotrexate and cisplatinum, presented at the Conference on Chemical Modifiers of Cancer Treatment, Clearwater, Florida, October 1985.

either by prior irradiation or prior exposure to c-DDP can reduce the clearance of a subsequent dose of c-DDP. The tissues and organs in pretreated animals (or patients) would consequently receive higher total drug exposures than in control animals. We are currently investigating the clearance of c-DDP in preirradiated and control mice. Preliminary data would suggest that pharmacokinetic changes may partly explain the increase in renal damage which were observed in the present study when c-DDP was given to previously irradiated animals.

Alteration in Time of Expression of Radiation Injury. c-DDP has been shown to stimulate proliferation in the kidneys (21), which could lead to a more rapid expression of radiation injury, resulting in increased damage at any given time after treatment. In the present studies, however, the largest increase in renal damage occurred when c-DDP was given at 6 months, *i.e.*, after the onset of radiation injury. If stimulated proliferation (as a result of the c-DDP injection) caused a more rapid expression of the radiation injury then this should have had the most pronounced effect for drug given at early times, before the expression of radiation injury. It therefore seems unlikely that a drug induced precipitation of latent radiation damage can explain the present data.

c-DDP given many months after renal irradiation led to a large increase in damage compared with that seen after either agent alone. This may be partly due to additional cell killing by the drug, leading to the expression of subclinical radiation effects. Indeed it has been suggested that such an addition of X-rays and drug damage is likely to occur in slow turnover tissues (22). Alterations in the pharmacokinetics of c-DDP in previously irradiated kidneys is also a strong possibility and has been demonstrated by other workers.[6]

Whether or not there was more drug induced cell killing with increasing time after renal irradiation is not completely resolved. It is clear, however, that c-DDP given many months after renal irradiation caused more damage than was predicted on the basis of results from experiments giving drug and X-rays in close sequence (14). This could have important clinical implications where chemotherapy involving the use of c-DDP is planned after previous renal irradiation. The resulting nephrotoxicity could be more severe than expected, even if renal function prior to chemotherapy was apparently normal.

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REFERENCES

1. Peckham, M. J., and Collis, C. H. Clinical objectives and normal tissue responses in combined chemotherapy and radiotherapy. Bull. Cancer (Paris), *68:* 132–141, 1981.
2. Yarnold, J. R., Horwich, A., Duchesne, G., Westbrook, K., Gibbs, J. E., and Peckham, M. J. Chemotherapy and radiotherapy for advanced testicular nonseminoma. 1. The influence of sequence and timing of drugs and radiation on the appearance of normal tissue damage. Radiother. Oncol., *1:* 91–99, 1983.
3. Phillips, T. L., and Fu, K. K. Quantification of combined radiation therapy and chemotherapy effects on critical normal tissues. Cancer (Phila.), *37:* 1186–1200, 1976.
4. Phillips, T. L., Wharam, M. D., and Margolis, L. W. Modification of radiation injury to normal tissues by chemotherapeutic agents. Cancer (Phila.), *35:* 1678–1684, 1975.
5. Cassady, J. R., Richter, M. P., Piro, A. J., and Jaffe, N. Radiation-Adriamycin interactions: preliminary clinical observations. Cancer (Phila.), *36:* 946–949, 1975.
6. D'Angio, G. Clinical and biological studies of actinomycin D and roentgen irradiation. Am. J. Roentgenol., *87:* 106–109, 1962.
7. Liebner, E. J. Actinomycin and radiation therapy. *Am. J. Roentgenol., 87:* 94–105, 1962.
8. Rosen, G., Suwansirikul, S., Kwon, C., Tan, C., Wu, S. J., Beattie, E. J., and Murphy, M. L. High-dose methatrexate with citrovorum factor rescue and Adriamycin in childhood osteogenic sarcoma. Cancer (Phila.), *33:* 1151–1163, 1974.
9. Ang, K. K., Landuyt, W., Rijnders, A., and Van der Schueren, E. Differences in repopulation kinetics in mouse skin during split course multiple fractions per day (MFD) or daily fractionated irradiations. Int. J. Radiat. Oncol. Biol. Phys., *10:* 95–99, 1984.
10. Denekamp, J. Changes in the rate of repopulation during multifraction irradiation of mouse skin. Br. J. Radiol., *46:* 381–387, 1973.
11. Withers, H. D., Chu, A. M., Reid, B. O., and Hussey, D. H. Response of mouse jejunum to multifraction radiation. Int. J. Radiat. Oncol. Biol. Phys., *1:* 41–52, 1976.
12. Denekamp, J., Stewart, F. A., and Hirst, D. G. Compensatory proliferation in normal tissues and tumours after irradiation. *In:* H. E. Edwards, S. Navaratnan, B. J. Parson, and G. O. Phillips (eds.), Proceedings of Meeting of Association of Radiation Research, 1979, pp. 261–271. Amsterdam: Elsevier/North-Holland Biomedical Press, 1979.
13. Stewart, F. A., Denekmap, J., and Hirst, D. G. Proliferation kinetics of the mouse bladder after irradiation. Cell Tissue Kinet., *13:* 75–89, 1980.
14. Stewart, F., Bohlken, S., Begg, A., and Bartelink, H. Renal damage in mice after treatment with cisplatinum alone or in combination with X-irradiation. Int. J. Radiat. Oncol. Biol. Phys., *12:* 927–933, 1986.
15. Chantler, C., Garnett, E. S., Parsons, V., and Veall, N. Glomerular filtration rate measurements in man by the single injection method using ^{51}CrEDTA. Clin. Sci., *37:* 169–180, 1969.
16. Stewart, F. A., Soranson, J. A., Alpen, E. L., Williams, M. V., and Denekamp, J. Radiation-induced renal damage: the effects of hyperfractionation. Radiat. Res., *98:* 407–420, 1984.
17. Williams, M. V., and Denekamp, J. Sequential testing of radiation-induced renal damage in the mouse. Radiat. Res., *94:* 305–317, 1983.
18. Brown, J. M. Drug or radiation changes to the host which could affect the outcome of combined modality therapy. Int. J. Radiat. Oncol. Biol. Phys., *5:* 1151–1163, 1979.
19. Litterst, C. L., and Schweitzer, V. G. Increased tissue deposition and decreased excretion of platinum following administration of cisplatin to cisplatin-pretreated animals. Cancer Chemother. Pharmacol., *12:* 46–49, 1984.
20. Reece, P. A., Stafford, I., Russel, J., and Gill, P. G. Cisplatin pretreatment reduces unbound platinum renal clearance in patients. Proc. Am. Assoc. Cancer Res., *26:* 351, 1985.
21. Kovacs, C. J., Braunschweiger, P. G., Schenken, L. L., and Burholt, D. R. Proliferative defects in renal and intestinal epithelium after *cis*-dichlorodiammine platinum (II). Br. J. Cancer, *45:* 286–294, 1982.
22. Rubin, P. Late effects of chemotherapy and radiation therapy: a new hypothesis. Int. J. Radiat. Oncol. Biol. Phys., *10:* 5–34, 1984.