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Renal Damage in the Mouse: The Effect of d(4)-Be Neutrons

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A further study on the response of the mouse kidney to d(4)-Be neutrons ($\bar{E}_N$ = 2.3 MeV) is described. The results confirm and augment the work published previously by Stewart *et al.* [*Br. J. Radiol.* **57**, 1009–1021 (1984)]; the present paper includes the data from a "top-up" design of experiment which extends the measurements of neutron RBE (relative to 240 kVp X rays) down to X-ray doses of 0.75 Gy per fraction. The mean RBE for these neutrons increases from 5.8 to 7.3 as X-ray dose per fraction decreases from 3.0 to 1.5 Gy in the kidney. This agrees with the predictions from the linear quadratic (LQ) model, based on the renal response to X-ray doses above 4 Gy per fraction. The mean RBE estimate from a single dose group at 0.75 Gy per fraction of X rays is, however, 3.9. This is below the LQ prediction and may indicate increasing X-ray sensitivity at low doses. Data from this study and from those published previously have been used to determine more accurately the shape of the underlying response to d(4)-Be neutrons; an α/β ratio of 20.5 ± 3.7 Gy was found. The best value of α/β for X rays determined from these experiments was 3.04 ± 0.35 Gy, in agreement with previous values.

INTRODUCTION

An important question in clinical neutron radiotherapy is the relationship between the RBE for late and early responding tissues (e.g. (*1*)). Although there does not appear to be a consistent difference in the value of RBE on the basis of classification into acute and late response, it is now apparent that the RBE increases *faster* with decreasing dose per fraction for the late than for the acutely responding tissues (*1–4*).

In a previous study on the mouse kidney, Stewart *et al.* (*5*) compared the response of d(4)-Be neutrons and 240 kVp X rays using conventional fractionation with one, two, four, and eight equal dose fractions. These data showed an increase in the RBE from 2.3 to 4.5 as the X-ray dose per fraction decreased from 14.4 Gy to 4.7 Gy, which was the smallest dose per fraction used. When compared with acute skin reactions on mouse feet, the RBE for kidney was slightly less than for skin over this dose range. However, the RBE for kidney was predicted (by the LQ model) to rise steeply and exceed the RBE for acute skin damage at X-ray doses per fraction below 3.9 Gy, with a limiting RBE possibly as high as 20–26 at very low doses.

The experiments described here were designed to test the previous measurements of RBE in the range of 4 to 14 Gy per fraction and the predictions of RBE at lower doses per fraction. To achieve this, we have irradiated using 1, 2, 5, or 10 equal dose

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fractions and also used 10 fractions plus a "top-up" dose of neutrons to measure RBE in the lower X-ray dose range of 0.75–3.0 Gy per fraction.

At the Gray Laboratory, d(4)-Be neutrons are used specifically as a radiobiological tool to study the response of normal tissues and tumors to a wide variety of different X-ray treatment schedules. The use of these neutrons as a top-up radiation enables such studies to be carried out with a considerable saving in time and expense. This is because the effect of X-ray dose fractions can be assessed using a reduced number of radiation fractions (as in the studies reported here) compared with standard single course fractionation techniques (e.g., (*6, 7*)). It is then also possible to study low doses per fraction, where otherwise conventional multiple fractionation would need an impossible number of radiation fractions to be given, e.g., >100 (*8*). To plan top-up experiments reliably, it is necessary to know accurately the sensitivity of tissues to the d(4)-Be neutron top-up radiation. The studies described here also address this question for renal damage in the mouse.

MATERIALS AND METHODS

Mice and irradiation schedule. Female CBA/Ht/GyfBSVS mice were 12–16 weeks old at the time of treatment (19–25 g weight) and were irradiated in air without anesthetic. Six animals were allocated to each dose group within all fractionation schedules, which were carried out in a maximum overall treatment time of 11 days. Dose groups allocated to the top-up arm received a top-up dose of d(4)-Be neutrons 3 days after the last fraction in a 10-fraction schedule, to give an overall treatment time of 14 days for these groups. Mice were irradiated bilaterally using a 20 × 13 mm field set up to include the whole of both kidneys. Each dose of neutrons or X rays was split into two immediately consecutive halves, with the mice rotated 180° in between, to improve dose uniformity.

X rays. The irradiation system has been described previously (*5, 9, 10*). X rays were 240 kVp, filtered with 1.0 mm Al and 0.25 mm Cu, giving an HVL = 1.3 mm Cu. Dose rate was 1.7 Gy min^{-1}. X rays were given as single doses and as 2, 5, and 10 fractions with interfraction intervals of 10, 2, and 1 day, respectively. Additionally, blocks of three dose groups were each given 10 fractions of the same X-ray dose, selected from the range 0.75–3 Gy per fraction. Three graded top-up doses of d(4)-Be neutrons were given subsequently to each block of dose groups to produce a complete dose–response curve for each small X-ray dose studied according to the method of Joiner *et al.* (*7*).

Neutrons. Neutrons were produced from the Gray Laboratory van de Graaff accelerator by bombarding a thick beryllium target with 4 MeV deuterons. Under irradiation conditions, this produces neutrons with a mean energy of 2.3 MeV and a dose mean lineal energy of 65.6 keV/μm, giving a half value thickness of ~3.0 cm ICRU muscle (*11, 12*). Four 25-cm diameter apertures cut in a steel and paraffin wax collimator (*11*) were further restricted to give a rectangular field 20 × 13 mm by using specially constructed collimator inserts made from 80% steel powder in epoxy resin (Devcon Ltd., Shannon, Ireland). Dosimetry was according to the European Protocol (*13*) and all doses are quoted as neutron dose alone, excluding the γ contamination which was measured as 12.2% of the total neutron plus γ dose. The mean neutron dose rate was 0.50 Gy min^{-1} at a target to skin distance of 42.7 cm. Neutrons were given as single doses and as 2, 3, 5, and 10 fractions all in the same overall time of 11 days, with interfraction intervals of 10, 4, 2, and 1 day, respectively. Additionally, 10 small neutron fractions in the dose range 0.2–0.45 Gy per fraction were given daily, followed by a top-up dose of neutrons according to the same protocol as used for the X-ray + top-up groups.

Assays. Three assays of renal damage have been used. These are clearance of ^{51}Cr-EDTA, urine output, and hematocrit. These procedures have been discussed extensively[1] (*5, 9, 10, 14*) and are described here briefly. Mice were assayed every 3 or 4 weeks from 15 to 32 weeks postirradiation.

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[1] M. V. Williams, Modification of the response of the kidney and an experimental tumour to ionising radiation. M.D. thesis, Cambridge University, 1983.

Isotope clearance. The clearance of EDTA is a measure of glomerular function. A radiolabeled compound (^{51}Cr-EDTA) was used to assess clearance by determining the blood concentration 1 h after an intraperitoneal injection of 10 μCi (~200 μg EDTA), given as 0.1 ml of a solution with activity 100 μCi/ml. Single 70-μl blood samples were taken from the orbital sinus. Following centrifugation for 3 min at 12,000 *g*, 20-μl samples of plasma were taken and counted for residual activity in an LKB 1282 autogamma counter. Results are expressed as the percentage of injected activity per milliliter of plasma at 1 h after injection.

Hematocrit. This was measured after centrifugation in the blood sample taken during the isotope clearance assay. Renal damage results in a progressive decrease in hematocrit after irradiation. Although the mechanism for this has not been proved, it has been suggested that this might result from loss of the juxtaglomerular cells which produce erythropoietin (*14*).

Urine output. Urine production has been found to increase after renal irradiation in mice (*9*). We have again confirmed the observation of Williams[1] that the increase in urine excreted may be determined conveniently by the frequency of urination, since there is a direct correlation between these two measurements until the mice enter an acute anuric phase of damage at which time they are sacrificed. Mice were housed in individual wire-bottomed cages for 24-h periods. Urine was collected on a paper roll moving at 15 cm h^{-1} below the bottom of the cages. The data were expressed as the urination frequency, in urination events per 24-h period.

RESULTS

Stewart *et al.* (*5, 10*) have shown that renal damage in mice develops with time in a qualitatively similar way after neutrons and X rays. The only difference in the response to the two radiations appears to be the amount of dose necessary to cause a given biological effect. In common with previous authors using these assays (*5, 10, 15*) we have therefore chosen to analyze the data at the time where we obtain maximum resolution and differentiation of the dose–response curves. These data are plotted in Figs. 1, 2, and 3, and dose–response curves were drawn by eye through the data as previously described (*2, 5, 9, 10, 14, 15*).

Figure 1 shows the data for the isotope clearance assay obtained at 24 weeks postirradiation. There is clear separation of the curves for 1, 2, 5, and 10 fractions of X rays (Fig. 1a). This contrasts with the data for neutron irradiation where all the curves for the different fractionation schemes lie virtually on top of each other. This reflects the almost linear underlying response to these neutrons and the consequent lack of dose sparing with increasing fractionation compared with X rays (e.g., Ref. (*16*)). The dose–response curves for single doses of X rays and neutrons are truncated. This is because some radiation damage to gut is unavoidable using fields designed to irradiate both kidneys in the mouse (e.g., Ref. (*10*)). The highest single doses plotted therefore represent the acute tolerance of a few centimeters of gut to local irradiation. Where these doses were exceeded in single irradiations, animals died within 8 weeks.

Figures 1b and c summarize the data from the top-up experiments carried out concurrently with the conventional fractionation study. The data are plotted here according to the methods of Joiner *et al.* (*7*). Each dose–response curve is composed of dose groups all given the same initial ten small doses of either X rays (Fig. 1b) or neutrons (Fig. 1c) and then given graded single top-up doses of neutrons. Thus full dose–response curves are obtained when residual activity of ^{51}Cr-EDTA is plotted against neutron top-up dose.

By increasing the size of the dose per fraction given in the initial treatments, the final dose–response curve obtained is shifted further left toward *lower* top-up doses.

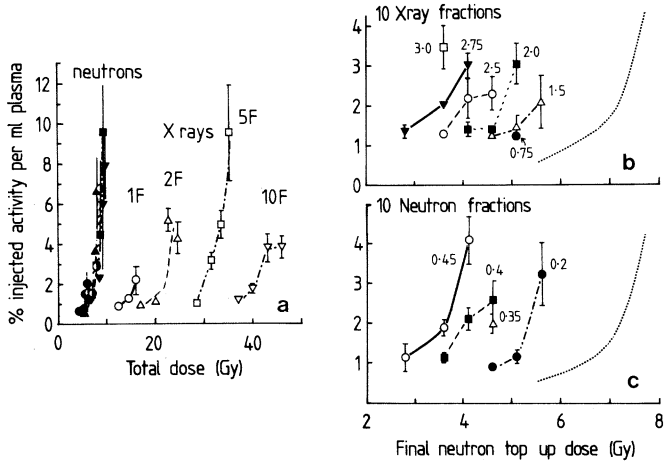

FIG. 1. Residual activity of ^{51}Cr-EDTA measured in plasma samples 1 h after injection. Assay at 24 weeks postirradiation. (a) Plotted against total dose of neutrons or X rays, given as 1 (○, ●), 2 (△, ▲), 3 (◑), 5 (□, ■), or 10 (▽, ▼) equal dose fractions. (b) and (c) Plotted against the final top-up dose of neutrons; each dose–response curve is for 10 small doses per fraction, as marked, of either X rays (b) or neutrons (c) followed by a neutron top-up dose. The curve on the far right (dashed) shows the response to two fractions of neutrons alone, used as a reference (see text).

This indicates an increasing subthreshold effect of the 10 small priming treatments with increasing dose per fraction, as expected.

The dose–response curve on the far right of Figs. 1b and c (dashed) is the response to two fractions of neutrons *alone* (no initial pretreatment) reproduced from Fig. 1a. This provides a "reference" curve for zero pretreatment against which to measure the leftward shift (i.e., underlying effect) of the other dose–response

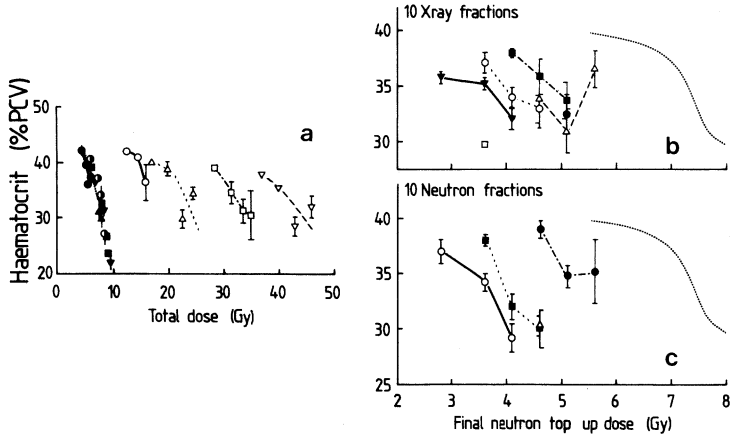

FIG. 2. Hematocrit at 28 weeks postirradiation. The description of the curves is as for Fig. 1.

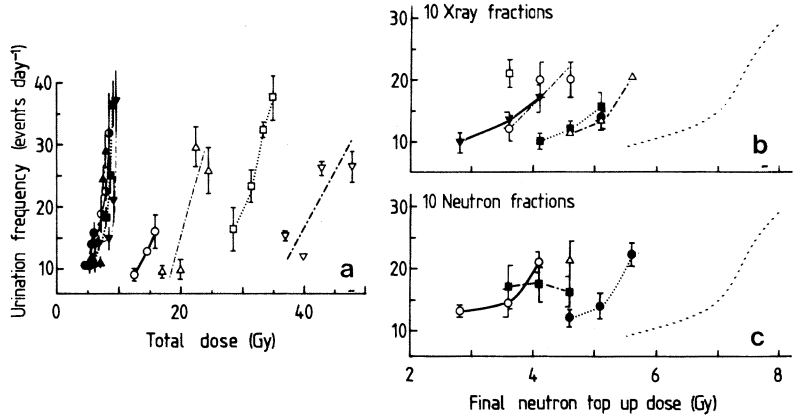

FIG. 3. Urination frequency at 20 weeks postirradiation. The description of the curves is as for Fig. 1.

curves for the different priming doses per fraction (see later). Of course, it would have been logical to use the response to a *single* neutron dose for this reference curve, since the top-up doses were also given as single neutron doses. However, this was not possible since full dose–response curves were not obtained for the single doses for the reason discussed above.

The data for the hematocrit assay are shown in Fig. 2. The treatments are identical to those in Fig. 1, but the data presented are at 28 weeks postirradiation since this is the time at which the best differentiation of the dose–response curves was obtained.

Figure 3 shows the data for urination frequency at 20 weeks. The resolving power of this assay is not as good as for isotope clearance and hematocrit. Poor data were obtained for the top-up experiments (Figs. 3b and c) where the dose–response curves are not well separated, and for 0.4 Gy per fraction of neutrons a dose–response relationship was not obtained.

RBE in the High Dose Range

The relative biological effectiveness of d(4)-Be neutrons can be measured from Figs. 1a, 2a, and 3a in a straightforward manner by calculating the ratio of the total doses of X rays and neutrons needed as 1, 2, 5, or 10 fractions to achieve the same functional effect (*5, 16*). In this way, it is possible to measure the RBE in the X-ray dose range of 3.9–16 Gy per fraction. These data are shown in Fig. 4. This diagram summarizes the RBE measurements for all three assays: EDTA clearance at 24 weeks, hematocrit at 28 weeks, and urine output at 20 weeks. Each data point represents an assessment of RBE for a particular assay at a single level of effect in Figs. 1, 2, and 3. The string of data points for each assay in each fractionation scheme shows the RBE over the full range of effect available for that pair of X-ray and neutron dose–response curves. The average fractional error (SEM) on individual RBE assessments, due to the errors on the original dose–response curves (Figs. 1, 2, and 3), was estimated as $\sim \pm 6\%$. The three assays all give very similar results, and although there is a trend toward slightly higher RBE values at later times, this is not significant at the $P = 0.05$

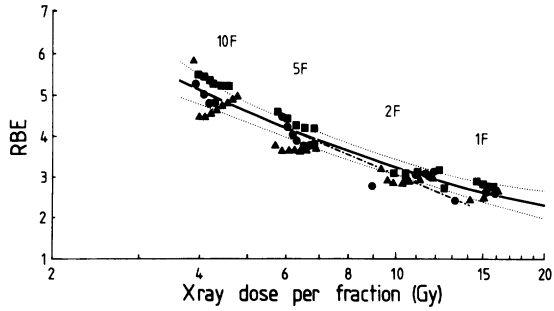

FIG. 4. RBE (X-ray dose/neutron dose) for 1, 2, 5 and 10 fractions of radiation. EDTA clearance (●), hematocrit (■), urine output (▲). Each point represents RBE measured at a different level of functional effect in Figs. 1, 2, and 3 and for each fractionation schedule the full range of functional effect is represented. Solid line: fitted to the data using Eq. (1) (see Discussion), the broken lines are the 95% confidence limits on the expected (mean) RBE from this fit. Dashed line: reproduced from Stewart *et al.* (*5*).

level. This confirms previous observations (*5*) that neither the time of assay nor which assay is actually used has any significant influence on the RBE values observed for renal damage in mice.

A solid line has been drawn through the data in Fig. 4. This line represents the expected (mean) RBE as a function of dose per fraction, determined from the fit of Eq. (1) (discussed later) to these data. The 95% confidence limits are also shown. The solid line is indistinguishable from the predicted RBE derived from the values of α and β in the linear-quadratic equation, fitted to only the data for EDTA clearance at 24 weeks (see Discussion). The EDTA assay therefore gave the most representative data for the response to conventional fractionation in this study. The RBE was 2.57 (95% CL = 2.34–2.80) for a single X-ray dose of 16 Gy, increasing to 5.16 (4.79–5.53) at 3.9 Gy per fraction (10 fractions). The dashed line in Fig. 4 is reproduced from a similar plot by Stewart *et al.* (*5*), their Fig. 7a. Agreement with the present data is excellent although we measured slightly higher (but not significantly different) RBEs at the higher doses. The data in Fig. 4 also extend to slightly lower doses since we gave 10 fractions compared with 8 given by Stewart *et al.* (*5*).

Extension of RBE to the Low Dose Range

We have utilized the data from the top-up experiments (Figs. 1b, c; 2b, c; 3b, c) to obtain RBE values at lower X-ray doses per fraction (0.75–3.0 Gy) using the equivalent remembered dose technique (*7*). The equivalent remembered dose (ERD) is the horizontal displacement of each dose–response curve in panels b and c of Figs. 1, 2, and 3 relative to the reference line (dashed), which in this case is the response to two fractions of neutrons alone. The ERD is a measure of the underlying effect of the initial small dose fractions, in units of equivalent neutron dose. ERD is plotted against dose per fraction in Fig. 5 for 10 X-ray fractions and 10 neutron fractions derived from panels b and c, respectively, of Figs. 1, 2, and 3.

To obtain an RBE measurement at a particular X-ray data point in Fig. 5, it is necessary to determine from the graphs the *neutron* dose per fraction which would

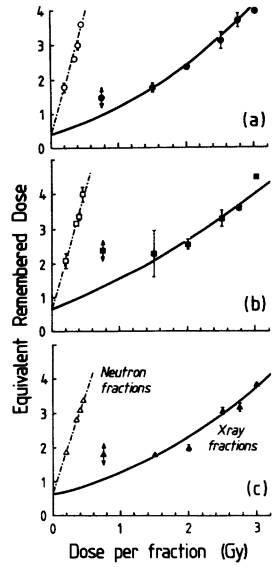

FIG. 5. Mean ERD, derived from the lateral displacement of the dose–response curves in Figs. 1–3. Open symbols, 10 neutron priming fractions + neutron top-up. Closed symbols, 10 X-ray priming fractions plus neutron top-up. (a) EDTA clearance from Figs. 1b, c. (b) Hematocrit from Figs. 2b, c. (c) Urination frequency from Figs. 3b, c. The points show the mean ± standard deviation of the values of ERD measured over the complete range of functional effect determined by each dose–response curve in Figs. 1b, c–3b, c. The double arrows on the ERD estimates at 0.75 Gy indicate indeterminate confidence limits (see text). Dashed lines: least-squares linear regression through the neutron fractionation data. Solid lines: Linear quadratic fits to the X-ray fractionation data, excluding the points at 0.75 Gy, using the analysis of ERD described by Joiner *et al.* (*7*).

produce an identical value of ERD. RBE is then the ratio of X-ray to neutron dose per fraction at this level of ERD (*7*). The RBE values obtained in this way are shown in Fig. 6. The neutron dose per fraction, corresponding to any level of ERD, was determined from a straight line fitted through the neutron data in Fig. 5 using least-squares linear regression. The positive intercept of this line on the ERD axis reflects the slight dose-sparing as a single neutron dose is split into two fractions (Figs. 1a, 2a, and 3a), since all top-up doses in these studies were given as single neutron doses but a two-fraction neutron reference was used to measure ERD. It is unlikely that the true relationship of ERD to neutron dose per fraction (Fig. 5) departs significantly from linearity at these low doses, as evidenced by the superposition of the neutron curves in Figs. 1a, 2a, and 3a. Indeed the whole range of neutron dose per fraction in Fig. 5 is less than 2.2% of the α/β ratio for neutrons (see Fig. 7), so that any deviation of the ERD from a linear relationship cannot exceed 2.2%, which would be extremely difficult to detect experimentally.

The values plotted in Fig. 6 show a continuing rise in RBE with decreasing dose per fraction. All the data points lie above the RBE for mouse skin at X-ray doses less than 4 Gy per fraction, confirming the original predictions made by Stewart *et al.* (*5*) from X-ray doses >5 Gy per fraction. In the present experiments, there was possibly a *decrease* in RBE for kidney at the lowest X-ray dose per fraction (0.75 Gy), reflect-

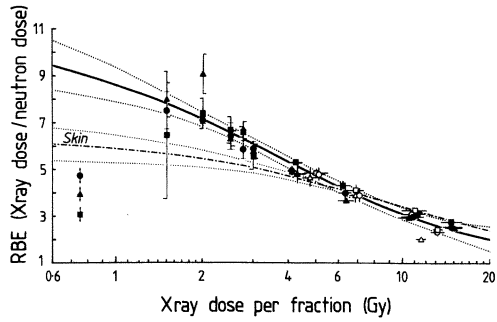

FIG. 6. RBE versus dose per fraction, from full course fractionation (>4 Gy per fraction) and from the top-up experiments (<3 Gy per fraction). Closed symbols: present data; open symbols: data from Stewart *et al.* (*5*). EDTA clearance (●, ○); hematocrit (■, □); urine output (▲, △). Error bars are standard deviation. Solid line: Least-squares regression fit to all the data except at 0.75 Gy, using Eq. (1). Dashed line: RBE for mouse skin (*7*). The arrowed lines attached to the points at 0.75 Gy indicate indeterminate error estimates. These RBE values have *not* been included in the fit of Eq. (1) to the data (see text). The broken lines indicate 95% confidence limits on the expected (mean) RBE values for kidney and skin, derived from the fit to Eq. (1).

ing a possible increase in *X-ray* effectiveness as measured by ERD (Fig. 5). However, this one RBE value and its associated ERD have been derived from only a single dose group (Figs. 1b, 2b, and 3b) and so must be less reliable, with indefinable confidence limits as shown in Fig. 6. In spite of this uncertainty, this observation is interesting in the light of similar previous results on mouse skin (*7*) where neutron RBE was also found to decrease below X-ray doses of 1 Gy per fraction.

DISCUSSION

Fitting the Linear Quadratic Model to the Data

The shape of the underlying dose-fractionation response to X rays is of major experimental importance because of its clinical relevance and because of current inter-

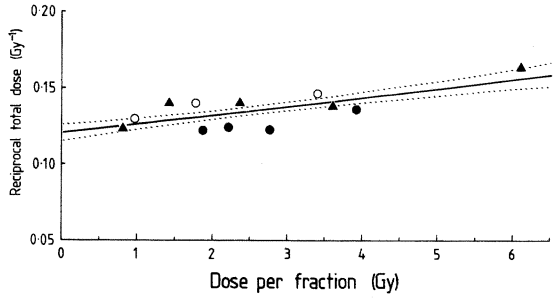

FIG. 7. Reciprocal total dose versus dose per fraction, for neutrons. Assay is EDTA clearance at 24 weeks (closed symbols: present data) and 25 weeks (open symbols: data from Stewart *et al.* (*5*)). Single course fractionation (▲, ○). Top-up data (●). Top-up data are included using the method of Joiner (*20*). Dashed lines indicate the estimate of the standard error limits.

est in its properties at low doses (*6, 7*). The shape of the underlying neutron response, and its relationship to the effect of X rays, is also of interest for the planning of future top-up experiments and for the prediction of RBE with changing dose per fraction. These underlying responses may be conveniently described by the ratio of the constants in the dose and dose-squared terms of the linear quadratic equation, i.e., the α/β ratio (e.g., Ref. (*17*)). If α/β is known for both neutrons and X rays (i.e., α_N/β_N and α_X/β_X) then the RBE relationship between the two radiations can also be determined at all doses if either α_N/α_X or $\sqrt{\beta_N/\beta_X}$ is known. These two terms represent the limiting RBE at extremely low or high doses, respectively.

We have shown previously (*7, 16*) that the LQ model still provides a good description of the underlying neutron dose–response relationship (or "cell survival" curve) even though this may be almost linear in shape, as in mouse skin. This simply means that α/β is large and under such conditions it is impossible to demonstrate the clear superiority of any one particular model. The LQ model is usually selected because of its simplicity. Stewart *et al.* (*5*) showed that the LQ model could be used as a good description of the renal response to neutron as well as photon irradiation. Figure 7 of this paper (described later) verifies this.

In common with previous authors (*5, 10*), we first derived the α/β ratio from a plot of the reciprocal of the isoeffective doses against the corresponding doses per fraction at fixed levels of functional effect for the three assays. The slope (proportional to β) and intercept (proportional to α) were obtained from least-squares regression analysis, and their ratio (α/β) was calculated for X rays and neutrons as well as the ratio of α_N/α_X. Errors and confidence intervals on these ratios were calculated from the elements of the covariance matrix, obtained from the regression analysis, and Fieller's theorem (*18, 19*). We applied this method to the single course fractionation data of Figs. 1a, 2a, and 3a and obtained the ratios shown in Table I. To obtain α/β estimates from the data for *neutron* priming plus neutron top-ups (Figs. 1c, 2c, and 3c) we have used the extension of the reciprocal dose plot suggested by Joiner (*20*). Estimates of α/β for the small X-ray priming fractions (Figs. 1b, 2b, and 3b) were obtained from fitting curves to the ERD values of Fig. 5 (excluding the data point at 0.75 Gy) using least-squares regression (*7*). These α/β values are also shown in Table I.

We were not satisfied with the α/β estimates for X rays determined by these methods, as summarized in Table I, for three reasons. First, all the values obtained appear to be higher than the accepted value of 3–3.5 determined from a large scale hyperfractionation study in the mouse kidney (*10*). However, the differences are not significant at the $P = 0.05$ level (t test), and in fact Lebesque *et al.* (*21*) have reanalyzed this set of data (*10*), calculating an α/β ratio of 5.0 ± 0.5 for 16, 32, and 64 fractions (dose per fraction range 1–4 Gy). Second, we found that it was not possible to obtain a good fit to the RBE data of Fig. 6 over the whole dose range by using any of, or a combination of, the values in Table I. Third, regression analysis on reciprocal dose plots is not a strictly valid statistical procedure (*17, 18, 21, 22*). It is possible to obtain aberrant results, and Thames *et al.* (*18*) have shown in simulations that in 18% of small scale studies and 8% of large scale studies the 95% confidence limits on estimates of α/β would not include the true value. The estimates of α/β for neutrons (full course fractionation data and the top-up data) and the estimates of α/β for X rays (full course fractionation data) in Table I may therefore be criticized on these grounds. The esti-

TABLE I

Ratios of α/β Derived from Reciprocal Dose Plots (Full Course Fractionation Study) or from the Analysis of ERD Using the Method of Joiner *et al.* (*7*) (Top-up Study)[a]

Assay: *Level of effect:*	*EDTA clearance* *week 24* *2%*	*Hematocrit* *week 28* *36%*	*Urine output* *week 20* *16 events day^{-1}*
Full course fractionation: 4–16 Gy per fraction			
α_X/β_X	4.0 ± 0.7 (0.2–7.8)	4.8 ± 0.5 (2.6–6.9)	5.5 ± 1.9 (0–14.0)
α_N/β_N	20.0 ± 5.8 (9.8–∞)	29 ± 18 (8.9–∞)	13.9 ± 4.0 (6.6–∞)
α_N/α_X	9.9 ± 1.7 (2.7–17.0)	9.4 ± 1.0 (5.7–13.2)	7.0 ± 1.7 (0–14.3)
Top-up study: 1.5–3 Gy per fraction			
α_X/β_X	4.0 ± 0.8 (1.8–6.2)	6.6 ± 7.0 (0–21.5)	3.9 ± 1.7[c] (0.2–7.6)
α_N/β_N	16.4 ± 5.1 (6.4–∞)	∞	[b]
α_N/α_X	8.9 ± 1.0 (6.1–11.6)	13.0 ± 4.8 (0–27.9)	10.3 ± 2.6[c] (4.6–16.0)

[a] SEM (± values) and 95% confidence intervals (parentheses) were estimated from the regression analyses (see text).

[b] Analysis not possible with only two data points.

[c] Obtained using information from neutron full course fractionation and X-ray top-up study.

mates of α/β for X rays obtained from the top-up studies may also be unreliable since the range of X-ray dose per fraction over which they are derived is small (<1.5 Gy), although the method of their derivation (*7*) is more mathematically sound.

To overcome these problems we have obtained estimates of α/β and confidence limits by fitting directly all the RBE data of Fig. 6 to a theoretical equation for RBE as a function of dose per fraction (e.g., Ref. (*23*)), using non-linear least-squares regression by the method of Marquardt (*24*). There are several ways of expressing the RBE equation, but for curve fitting purposes it is important to minimize the occurrence of terms containing the quadratic β_N values since these always have a larger fractional error attached to them for d(4)-Be neutrons where α/β is large. In terms of a reciprocal dose plot for these neutrons, there is always more fractional error attached to the slope of the line than to the intercept. Taking this into account, we have expressed the RBE relationship as

$$\text{RBE} = \frac{K + \sqrt{K^2 + 4Ld(1 + Md)}}{2(1 + Md)}, \tag{1}$$

where d is the X-ray dose per fraction, $K = \alpha_N/\alpha_X$, i.e., the limiting RBE at very low doses, $L = \beta_N/\alpha_X$, $M = \beta_X/\alpha_X$.

TABLE II

Direct Fit of the RBE versus Dose per Fraction Relationships (Equations (1 and 2)) to the Data of Fig. 6[a]

	Present data	*Present data + data of Stewart* et al. (5)	
Parameter	*Eq. (1)*	*Eq. (1)*	*Eq. (2)*
$K = \alpha_N/\alpha_X$	11.33 ± 1.37	11.03 ± 0.93	11.65 ± 0.69
	(8.52–14.15)	(9.12–12.94)	(10.26–13.04)
$L = \beta_N/\alpha_X$	0.49 ± 0.62	0.24 ± 0.35	
	(0–1.75)	(0–0.96)	
$1/M = \alpha_X/\beta_X$	3.26 ± 1.04	3.56 ± 0.80	3.04 ± 0.35
	(1.11–5.41)	(1.92–5.20)	(2.33–3.75)
α_N/β_N	23	45	
	(7–∞)	(12–∞)	

[a] For the fit to Eq. (1), α_N/β_N is given by K/L. For the fit to Eq. (2), α_N/β_N is fixed at 20.5 (see text). Errors are ± standard errors of the mean; 95% confidence intervals are given in brackets.

If K and L are known, then $\alpha_N/\beta_N = K/L$. Thus the three parameters above, K, L, and M, constitute the required description of the X-ray and neutron responses and their relationship to each other.

This approach of fitting the RBE versus dose relationship directly has several advantages over the reciprocal dose plot and associated methods using isoeffect doses (*17*). First, the RBE at any number of different levels of functional effect can now be included in a single analysis. This maximizes the use of the raw data in the dose–response curves (Figs. 1, 2, and 3) as well as allowing the direct intercomparison between different experiments with non-overlapping ranges of effect. Second, all the assays may be included in the same single analysis if, as in the case of the kidney, there is no evidence that the α/β values for either neutrons or X rays, or the RBE values, are significantly different for the three endpoints EDTA clearance, hematocrit, and urine output (*5, 10*). Third, and most importantly, the data from the full course fractionation work and from the top-up experiments may be included together within one analysis. A slight disadvantage of the approach is the need to fit three independent parameters (K, L, M), and so the value of L (i.e., β_N/α_X, the parameter with the greatest error contribution) will be most difficult to determine reliably. This leads to a large error in the value for α_N/β_N and is because β_N cannot be determined accurately when α_N/β_N is large, but this is generally unavoidable in neutron experiments.

Table II (column 2) shows the least-squares fit of Eq. (1) to all the RBE data from these experiments, as shown by the closed symbols in Fig. 6 but excluding the values at 0.75 Gy which have indeterminate confidence limits, making a total of 27 data points. This gave an α/β value for X rays of 3.26 ± 1.04 Gy (SEM) and a limiting RBE at extremely low doses (α_N/α_X) of 11.33 ± 1.37. Note that the limiting RBE depends only on α_X and α_N and is not subject to the uncertainties associated with the parameters containing β values (L and M). The third column of Table II shows the fit when the data of Stewart *et al.* (*5*) are added in (the open symbols in Fig. 6), making

a total of 39 data points. The value of α/β for X rays and the limiting low-dose RBE are changed only slightly while the errors are reduced. The solid curve in Fig. 6 shows the excellent fit to the kidney data using these parameters. The fitted curves for kidney and skin show that the RBE for renal damage increases *above* the RBE for skin at doses per fraction less than 6.3 Gy. These data demonstrate statistical significance ($P = 0.95$) for this difference in RBE at doses per fraction less than 4.6 Gy.

Since the parameter L ($=\beta_N/\alpha_X$) and hence α_N/β_N ($=K/L$) has a large uncertainty from the fit of Eq. (1) to the RBE data, it is useful to observe the effect on α_X/β_X and α_N/α_X of setting α_N/β_N to a fixed value, C. Since $L = K/C$, Eq. (1) then reduces to

$$\text{RBE} = \frac{K + \sqrt{K^2 + 4Kd(1 + Md)/C}}{2(1 + Md)} \tag{2}$$

with only two parameters to fit, $K = \alpha_N/\alpha_X$ and $M = \beta_X/\alpha_X$. The constant C was chosen from a reciprocal dose plot (Fig. 7) of isoeffective neutron doses from the EDTA clearance assay, measured at the 2% level of retention. This particular assay and effect level were selected to maximize the number of data points in Fig. 7. The data from the top-up experiments have been included by using the method of Joiner (*20*). The value of C (α_N/β_N) was 20.5 ± 3.7 Gy measured from Fig. 7. Fitting Eq. (2) to all the data of Fig. 6 (excluding the 0.75 Gy values) gives α/β for X rays equal to 3.04 ± 0.35, in good agreement with Stewart *et al.* (*10*). The limiting RBE at low doses is 11.65 ± 0.69; this is very close to the value of 11–13 predicted by Stewart *et al.* ((*5*), their Table III) when they compared their neutron data with historical X-ray data.

CONCLUSIONS

The RBE for 2.3 MeV neutrons increases continuously as the X-ray dose per fraction is reduced from 15 Gy to 1.5 Gy in mouse kidneys. This is due to the low value of α/β for X rays, i.e., the continued curvature of the dose–response relationship down to 1.5 Gy.

The RBE was higher than for mouse skin at all X-ray doses per fraction below 6.3 Gy, and the limiting RBE for kidney is calculated as 11.65 ± 0.69, compared with 6.66 ± 0.41 determined previously for mouse skin (*7*).

The best value of α/β for X rays was 3.04 ± 0.35 Gy, with α/β for neutrons equal to 20.5 ± 3.7.

Direct fitting of the RBE versus dose relationship is an alternative to reciprocal dose plots for obtaining the α/β ratio for X rays and enables confidence intervals on the values to be reduced by maximizing the use of the raw data.

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