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THE EFFECT OF OVERALL TIME ON THE L.D.₅₀ OF
GRAIN WEEVILS (SITOPHILUS GRANARIUS) IRRADIATED
WITH CONTINUOUS OR FRACTIONATED DOSES OF
⁶⁰CO γ -RAYS.

BY

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see. p. 199.

ABSTRACT

The investigation had two objectives:

1. To study, in an organised normal tissue, the form of relationship between the iso-effect dose and the overall irradiation time of fractionated regimes of radiation.
2. To test the general formula for equating protracted and acute regimes of radiation (Liversage, 1969). This formula had previously been shown to be approximately true for two mammalian tissues.

The LD 50₍₃₀₎ for grain weevils has been determined for 63 different irradiation regimes ranging from single doses delivered in 24 minutes or continuously at low dose-rate over periods up to 106 hours to fractionated acute regimes involving 1 to 20 fractions delivered in overall times varying from 0 to 28 days. The use of 68200 weevils has enabled iso-effect data to be established which are probably more comprehensive and accurate than those obtained for any other tissue.

Weevils were housed and irradiated at constant temperature and at constant humidity. A ⁶⁰Co γ -ray beam unit was used for all irradiations.

Over the whole range investigated the general formula for equating protracted and acute regimes of radiation has certainly been found to be valid, to the degree of accuracy ($\pm 10\%$) claimed by Liversage (1969).

For fractionated regimes, the relationship between the LD 50 for weevils and overall time is not consistent with published iso-effect formulae but a new relationship has been established which fits the experimental data, provided the radiation is delivered in equally spaced fractions of equal magnitude. Unexpected results were obtained using unequally spaced fractions or doses of unequal magnitude. These results suggest that the rate of cellular repopulation at any instant of time is a function of the damage present.

The implications of these results in radiobiology and radiotherapy are discussed. It is suggested that the effects of cellular repopulation in

normal tissues, such as skin, may have been under-emphasized and those of intracellular recovery over-emphasized in recent years.

THE EFFECT OF OVERALL TIME ON THE L.D.50 OF GRAIN WEEVILS
(SITOPHILUS GRANARIUS) IRRADIATED WITH CONTINUOUS OR FRACTIONATED
DOSES OF ^{60}Co γ -RAYS

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I N T R O D U C T I O N

Low dose-rate radium treatment for carcinoma of the cervix is being replaced by a new technique involving fractionated high dose-rate irradiation. Optimal treatment regimes for the new technique need to be established through clinical experience.

Theoretical considerations led the present author to suggest a general formula for equating protracted and acute regimes of radiation (Chapter 4). It was hoped that this formula would help to bridge the gap between the old and the new treatment techniques and thus provide some guidance as to the dose levels and fractionation regimes which might be effectively employed in early clinical trials.

Within the limits of accuracy of available experimental and clinical data, the formula had been shown to be true for two very different effects in mammals, namely, skin reactions in patients and lethality of mice (4.4). The present investigation was designed to test the formula more rigorously. The use of insects not only helped to test the general nature of the formula but also enabled much more data to be obtained with greater accuracy than would have been possible if a third mammalian tissue had been used instead.

Preliminary experiments revealed that the chosen system exhibited a much larger amount of long term repair (often assumed to be due to repopulation of cells) than that shown by mammalian tissues. Although several different formulae (Chapter 5) had been suggested to enable the magnitude of such effects to be predicted in some organised mammalian tissues, the exact type of relationship governing this effect had not been determined. The reason for this was that the available experimental mammalian data were

generally insufficiently precise to distinguish clearly between the small changes in iso-effect dose predicted by the different formulae.

In order to achieve the primary objective of this investigation it was necessary, amongst other things, to make a partial study of the repopulation effect in weevils. This study was therefore extended in the hope of obtaining some insight into the nature of the laws governing this effect in an organised tissue.

SECTION 1

THE NEED FOR AN EXPERIMENTAL STUDY OF CERTAIN ISO-EFFECT RELATIONSHIPS

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CHAPTER 1

THE INTRODUCTION OF HIGH DOSE-RATE INTRACAVITARY X-RAY THERAPY FOR THE TREATMENT OF CARCINOMA OF THE CERVIX

1. 1. The existing low dose-rate radium technique

For many years carcinoma of the cervix has been treated by inserting X-ray emitting applicators into the uterine canal and lateral fornices of the patient (e.g. Tod and Meredith, 1953). Techniques vary from centre to centre but in general each patient has one, two or three insertions and the radium remains in situ for periods of 24 to 120 hours depending upon the activity of the radium and the number of insertions used at the centre concerned. Due to the rapid fall off in dose in the vicinity of the applicators it is difficult to specify the dose delivered in terms of a single figure. Thus doses are usually specified in terms of the dose delivered to point A which is defined as a point 2cm lateral to the centre of the uterine canal and 2cm from the mucous membrane of the lateral fornix. Optimum dose levels for these treatments are well established. At the Charing Cross Group of Hospitals the standard procedure has been to deliver a total dose of 6000 rads to point A in three 24 hour insertions spaced one and two weeks apart. One of the main dangers of this treatment is that the rectum may receive too high a dose and in order to avoid this, gauze packing material is inserted beneath the radium applicators (i.e. the vaginal ovoids). Measurements of the dose-rate in the rectum are made before the patient leaves the operating theatre and if the dose-rate is too high the packing is adjusted or the treatment time is reduced. Joelsson and Bäckström (1969) have shown however that the dose-rate in the rectum usually increases during the period of the insertion due to the maceration of the gauze packing material, that this increase in dose-rate could be in excess of 50% and on average amounted to 26%.

1. 2. The new high dose-rate technique

Instead of using low dose-rate radium applicators, similar treatment may now be given using high dose-rate ^{60}Co sources inserted by remote control as described by O'Connell, Joslin, Howard, Ramsey and Liversage (1967). The geometrical arrangement of the sources and their relative strengths have been kept identical to those used in the radium technique of Tod and Meredith (1953) but the sources are clamped in position and the vaginal ovoids are separated from the posterior wall of the vagina by solid perspex spacers. These improvements are made possible by the fact that each treatment lasts only a few minutes as the dose-rate delivered to point A is now of the order of 180 rads/min instead of the 1.4 rads/min which is delivered to point A during a corresponding radium insertion.

The patient receives several insertions but optimum treatment regimes have yet to be established.

1. 3. The advantages of the new technique

- (a) The accuracy of the treatment is improved as the sources can be positioned with much greater precision and there is no danger of over-dosing the rectum due to the applicators moving during treatment.
- (b) Radiation hazard to staff and other patients is virtually eliminated.
- (c) Many patients may be treated as out-patients.

1. 4. Disadvantage of the new technique

The use of high dose-rate intracavitary treatment is a radical departure from the well established radium technique so that the radiobiological implications of such a change need to be considered.

CHAPTER 2

RADIOBIOLOGICAL PROBLEMS ASSOCIATED WITH THE NEW TECHNIQUE

2. 1. The choice of a suitable treatment regime

In time, clinical trials should show whether the new technique is superior to the old but the radiotherapist initiating a trial needs to decide how many fractions and what total dose should be received by the patients. An unwise choice of either of these factors could lead to a worsening of the clinical results which could more than cancel out the expected improvement due to better and firmer positioning of the applicators. Thus before starting clinical work it was natural to enquire as to whether any guidance could be obtained from radiobiology. The aim of the new technique is to produce an equal or greater effect on the tumour and an equal or less effect on the surrounding tissues than the existing technique.

2. 2. Variation of biological effect with dose-rate

A study of the published data reveals that the biological effectiveness of continuous irradiation varies with the period of irradiation. The relative biological efficiency (R.B.E.) of radiation delivered at high dose-rate, in the course of a few minutes, compared with that given at low dose-rate, in say a period of 70 hours, is liable to vary appreciably from tissue to tissue. For example, under these conditions the R.B.E. for the healing of small skin tumours is of the order of 2.2 whilst the corresponding value for $LD_{50(30)}$ in mice is only about 1.75. (Fig 2a) It thus appears that if a 70 hour radium treatment were to be replaced by a single high dose-rate insertion lasting only a few minutes, to produce the same effect on the irradiated tissues the dose delivered would need to be reduced by a factor of about two but that this factor could vary appreciably from tissue to tissue.

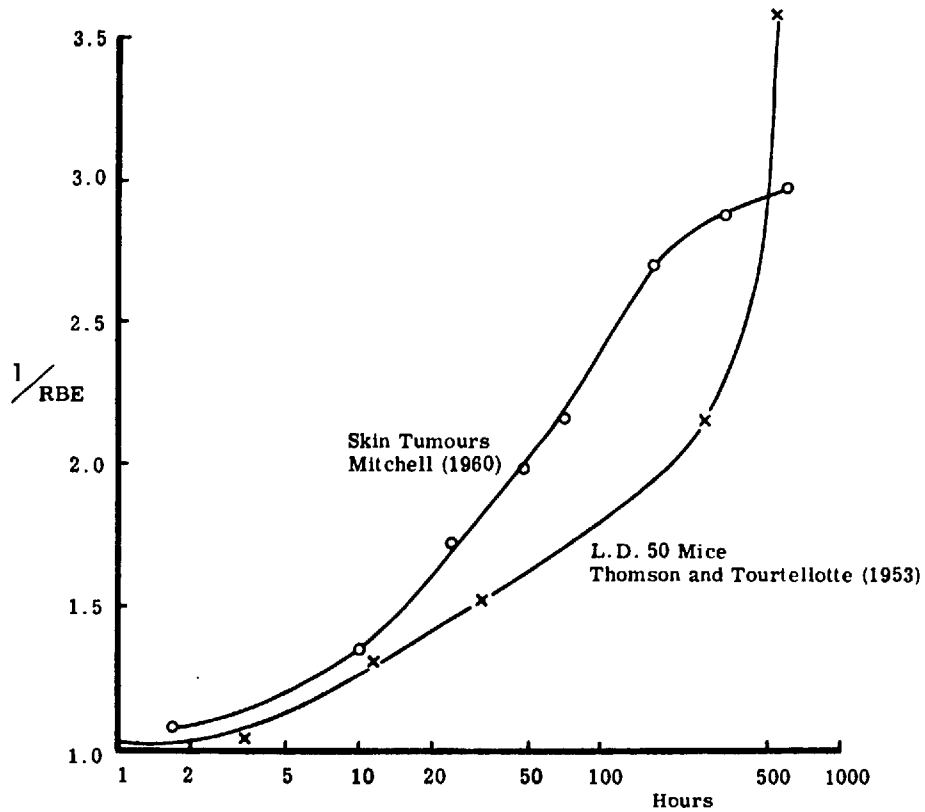


Fig.2a The increase in iso-effect dose
with increase in continuous
irradiation time.

2. 3. Variation of biological effect with fractionation

It is also known that if a single dose (at high dose-rate) is replaced by a number of daily doses, in order to obtain the same effect on the irradiated tissues, the total dose delivered must be increased. Fig 2b shows for example that if 20 daily fractions are given instead of a single dose, the total dose needs to be increased by a factor of about 2 but that the exact amount of the increase varies from tissue to tissue. For skin reactions the factor appears to be about 2.7 whereas for $LD_{50/30}$ in mice it is only about 1.8.

2. 4. Difficulty of devising a useful experiment to solve these radiobiological problems

Clearly there is no unique answer to the question "What dose D_{NT} given at high dose-rate in N fractions in an overall time T days will be equivalent to a continuous irradiation delivering D_t rads in a treatment time of t hours?" Not only will the answer depend upon the values of N , T , and t but, for any given values of N , T and t , the answer is likely to depend upon the effect being studied and the type of tissue being irradiated.

Under these circumstances it is difficult, if not impossible, to devise an animal experiment which will be relevant to the problem of the radiotherapist about to initiate high dose-rate intracavitary treatment. After much effort an iso-effect relationship applicable to say skin reaction in rabbits might be found but this would not necessarily provide information applicable to carcinoma of the cervix or to the normal tissues, such as the rectum and the vaginal epithelium, which are of interest to the radiotherapist. It would provide no information at all as to how the therapeutic ratio is likely to be affected by his choice of treatment regime.

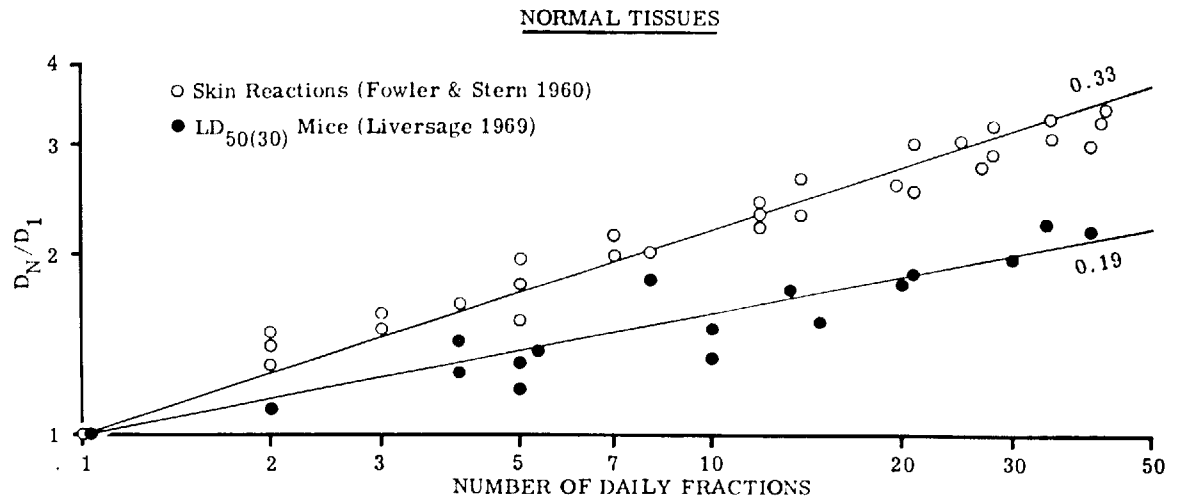


Fig.2b The increase in iso-effect dose with increase in the number of daily fractions of acute irradiation.

These considerations led the author to examine the problem from a more fundamental point of view. The increases in iso-effect dose resulting from either the protraction of a continuous irradiation or from the fractionation of a high dose-rate regime are both believed to be due mainly to the repair of intracellular sub-lethal damage. In the next two chapters these effects are examined and a general formula is derived to show how the increase occurring in a given tissue with protraction is related to the increase occurring in the same tissue with fractionation. Subsequent chapters describe the testing of this relationship in a biological system known to exhibit large fractionation effects.

CHAPTER 3

THE APPLICATION OF CELL SURVIVAL THEORY

3. 1. Cell Survival Curves

Cell survival curves, obtained by plotting the surviving fraction of cells (on a logarithmic scale) against dose (on a linear scale), usually display an initial shoulder which is frequently followed by a more-or-less straight portion. The intercept D_Q (Fig 3a) is known as the quasi-threshold dose (Alper, Fowler, Morgan, Vonberg, Ellis and Oliver, 1962). Survival curves which do not attain a constant slope have also been observed (e.g. Barendsen, Beusker, Vergroesen and Budke, 1960). The slope of the curve after a given dose of radiation is a measure of the sensitivity of the cells to further irradiation and it will be seen that in the shoulder region of the curve the sensitivity increases with the dose delivered. To explain this effect it is necessary to assume that the cells may suffer sub-lethal damage, i.e. a degree of damage which by itself is insufficient to kill the cells but which renders them more susceptible to subsequent irradiation.

3. 2. Recovery from sub-lethal damage

Experiments, such as those of Elkind and Sutton (1960), in which two doses of radiation have been given at varying hours apart, (sometimes referred to as split-dose experiments), have shown that cells recover from this sub-lethal damage in the space of a few hours. If N daily doses of D rads are given, the shoulder of the cell survival curve is restored between one dose and the next and, according to simple theory, the surviving fraction of cells S should be given by:

$$S = (S_D)^N \quad \text{-----} \quad (1)$$

where S_D is the surviving fraction after a single dose of D rads.

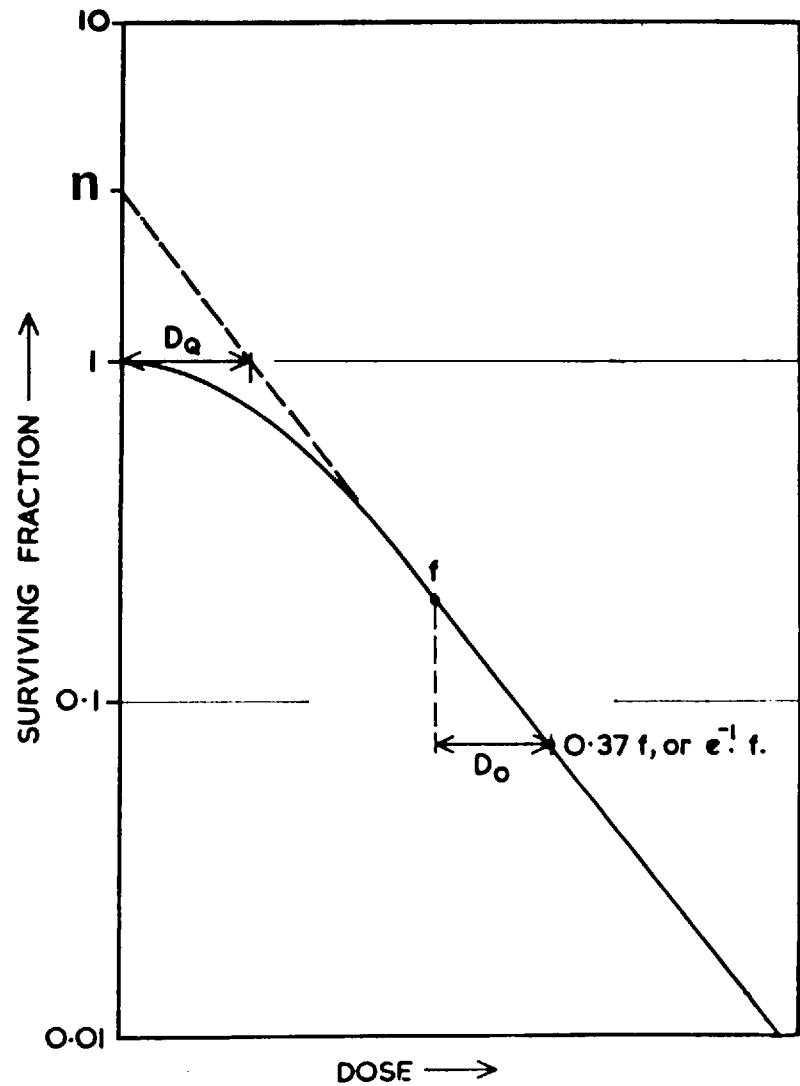


Fig. 3a A cell survival curve (from Alper, Fowler, Morgan, Vonberg, Ellis and Oliver, 1962) illustrating the meaning of the quasi-threshold dose D_Q . D_Q is the intercept which the extrapolated exponential portion of such a curve makes with an abscissa through 100 per cent survival.

In Fig.3b which is taken from Elkind and Sutton (1960) it will be observed that the survival curves A, B, C, D etc applicable to each dose, are parallel to each other and are separated by the quasi-threshold dose D_Q . Thus on this simple theory the total fractionated dose necessary to kill a given fraction of the cells will be $(N-1)D_Q$ rads higher than the corresponding single dose.

It is generally believed that this simple theory does not agree with clinical or experimental observations for two reasons:

1. Cell proliferation occurs between fractions.

2. A dose of radiation tends to produce a partially synchronised population of surviving cells which subsequently progresses through the different stages of the cell cycle which have different sensitivities to radiation. It is often possible to correct for the effects of cell proliferations (see later) but only in exceptional cases is it possible to make any allowance for the effect of cell synchrony. These effects are described and discussed by Young and Fowler (1969) and Fowler (1968) who suggested that the effects of synchrony tend to become smoothed out as the number of fractions is increased and therefore believed that there would be no great difference between simple theory and experimental observations when more than 4 or 5 fractions were involved.

3. 3. The Theory of Lajtha and Oliver

In order to describe the recovery from sub-lethal damage mathematically and to apply the theory to continuous low dose-rate irradiation, Lajtha and Oliver (1961) and Oliver (1964) introduced the concept of sub-lethal damage dose equivalent. This may be defined as that dose of radiation D_g which given instantaneously to the cells would bring them to the same state of sub-lethal damage, and hence the same state of sensitivity, as that in which the cells find themselves at the

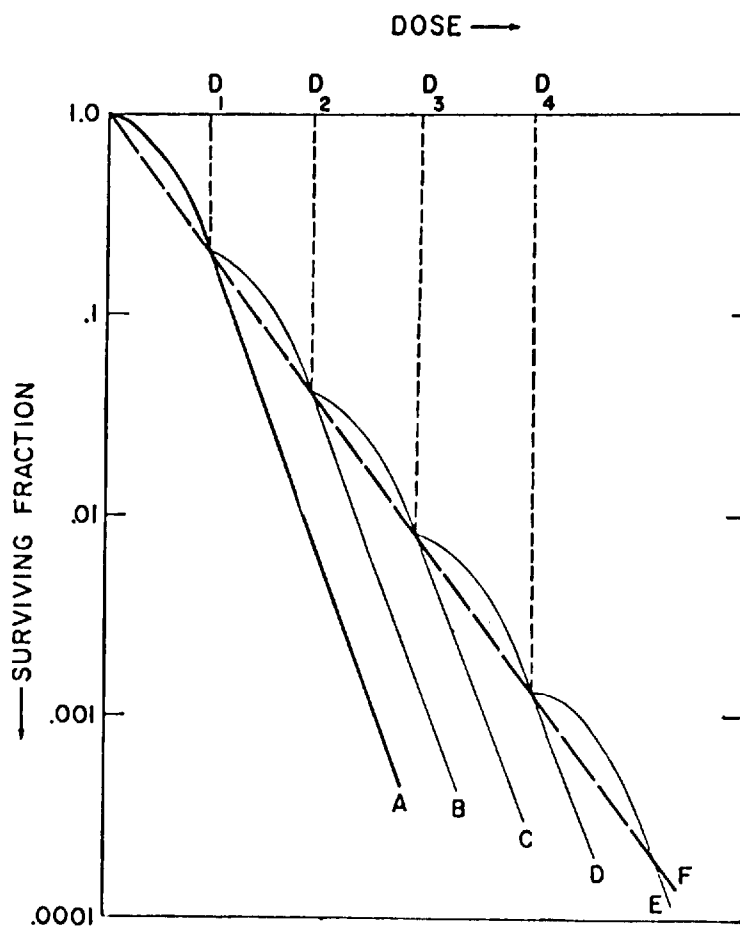


Fig.3b An idealized experiment taken from Elkind and Sutton(1960). Curve A is the survival curve for single acute exposures of X-rays. Curve F is obtained if each dose is given in a series of small fractions of size D_1 separated by intervals of sufficient duration to enable complete recovery from sub-lethal damage to occur between fractions.

given moment. Lajtha and Oliver inferred from the in vitro data of Elkind and Sutton (1959) that the dose equivalent of sub-lethal damage faded exponentially with a half-life, τ , of 1.5 hr, i.e. they assumed that t hours after an acute dose D_1 , the irradiated cells would have the same amount of sub-lethal damage present, and hence the same sensitivity, as they would have had immediately after an acute dose of D_E rads where:

$$D_E = D_1 e^{-\mu t} \quad \text{--- (2)}$$

where $\mu = 0.693/\tau$

On the above basis, Lajtha and Oliver showed that the slope of the protracted cell survival curve after irradiating cells for t hours at d rads/hour should be the same as the slope of the corresponding acute cell survival curve when a dose of D_E rads has been delivered, where D_E is now given by

$$D_E = \frac{d}{\mu} (1 - e^{-\mu t}) \quad \text{--- (3)}$$

As t becomes large compared with τ it will be observed that D_E tends to a limiting value equal to $\frac{d}{\mu}$, i.e. an equilibrium condition is established where the rate of production and the rate of repair of sub-lethal damage are equal. Thus the lower the dose-rate d , the smaller will be the amount of sub-lethal damage present and the lower will be the sensitivity of the cells. Lajtha and Oliver were thus able to explain the decrease in effectiveness of X- and γ -irradiation with decrease in dose-rate in terms of the continuous repair of sub-lethal damage occurring during the protracted low dose-rate irradiation.

3. 4. The application of cell survival curve theory to intracavitary therapy

Liversage (1966) attempted to use cell survival curve theory to predict regimes of high dose-rate treatment which would be equivalent to the existing low dose-rate radium treatment referred to in Chapter 1. The problem basically was to find what total dose D_N delivered in N fractions in T days using a high dose-rate would be equivalent to 6000 rads delivered in three 24 hour irradiations in an overall time of three weeks. The starting point for the calculations involved assuming that a certain cell survival curve was applicable to the tissues of interest. As several tissues were of interest and there was little or no evidence available to suggest which of the available cell survival curves was most applicable to any particular tissue, the calculations were performed for the seven very different cell survival curves detailed in the caption to Fig 3c.

Using the method of Lajtha and Oliver (1961) the fraction of cells surviving the radium regime was calculated. Then using equation (1) the value of D_N which would give the same surviving fraction was calculated for various values of N ranging from 3 to 20. In an attempt to eliminate the effects of cell proliferation it was suggested that the N fractions of acute irradiation should be given in the same overall time as that used for the radium treatment, i.e. 3 weeks. The results of these calculations are shown in Fig 3c. It will be seen from this diagram that if a small number of fractions are given the predicted iso-effect dose varies greatly with the particular shape of cell survival curve chosen but, if between 15 and 18 fractions are given, all the cell survival curves studied predict the same iso-effect dose to an accuracy of $\pm 5\%$.

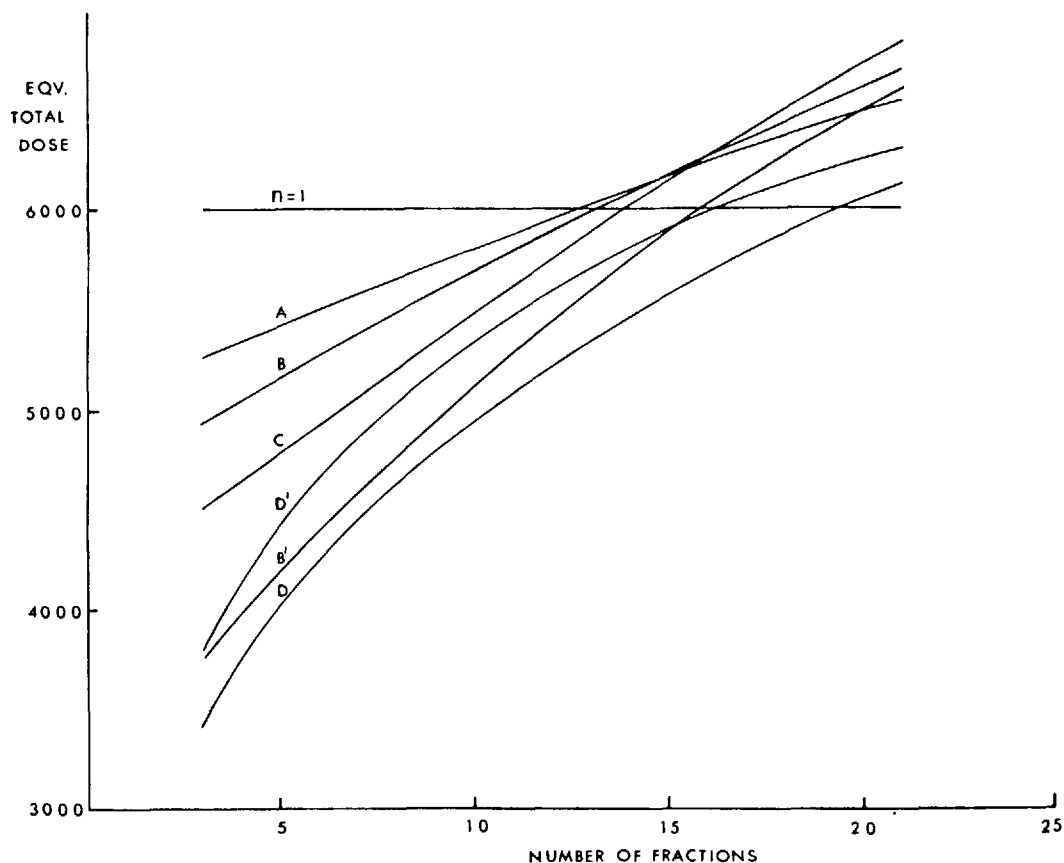


Fig.3c Total fractionated acute dose D_N rads equivalent to three 24-hour doses of 2000 rads plotted against number of fractions, as calculated by Liversage(1966) by applying the method of Lajtha and Oliver(1961) to the following seven cell survival curves:

- A a multi-target curve($n=1.6$; $D_0=180$ rads)
- B " " " " ($n=2.0$; $D_0=160$ rads)
- C " " " " ($n=2.8$; $D_0=140$ rads)
- D the experimental curve of Barendsen et al (1960) for human kidney cells
- B' and D' the same as B and D above but dose modified by a factor of 2.5 to obtain predictions for hypoxic cells
- $n=1$ any survival curve with unit extrapolation number such as that which was observed by Hall, Bedford and Oliver(1966) for cells irradiated under conditions of extreme hypoxia.

If the cells of different tissues have different survival curves, which has not been proved but which would seem likely a priori, then the above findings have important implications in radiotherapy. The theory was therefore examined to see if there was some fundamental reason why all the predictions should be in such close agreement in the range 15 to 18 fractions. This led to the derivation of the general formula discussed in the next chapter.

CHAPTER 4

A GENERAL FORMULA FOR EQUATING PROTRACTED AND ACUTE REGIMES OF RADIATION

4. 1. The causes of the increase in iso-effect dose with fractionation and protraction

According to the theory discussed in the ^{previous} ~~last~~ chapter, the dose D_{NT} necessary to produce a given effect increases with the number of fractions N and the overall irradiation time T days for two reasons:

- (a) Intracellular sub-lethal damage is repaired between fractions and a quantity of radiation which we will call R_{D_N} is used up in re-creating this sub-lethal damage.
- (b) Cell proliferation increases the surviving fraction of cells during the irradiation time T and an additional quantity of radiation R_{D_T} needs to be given to compensate for this repopulation effect.

These effects may be expressed mathematically,

$$D_{NT} = D_1 + R_{D_N} + R_{D_T} \text{ ————— (4)}$$

where D_1 is the single dose (at high dose-rate) necessary to produce the given effect.

Also according to the theory discussed in the last chapter (3.3) the protracted dose D_t necessary to produce a given effect, if delivered continuously over a period of t hours may be represented mathematically by the following relationship

$$D_t = D_1 + R_{D_t} + R_{D_t} \text{ ————— (5)}$$

where R_{D_t} is the dose "wasted" due to the repair of intracellular sub-lethal damage occurring during the irradiation and R_{D_t} is the additional quantity of radiation necessary to compensate for repopulation occurring during the irradiation period of t hours.

Fowler (1968) showed for pig skin that R_{D_N} was of the order of $(N-1)$ times several hundred rads whereas R_{D_T} was only of the order of T times 25 rads. (Eqn 4). Thus Fowler's pig skin experiments show that in this mammalian tissue the iso-effect dose increases with the number of daily fractions due mainly to the increase in the number of fractions rather than the increase in overall time; i.e. R_{D_N} is in this case much greater than R_{D_T} .

To produce equal skin reaction on patients the data (Fig 2a) of Mitchell (1960) show that 3600 rads in 70 hours produces the same effect as 1800 rads in 1.7 hours. If we assume that patient's skin repopulates at a rate equivalent to something of the order of 25 rads/day it will be seen from equation (5) that R_{D_t} is of the order of 1700 rads whereas R_{D_t} is probably less than 100 rads.

It will thus be seen that for skin reactions the increase in iso-effect dose with fractionation and with protraction are largely due to recovery from sub-lethal damage although repopulation plays a small but significant part in the former case and a very small probably insignificant part in the latter case.

4. 2. The derivation of the general formula

Liversage (1969), using the theory of Lajtha and Oliver (1961) showed that irrespective of the shape of the applicable cell survival curve, R_{D_N} in equation (4) should be approximately equal to R_{D_t} in equation (5) if N and t were related by the equation

$$N = \frac{\mu t}{2 \left[1 - \frac{1}{\mu t} (1 - e^{-\mu t}) \right]} \quad (6)$$

In other words, for any given tissue, it was suggested that a given dose would produce equal effects irrespective of whether it was given in N fractions in T days or continuously over a period of t hours provided:

(a) N and t were related by equation (6) and

(b) negligible repopulation of cells occurred during the periods of T days and t hours.

If the effects of repopulation were not negligible then instead of D_{NT} needing to be equal to D_t in order to produce an equal effect the following relationship would be obeyed:

$$D_{NT} = D_t + \frac{R_{D_T} - R_{D_t}}{\mu} \quad (7)$$

Liversage suggested for skin reactions that

$$R_{D_T} - R_{D_t} = C (T - t/24) \quad (8)$$

where $C = 25$ rads/day approximately.

The basis of the argument used by Liversage (1969) is illustrated in Fig.4a. Using equation (3) it may be shown that the average value of the dose-equivalent of sub-lethal damage during a protracted irradiation lasting t hours is given by:

$$\begin{aligned} D_{Eave} &= \frac{1}{t} \int_0^t \frac{d}{\mu} (1 - e^{-\mu t}) dt \\ &= \frac{d}{\mu} \left[1 - \frac{1}{\mu t} (1 - e^{-\mu t}) \right] \quad (9) \end{aligned}$$

Thus the average slope of the protracted cell survival curve during an irradiation lasting t hours is assumed to be equal to the slope of the acute survival curve at the point D_{Eave} and this is assumed to be equal to the average slope of the acute survival curve from 0 to $2D_{Eave}$. Thus protracted irradiation at d rads/hour is assumed to be as effective as acute irradiation given in fractions of magnitude $2D_{Eave}$. Thus N doses of $2D_{Eave}$ should kill the same number of cells as irradiation given at d rads/hour for t hours provided the same total dose is given in each case.

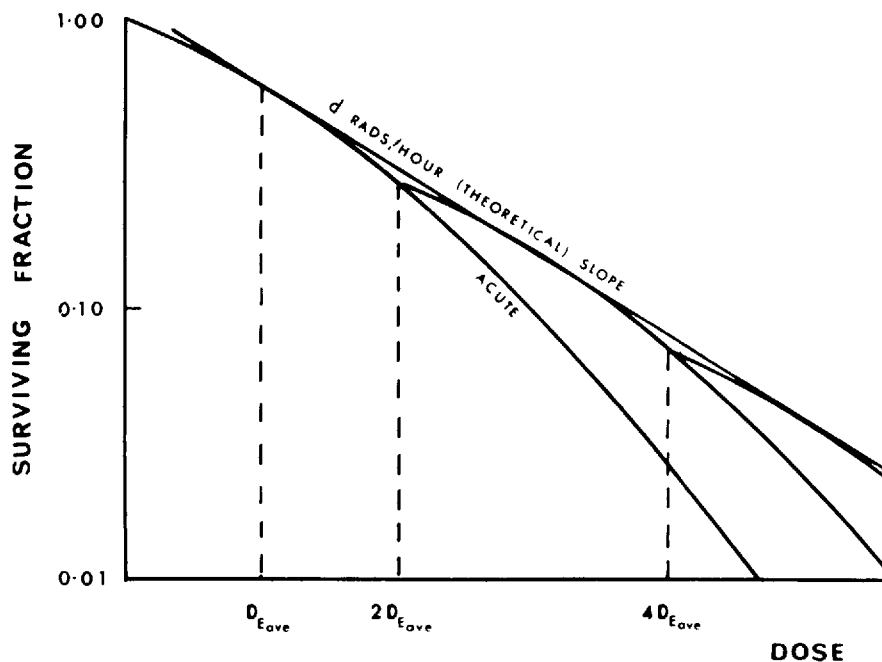


Fig.4a The average slope of the low dose-rate (drads/hour) survival curve is assumed by Liversage(1969) to be equal to the slope of the acute cell survival curve at the point $D_{E_{ave}}$ (Eqn.9). This slope is then assumed to be equal to the average slope of the acute curve from 0 to $2D_{E_{ave}}$. Thus protracted irradiation at d rads/hour is assumed to be as effective as acute irradiation given in fractions of magnitude $2D_{E_{ave}}$

For this to be so

$$2 D_{\text{Eave}} N = t d$$

or

$$N = \frac{\mu t}{2 \left[1 - \frac{1}{\mu t} (1 - e^{-\mu t}) \right]} \quad (\text{see equation 6})$$

The above derivation involves a number of approximations (e.g. it assumes that the tangent to the curve at the point D_{Eave} has the same slope as the chord from 0 to $2 D_{\text{Eave}}$ and for this reason Liversage (1969) suggested that the relationship was only likely to be true to an accuracy of $\pm 10\%$.

4. 3. The value of the parameter μ

Apart from N and t , the only unknown parameter in the above equation is μ which is equal to $\frac{0.693}{\tau}$ where τ is the half-life of the dose equivalent of sub-lethal damage. Lajtha and Oliver had inferred from the work of Elkind and Sutton (1959) that τ was equal to 1.5 hours but Liversage (1969a) reviewed published experimental data and came to the conclusion that it was not possible to determine τ precisely for any of the data he analysed. He found, however, that all the evidence reviewed suggested that τ is of the order of 1 or 2 hours for mammalian systems irradiated at normal body temperatures and that, in such systems, little error is produced in dosage calculations by assuming a value of 1.5 hours to be applicable, as originally suggested. Values of N calculated from equation (6) for different values of t , assuming $\tau = 1.5$ hours, are given in Table 1.

4. 4. Validity Checks

In order to test his formula, Liversage (1969) used equations (6), (7) and (8) above to predict, from published data for protracted irradiation, how the iso-effect dose would vary with the number of

TABLE 1

t	N
1	1.16
2	1.33
3	1.51
4	1.70
5	1.89
6	2.09
7	2.30
8	2.51
9	2.72
10	2.94
12	3.38
15	4.05
20	5.18
24	6.09
30	7.47
40	9.8
48	11.6
60	14.4
72	17.2
96	22.7
120	28.2
144	33.8
168	39.3
200	46.7
400	92.9
600	139.1

The radiation "wasted" due to the repair of intracellular sub-lethal damage during a fractionated regime involving N acute fractions should be approximately equal to that "wasted" due to the same cause during a continuous irradiation lasting t hours provided N and t are related by equation (6). The above table gives values of N calculated from this equation assuming the half-life of the dose-equivalent of sub-lethal damage is 1.5 hours (Chapter 4.3).

daily fractions

(a) For skin reactions in patients

(b) For $LD_{50(30)}$ in mice.

He was able to show that over the whole range of continuous irradiation times tested, namely from 0 to 600 hours, the predictions agreed with the data for the corresponding fractionation regimes to an accuracy equal to that of the spread of the published data, which was usually of the order $\pm 10\%$.

4. 5. Radiotherapeutic implications of this formula

If a radiotherapist decides to replace a continuous low dose-rate regime lasting t hours by a fractionated regime involving N fractions where N and t are related by equation (6) (see Section 4.2) the overall treatment time will almost certainly be increased from t hours to at least $(N - 1)$ days. Liversage (1969) claims that as his formula should apply to all tissues, such a change will have little or no effect on the therapeutic ratio. There are two factors which could invalidate his argument

1. As the overall time is increased, both the tumour and the surrounding normal tissue will have more time to repopulate. Differing rates of repopulation could thus produce advantageous or adverse changes depending on whether the tumour repopulated more slowly or more rapidly than the surrounding normal tissue. In view of the fact that $\dot{D}_T$ is relatively small (i.e. of the order of 25 rads/day) the difference between $\dot{D}_T$ for tumour and for normal tissue is also likely to be small unless the tumour is a particularly fast growing type.
2. In deriving the formula it was assumed that the oxygen tension of the various cells remains constant throughout the treatment regime.

In practice the oxygen status of tumour cells, initially hypoxic, is liable to improve during a treatment regime lasting $(N - 1)$ days but is unlikely to improve to the same extent during irradiation lasting only t hours. For this reason the fractionated regime might show an improved therapeutic ratio.

4. 6. Need for further testing

In view of the above considerations, the present author claims that there is no inherent advantage in low dose-rate irradiation compared with high dose-rate irradiation provided the latter is fractionated in accordance with equation (6). He considers that the good results obtained with radium are due to geometrical considerations which can be maintained, and even improved, when low dose-rate intracavitary treatments are replaced by high dose-rate intracavitary treatments. This conclusion relies entirely on Liversage's claim that his formula is of a general nature and applies equally to all tissues. Admittedly he has shown it to be true to within stated limits for two very different mammalian tissues namely skin reactions in patients and $L D 50_{(30)}$ in mice. These two tissues are known to exhibit very different iso-effect relationships (See Fig.2a and 2b) but it would be advantageous if the formula could be shown to be true for some completely different type of tissue - perhaps even a non-mammalian tissue.

CHAPTER 5

THE VARIATION OF ISO-EFFECT DOSE WITH THE OVERALL TIME OF A FRACTIONATED IRRADIATION

5. 1. The need for an iso-effect formula

In order to apply or to test the general formula referred to above (equation 7) it is necessary to be able to estimate how the iso-effect dose varies with the overall time of a fractionated regime of radiation involving a constant number of fractions. A satisfactory method of making such estimates would be invaluable to radiotherapists in general (e.g. a patient's prescribed course of treatment may be interrupted for one reason or another and the radiotherapist will then have to decide what additional dose should be given to allow for the prolongation caused by this interruption).

Several formulae which claim to relate iso-effect dose and overall treatment time have been published but it should be stressed that the exact form of this relationship is not known. Fortunately in radiotherapy, the variation in iso-effect dose with overall time is generally small and a large error in estimating this small effect is usually tolerated. It is because these effects are so small in normal clinical practice that they are imprecisely known but dangers arise if existing iso-effect formulae are used to predict large changes due to radical changes in the overall duration of treatment regimes.

5. 2. Simple formulae relating iso-effect dose and overall time

1. According to the formula of Ellis (1967)

$$\frac{D_{NT_1}}{D_{NT_2}} = \left(\frac{T_1}{T_2} \right)^B \quad \text{---(10)}$$

where B is a constant.

Ellis suggests that for normal tissues $B = 0.11$.

2. According to the formula of Cohen (1968)

$$D_{NT_2} - D_{NT_1} = C (T_2 - T_1) \text{ —————(11)}$$

where C is a constant.

Cohen suggests that for skin reactions in patients $C = 30$ rads/day.

3. According to the formula of Liversage (1971)

$$D_{NT} - D_{NT_F} = C (T - T_F) \text{ —————(12)}$$

where T_F is the time required to give N fractions at five fractions a week and C is a variable which for skin reactions may be taken as 30 rads/day if $T - T_F < 20$ days and as 20 rads/day if $20 < (T - T_F) < 30$ days.

5. 3. More complicated formulae

Cohen and Scott (1968) modified the formula of Cohen (1968) by applying the logistic model of Sacher and Trucco (1966). Thus instead of assuming a constant rate of repopulation (expressed in rads per day) they assumed that the instantaneous rate of repopulation at any instant of time during the course of a fractionated regime of treatment was equal to $C(H - S)$ rads/day where C is a constant, S is the surviving fraction of cells at that instant of time and H is the maximum fraction of the original population of cells which could be restored by repopulation in an infinite time. Cohen and Scott suggested that H diminishes in value during the course of a fractionated irradiation due to the accumulation of irreparable injury. However, as there are no data available regarding the probable value of H, they assumed it to have a constant value of unity. By doing this Cohen and Scott at least introduced a factor to allow for homeostatic

influences which should presumably prevent the cells from repopulating to a level higher than that normally present in unirradiated tissue. It will be seen that in order to apply the above formula it is necessary to evaluate the surviving fraction of cells present at different times during the course of treatment. Cohen and Scott have a computer program which enables this to be done on the basis of a three-component cellular lethality model involving at least six parameters. Each of these parameters needs to be evaluated by supplying the computer with all the iso-effect data available for the effect being considered and computing the values of parameters which provide the best fit. Lack of precision in available clinical data throws considerable doubt on the accuracy of the values computed for the various parameters.

It should, in fairness, be pointed out that the modification of the Cohen model by the introduction of the logistic factor will make little difference to the estimated amount of repopulation occurring during a conventional radiotherapy treatment. A single dose of X-Rays of 600 rads is sufficient to reduce the surviving fraction of human cells to below 0.1 (Barendsen, Beusker, Vergroesen and Budke, 1960) and Cohen (1968) estimates that at the end of a successful radiotherapy treatment, the surviving fractions of cells are of the order of 10^{-5} and 10^{-6} for normal skin and epidermoid cancer respectively. Thus during the majority of a fractionated course of radiotherapy, if H is taken as unity, the value of $(H - S)$ will also be close to unity. The logistic factor could be important however, when the effects of prolonged interruptions of treatment are being considered.

5. 4. Existing experimental evidence on the relationship between
Iso-effect dose and overall time

Many experiments on the skin of animals have been designed to evaluate the values of B and C in equations (10) and (11) or to try and establish the true form of the relationship between iso-effect dose and overall time. The results of these experiments are reviewed by Fowler (1971). The values of B and C obtained from these experiments are somewhat variable and Liversage (1971) has suggested that B and C are factors which vary with overall time or fraction spacing.

Although much of the experimental work is very accurate, the effect being examined is so small that the precision of the results is not good enough to determine which of the above iso-effect formulae is closest to the truth. The accuracy of the existing experimental data is certainly insufficient to enable the true form of the relationship between iso-effect dose and overall time to be established. To establish the form of this relationship further experimental work is required, preferably using a system in which the magnitude of the effect is greater than that found in the skin of animals.

SUMMARY OF SECTION 1 (CHAPTERS 1 - 5)

In the treatment of carcinoma of the cervix, the substitution of fractionated high dose-rate intracavitary therapy for low dose-rate radium treatment, delivering D_t rads in t hours, introduces several advantages but raises radiobiological problems. The main problem is to decide how many fractions N , what overall time T and what total dose D_{NT} should be used in clinical trials designed to compare the old and new techniques. As the geometrical dose distributions are the same in both types of treatment, it is natural to try to relate the dose necessary in the new technique to that used, and found to be effective, in the old.

A study of available clinical and experimental data reveals that, for given values of N , T and t , the magnitude of the dose D_{NT} which will produce the same effect as the dose D_t will vary from tissue to tissue. Iso-effect doses predicted by cell survival curve theory vary from one cell line to another depending upon the shape of the survival curve for the cell line concerned. As the cells of different tissues are likely to have different survival curves this could explain why different equivalent doses are required for different tissues. Cell survival curve theory predicts however that, provided N and t are related by the general formula described in Chapter 4, the value of D_{NT} (corrected to allow for the effect of cell proliferation during time T) will be approximately equal to D_t (corrected to allow for the effect of cell proliferation during time t) irrespective of the shape of the relevant cell survival curve.

To test whether this general formula holds true for gross effects in different tissues, it was tested against published clinical and experimental data for skin reactions in patients and L D 50 in mice. The formula was found to be a good approximation to the truth for these two very different effects in mammalian tissues. If the formula is really of general

application it could be a useful guide in radiotherapy both for estimating the dose levels to be given and for estimating the number of fractions to be used in the new technique to ensure that the therapeutic ratio will be at least as good as that obtained with the old technique.

Although this formula has been shown to be approximately true for the two very different mammalian tissues mentioned above, it would be useful if the general nature of this formula could be tested more rigorously by experiments with some completely different type of tissue - not necessarily a mammalian tissue. The chosen tissue must however show appreciable effects due to the repair of sub-lethal damage.

In order to apply, or to test, the general formula it is necessary to be able to correct iso-effect doses for the effects of repopulation occurring during the period of the treatment regime. A number of different iso-effect formulae enabling this to be done are referred to briefly in Chapter 5 but the exact form of this relationship is not known. In order to determine the nature of this relationship a comprehensive set of very accurate iso-effect data is required for one particular end-point. For this purpose it would be preferable if the end-point chosen could be one in which the magnitude of the repopulation effect is much larger than that observed in the skin of animals.

SECTION 2.

EXPERIMENTAL DESIGN AND TECHNIQUE.

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THE DESIGN OF EXPERIMENTS TO TEST THE ABOVE FORMULAE.

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- 6. 2. Choice of end-point.
 - 6. 2. 1. Part or whole-body irradiation?
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THE SUITABILITY OF LD 50 FOR GRAIN WEEVILS AS AN END-POINT.

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DOSIMETRY.

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(thermoluminescent dosimetry) by Mr R.G.Dale.

SUMMARY OF SECTION 2 (CHAPTERS 6 - 9).

CHAPTER 6

THE DESIGN OF EXPERIMENTS TO TEST THE ABOVE FORMULAE

6. 1. Data required to test the General Formula

To test the general formula it is first necessary to determine the value of γ , the half-life of the dose equivalent of sub-lethal damage, applicable to the chosen end-point. This may be estimated from a split-dose recovery curve. It is then necessary to find how the iso-effect dose of continuous irradiation varies with irradiation time t over an appropriate range and how the iso-effect dose of fractionated irradiation varies with fraction number over a corresponding range. (4.3 Table 1). To correct for the effects of repopulation it is also necessary to find how the iso-effect dose for a constant number of fractions increases with overall irradiation time T and whether this increase is a function of the number of fractions employed. This information may also be used to test the formulae referred to in Chapter 5.

The ranges of the required data will now be considered.

Liversage (1969) showed that recovery should be 99% complete in a period of 7γ which is usually of the order of 10 hours but which could be appreciably larger or smaller in some uninvestigated non-mammalian tissue. To cover the whole of the likely recovery period it was decided that the iso-effect dose should be determined for eleven split-dose intervals ranging from 0 to 25 hours.

Gynaecological radium is usually inserted for periods of 24, 48, or 72 hours but occasionally for longer periods. According to the General Formula, the radiation "wasted" due to the repair of intracellular sub-lethal damage during 24, 48 or 72 hours should be equal to that "wasted" due to the same cause occurring in the intervals

between 6, 12 or 17 fractions of acute irradiation (4.3 Table 1). The experiments should at least span the above ranges. The longest continuous period for which the ^{60}Co unit could be made available for these experiments was 106 hours (Maundy Thursday until Easter Tuesday) and it was decided to utilise the whole of the range available during one such annual occasion.

The initial fractionation experiments were designed to cover the range 1 to 20 "daily" fractions (Five fractions per week). In order to correct for long term repair (repopulation) it was therefore necessary to study how the iso-effect dose, for a constant number of fractions, varies with overall treatment time for periods up to 28 days. The above considerations governed the initial design of the experiments and are summarised below:

In order to test the General Formula over a reasonable working range it was decided to find for one specific end-point how the iso-effect dose varies with

- (a) split-dose interval (11 regimes, 0 to 25 hours)
- (b) continuous irradiation time (13 regimes, 1 to 106 hours)
- (c) number of "daily" fractions (7 regimes, 1 to 20 fractions)
- (d) overall irradiation time using 4 fractions (6 regimes, 3 to 28 days)
- (e) overall irradiation time using 9 fractions (3 regimes, 10 to 28 days).

Thus initially it was estimated that the iso-effect dose would need to be determined for 40 different irradiation regimes. As the work progressed the need and opportunity to obtain additional data arose. The iso-effect dose was in fact determined for 63 different irradiation regimes (Chapter 11). Most of this increase was due to the need for

additional data regarding the long term repair process which was found to be more important and more complicated than originally envisaged. The remainder was due to repeating the daily fractionation regimes at a higher dose-rate made possible by the installation of a new ^{60}Co unit.

6. 2. Choice of end-point

6. 2. 1. Part or whole-body irradiation?

When comparing the effects of continuous irradiation with those of fractionated irradiation it is clearly advantageous if the geometrical dose distribution can be kept constant. The only practical methods of continuously irradiating part of an animal for 100 hours involve the use of interstitial, intracavitary or surface applicators. The geometry of such devices is extremely critical and difficult to simulate with fractionated high dose-rate irradiation. With whole-body irradiations these problems do not arise. Both low and high dose-rate irradiations may be delivered by the same ^{60}Co γ -ray unit, using similar geometry but with or without the insertion of a lead absorber.

6. 2. 2. Statistical considerations

The experiments are intended to show small variations in iso-effect dose with changing conditions and it is therefore essential that for each regime the iso-effect dose should be determined with a high degree of accuracy. It was decided to aim at a somewhat arbitrary accuracy of $\pm 3\%$. To obtain this degree of accuracy, using whole-body irradiation and lethality as an end-point it was estimated that several hundred animals would be required for each of the 40 or more irradiation regimes. Thus the total number of animals required would be of the order of tens of thousands. (The number actually used was 68200). Housing such large numbers

of small mammals and irradiating many of them for continuous periods of up to 100 hours would require resources which are beyond most of our research institutes. These problems are greatly reduced if small animals such as insects are used.

6. 2. 3. Other criteria affecting choice

The design of an experiment is greatly simplified if previous work has already revealed some information regarding the effects to be studied. For example, if the LD 50 for an animal is being used as the end-point it is useful to have some idea of the value of the LD 50 for single dose irradiation before planning the experiments. If the effects of fractionation are being studied it is advantageous to use an experimental system in which such effects are known to be large.

Other practical points which need to be considered are life span, ease of handling, habits and required living conditions.

CHAPTER 7

THE SUITABILITY OF L D 50 FOR GRAIN WEEVILS AS AN END-POINT

7. 1. The advantages of using grain weevils

The main formula to be tested has already been shown to be valid to a limited degree of accuracy for two mammalian tissues (Chapter 4.4.) Thus, experiments with insects should help in testing the general nature of the formula more effectively than the use of yet another mammalian tissue.

An adult grain weevil weighs approximately 2.5 mg and is approximately 5 mm long. Forty such weevils can live quite happily for more than 28 days in a 2.6 cm diameter perforated plastic container, in a warm moist atmosphere, provided adequate food is supplied. Housing and irradiating the required large number of specimens thus presents few problems.

Considerable data regarding the susceptibility of grain weevils to γ -radiation are available. This helps in designing the experiments and enables precautions to be taken to avoid inconsistencies which might otherwise result from insufficient control of factors which influence their susceptibility. The published data which have an important bearing on the design of these experiments are briefly described in 7.2.

The average life of a grain weevil housed at 70% RH and 25° C is of adequate duration, being about 110 days if kept on wheat and about 190 days if kept on flour (Coombs and Woodroffe, 1964). Grain weevils do not fly and can only climb a vertical glass wall with difficulty. They are thus easily handled (Chapter 8.3).

Insects, despite their smallness, are a highly developed species having respiratory, circulatory, digestive, excretory, reproductive and nervous systems (Wigglesworth 1956). Weevils have a remarkable

ability to recover from radiation injury as was shown by Jefferies and Banham, (1961). These workers found that grain weevils given two doses of 4000r 10 days apart showed the same percentage mortality as grain weevils given a single dose of 4000r. They claimed that this demonstrated that complete recovery from 4000r occurred in a period of ten days.

In organised tissues, the long term repair of radiation injury is generally considered to be influenced by homeostatic forces but the rate of repair is much slower, and hence more difficult to measure, in mammals than in weevils. The latter would appear to be good experimental subjects in which to study the form of relationship, if any, between the rate of repair and the amount of damage present at any instant of time.

7. 2. Published data on the radiation susceptibility of grain weevils

Grain weevils breed in grain and in experimental work are normally housed on grain. The female lays her eggs in the grain and Fig 7a taken from Cornwall and Morris, (1959), shows the developmental stages existing between oviposition and the emergence of the adult weevil some 33 days later, if housed at 26° C.

Jefferies and Banham (1961) using ^{60}Co γ -rays found the LD 50₍₂₈₎ for grain weevils to be 4470r using single doses and 6780r using 4 fractions in 3 days at dose-rates of 7300 to 10000 r/hr. Their weevils were housed at 26° C and 76% R.H but were irradiated at temperatures which varied between 25° and 35° C.

Martin, Burson, Bull and Cornwall (1962) studied the effect of culture density on susceptibility of grain weevils reared and housed in a room kept at 26°C, 75% R.H. They showed that if grain weevils

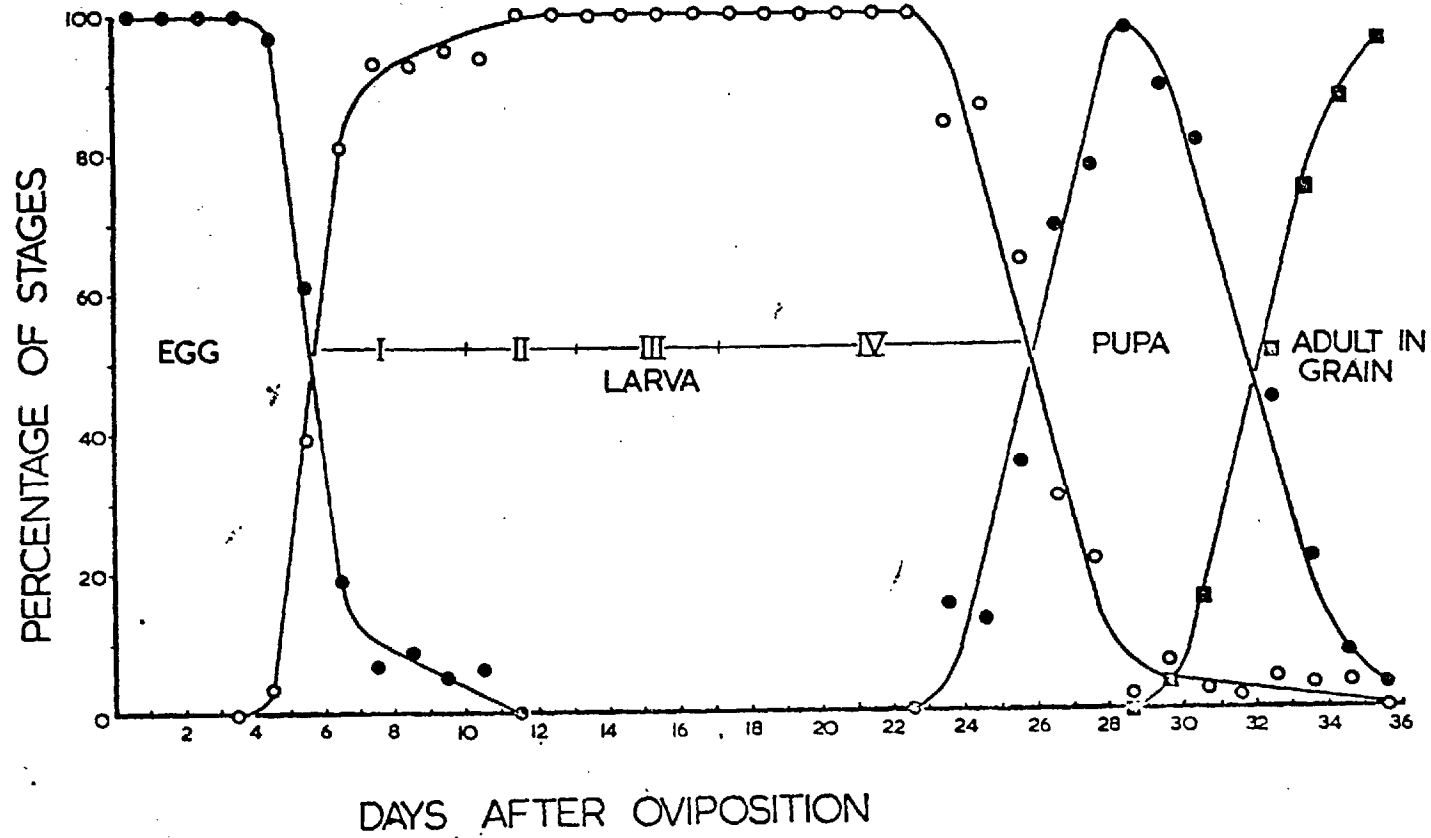


Fig. 7a Percentages of development stages in grain infested by grain weevils at 26°C and 77% R.H. Taken from Cornwell and Morris, (1959)

were cultured on wheat housed in glass jars, the single dose LD 50₍₄₂₎ for the emerged adult decreased from 6750r to 5000r as the culture density was increased from 10 to 1000 parents/800g of wheat.

They also showed that the radiation mortality (i.e. the mortality observed after irradiation but appropriately corrected for natural mortality) remained constant from 14 to 56 days after irradiation.

These authors showed that during the developmental stages, the temperature of the dense cultures rose by as much as 6°C above room temperature due to metabolic activity. They suggested that the LD 50 for grain weevils was very dependent on the temperature at which they had been kept during their development from egg to adult.

Further work by Pendlebury, Banham, Cooper and Bland (1962) investigated how the LD 50 varied depending on whether weevils were kept at 15°C or 30°C before, during or after irradiation. They found that keeping the weevils at 30°C instead of 15°C before irradiation increased their sensitivity by 50%, during irradiation decreased their sensitivity by 20% and after irradiation had little effect on the ultimate percentage kill but decreased the time for radiation deaths to reach completion from 56 to 14 days.

7. 3. Overcoming difficulties

7. 3. 1. The disadvantages of grain

In all the irradiation experiments referred to above the weevils had been reared, irradiated and subsequently housed on grain.

In order to count the surviving weevils in any batch it is first necessary to sieve the grain to separate the weevils from it.

This process is very time consuming as the weevils burrow into the grain to feed and even after sieving for 15 minutes some weevils may still lie hidden inside the grain.

In prolonged experiments the grain needs to be changed at least every 28 days to prevent the emergence of offspring. Between changes, the metabolic activity of the developmental stages of these offspring may well affect the temperature of the batch under test. To reduce these difficulties it was decided to keep the weevils on flour at 70% R H. Sieving is then much easier and offspring do not interfere with the experiment for the simple reason that the female weevil does not normally lay eggs in flour. Weevils require a moist atmosphere but flour tends to cake and grow mould if stored at a relative humidity above 70% R H.

7. 3. 2. Constant Temperature Requirement

The published data referred to above clearly indicate that the temperature must be very carefully controlled during all stages of this investigation and the building of a constant-temperature, constant-humidity, irradiation cabinet and storage room (Chapter 8) were essential.

In several of the investigations referred to above, the weevils had been irradiated and stored at 26°C and, to obtain comparable results, these conditions should be replicated as closely as possible. In hot weather, however, the irradiation cabinet would probably require to be cooled to keep its temperature below 27°C. To reduce the risk of such an eventuality it was decided that during all the present experiments the weevils would therefore be irradiated and stored at 27°C.

Because the susceptibility of weevils is so critically dependent on temperature before and during irradiation it was decided that every experimental specimen should be accompanied by

specimens taken from the same culture to act as controls. Some controls would be unirradiated whilst others would receive single doses of varying magnitude to enable the single dose LD 50 to be determined for each culture (Chapter 8.3 and 11.1)

7. 3. 3. Dose control

In order to cope with the very large numbers of weevils and irradiation regimes involved it was essential to design the irradiation cabinet in such a way that it could be used in conjunction with a ^{60}Co γ -ray beam unit to irradiate several hundred batches of 40 weevils simultaneously to many different doses.

The constant-temperature constant-humidity irradiation cabinet would require time to reach the correct condition each time the door was opened to insert or remove specimens. Simultaneous irradiation of specimens to different doses could therefore only be achieved by keeping the irradiation time constant and controlling the dose delivered to the specimen by placing it in a position within the cabinet where it would receive the required dose-rate. The choice between irradiating at constant dose-rate or for a constant irradiation time per fraction was therefore dictated by technical rather than scientific considerations. From the purely scientific point of view the author would however have made the same choice on the grounds that the proportion of radiation "wasted" due to the repair of intracellular sub-lethal damage during an irradiation is a function of the time available for such repair to occur. Ideally, for the acute regimes, the irradiation time should be sufficiently short for such repair to be negligible during the actual irradiation.

CHAPTER 8

EXPERIMENTAL TECHNIQUE

8. 1. The Constant-Temperature Room

8. 1. 1. Layout

An area of 2.5 X 1.6 metres in a basement room having no windows or exterior walls was partitioned off to make a constant-temperature constant-humidity room. The walls were lined with 2.5 cm thick expanded polystyrene and the partition, which included a sliding door, was made of a wooden framework lined both sides with expanded polystyrene. The humidifier and heating elements described below were mounted on the floor, opposite the door, under shelf T (Fig 8a). On each side of the room were built three shelves numbered from floor to ceiling L1, L2 and L3 on the left and R1, R2 and R3 on the right. The corner of the room and part of shelves R1, R2 and T are shown in Fig.8b.

8. 1. 2. Control Apparatus

The humidifier consisted of a boiler fitted with an electric kettle element and supplied with water from a constant-level cistern. A humidistat (Teddington Autocontrols Ltd, Type HS) was used in conjunction with a hot wire vacuum relay (A.E.I.Sunvic Type H.V.R.) to switch on or off full power to the kettle element as the room humidity fell or rose above that required. Reduced power was initially supplied continuously to the kettle element via an energy regulator (A.E.I.Sunvic Type E.R.C.) but after the room had been conditioned for a few weeks it was found preferable to dispense with this facility (8.1.3.)

CONSTANT TEMPERATURE AND HUMIDITY ROOM

Fig.8a Plan of constant-temperature, constant-humidity room.

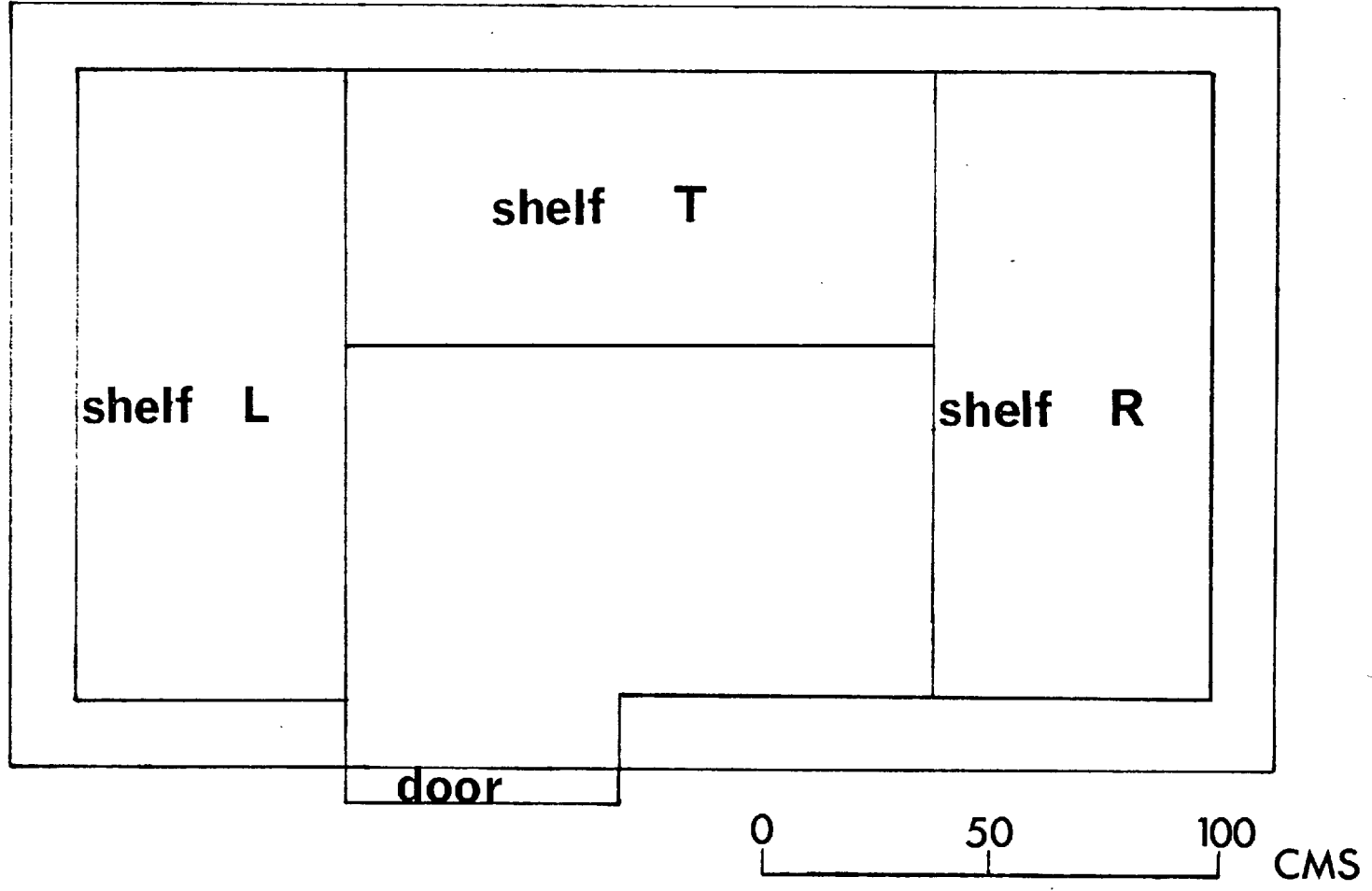




Fig. 8b One corner of the constant-temperature room. The glass honey-jars containing irradiated weevils and flour can be seen standing on small platforms in the oil filled trays on the right hand shelves. The perspex nest of shelves in the centre of the picture was used to house specimens during irradiation in the specially designed irradiation cabinet described later (8.2).

Temperature control was maintained by means of a modified domestic fan heater. The two 1 Kw heating elements were connected in series, instead of in parallel, so as to reduce the power to 500 watts. The electrical connections were rearranged so that the fan ran continuously but the heating elements were only energised via a thermistor temperature controller (A.E.I. Sunvette Type T.H.I.) The fan heater was mounted in such a position that it blew the water vapour rising from the boiler in the direction of the door. As a safety measure, a room thermostat (A.E.I. Sunvic Type SR 2) was fitted and adjusted so as to switch off all power supplies to the humidifier and the fan heater should the room temperature rise to above 29°C.

8. 1. 3. Conditions obtained

Thermohygrographs (Casella) were used to record the temperature and relative humidity in the room. These were calibrated at the beginning and end of each batch of experiments by comparison with a whirling Hygrometer (Casella). Under normal operating conditions the thermohygrographs showed that the humidistat switched on and off the power to the kettle element 6 or 7 times per hour causing rapid fluctuations in the relative humidity. The magnitude of these fluctuations varied with position having a minimum value of $\pm 1\%$ (Shelf R1) and a maximum value of $\pm 3\%$ (Shelf L1). The relative humidity existing on any shelf, at any time, was considered to be the mean of that recorded as the moisture content of wheat or flour would be unlikely to follow such rapid fluctuations as in-air conditions. When the energy regulator was used the fluctuations occurred less frequently which was the main reason why this device was dispensed with.

Periodic fluctuations in temperature due to the heater being switched on and off were hardly detectable being less than $\pm 0.2^{\circ}\text{C}$.

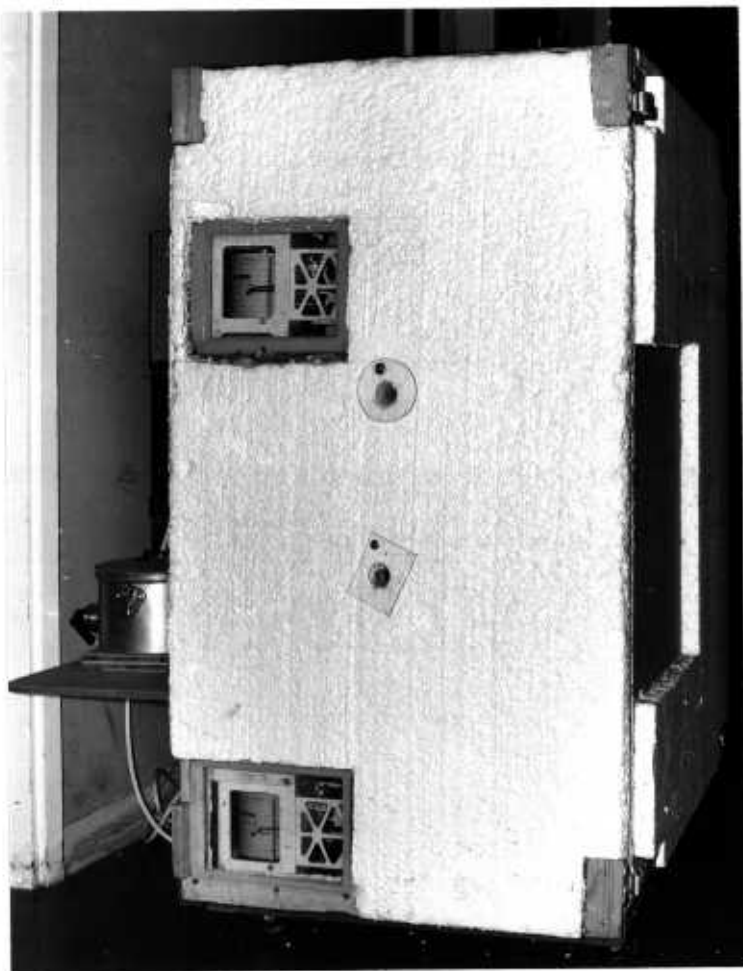
The three thermohygrogaphs available were placed in twelve different positions in the room over a nine day period to record the variation in temperature and humidity with position and to some extent with time. At all times and in all positions the relative humidities and temperatures observed were found to lie within the ranges $71 \pm 4\%$ R.H. and $27 \pm 1.5^{\circ}\text{C}$. The monitor thermohygrograph situated on Shelf T (later moved to Shelf L2) recorded average values of 70% R.H. 27.0°C and showed a day to day variation over a number of months of $\pm 2\%$ in relative humidity and less than $\pm 0.5^{\circ}\text{C}$.

It was found that the average temperature on the shelves on the left hand side of the room was 1°C higher than those on the right. The effects of this difference were largely eliminated by distributing specimens from the same batch uniformly round the room (8.3.4.)

8. 2. The Irradiation Cabinet

8. 2. 1. Constuction

The cabinet, 102 cm long, 66 cm wide, and 120 cm high, was made of wood lined with 2.5 cm thick expanded polystyrene. An insulated partition, having a vent at top and bottom, divided the cabinet into a specimen compartment at one end and an air-conditioning compartment at the other. A removable door was fitted at each end. Figs 8c and 8d show the conditioning end of the cabinet



Figs. 8c and 8d The air-conditioning end of the irradiation cabinet showing the door closed and open. The apparatus controlling the temperature and humidity is described in chapter 8.2.2.

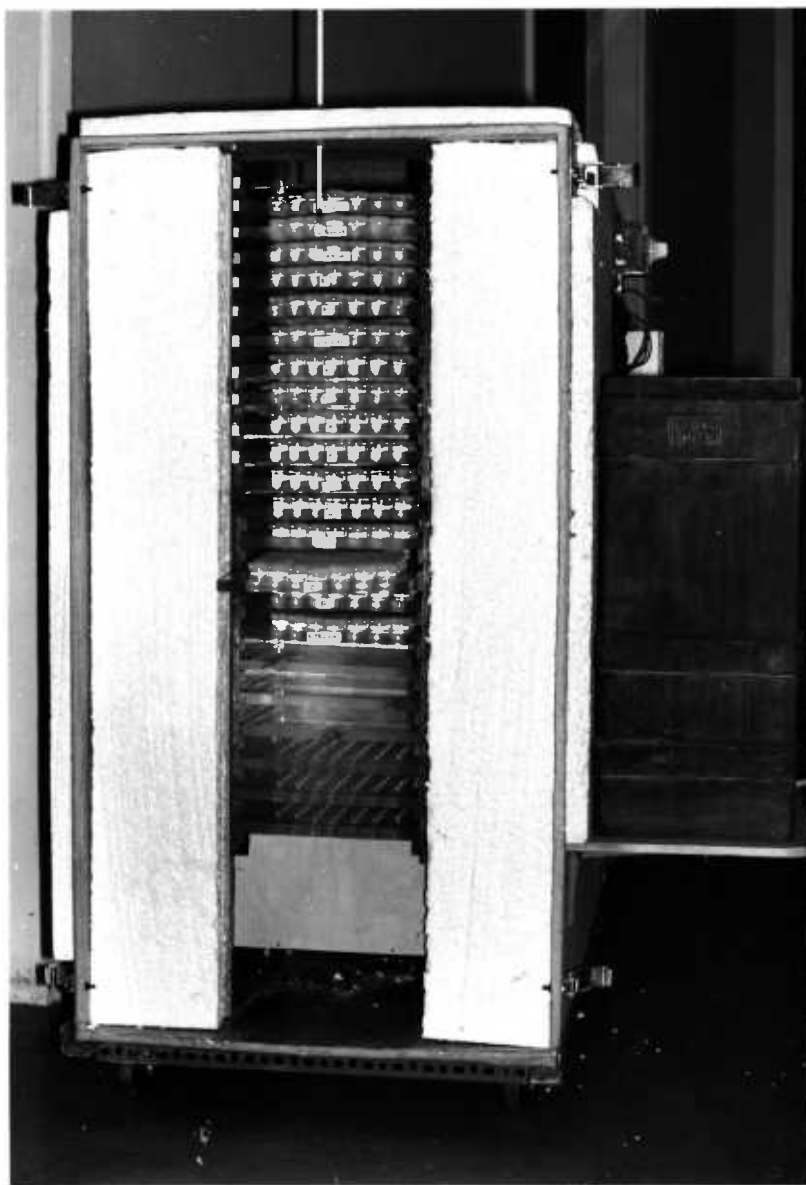


Fig. 8e The specimen compartment of the irradiation cabinet with the door open to show the nest of shelves housing the plastic pots containing weevils for irradiation.

with and without the door whilst Fig 8e shows the specimen end without door.

The specimen compartment housed a perspex nest of 23 removable 12.7 mm thick perspex shelves. Each shelf contained 37 ^{my}symmetrically arranged holes (Fig 9a) which during irradiation were filled with plastic pots containing either 40 weevils plus flour or flour only. In either case, the amount of flour in the pot was adjusted so that the weight of the pot and its contents was equal to the weight of perspex displaced. When exposed to a vertically downward ^{60}Co γ -ray beam the dose-rate received by the weevils was determined by their position in the nest (Chapter 9). Thus several hundred pots of weevils could be irradiated to different doses simultaneously.

8. 2. 2. Control Apparatus

Temperature and humidity control for the irradiation cabinet was obtained using a similar technique to that employed in the constant temperature room. The control circuits were essentially the same but three 15 watt light bulbs were used to supply heat and the kettle element of the water boiler was only energised through the energy regulator. Water vapour from the boiler, which was mounted outside the cabinet, was fed into the cabinet through a lagged pipe (Fig 8d). Air was continuously circulated from the air conditioning compartment to the specimen compartment by a fan. Two thermohygrographs were used to monitor the air entering and leaving the conditioning compartment and the temperature of the specimen compartment could be observed directly on a thermometer.

8. 2. 3. Conditions obtained

The cabinet was conditioned for two or three weeks before starting experiments. The relative humidity in the cabinet then decreased so slowly that several hours would elapse between successive

operations of the humidistat. During this period the humidity fell from 2% above to 2% below that required. For continuous irradiation lasting many hours average conditions would be close to those required but a fine control was necessary for acute irradiations. This was provided by installing small adjustable vents in the conditioning compartment and inserting a low power electric cup heater in the boiler. These refinements allowed manual adjustment of the conditions.

Except in the case of the split dose experiments specimens were inserted in the cabinet at least one hour (usually several hours) before irradiation to allow equilibrium conditions to be obtained. It was found that the temperatures recorded by the two thermohygrographs were never more than 1°C different and averaged $27 \pm 0.5^{\circ}\text{C}$ during every irradiation except for the split-dose experiments where they averaged $27 \pm 1^{\circ}\text{C}$. The temperature of the thermometer in the specimen compartment was observed at the beginning and end of each irradiation and was usually found to be $27 \pm 0.1^{\circ}\text{C}$.

The upper thermohygrograph usually recorded a humidity some 3% higher than the lower thermohygrograph. During each irradiation the average value of the recorded humidities was $70 \pm 2\%$ except for the split-dose experiments where it was $70 \pm 3\%$.

8. 3. Care and cultivation of grain weevils

8. 3. 1. Production of stock cultures

The original culture containing 50 adult weevils (*sitophilus granarius*) on approximately 200g of grain, was kindly supplied by the Pest Infestation Laboratory at Slough and housed in the C.T.Room. Weevils emerging from this culture were used to make up six stock cultures.

Standard stock cultures were made by placing 120 adult weevils on 200g of Argentine Plate Wheat which had been disinfested by heating to 60°C for four hours and then conditioned by allowing it to stand in the C.T. Room for at least one week. The wheat was contained in glass jam jars 7.8 cm diameter sealed with filter paper fixed by wax and two layers of cotton cloth held by rubber bands.

Throughout these experiments, any jar containing a culture, flour, grain or weevils was stored on its own individual platform in a tray of oil on one of the shelves in the C.T. Room. The oil prevented any weevils which might escape from reaching other specimens and helped to reduce the risk of specimens becoming infested with other species of insects attracted by the food and the environment. No new weevils would emerge from the stock culture for 33 days but, by 36 days the rate of emergence exceeded 100 weevils per day. Young weevils could be separated by sieving the culture at frequent intervals during the next three or four weeks. Weevils pass through a British Standard No 12 sieve but grain does not. Stock cultures were prepared every six weeks.

8. 3. 2. Production of weevils for irradiation

Special cultures were prepared to produce weevils for experiments. The grain was prepared as for stock cultures but only 100 weevils (obtained from standard stock cultures) were placed to oviposit on each jar of grain. The parents were removed by sieving 15 $\pm$ 1 days later. Between 10 and 20 such cultures were prepared approximately 60 days before the first irradiation in any group of experiments. The cultures were sieved again 24 days and 14 days before the first irradiation. The weevils obtained from the second sieving were

used in the experiments and were thus all 14 to 24 days old on the first irradiation day. Each such culture produced approximately 1000 weevils. Half of these were stored in a jar marked X and the remainder in a jar marked Y. Each of these jars contained 40g of disinfested, conditioned, whole wheat flour which had been sieved through a British Standard No 30 sieve. The jars were sealed in the same way as the cultures and were stored on shelf R2 until required (8.3.3.) All cultures were stored on shelf L3 except for the early experiments. The cultures used to produce weevils for the pilot experiment and split-dose experiment were stored on shelf R2. (It is thought that the difference in temperature between these two shelves may have been partly responsible for the weevils from the early cultures being slightly more resistant to irradiation).

8. 3. 3. Bottling of Weevils for irradiation

Each group of experiments, designed to determine the L D 50 for a number (about 10) different irradiation regimes, required a total of about 300 plastic pots of 40 weevils. The plastic pots were 2.7 cm high and 2.6 cm in diameter. Two weeks before their first irradiation, these pots were loaded with the required amount (approximately 4.7g) of disinfested flour and stored in the C.T.Room. The 30 or so pots to be used for a given irradiation regime were allocated consecutive numbers. Loading the 300 or so pots with weevils occupied two full days work and this task was usually started 5 days before the first irradiation. Pots with numbers ending in zero were loaded first and those ending in nine last. On the first loading day the weevils from all the jars marked X (8.3.2.)

were pooled and used for loading half the pots. Weevils from the jars marked Y were used on the second day.

The problem of handling and counting the thousands of weevils involved was solved surprisingly easily. If a thousand or more weevils are placed in a large upturned beaker they tend to walk out of the spout, more or less in a single file, and are easily picked up by a "pouter". The latter is basically a miniature vacuum cleaner comprising a glass or perspex vessel 10cm long and 2.5cm diameter which is sealed by a rubber bung. Inlet and outlet glass tubes pass through the rubber bung. The outlet tube, whose entrance is covered by a wire gauze, is connected to a vacuum pump. The inlet tube is bent through a right angle and has a small hole in one side. When this is covered with a finger and the end of the inlet tube is placed near a weevil the latter is sucked up into the "pouter". In this way 40 weevils may be counted and collected in as many seconds, and then transferred to one of the plastic pots which are fitted with perforated polythene lids.

8. 3. 4. Storage of specimens, before, during and after irradiation

Before and after irradiation, the pots containing the weevils were housed in the C.T.Room on shelves L1, R1, L2 and R2 in a definite sequence which depended primarily on the last digit of their allocated reference number. Thus, pots whose number ended in zero were housed at the door end of shelf L1 whilst those ending in nine were housed at the far end of shelf R2. In this way, any variation in irradiation susceptibility produced by positional factors or loading sequence would be reflected in the results observed for any given irradiation regime but would affect all regimes equally.

Preliminary experiments (10.1) revealed that weevils were capable of enlarging the perforations in the polythene lids sufficiently to escape. To prevent this happening each plastic pot was stored before and after irradiation in a somewhat larger plastic container also fitted with a plastic perforated lid. The lids of the plastic pots were inspected daily and replaced when necessary.

Weevils receiving single doses of irradiation were transferred to glass honey jars 6.6cms in diameter 7 days after irradiation. These jars had been filled with 35g of disinfested sieved whole wheat flour and housed in the C.T.Room for at least 7 days. Weevils receiving protracted or fractionated regimes of treatment were similarly transferred 7 days after Day 0, defined below.

8. 4. Definition of Day 0

Preliminary experiments showed that weevils killed by single doses of radiation usually died about 16 days later and that weevils surviving more than 23 days suffered a mortality similar to that of unirradiated weevils. (Chapter 13.2). Thus the majority of deaths occurring within 30 days could be ascribed to the effects of radiation whilst those occurring after 30 days were probably due to other causes. The $L D_{50(30)}$ was therefore considered to be a good yardstick by which to measure the susceptibility of weevils to single doses of irradiation. When comparing the effects of fractionated irradiations with those produced by single doses, the question arose as to how one should define Day 30. It could be argued that the 30 day period could be counted from either half way through the irradiation regime or alternatively from the end of the irradiation regime. In view of this uncertainty it was decided to define Day 0 as the day which was three-quarters through the irradiation

regime and Day 30 as being 30 days later. This was a somewhat arbitrary choice which had to be made in order to design the experiments. The error in the estimation of the iso-effect doses due to defining Day 0 incorrectly is discussed in section 13.2.

8. 5. Counting and scoring the survivors

The number of weevils surviving was counted 5 or 6 times between Day 0 and Day 37. Counts were invariably made on Day 7, when the weevils were transferred from plastic pots to honey jars and on Day 30. At each count the weevils in any specimen were separated from the flour by sieving with a British Standard Sieve No 25. The weevils were then placed on a white plastic table top. Those which moved were picked up by the "pouter"; those which did not move were placed on a sheet of metal which was then gently heated. Weevils which failed to respond to this stimulus were considered dead. Both the numbers of dead and alive weevils in any sample were counted in order to detect counting errors, which were found to be very rare. Dead weevils were discarded; live weevils were resealed in their respective jars.

CHAPTER 9

DOSIMETRY

9. 1. Irradiation conditions

Weevils were irradiated in the specimen compartment of the irradiation cabinet described above (8.2.)

Until September 1971 the vertically downward beam of an Orbitron Cobalt-60 Unit (A.E.I.Ltd) giving a 22cm square field at a source-cabinet distance of 60cm was used for all the irradiations. Each acute irradiation lasted between 71 and 79 minutes but the doses delivered to the weevils varied between 700 and 7500 rads depending upon their position within the cabinet. During protracted continuous irradiation, lasting between 6 and 106 hours, the dose-rates in the cabinet were reduced by introducing a 2cm thick lead absorber into the beam at a distance of 47cm from the source.

After September 1971, the Orbitron was replaced by a Mobaltron 80 Unit (T.E.M.Instruments Ltd). This gave a 22.6cm or 13.3cm square field when operated at a source-cabinet distance of 72.5cm or 42.5cm respectively. The higher dose-rates obtainable with this unit enabled some of the acute irradiation experiments to be repeated using shorter irradiation times (24 to 26 min.)

The shelves in the specimen compartment were lettered A to W in descending order and the position of each pot on each shelf was numbered in accordance with the key diagram (Fig 9a). The dose-rate on any shelf was expected to be approximately 15% less than that on the shelf immediately above and it was anticipated that there would be a gradual decrease in dose-rate toward the periphery of each shelf. The design of the experiments frequently required 5 pots to be irradiated to the same

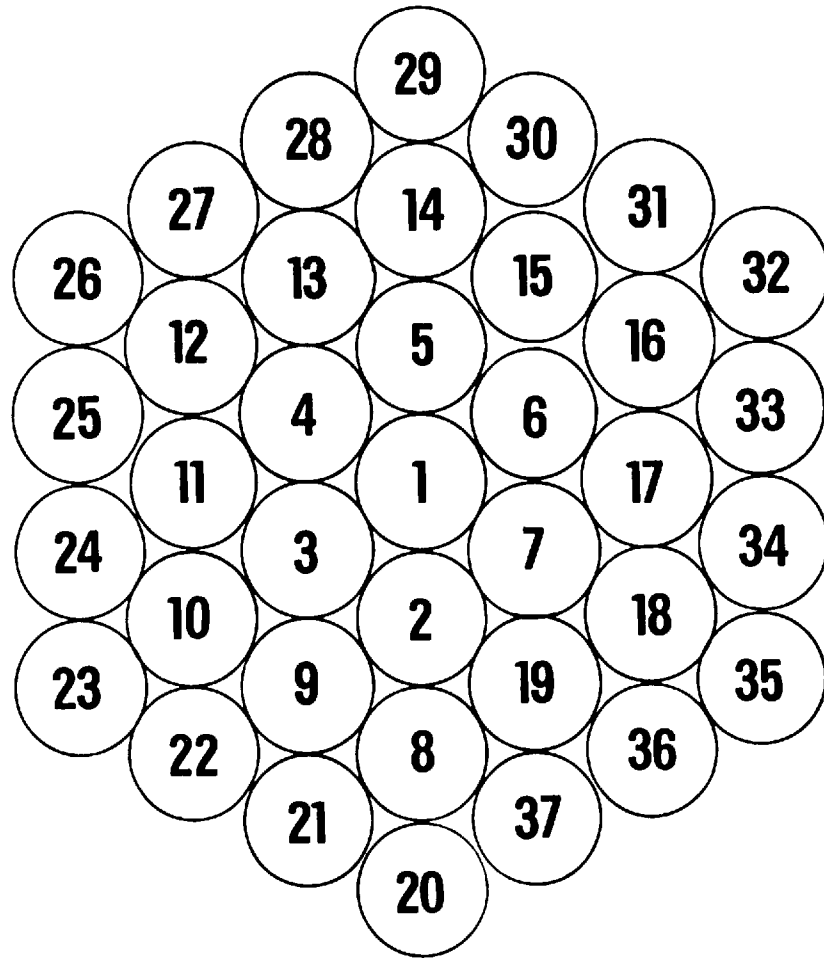


Fig. 9a Plan view of the 37 plastic specimen pots housed on each shelf of the irradiation cabinet. The holes into which the specimen pots fitted were numbered as shown above and the dose-rates received by pots in different positions on each shelf are recorded in Table 2.

dose. Such pots were therefore positioned on the same shelf in holes lying on concentric arcs i.e. they were allocated to one of the five groups of holes referred to in Table 2 (9.2).

9. 2. Ionization chamber measurements

The exposure-rate on the surface of each shelf at the centre of each of the above arcs was measured using an Ionex Mark III Dose-rate meter (I.D.L.Ltd). This had been calibrated against a Farmer Sub-standard Dose-meter (I.D.L.Ltd) which had been calibrated by the National Physics Laboratory.

To facilitate this measurement a special perspex shelf was designed. This consisted of a number of rectangular perspex slabs, one of which contained a hemi-cylindrical cavity in which the ionization chamber plus build-up cap could be half-immersed. These slabs could be fitted together in such a way that they formed a solid perspex shelf of the same dimensions as those in the irradiation cabinet. By rearranging the positions of the slabs, the centre of the half-immersed ionization chamber could be made to coincide with the position normally occupied by the centre of any of the 37 pots on that particular shelf.

Weevils housed in plastic pots were generally found to remain on the surface of the flour which was 6.5mm above the upper surface of the perspex shelf. Thus an appropriate inverse square law correction averaging 1.6% was necessary to convert exposure-rate on the shelf surface to exposure-rate on the flour surface. The observed Ionex exposure-rates were also corrected for calibration factor, temperature and pressure. Thus the exposure-rates in various positions on each shelf were determined.

A small discrepancy between the above measurements and confirmatory

measurements using thermo-luminescent dosimetry (9.4) resulted in further ionization chamber measurements being made. The chamber complete with build-up cap was half immersed in the special perspex shelf in the position of shelf A in the same way as during the original measurements. Under this condition the dose-meter should record the exposure-rate which would exist at the centre of the chamber if the whole of the build-up cap and chamber were replaced by air. The build-up cap was then removed and the chamber retained in the same position using sellotape to suspend it on the axis of the hemicylindrical cavity. The change in ionization current observed was negligible (an increase of 0.2%) indicating that adequate build-up material was provided by the perspex nest of shelves. The air cavity below the ionization chamber was then filled with flour. This caused the ionization current to increase by 1.4% indicating that the true exposure-rate on the surface of the flour was 1.4% higher than that calculated from the original measurements.

To confirm the above correction the naked ionization chamber was mounted half-immersed in the surface of the flour in a plastic pot in the same position on shelf A. With or without a lid on the pot, the ionization current observed was now found to be 3% higher than that observed when the chamber with build-up cap was half-immersed in the perspex shelf. It was then realised that in addition to making a 1.6% correction for inverse square law a further 1.4% correction was necessary to allow for back-scattered radiation from the flour immediately below the point of interest.

The dose-rate (rads/min in water) received by weevils in a given group of pots on a given shelf was assumed to be the exposure-rate observed at the centre of the arc on which the pots were situated, corrected as described above and multiplied by a rad/roentgen conversion factor

of 0.965 (I.C.R.U.1964). An exception to the above procedure was made in the case of pots in Group 1 (Table 2) where exposure-rates on one side of the arc were found to be 1% (occasionally 2%) higher than those on the other. In this case the average of the exposure-rates observed in positions 3, 4, 6, and 7 was assumed to apply to all the pots in positions 2 to 7.

Table 2 shows the dose-rates received during irradiation with the Orbitron Unit without lead absorber. Similar tables were obtained for the alternative irradiation conditions referred to above.

9. 3. Consistency of Ionization Measurements

The dose-rates received, particularly on the lower shelves of the cabinet, would vary appreciably with time if the absorption and scattering characteristics of the flour in the pots varied appreciably with time. It was therefore decided to repeat the ionisation measurements at a number of selected sites after the cabinet had been in use for three months. Appropriate corrections for source decay were of course applied. Of the 36 repeat measurements made without lead 5 differed from the original measurements by more than 0.8% but none by more than 1.0%. Of 51 repeat measurements with lead 6 differed by more than 2% but none by more than 2.5%. The reduced consistency found with lead was probably due to reduced measurement accuracy at the lower exposure-rates, which at some sites were as low as 2R/min. In the case of the measurements with lead the 51 chosen sites were the sites which had been used to irradiate weevils. The doses received by these weevils were therefore calculated from the average of the two series of measurements.

TABLE 2

Dose-rates (rads/min. in water) in Irradiation Cabinet using Orbitron Unit without lead at 60cm S.C.D. on 7.5.1970

SHELF	GROUP 1 HOLES 2, 3, 4, 5, 6, 7.	GROUP 2 HOLES 9, 10, 11 12, 13.	GROUP 3 HOLES 15, 16, 17, 18, 19.	GROUP 4 HOLES 21, 22, 24, 25, 27, 28.	GROUP 5 HOLES 30, 31, 33, 34, 36, 37.
A	96. 4	95. 4	95. 3	94. 5	92. 1
B	85. 2	84. 6	84. 0	82. 9	81. 1
C	74. 8	74. 2	73. 9	72. 4	71. 2
D	65. 3	64. 3	64. 7	62. 5	61. 7
E	56. 8	56. 3	56. 2	53. 7	52. 7
F	48. 5	47. 7	47. 8	45. 4	44. 4
G	41. 3	40. 6	40. 4	38. 3	37. 7
H	35. 6	34. 6	34. 2	32. 9	32. 7
I	30. 4	29. 2	29. 0	28. 2	28. 2
J	26. 1	24. 5	24. 6	24. 3	24. 5
K	22. 3	21. 1	21. 0	21. 2	21. 2
L	19.15	17. 6	18. 0	18.35	18. 5
M	16.45	15.05	15. 4	15. 8	15. 6
N	14. 2	13. 2	13.25	13. 5	13. 5
O	12.25	11. 4	11. 5	11. 7	11.65
P	10.55	9. 9	9. 9	10. 2	10. 0

9. 4. Confirmatory measurements using T.L.D. (thermoluminescent dosimetry)

by Mr R G Dale

Tissue equivalent Lithium Borate powder (Jayachandran, West and Shuttleworth, 1968 and Jayachandran, 1970) kindly supplied by the Medical Physics Department at Hammersmith Hospital, was used in conjunction with a Con-Rad Model TLD 5a reader to check the doses delivered on the surface of the flour in the specimen pots on a number of shelves in the irradiation cabinet.

Gelatin capsules approximately 4mm diameter and 11mm long containing Lithium Borate powder were half immersed in the surface of the flour in pots numbered 3, 5 and 7 on shelves A, D, H and L and 2, 4 and 6 on shelves B, F and J. The Orbitron Unit operating under the conditions specified above (9.1) was then used to irradiate the cabinet for 5 minutes. Three additional capsules, used as standards, were irradiated in a Mix D wax (Jones and Raine 1949) phantom with 500 rads of ^{60}Co γ -radiation. This dose was determined from a measurement made in the phantom using the Ionex dosimeter referred to above. The observed exposure-rate in the phantom, appropriately corrected for calibration factor, temperature and pressure was converted to dose-rate (rads/min in water) using a conversion factor of 0.95 in accordance with the Code of Practice for Megavoltage Dosimetry (1969) published by the Hospital Physicists' Association.

As the dose response of Lithium Borate on the previous day had been shown to be linear over the range 0 to 500 rads, the doses received by the capsules in the irradiation cabinet were found by comparing their thermoluminescence with that of the standard capsules.

In Fig 9b the dose-rates, with limits of $\pm$ one standard deviation, as measured using T.L.D. are plotted against shelf position and compared with the dose-rates for the same group of pots as measured using the ionization chamber (9.2.). Although the latter dose-rates have been corrected for the back-scatter effect from the flour referred to above, the T.L.D. values are on average 2.1% higher than the ionization chamber values. This figure is greatly influenced by the sites having the largest uncertainties and is reduced to 1.1% if the percentage differences at the individual sites are weighted in inverse proportion to the standard deviations of their T.L.D. readings. It was therefore considered that the general agreement was satisfactory.

A re-examination of the dose response curve for Lithium Borate revealed that the best straight line passing exactly through the origin and through the point at 500 rads also passed through all the 9 intervening points provided they were given limits of $\pm$ two standard deviations.

The best curved line passing through the experimental points was coincident with this straight line at 0 and 500 rads but in the region 200 to 300 rads the curved line would have indicated doses 2% to 3% less than those obtained assuming strict linearity.

This possible slight departure from linearity could have accounted for the slight discrepancy reported above.

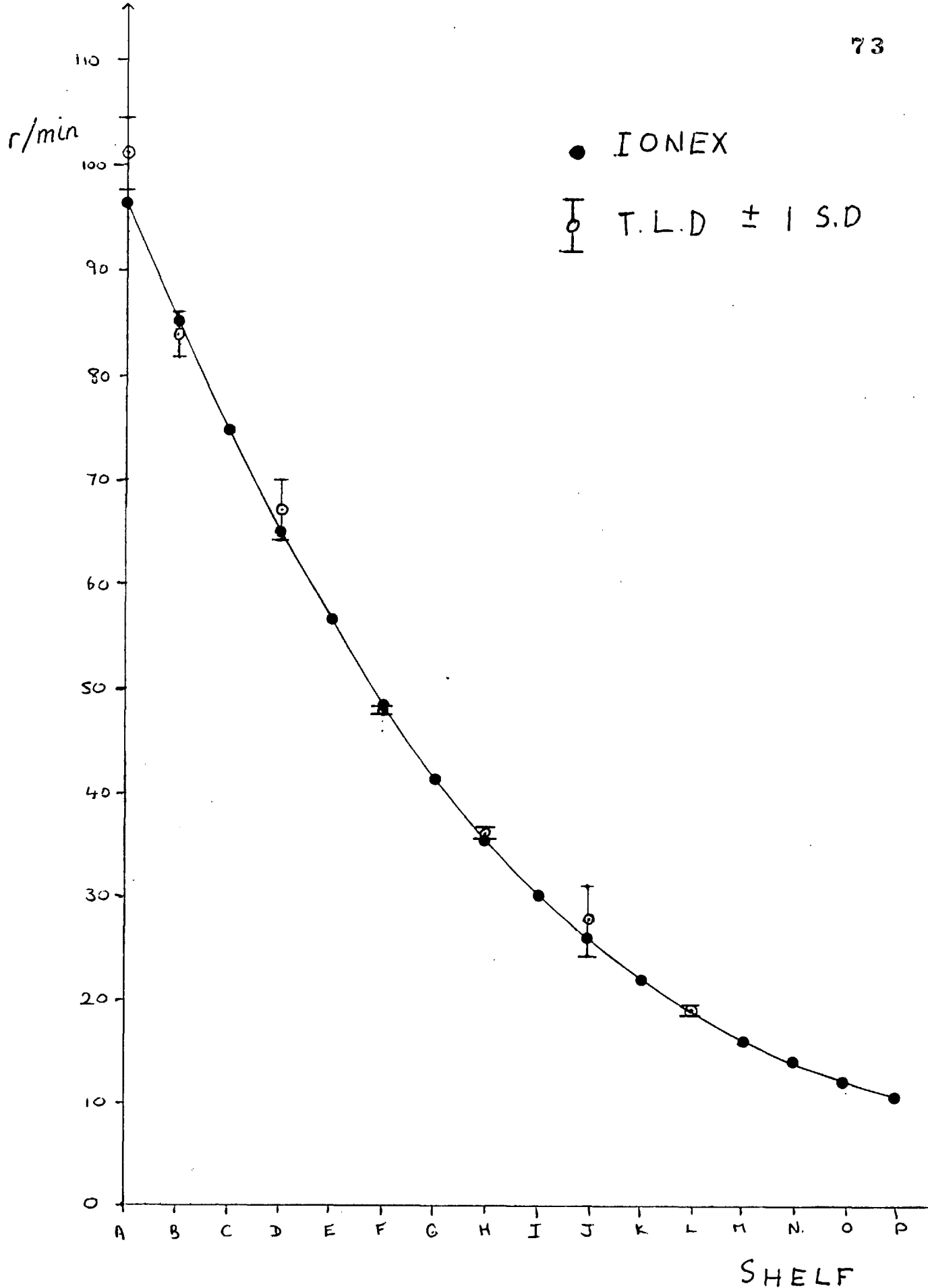


Fig. 9b Variation of dose-rate from shelf to shelf in irradiation cabinet. Comparison of thermoluminescent dosimetry (T.L.D.) and ionization chamber (Ionex) measurements.

SUMMARY OF SECTION 2 (CHAPTERS 6 - 9)

Further testing of the General Formula (Section 1) required the obtaining of a large body of accurate iso-effect dose data for one particular end-point. It would be preferable if these data could be obtained from experiments on a tissue which was very different from the mammalian tissues previously used for testing the formula and it was estimated that the iso-effect doses would need to be determined for at least 40 different irradiation regimes. (In practice 63 regimes were investigated). Some of these regimes would involve fractionated high dose-rate irradiations whilst others would involve continuous low dose-rate irradiation for periods up to and including 100 hours. Whole-body irradiation may be delivered to small animals and a comparison of the doses delivered at high and low dose-rates can be made with a degree of accuracy far greater than that obtainable if only part of an animal is irradiated. It was therefore decided that the LD 50 of some small animal would be used as the end-point. Statistical considerations suggested that tens of thousands of such animals would be required. To overcome the problems of housing and irradiating such large numbers it was decided to use insects. The grain weevil was chosen because it was known to exhibit appreciable recovery from radiation injury. Published data on the radiation susceptibility of this insect were available for designing the experiments. Because the grain weevil only thrives in a humid environment and its radiation susceptibility is very temperature dependent it was necessary to build a constant-temperature, constant-humidity storage room and irradiation cabinet. Both were maintained at 27°C, 70% R.H. Weevils were cultured on grain but, in order to overcome certain practical difficulties, were housed and irradiated on flour.

The irradiation cabinet used in conjunction with a ⁶⁰Co beam unit, enabled several hundred plastic pots, each containing flour and 40 weevils, to be

irradiated simultaneously to different doses depending on their chosen position within the cabinet. A lead absorber was used to reduce the dose-rates received during protracted continuous irradiation.

The doses received by the weevils in different positions in the cabinet were determined from ionization chamber measurements. These agreed within the limits of experimental accuracy with measurements made by Mr R.G.Dale using thermoluminescent dosimetry.

SECTION 3.

THE EXPERIMENTS AND THEIR RESULTS.

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- 10. 2. Pilot Irradiation Experiments.
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 - 10. 2. 4. Discussion.
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THE RESULTS.

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SUMMARY OF SECTION 3. (CHAPTERS 10 - 12).

CHAPTER 10

PRELIMINARY EXPERIMENTS

10. 1. Studies of survival under different conditions of storage

Weevils receiving prolonged fractionated regimes would need to live in the 2.6 cm diameter plastic pots (8.3.3.) for several weeks instead of in the more spacious honey jars in which they are normally housed. In both cases the weevils would be kept on disinfested, sieved and conditioned wholewheat flour but the surface of the latter would become more polluted with excreta in the plastic pots than in the honey jars. A preliminary experiment was done using 600 weevils of age 0 to 6 days to find out to what extent the natural mortality of the weevils would be affected by keeping them in plastic pots rather than honey jars. The weevils were divided into 3 batches.

Batch A consisted of 5 plastic pots each containing 40 weevils and 4.2g of flour. These pots were left completely undisturbed in the constant-temperature room.

Batch B was identical to batch A but the pots were shaken every 2 - 3 days to bring some of the clean underlying flour to the surface. The weevils in this batch were transferred to honey jars after 49 days.

Batch C consisted of 5 honey jars, each containing 40 weevils and 50g of flour, sealed with filter paper and two layers of cotton cloth.

Surviving weevils in batches B and C were counted, as described above (8.5) every 7 days but all batches were inspected each day. At 20 days after sealing, it was found that the weevils in two of the five pots in batch A had enlarged the perforations in their lids sufficiently to escape. Those which escaped died in the oil surround. As these pots could no longer be left undisturbed the survivors and dead were counted. The natural mortality for batch A at 20 days was 1.5% i.e. the same as that observed for batches B and C.

No differences in survival were observed between batch B and C within the limits of statistical accuracy. The percentage survivals of the two batches were 94% and 94% at 70 days, 51% and 45% at 135 days, 25% and 27% at 154 days and 0.5% and 1.5% respectively at 218 days. It should be mentioned that although no escape of weevils occurred, the holes in two of the perforated lids in batch B were enlarged sufficiently by the 28th day to make escape appear imminent. These lids were therefore replaced.

It was concluded from this experiment that the natural mortality of weevils would not be greatly affected by storing them for periods up to seven weeks in the small plastic pots, provided these were shaken every 2 or 3 days. It was realised that the perforated lids might need changing occasionally and that it would be advisable to store each pot in a somewhat larger plastic container (8.3.4)

10. 2. Pilot Irradiation Experiments

10. 2. 1. Objectives

Each of the seven groups of main experiments (11.2 to 11.8) would involve the use of approximately 10,000 weevils and a considerable amount of experimental work spread over a period of approximately 5 months. If the true LD 50 lay outside the range of dose levels chosen or if too large a range of dose levels was employed much time and effort would be wasted. It was therefore essential that pilot experiments should be carried out which would enable appropriate ranges of dose levels to be chosen for the main experiments.

The pilot experiments were therefore designed to obtain approximate values of the LD 50 for weevils for a small number of important irradiation regimes under the exact experimental conditions to be employed. These experiments would serve the additional objective of testing, in practice, the proposed experimental technique

(Chapter 8). It had been decided that the age of weevils employed in the main experiments should be 14 to 24 days. The pilot experiments were therefore also designed to see whether there was any gross difference in susceptibility of weevils of different ages within this range. Thus in the pilot experiments each batch of weevils employed was sub-divided into groups aged 14-18, 18-21 and 21-24 days.

10. 2. 2. Irradiation Regimes

For the pilot experiment it was decided to find the approximate LD 50 for the following regimes

- (a) Single doses
- (b) 4 fractions in 3 days
- (c) 4 fractions in 21 days

The results of (a) and (b) should enable reasonable estimates to be made of how the LD 50 should increase with the number of daily fractions whilst the results of (b) and (c) should enable estimates to be made of how the LD 50 for a given number of fractions should increase with overall time.

For each of the above regimes the expected LD 50 was predicted from the results of Jefferies and Banham, (1961) (7.2) and dose levels chosen which were considered to span the predicted LD 50 by an adequate range of values.

Three pots (one for each age group) each containing 40 weevils were irradiated at each dose level and three kept unirradiated to act as controls for each regime.

The dose levels were as follows:

Single doses, 8 dose levels	(2360-6580r)
4 fractions in 3 days, 7 dose levels	(4370-9440r)
4 fractions in 21 days, 7 dose levels	(6840-17580r)

10. 2. 3. Results

Figs 10a and 10b show the number of survivors plotted against time for two dose levels for the single dose and the 4 fraction in 21 days regimes.

Fig.10c shows the number of survivors plotted against dose for each of the three regimes. From these graphs it was estimated that the LD 50₍₃₀₎ for each regime was as follows

Single dose, $6200 \pm 200r$

4 fractions in 3 days, $10800 \pm 800r$

4 fractions in 21 days, $16100 \pm 500r$

10. 2. 4. Discussion

The LD 50's observed for single doses and 4 fractions in 3 days were much higher than the corresponding values of 4470r and 6780r found by Jefferies and Banham (1961). The latter workers had housed and irradiated their weevils on wheat rather than flour, had employed much higher culture densities and had not controlled their irradiation temperature. The differences in the observed results are therefore not surprising, especially when the changes in susceptibility known to be produced by changes in culture density or temperature (7.2.) are considered.

It will be observed that the LD 50 for 4 fractions in 3 days lies outside the range of dose levels used for this regime. The value of the pilot experiments may be judged from the fact that this state of affairs never arose in any of the 63 different irradiation regimes for which the LD 50 was measured in subsequent experiments.

Analysis of the results of the pilot experiments failed to show any significant or consistent variation in susceptibility with age over the range studied. The effect of age is discussed more

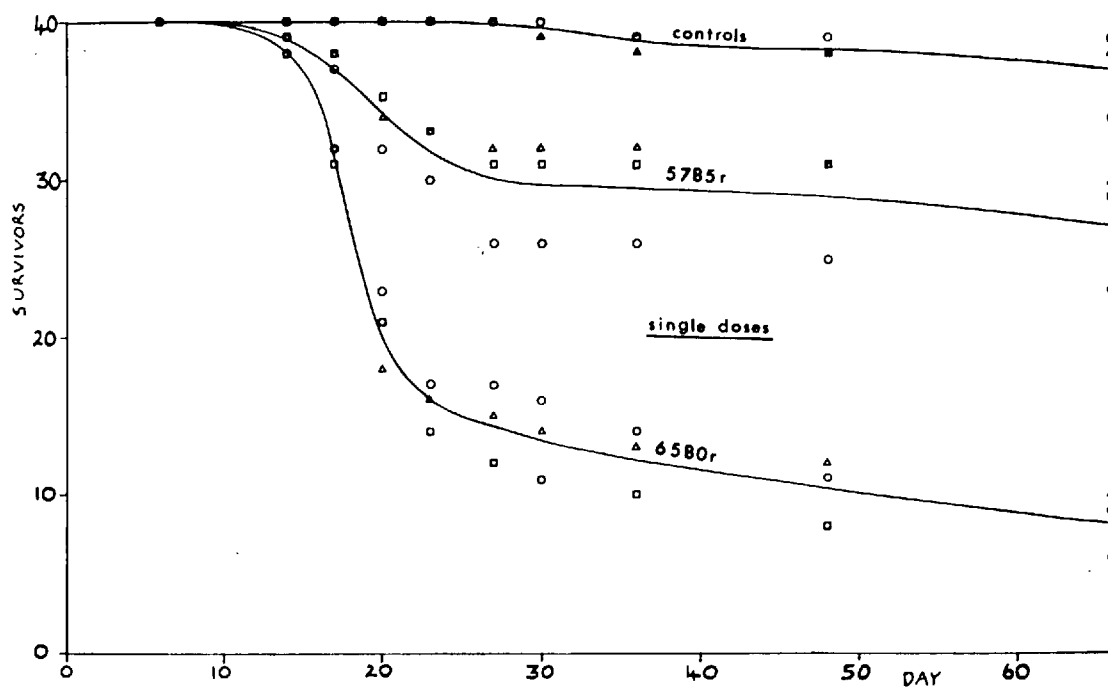


Fig. 10a Pilot Experiments. The number of survivors plotted against time after single doses of 5785 or 6580 rads

- 40 weevils 14-18 days old
- 40 weevils 18-21 days old
- △ 40 weevils 21-24 days old

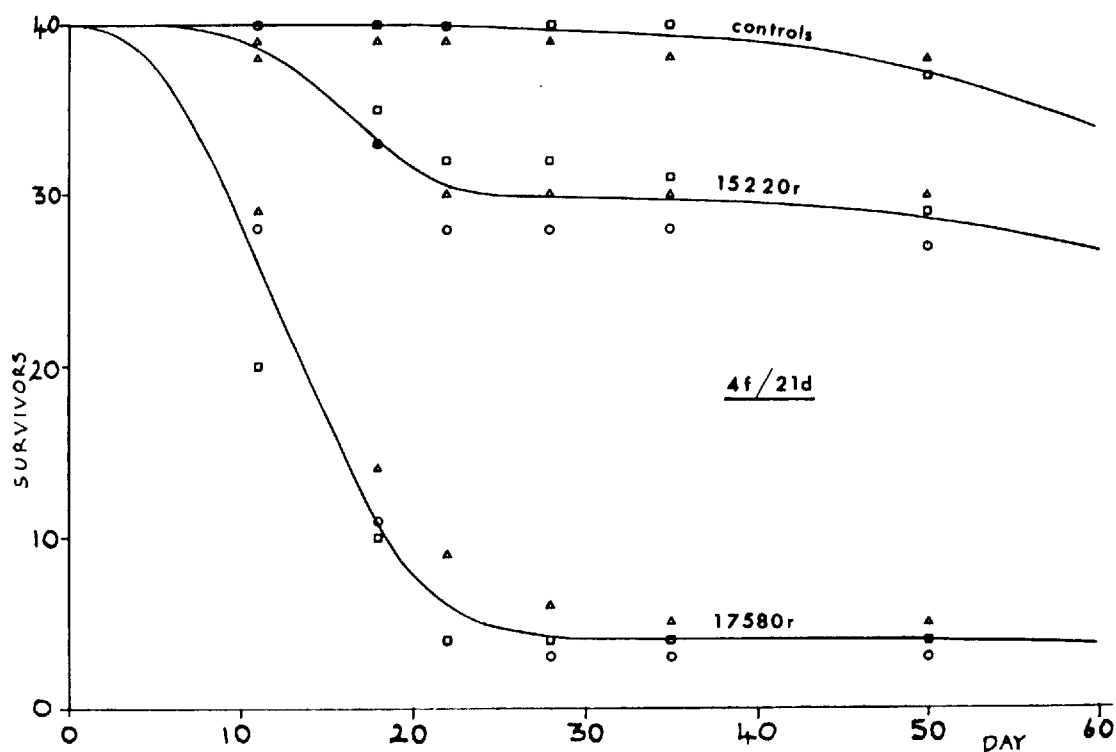


Fig. 10b Pilot Experiments. The number of survivors plotted against time after total doses of 15220 or 17580 rads delivered in 4 fractions in 21 days. Day 0 was taken as the day which was three-quarters through the irradiation regime, as previously discussed (8.4)

- 40 weevils 14-18 days old
- 40 weevils 18-21 days old
- △ 40 weevils 21-24 days old

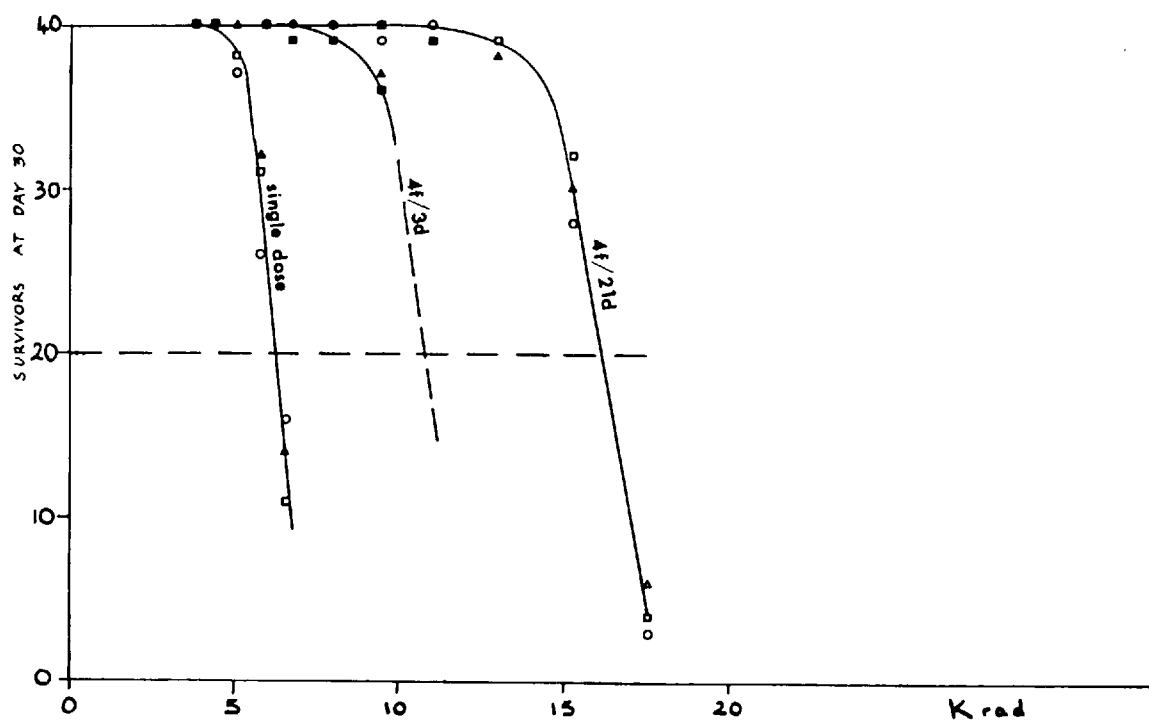


Fig 10c Pilot Experiments. The number of survivors at day 30 plotted against total dose for regimes involving either single doses, 4 fractions in 3 days or 4 fractions in 21 days.

○ 40 weevils 14-18 days old

□ 40 weevils 18-21 days old

△ 40 weevils 21-24 days old

fully later (13.3.)

The pilot experiments suggested that the LD 50 increased with fractionation at the rate of approximately 1500 rads per daily fraction and this provided a useful guide to the dose levels to be given in the main daily fractionation experiments. These showed the increase to be 1900 rads per fraction on changing from 1 to 2 daily fractions but only 1320 rads per fraction on changing from 14 to 20 daily fractions (Chapter 17).

The pilot experiments also suggested that, for a constant number of fractions, the LD 50 increased with overall irradiation time at a rate of approximately 300 rads per day. This provided a useful guide to the dose levels to be given in the main fractionation experiments employing long intervals (up to 9 days) between fractions. The results of the main experiments (Chapter 16) were in close agreement with the value of 300 rads per day when the interval between fractions was increased from 1 to 7 days but gave values close to 600 rads per day when the interval between fractions was only increased from 1 to 2 days. Fortunately, in the latter case, the total increase was relatively small and in estimating the range of dose levels for the main experiments a suitable margin was included to allow for some uncertainty in prediction.

10. 3. Decision as to whether work should continue or not.

The ideal system for testing the General Formula (Chapter 4) would be one exhibiting a large effect due to the recovery of sub-lethal damage and no effect due to repopulation. The pilot experiments suggested that the proposed system exhibited a large effect due to fractionation (1500 rads per daily fraction) and a relatively small but not insignificant effect due to repopulation (300 rads per day). Thus, the proposed system seemed likely to provide a practical and suitable

means of testing the General Formula provided appropriate corrections for repopulation could be accurately determined.

The pilot experiments employing four fractions showed that increasing the overall time from 3 to 21 days caused an increase in the LD 50 of 49%. This is a much larger increase in iso-effect dose than that found for skin reactions by Fowler, Morgan, Silvester, Bewley and Turner (1963). These workers found an increase of only 17% on changing from 5 fractions in 4 days to 5 fractions in 28 days. The proposed system therefore appeared to be an excellent one in which to study the form of relationship between iso-effect dose and overall time (5.4).

In the light of the above findings it was decided that the main experimental work should continue as planned but additional experiments to elucidate the exact nature of the repopulation correction were carried out.

CHAPTER 11

THE MAIN EXPERIMENTS

11. 1. General Considerations

11. 1. 1. Planning of dose levels for different regimes

In order to choose appropriate dose levels for each of the irradiation regimes studied it was necessary to predict the LD 50 for each regime. For the fractionated experiments this was done by interpolating and extrapolating the results of the pilot experiments (and others as they became available). Two alternative assumptions were made. Firstly it was assumed that the curves obtained by plotting either iso-effect dose against number of daily fractions or iso-effect dose (for a constant number of fractions) against overall time would be straight lines on linear graph paper. Secondly, it was assumed that straight lines would be applicable if double logarithmic graph paper were used instead (Ellis 1967). For the preliminary continuous irradiation experiments (11.4.1.) predictions were based on the application of the General Formula to the fractionated results already obtained. For this purpose the half-life of the dose equivalent of sub-lethal damage was assumed to be 1, 1.5 or 2 hours. For the main continuous irradiation experiments (11.5) the results of the preliminary experiments (11.4.1) were also used.

For each regime a range of dose levels was then chosen which would overlap the predicted values of the LD 50 by a margin of approximately $\pm 18\%$. This margin was chosen to take into account inherent uncertainties (say $\pm 5\%$) and the desire to obtain information at the 10% and 90% levels of survival. The latter required a spread of $\pm 13\%$ in dose as judged from the pilot experiments (Fig.10c) It was generally found that the range of dose levels could be obtained by irradiating weevils on 5 consecutive shelves of the

irradiation cabinet. This number was reduced to 3 where there was little uncertainty involved and increased to 7 where there was a large uncertainty in the predicted LD 50. To obtain the required degree of statistical accuracy 5 pots of 40 weevils were irradiated at each dose level. When only 3 shelves were needed the number of dose levels was sometimes increased by irradiating 5 pots in each of two positions on the intermediate shelf (e.g. 5 pots in Group 1 holes and 5 in Group 5 - Table 2, Chapter 9).

11. 1. 2. Numbers involved and timing

From the above it will be observed that for each irradiation regime approximately 1000 weevils (25 pots of 40) would be required.

The constant temperature room would house up to 330 honey jars (13,200 weevils) so that the number of regimes which could be investigated at any one time was approximately 13. The experiments were therefore split into a number of groups detailed below.

(11.2 to 11.8) The irradiation regimes studied (77 including replicates) are listed in Chapter 12.3. (Table 3).

The special cultures required to produce weevils for irradiation needed to be prepared approximately 60 days before irradiation (8.3.2.), irradiation regimes lasted up to 28 days and counting continued for at least another 35 days. Thus approximately 4 months elapsed between initiating a group of experiments and obtaining their results.

11. 1. 3. Single dose controls for every irradiation

Because it had been decided (7.3.2.) that the single dose LD 50 should be determined for each batch of cultures, an appreciable number of weevils in each group of experiments were used as irradiated controls. These weevils required single doses of the

order of 6000r and required to be placed on the upper shelves of the irradiation cabinet. During multiple fraction experiments these weevils were sub-divided; some being irradiated during each irradiation period. In this way any large and influential variation in irradiation conditions from day to day would be apparent from the survival of the irradiated controls.

11. 1. 4. Irradiation Schedules

To ensure that the ^{60}Co Unit and the requisite space in the irradiation cabinet would be available at the required time detailed advanced planning of these experiments was essential. Irradiation schedules, showing the date, exposure time and the position to be occupied by each specimen pot, were prepared two or three months in advance (i.e. before preparing the necessary cultures) (8.3.2.)

11. 2. The June 1970 Experiments: The split-dose recovery curve.

This group of experiments involving 11,320 weevils had a single aim - namely the production of a split-dose recovery curve. This was needed for the reasons already discussed (6.1) and involved finding the LD 50 for weevils given two equal doses of radiation separated by intervals which varied, in 2.5 hour increments, from 0 to 25 hours. The number of weevils involved was too large to permit the first of these two doses being delivered simultaneously to all weevils. By carefully planning the irradiation schedules, it was found that all weevils could receive their required doses by placing them in the cabinet during two out of a total of six irradiation periods. The six irradiations were performed at 0, 5, $7\frac{1}{2}$, $12\frac{1}{2}$, $22\frac{1}{2}$ and 25 hours after the start of the experiment., (e.g. the weevils requiring a 15 hour split-dose interval were irradiated during the 3rd and the 5th periods).

The results of all the experiments are presented in Table 3 (12.3) and discussed in Section 4.

11. 3. The November 1970 Experiments

This group of experiments involving 13,160 weevils was designed to study the three effects referred to below.

11. 3. 1. Variation of LD 50 with "daily" fractionation

These experiments were designed to determine how the LD 50 varied with the number of fractions when these were given at the rate of five fractions per week. It was decided to use 6 irradiation regimes namely 1, 4, 6, 9, 14 and 20 fractions; the LD 50 for 2 fractions being obtainable from the split-dose experiment (11.2). The need for this information has already been discussed (6.1.).

11. 3. 2. Variation of LD 50 with overall time

These experiments were designed to determine how the LD 50 for either 4 or 9 fractions varied with overall time (6.1.) It was decided to use six 4-fraction regimes having overall times of 3, 7, 10, 14, 21 and 28 days and to use three 9-fraction regimes having overall times of 10, 18 and 28 days. It was also decided to find the LD 50 for 2 fractions in 3 days so that the 2-fraction data (11.2) could be extended.

11. 3. 3. Variation of LD 50 with age and pre-storage

It was realised that in the experiments referred to above (11.3.1 and 11.3.2) some of the weevils would be 28 days older and would have been stored in plastic pots for 28 days longer when they received their last irradiation than weevils which received all their dose on the first day. In order to determine whether the susceptibility of weevils was affected by age or pre-storage ov

the above range an exceptionally large number (2040) irradiated controls were used in these experiments. 800 of these were given single doses during the first five days, a similar number during the last five days, and the remainder on the intermediate days of the 28 day irradiation period.

11. 4. The January 1971 Experiments

This group of experiments involving 7,800 weevils was designed to study the two effects referred to below.

11. 4. 1. Preliminary low dose-rate continuous irradiation

The main low dose-rate experiments which were planned for Easter 1971 (11.5) would not be repeatable until Easter 1972 when the ^{60}Co Unit would again be available for the necessary period of 106 hours. It was therefore important that the dose levels chosen for the main experiments should cover the required ranges by a safe but not extravagant margin. If too many dose levels were employed for one regime the capacity of the irradiation cabinet would restrict the number of regimes which could be investigated during the Easter period.

It was therefore decided to do preliminary experiments using continuous irradiation to determine the LD 50 precisely for irradiation periods of 7, 15, 36 and 58 hours, the latter being the longest period for which the ^{60}Co Unit would be available during a normal week-end. (The unit is used for treating patients on week days).

11. 4. 2. The effects of post-storage

Because of the way Day 0 was defined (8.4) weevils receiving irradiation regimes of 28 days duration could not be transferred to honey jars until Day 7. In order to adopt a standard

procedure weevils were always transferred on Day 7 with the exception of those used in the pilot study and in the split-dose recovery curve experiments which had been completed before this limitation had been realised. In these experiments weevils had been transferred to honey jars within three days of their irradiation regimes being completed. To determine whether the single dose LD 50 was affected by the duration of post-irradiation storage in plastic pots 3800 weevils, including unirradiated, controls were used. These were sub-divided into four groups which were transferred to honey jars on Day 1, Day 4, Day 7 and Day 14. The LD 50 for each of these groups was determined separately. The weevils transferred on Day 7 were used as irradiated controls for the continuous experiment (11.4.1)

11. 5. The Easter 1971 experiments: The main low dose-rate irradiation

This group of experiments involving 6000 weevils was designed to determine how the LD 50 varied with the duration of continuous irradiation over the range 1 to 106 hours (6.1). Weevils were sub-divided into groups and irradiated to appropriate dose levels for 1.25, 6, 14, 22, 38, 54, 68, 84, or 106 hours.

11. 6. The April 1971 Experiments

This group of experiments involving 4920 weevils was designed to answer questions raised by the results of the earlier experiments.

11. 6. 1. Comparison of 4 fractions in 1 day with 4 fractions in 3 days

The experiments (11.3.2) to determine how the LD 50, using a constant number of fractions, increased with overall time was designed with the object of obtaining information which would enable the daily fractionation data to be corrected for the effect of repopulation. These experiments showed that the rate of the repopulation effect (expressed as rads per day)

decreased appreciably as the interval between fractions was increased. To determine the correction applicable to daily fractionation the observed data needed to be extrapolated backwards to zero time interval, and, when this was done, suggested that the initial rate of the repopulation effect was equivalent to about 800 rads/day. This was a larger correction than originally envisaged and unreliable in that the extrapolation had to be extended to a region likely to be greatly affected by radiation induced mitotic delay. To reduce the amount of extrapolation it was decided to extend the observed data to include fraction intervals of 8 hours. This was done by determining the LD 50 for 4 fractions in 1 day and comparing this directly with the LD 50 for 4 fractions in 3 days.

11. 6. 2. The effect of a gap at the end or in the middle of an irradiation regime

When the observed LD 50's for the daily fractionation regimes (11.3.1) corrected for the effect of repopulation using data obtained from (11.3.2.) were plotted against number of fractions, the point obtained for 6 fractions in 7 days was found to be 1200 rads lower than that estimated from the smooth curve passing through the other points. Examination of all the "daily" (i.e. 5 fractions/week) regimes revealed that the 6 fractions in 7 days regime was the only one used in which a 3-day gap (Friday to Monday) had occurred immediately before the final fraction. Several of the "daily" fractionation regimes involved gaps of 3 days but these were much more symmetrically distributed throughout the regimes and were always followed by a further 4 or 5 fractions. It was therefore decided that the LD 50 should be determined for 6 fractions in 5 days and 6 fractions in 7 days with a 3-day gap between the 3rd and 4th fractions instead of between the 5th and 6th fractions.

11. 6. 3. An additional daily fractionation experiment

The above experiment (11.6.2.) involved irradiating specimens every day for eight consecutive days. The opportunity was therefore taken of determining the LD 50 for 9 truly daily fractions (i.e. 9 fractions in 8 days).

11. 7. The October 1971 experiments

These experiments involved 12,800 weevils and had two objectives discussed below.

11. 7. 1. The LD 50 for 4 fractions in 9 hours

This experiment was included as a further extension of the 4 - fraction data referred to above (11.6.1.) It was hoped that this experiment would provide useful information regarding the repopulation effect, if any, occurring between 3 and 8 hours after irradiation.

11. 7. 2. Doses of unequal magnitude

The results of experiments (11.3.2.) and (11.6.1.) showed that the rate of the repopulation effect (expressed as rads/day) decreased exponentially with time (16.4.) The magnitude of the total effect occurring during a multi-fraction regime and the rate of the exponential decrease suggested indirectly that the instantaneous rate of repair (rads/day) might be approximately proportional to the damage present (expressed as rads). To test this simple hypothesis and to discover whether the rate of repair was a function of the damage present, experiments were designed to measure the rate of repair occurring after initial doses of different magnitudes.

Weevils were irradiated with an initial dose D_A rads and t_g days later subjected to a second dose D_B . By keeping D_A and t_g constant and varying D_B the total dose ($D_A + D_B$) necessary to kill 50% of the weevils in 30 days was determined. In this way the LD 50₃₀ was determined for five values of D_A ranging from 1000 to 5000 rads and

three values of t_g , namely 1.03, 3.83 and 8.14 days. The change in LD 50 with time interval enabled estimates to be made of the rate of repair occurring after each initial dose. From the above it will be seen that the LD 50 needed to be determined for 15 different irradiation regimes and this required a total of 12600 weevils.

11. 8. The January and February 1972 Experiments: The effect of shortening the irradiation time during acute fractionated regimes

This group of experiments involved a total of 8600 weevils.

The acute irradiation time of 71-79 minutes used in all the above experiments was longer than desirable as it was considered that an appreciable amount of sub-lethal damage recovery could have occurred during the course of each acute fraction. The installation of the Mobaltron 80 Unit (9.1) enabled LD 50 doses to be given in irradiation times of the order of 25 minutes. It was therefore decided that all the daily fractionation experiments referred to in (11.3.1.) should be repeated using the shorter irradiation time. The above regimes included 9 fractions in 10 days and, by also including 9 fractions in 28 days, it would be possible to assess the change that such a shortening would have on corrections for repopulation effects. Irradiated controls would be irradiated for both the long and the short irradiation times thus enabling the single dose LD 50 to be determined under both conditions. It was decided that all these experiments could be done as a single group. Unfortunately the supply of Argentine Plate Wheat which had been used for all cultures up to this time (8.3.) was running low and fresh supplies were no longer available. It was therefore decided that Australian Wheat would need to be used for some of the experiments and these would require their own irradiated and unirradiated controls. The regimes were therefore sub-divided into two batches - one to use Argentine Plate Wheat, the other Australian Wheat. These experiments were planned to

run concurrently in January 1972 but twelve days after being placed to oviposit on the cultures made from Australian Wheat the weevils involved were found to be dead - presumably due to insecticide contamination. Because of this, the second batch of experiments had to be delayed until February. Fresh cultures were prepared for the February experiments using Manitoba Wheat kindly supplied by the Pest Infestation Laboratory. The regimes employed in each batch are listed in Table 3 (12.3.)

CHAPTER 12

THE RESULTS

12. 1. The observed results

The survival at Day 30 in each pot was corrected for natural mortality using the results of the appropriate unirradiated controls and the formula of Abbott (1925)

For each irradiation regime the average corrected percentage survival at each dose level was plotted against the logarithm of dose on probability graph paper. The best fitting straight line (as judged by eye) was then drawn through the plotted points. The dose corresponding to 50% corrected survival on the eye-line will be referred to as the eye-line LD 50₍₃₀₎ and the values obtained are compared in Table 3 with those obtained using a computer program described below.

12. 2. The estimation of the LD 50 values by probit analysis

For each irradiation regime the probit regression line and the 95% fiducial limits of the LD 50 and slope were estimated using the method of maximum likelihood (Finney 1952). This is an iterative method which starts by accepting a provisional regression line (usually the eye-line) as the basis of the calculations. This provisional line determines the weighting factors to be allocated to the individual results. The first cycle of calculations enables a more accurate regression line to be found and this is used as the starting point for the next cycle. Provided the probits of the observed data plotted against the logarithm of dose lie close to a straight line there is little difficulty in deciding where the regression line should be drawn. Little change in the position of the regression line is produced by the first cycle of calculations and even less by the second.

The introduction of computer techniques enables a much larger number of cycles to be carried out without additional effort and eliminates any

subjective errors which might otherwise be caused by the investigators choice of eye-line.

The computer program written in basic language by Mr R G Dale consists of three parts. The first part (Fig 12a) determines the parameters of the most likely probit regression line, the second part (Fig 12b) performs a χ^2 test to determine how well the observed data fits this line and calculates the heterogeneity factor (Finney 1952), the third part (Fig 12c) calculates the 95% fiducial limits on both the LD 50 and the slope of the probit regression line.

The program assumes originally that the provisional regression line is a horizontal straight line. By repeating the cycle of calculations the computed regression line is moved closer to the line of maximum likelihood. Ten such cycles were used routinely but it was found from experience that four or five would probably have been sufficient as little or no change in the parameters of the regression line normally occurred after the fourth or fifth cycle.

All the observed data, including those for pots having zero or 100% survival, were used in obtaining the regression line. The χ^2 test is unreliable when applied to test the discrepancy between observation and prediction if, in any pot, the number of specimens expected to respond (or not to respond) is less than five (Finney 1952). Thus in part 2 of the program (Fig 12b) the results obtained with such pots were pooled with those of others irradiated to the same dose level, before applying the χ^2 test. If pooling all the data for the given dose level failed to raise the number to above 5, the program ignored the data for that dose level in compiling the value of χ^2 applicable to that regime and a warning was printed stating that this was so.

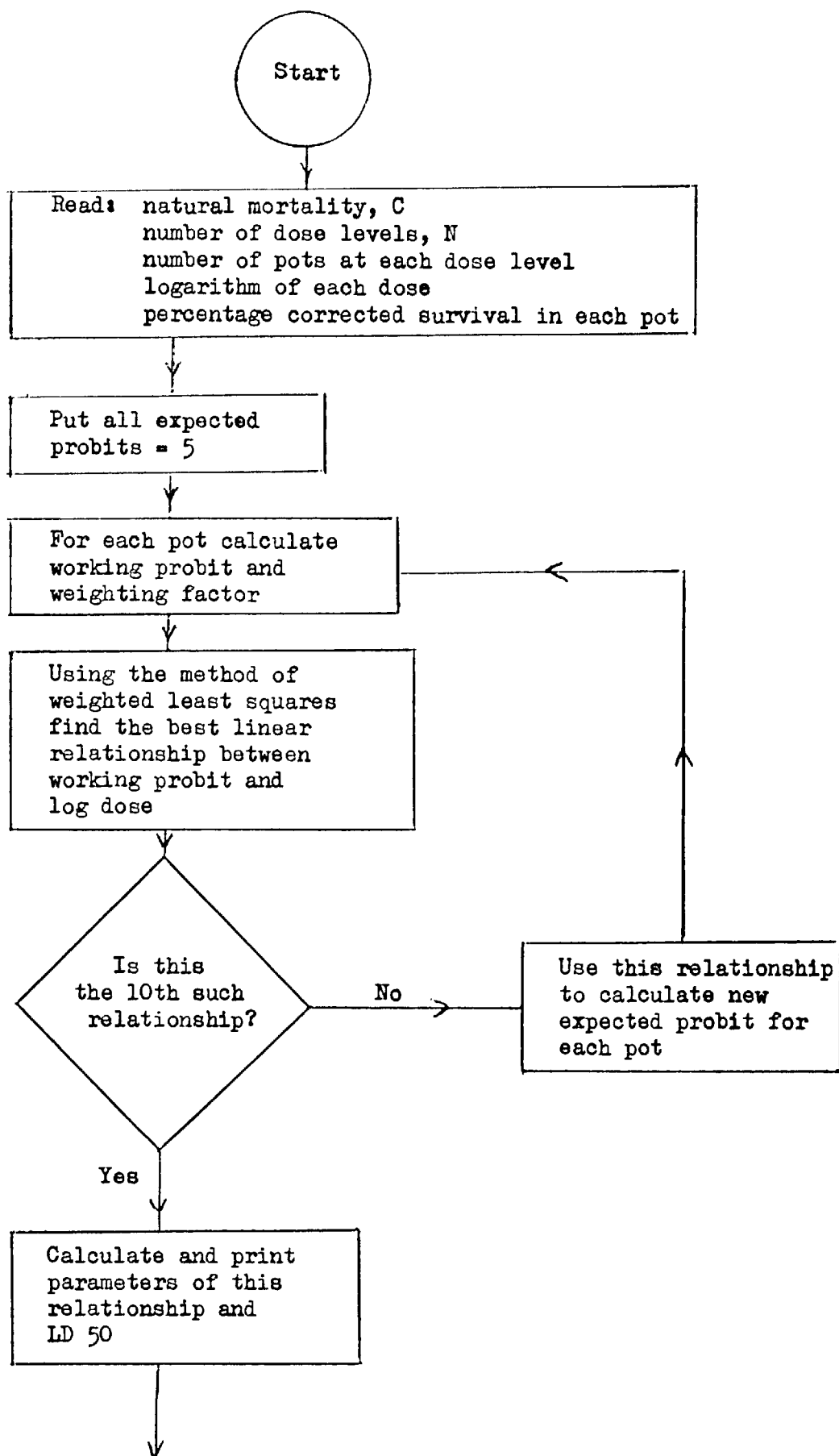


Fig 12a

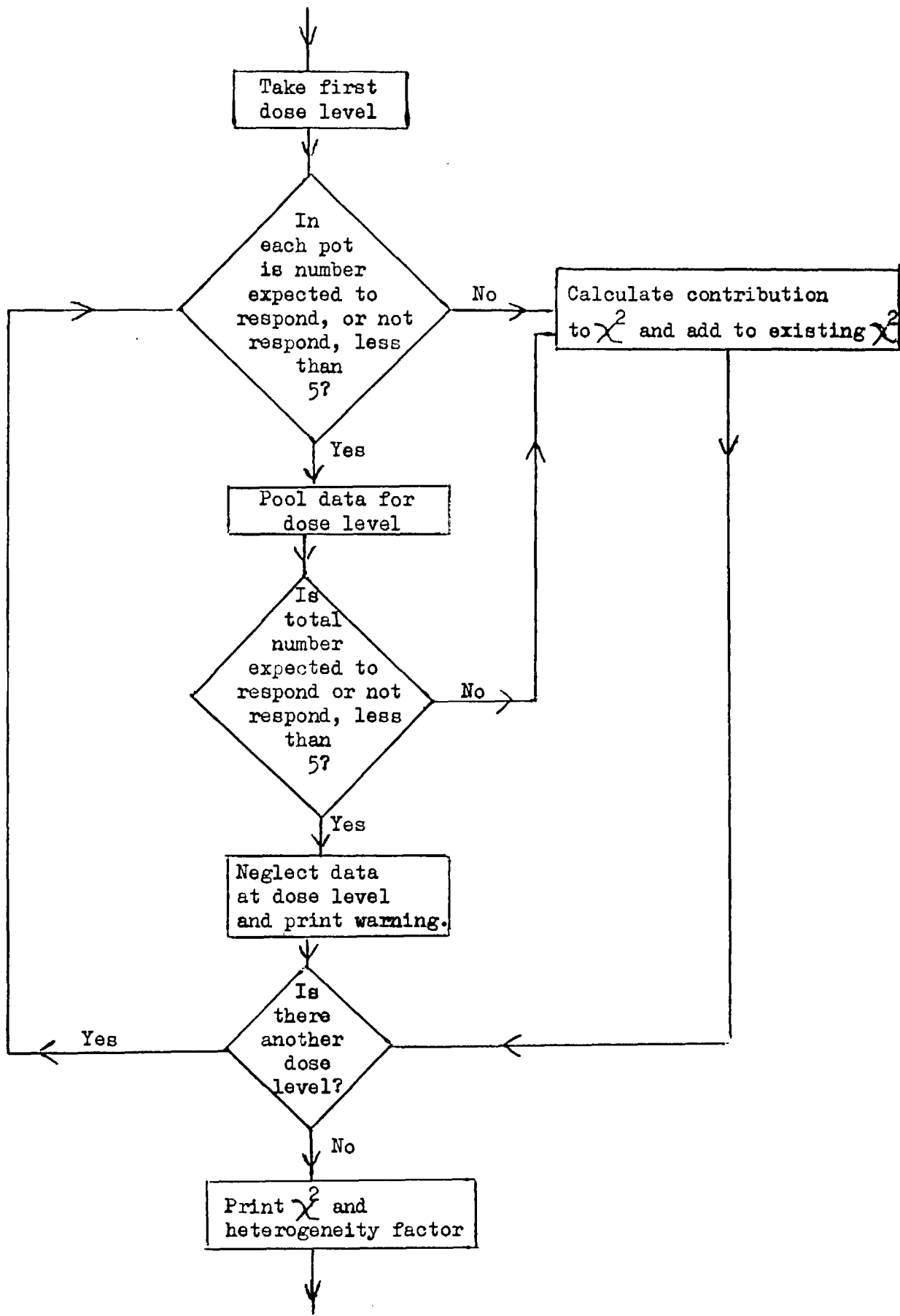


Fig 12b

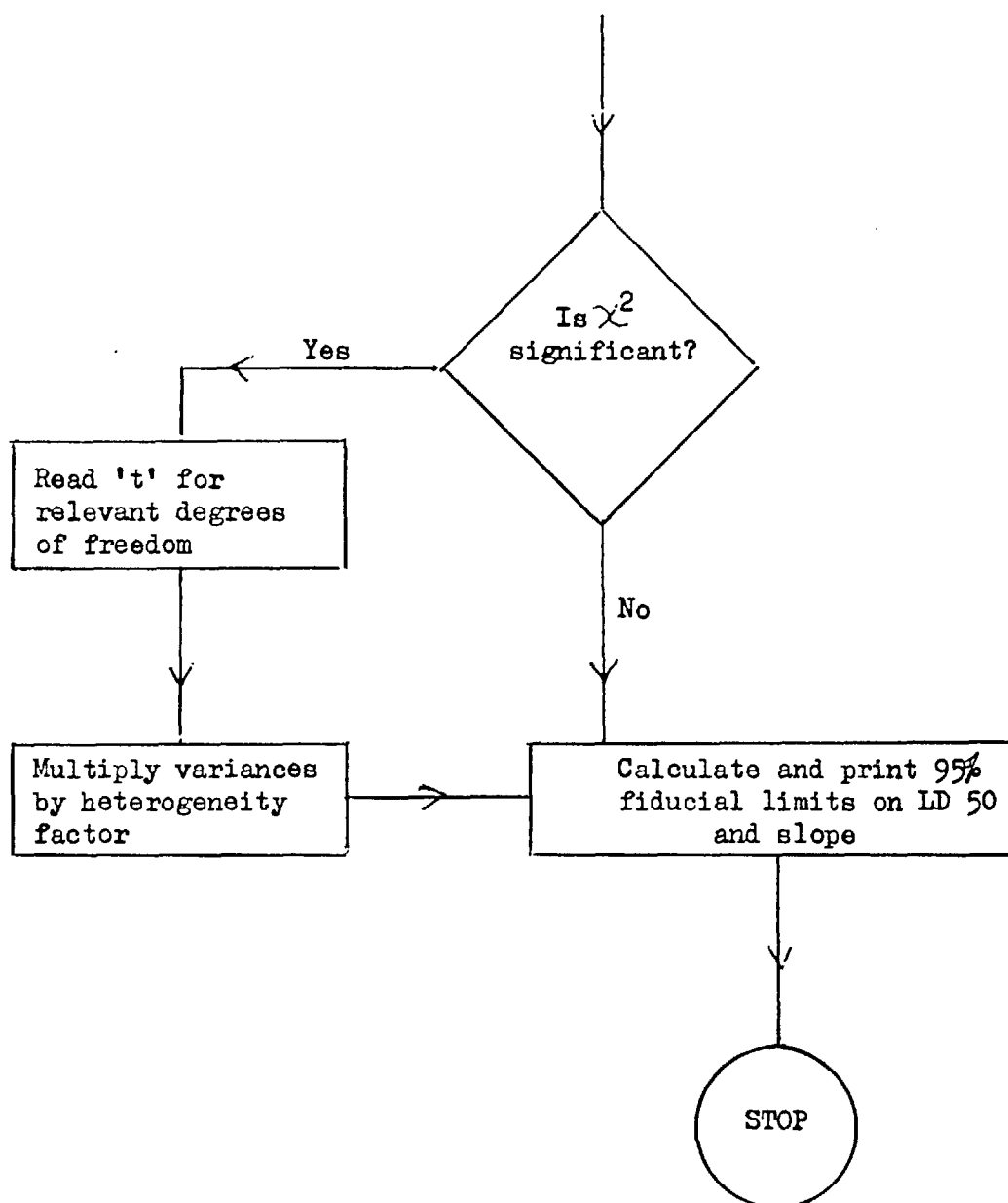


Fig 12c

All such data were then examined to see whether the discrepancy between predicted and observed survival was abnormally large. The greatest discrepancy found was for the 20 fraction in 25 days regime in which, at one of the six dose levels used, 24 weevils out of 200 died instead of the predicted 16. The latter number consisted of 12 which were expected to die from natural causes (by comparison with the unirradiated control group) and 4 which were expected to die from the radiation. The value of χ^2 for this individual result is 4.4. On statistical grounds a discrepancy of this magnitude would be expected to occur in 4% of the observations.

The 20 or 30 pots involved in each treatment regime had been uniformly distributed in the constant-temperature room which was known to have a temperature gradient from one side to the other (8.1.3.) In calculating the 95% fiducial limits it was essential to take into account any heterogeneity arising from this or other causes. Wherever χ^2 was found to be significant at the 95% confidence level all variances were therefore multiplied by the heterogeneity factor as suggested by Finney (1952). The third part of the computer program (Fig 12c) performed the appropriate calculation and printed out the requisite 95% fiducial limits.

The program was carried out on the Leasco Time Sharing System employing a Hewlett Packard 2000A Computer operated on line from a Westrex terminal. The data ^{were} fed in using punched tape.

12. 3. The LD 50 values obtained for each regime

The results of all the experiments described in Chapter 11 are presented in Table 3. Examination of this table shows that the maximum discrepancy between the eye-line LD 50₍₃₀₎ and the computed LD 50₍₃₀₎ for any regime is 1.1% (experiment No 76). In general, the

TABLE 3

Group	Ref No	Regime	LD 50(30)		95% F.L. %	Normalised LD 50(30) and 95% F.L.(rads)	Remarks
			Eye Line	Computed			
June 1970	1	S.D.	6280	6270	0.8	5900	The weevils in this group had 1 - 4 days post-storage in plastic pots instead of the usual 7 days (See Ref No 27-30).
	2	2.5 h split	7740	7740	2.3	7280 + 170	
	3	5.0 " "	8430	8400	3.0	7900 + 240	
	4	7.5 " "	8430	8480	2.8	7970 + 230	
	5	10.0 " "	8320	8290	1.5	7800 + 130	
	6	12.5 " "	7910	7880	1.3	7410 + 110	
	7	15.0 " "	8090	8150	1.2	7660 + 110	
	8	17.5 " "	7870	7850	3.1	7380 + 240	
	9	20.0 " "	8020	8030	1.3	7550 + 110	
	10	22.5 " "	8150	8140	2.8	7650 + 220	
	11	25.0 " "	8340	8390	3.7	7890 + 300	
Nov 1970	12	S.D.	5920	5910	1.3	5900	Irrad. 1st week Irrad. 4th week 3d gap at end
	13	S.D.	5750	5730	2.7	5900	
	14	2F/3d	8730	8750	3.5	8740 + 320	
	15	4F/3d	10420	10450	1.4	10440 + 200	
	16	6F/7d	13460	13420	3.1	13410 + 460	
	17	9F/10d	17420	17530	3.1	17680 + 730	
	18	14F/17d	23990	24040	1.2	24240 + 730	
	19	20F/25d	31260	31530	3.2	32130 + 1350	
	20	4F/7d	12710	12670	1.3	12660 + 230	
	21	4F/10d	13870	13810	1.4	13920 + 420	
	22	4F/14d	15100	15150	2.4	15280 + 550	
	23	4F/21d	16330	16310	4.0	16620 + 800	
	24	4F/28d	16870	16850	4.4	17160 + 890	
	25	9F/18d	22810	22900	4.5	23330 + 1210	
	26	9F/28d	24660	24620	3.6	25080 + 1130	
Jan 1971	27	S.D.	5860	5890	1.1	6110 + 200	1d post-storage 4d " " 7d " " 14d " "
	28	S.D.	5920	5930	4.0	6150 + 310	
	29	S.D.	5710	5690	3.1	5900	
	30	S.D.	5500	5490	4.8	5690 + 320	
	31	7h cont.	7930	7920	3.1	8210 + 360	
	32	15h "	9590	9550	2.6	9910 + 400	
	33	36h "	11330	11350	5.4	11760 + 730	
	34	58h "	13340	13330	4.4	13820 + 750	

TABLE 3 (CONTINUED)

Group	Ref No	Regime	L D 50(30)		95% F.L %	Normalised L D 50(30) and 95% F.L.(rads)	Remarks
			Eye Line	Computed			
Easter 1971	35	S.D.	6140	6130	2.4	5900	
	36	6h cont.	7910	7920	4.5	7620 $\pm$ 390	
	37	14h "	9860	9870	2.3	9490 $\pm$ 310	
	38	22h "	10500	10480	3.2	10080 $\pm$ 400	
	39	38h "	12680	12670	5.9	12190 $\pm$ 780	
	40	54h "	13680	13720	3.6	13200 $\pm$ 570	
	41	68h "	14960	14920	4.5	14360 $\pm$ 730	
	42	84h "	15100	15090	2.6	14520 $\pm$ 510	
	43	106h "	16710	16750	3.1	16120 $\pm$ 630	
April 1971	44	S.D.	5860	5850	1.7	5900	3d gap in middle
	45	4f/1d	9270	9260	2.1	9340 $\pm$ 250	
	46	4f/3d	10490	10520	2.4	10600 $\pm$ 310	
	47	6f/7d	14490	14480	3.1	14600 $\pm$ 510	
	48	9f/8d	16790	16830	1.0	16970 $\pm$ 340	
	49	6f/5d	13030	13040	1.3	13140 $\pm$ 280	
Oct 1971	50	S.D.	5740	5730	3.5	5900	
	51	4f/9h	9250	9260	2.9	9540 $\pm$ 430	
	52	2f/1.025d D _A = 1010	6520	6500	2.1	6690 $\pm$ 270	Normalised values D _A = 1040 D _A = 2130 D _A = 3480 D _A = 4660 D _A = 5240
	53	D _A = 2070	7410	7420	2.4	7640 $\pm$ 320	
	54	D _A = 3380	8050	8040	1.8	8280 $\pm$ 320	
	55	D _A = 4520	7890	7950	2.4	8190 $\pm$ 340	
	56	D _A = 5090	7230	7220	2.9	7430 $\pm$ 330	
	57	2f/3.83d D _A = 1000	6650	6650	1.9	6850 $\pm$ 270	Normalised values D _A = 1040 D _A = 2130 D _A = 3400 D _A = 4610 D _A = 5240
	58	D _A = 2070	7690	7610	3.2	7840 $\pm$ 370	
	59	D _A = 3300	8750	8730	1.9	8990 $\pm$ 360	
	60	D _A = 4480	9040	9040	2.3	9310 $\pm$ 390	
	61	D _A = 5090	8510	8550	2.7	8800 $\pm$ 390	
	62	2f/8.14d D _A = 1010	6950	6960	2.6	7170 $\pm$ 320	Normalised values D _A = 1040 D _A = 2130 D _A = 3450 D _A = 4580 D _A = 5130
	63	D _A = 2070	7870	7860	1.0	8090 $\pm$ 290	
	64	D _A = 3350	9020	9040	1.4	9310 $\pm$ 350	
	65	D _A = 4450	9460	9410	1.7	9690 $\pm$ 380	
	66	D _A = 4980	8320	8360	3.6	8610 $\pm$ 430	

TABLE 3 (CONTINUED)

Group	Ref	Regime	L D 50 (30)		95% F.I. %	Normalised L D 50 (30) and 95% F.L. (rads)	Remarks
			Eye Line	Computed			
Jan 1972	67	S.D. (72')	5610	5620	1.3	5900	Irrad 1st day
	68	S.D. (24')	5060	5070	3.3	5320 + 190	" 1st day
	69	S.D. (25.5')	5140	5140	2.0	5400 + 130	" 28th day
	70	9F/10d	16220	16380	5.0	17190 + 890	26' exp.
	71	9F/28d	22390	22470	3.7	23580 + 920	26' exp.
	72	14F/17d	21280	21340	2.4	22400 + 600	26' exp.
	73	20F/25d	27670	27680	3.5	29050 + 1070	26' exp.
Feb 1972	74	S.D. (72')	5850	5840	2.9	5900	Irrad 1st day
	75	S.D. (24')	5180	5180	3.7	5230 ± 250	" 1st day
	76	4F/3d	9860	9970	3.7	10070 ± 470	26' exp.
	77	6F/7d	13390	13390	3.9	13530 ± 660	26' exp.

computed LD 50₍₃₀₎ values are accurate to ± 3 or 4% at the 95% level of confidence.

In the penultimate column of Table 3, the normalised values, which are referred to in all subsequent analyses were obtained by multiplying the computed LD 50₍₃₀₎ by 5900 and dividing by the single dose LD 50₍₃₀₎ obtained in the same group of experiments. When experiments involved prolonged fractionation (e.g. Nov.1970) the single dose LD 50 was determined at the beginning and end of the group of experiments and an intermediate value (interpolated to Day '0' of the relevant fractionated regime) was used for the purposes of normalisation. Any error caused by this procedure was likely to be small as the variation observed in the single dose LD 50 between the beginning and end of a group of experiments was never more than 3%.

The 95% fiducial limits quoted in the penultimate column were obtained by taking the square root of the sum of the squares of the limits of the computed LD 50 for the relevant regime and those of the corresponding single dose LD 50.

12. 4. The slopes of the probit regression lines

For the 15 single dose experiments listed in Table 3, the average slope of the computed regression lines (probit of survival against logarithm of dose) was found to be 22.2 with a standard deviation of ± 2.8 . This slope is considerably steeper than the corresponding value of 10.8 ± 0.7 obtained by Jefferies and Banham (1961). The difference may in part be attributed to the improved conditions of culture and tighter control of temperature during the present investigation. The present author is indebted to the earlier workers for discovering the importance of these factors (7.2).

For the 12 low dose-rate continuous irradiation regimes the average slope of the computed regression lines was found to be 18.8 ± 2.4 . The corresponding average for the 35 fractionated regimes was found to be 24.9 ± 4.1 . There are theoretical reasons for expecting the former average to be lower and the latter average to be higher than that for single doses (Chapter 17).

SUMMARY OF SECTION 3 (CHAPTERS 10-12)

THE EXPERIMENTS AND THEIR RESULTS

Preliminary experiments demonstrated that the natural mortality of weevils was little affected by storage for 7 weeks in the small plastic pots to be used in the main experiments.

Pilot irradiation experiments, carried out under the conditions to be used in the main experiments, revealed that the single dose LD 50 was appreciably higher than that found by previous workers under somewhat different conditions. These experiments also provided information on how the LD 50 was likely to increase with the number of daily fractions and with the overall irradiation time. This information was used to predict the dose levels required in the main experiments. The latter were designed to obtain an accurate and comprehensive body of iso-effect data for fractionated acute and low dose-rate continuous irradiation. Particular attention was paid to the effect of repopulation and how this varied with fraction interval and the amount of damage present. Altogether, 63 different irradiation regimes were investigated.

For each regime the LD 50₍₃₀₎ was determined using both a simple graphical method of analysis and a computer program written by Mr R G Dale employing probit analysis (Finney 1952). The values of the LD 50₍₃₀₎ determined by the two methods agreed in every instance to within $\pm 1.1\%$. The main advantage of the computer analysis was that it greatly facilitated the determination of the 95% confidence limits. As will be seen from Table 3 (12.3) these were usually less than ± 3 or 4%.

SECTION 4ANALYSIS OF RESULTS.CHAPTER 13CONSIDERATION OF UNDESIRABLE EFFECTS WHICH MIGHT INFLUENCE
THE OBSERVED LD 50 VALUES.

- 13. 1. Variation of susceptibility between groups of experiments.
- 13. 2. Choice of Day 0 for prolonged treatment regimes.
 - 13. 2. 1. Variation of time of death with duration of regime.
 - 13. 2. 2. Estimated error in observed LD 50₍₃₀₎ due to choice of Day 0.
- 13. 3. Variation of LD 50₍₃₀₎ with age and pre-storage in plastic pots.
- 13. 4. Variation of LD 50 with period of post-storage in plastic pots.

CHAPTER 14.THE SPLIT-DOSE RECOVERY CURVE FOR WEEVILS.

- 14. 1. The observed results.
- 14. 2. Factors governing the shape of split-dose recovery curves.
- 14. 3. Comparisons of the split-dose curve for weevils with that obtained by Sinclair for Chinese hamster cells.
 - 14. 3. 1. The initial rise due to intracellular recovery.
 - 14. 3. 2. Fluctuations due to progression.
 - 14. 3. 3. The repopulation effect.
- 14. 4. Estimation of γ for the split-dose curve.

CHAPTER 15.THE VARIATION OF THE LD 50 WITH THE NUMBER OF FRACTIONS N
DELIVERED IN A CONSTANT OVERALL TIME T.

15. 1. Iso-effect animal experiments to show the effect
of varying N only.
15. 2. Weevil results demonstrating the dependence of
 D_{N_1T} / D_{N_2T} on the value of T.

CHAPTER 16.VARIATION IN LD 50 FOR CONSTANT N AND VARIABLE T.

16. 1. Attempt to fit results to an Ellis type formula.
16. 2. Attempt to fit results to the Cohen type formula.
16. 3. Attempt to fit results to the Liversage type formula.
16. 4. The search for the true relationship.
 16. 4. 1. The importance of fraction interval.
 16. 4. 2. The effects of mitotic delay.
 16. 4. 3. The shape of the relationship between
repopulation and $(t_s - t_m)$.
16. 5. Discussion.
16. 6. Conclusion.

CHAPTER 17.VARIATION IN LD 50 WITH FRACTION NUMBER.

17. 1. Daily Fractions.
17. 2. The increase in LD 50 due to intracellular recovery
between fractions.
17. 3. The effect of reducing the irradiation time per fraction.
17. 4. The derivation of a dose-response curve for the critical
cells of the weevil.
17. 5. Application of the theory of Lange and Gilbert.
17. 6. Variations of probit slope with regime.
17. 7. Conclusions.

CHAPTER 18.THE RELATIONSHIP BETWEEN THE RATE OF REPOPULATION AND THE DAMAGE PRESENT.

18. 1. Experiments using two doses of unequal magnitude.
18. 2. Predicted results assuming the rate of repopulation to be proportional to the damage present.
18. 3. Comparison of observed and predicted results.
18. 4. The anomalous result for 6 fractions in 7 days.
18. 5. Conclusions.

CHAPTER 19.TESTING THE GENERAL FORMULA.

19. 1. Variation in LD 50 with duration of low dose-rate continuous irradiation.
19. 2. The correction for repopulation.
19. 3. The application of the general formula to the above data.
19. 4. Conclusions.

CHAPTER 20.DISCUSSION OF THE RESULTS AND THEIR IMPLICATIONS IN RADIOBIOLOGY AND RADIOTHERAPY.

20. 1. Comparison of results with mammalian data.
20. 2. The relationship between the rate of repopulation and the damage present.
20. 3. The relationship between iso-effect dose and overall time of a fractionated regime.
20. 4. The relative importance of intracellular recovery and repopulation.
20. 5. The general formula for equating protracted and acute regimes of radiation.

SUMMARY OF CONCLUSIONS.

CHAPTER 13CONSIDERATION OF UNDESIRABLE EFFECTS WHICH MIGHT INFLUENCE THE OBSERVEDLD 50 VALUES13. 1. Variation of susceptibility between groups of experiments

The irradiated controls were used to determine the single dose LD 50₍₃₀₎ for the weevils involved in each group of experiments and was found to be 5900 rads $\pm$ 5% for every group except the June 1970 group which had an LD 50₍₃₀₎ of 6270 rads, (i.e. 6% high). The reason for this group being less susceptible than the average was considered to be mainly due to these weevils having been cultured on shelf R2 instead of shelf L3 (8.3.2.) because the temperature of the latter was 0.5 to 1°C higher than that of the former (The effect of temperature on the susceptibility of weevils is discussed in Chapter 7.2). The variation in storage conditions after irradiation (see below) (13.4) may also have been partly responsible for the relatively low susceptibility.

Variation in the susceptibility of weevils between batches was corrected for by the normalising procedure referred to above (12.3).

13. 2. Choice of Day 0 for prolonged treatment regimes13. 2. 1. Variation of time of death with duration of regime

Throughout the experiments, Day 0 had been defined (8.4.) as the day which was three-quarters through the irradiation regime. If this somewhat arbitrary definition had been well chosen then the interval between Day 0 and radiation death might be expected to be similar for all irradiation regimes. The survivals observed in 38 experiments (Ref Nos 12 - 49) were analysed to see whether this was so. The survivors had been counted at approximately 5 day intervals between Day 7 and Day 37.

For each regime, for the dose levels immediately above and immediately below the LD 50, graphs were drawn plotting survival against time. The interval between Day 0 (as defined above) and the day on which the radiation mortality was 50% of that occurring by Day 30 was determined from each of the 76 graphs. The intervals so determined were all found to lie between 6 and 18 days and 90% between 9.5 and 16.5 days. These results were examined to see whether there was any trend suggesting that this interval was dependent on the percentage surviving or the overall irradiation time. No consistent variation with percentage survival could be detected but it was found that this interval tended to decrease from 16 days to 10 days as the overall irradiation time increased from 0 to 28 days. This suggested that the effective Day 0 and Day 30 for prolonged fractionated regimes may have occurred up to 6 days earlier than had been assumed and that the effective Day 0 occurred closer to half through rather than three-quarters through, the irradiation regime.

13. 2. 2. Estimated error in observed LD₅₀(₃₀) due to choice of Day 0

All the above regimes having overall irradiation times in excess of 10 days were examined to determine the difference between their corrected percentage survivals at 24 days and at 30 days. The maximum difference found was 2.7%. The regimes showing the greatest changes were then further examined to determine the difference between their LD 50(₃₀) and LD 50(₂₄). The maximum difference so found was 0.8%. It was therefore considered that no appreciable error in the determination of the LD 50(₃₀) had resulted from the choice of Day 0.

13. 3. Variation of LD₅₀(₃₀) with age and pre-storage in plastic pots.

Experiments Nos 12 and 13 and Nos 68 and 69 show that there is less than 3% difference in susceptibility between weevils which have been housed in small plastic pots for approximately 4 days and those which have been so housed for 28 days longer and are 28 days older at the time of irradiation. Thus the LD 50(₃₀) observed for prolonged fractionation regimes should be little affected by changes in susceptibility due to ageing and housing during the course of the regime. The method used to normalise the observed LD 50's (12.3) should, however, compensate for any slight changes occurring due to this cause.

13. 4. Variation of LD 50 with period of post-storage in plastic pots

Experiments Nos 27 to 30 show that as the period of post-irradiation storage in plastic pots is increased from 1 to 14 days the observed LD 50(₃₀) decreases by approximately 400 rads (i.e. an average of 30 rads per day). In an attempt to eliminate this source of variation, weevils in subsequent experiments (Nos 31-77) were transferred from plastic pots to honey jars after the same period of post-irradiation storage as that employed in the main fractionation experiments (Nos 12-26) i.e. the weevils were transferred on Day 7.

CHAPTER 14

THE SPLIT-DOSE RECOVERY CURVE FOR WEEVILS

14. 1. The observed results

In experiments Nos 1 - 11 weevils were irradiated with two equal doses separated by intervals of 0 to 25 hours. The LD 50₍₃₀₎ and corresponding 95% fiducial limits determined for each of the split-dose intervals are presented in Table 3 (12.3.) The observed results, normalised in accordance with their appropriate single dose controls as previously described (12.3.) are plotted against interval in Fig 14a. The resultant split-dose recovery curve exhibits similar characteristics to those obtained with mammalian cells and tissues. Before attempting to interpret this curve, the factors which influence the shape of such curves will be considered.

14. 2. Factors governing the shape of split-dose recovery curves

Young and Fowler (1969) suggest that there are three factors which influence the shape of a recovery curve: intracellular recovery, progression and repopulation. Such curves generally show an initial rapid rise in the surviving fraction of cells which reaches a peak in a few hours and is considered to be due mainly to the repair of intracellular sub-lethal damage. The recovery curve may then show a number of peaks and troughs believed to be due to the progression through the division cycle of a partially synchronised population of cells surviving the first dose. When the split-dose interval exceeds the mitotic delay induced by the first dose, the curves show a gradual rise due to repopulation. The first and second effects and the second and third effects overlap and it is consequently difficult to analyse the relative effects of the three components.

Young and Fowler (1969) used a model incorporating experimental data for synchronous and asynchronous Chinese hamster cells (Sinclair,

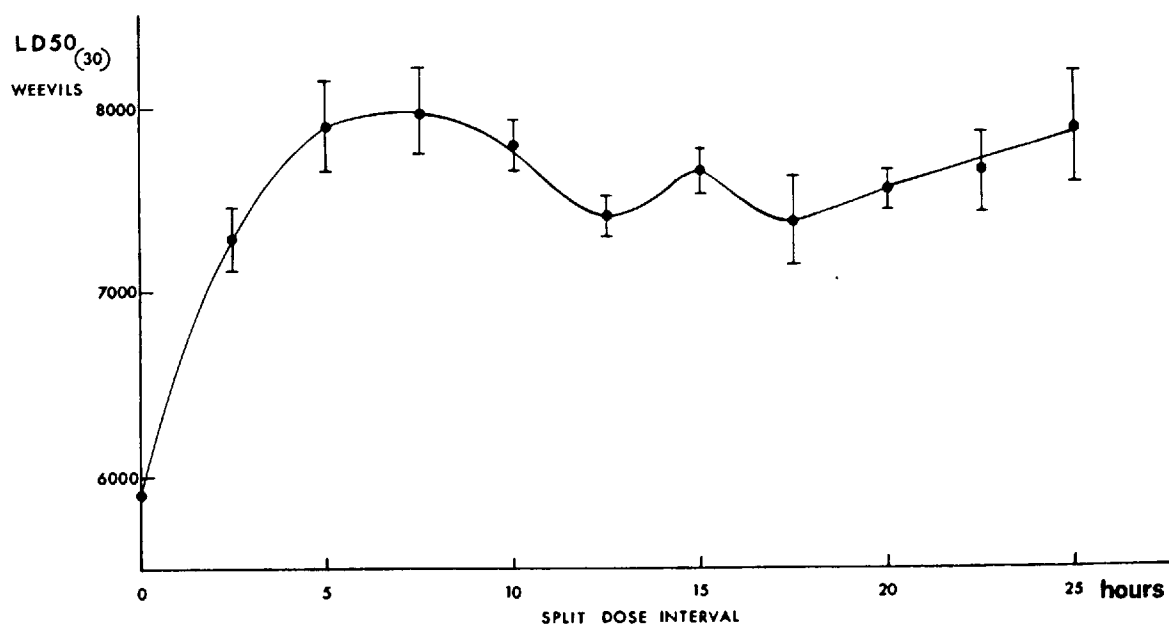


Fig. 14a The observed split-dose recovery curve. The total LD 50₍₃₀₎ for weevils irradiated with two equal doses is plotted against the interval between doses. The limits shown are the 95% fiducial limits estimated by probit analysis as previously discussed (12.2)

1967, 1968) to investigate the effects of progression and repopulation on split-dose experiments. This model assumed that full intracellular recovery occurs before the second dose is given. Thus their predicted split-dose recovery curves showed how the surviving fraction of cells should vary with interval due to progression and repopulation, but could not show the rise due to intracellular recovery. Provided suitable allowance is made for this factor the predictions of this model are in close agreement with the split-dose recovery curve obtained experimentally by Sinclair (1967, 1968) and therefore support the above theories regarding the factors governing the shapes of split-dose recovery curves.

By applying the theory of Lajtha and Oliver (1961), Liversage (1969a) predicted how the surviving fraction of cells in Sinclair's split-dose experiment should vary due to the repair of intracellular sub-lethal damage in the absence of any effect due to progression and repopulation. Fig 14(b) shows how the predictions of Young and Fowler (1969) may be combined with the predictions of Liversage (1969a) to obtain a theoretical split-dose curve which takes into account all three factors discussed above and which is in reasonably good agreement with experiment.

14. 3. Comparisons of the split-dose curve for weevils with that obtained by Sinclair for Chinese hamster cells

14. 3. 1. The initial rise due to intracellular recovery

Both curves show an initial rapid rise but whereas the cell survival curve reaches a peak of survival at between 2 and 3 hours the LD 50 for weevils does not reach a peak until 7 hours. (Figs 14a and 14b).

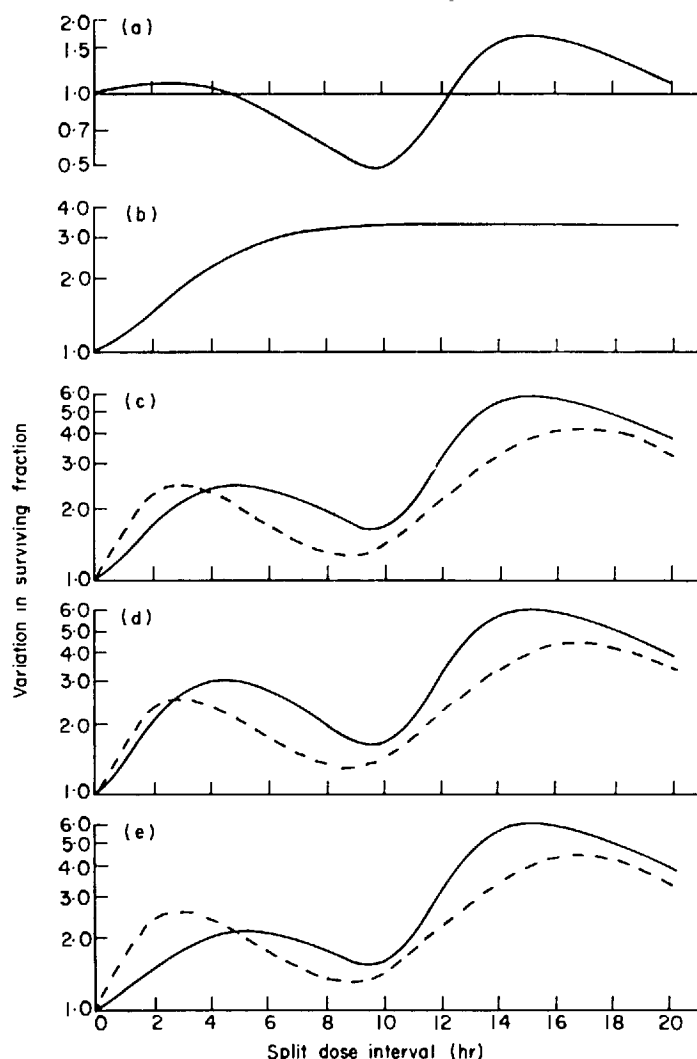


Fig. 14b Variation in surviving fraction of Chinese hamster (V79) cells with split-dose interval, for a split-dose of 710 + 710 rads. (Taken from Liversage, 1969a)

- (a) Theoretical effect due to progression and repopulation according to model of Young and Fowler (1969).
- (b) Theoretical effect due to intracellular recovery according to theory of Lajtha and Oliver (1961) assuming $\tau = 1.5$ hr.
- (c) Full line: theoretical curve obtained by combining (a) and (b) above, and assuming $\tau = 1.5$ hr. Dashed line: experimental curve obtained by Sinclair and Morton (1964).
- (d) The same as (c) above but the theoretical curve assumes $\tau = 1.0$ hr.
- (e) The same as (c) above but the theoretical curve assumes $\tau = 2.0$ hr.

14. 3. 2. Fluctuations due to Progression

After the peak, both curves fluctuate. The first trough occurs at 9 or 10 hours in the cell survival curve but at $12\frac{1}{2}$ hours in the LD 50 curve. In the latter curve there is a suggestion of a second trough five hours later. The magnitude of the fluctuations between peak and trough appears to be a smaller fraction of the total amount of recovery for the weevil LD 50 curve than for the Chinese hamster cell survival curve.

14. 3. 3. The repopulation effect

The effect of repopulation is more apparent in the cell survival curve than in the LD 50 curve, e.g. the second peak of the cell survival curve is appreciably higher than the first peak whilst the LD 50 curve is no higher at 25 hours than it is at 7 hours. The LD 50₍₃₀₎ for a split-dose interval of 3 days (Experiment No 13, Table 3) is however 850 rads higher, suggesting that repopulation becomes important at a later stage in the LD 50 curve. It will be shown later (Chapter 16) that a first dose of 4000 rads is estimated to induce a mitotic delay in the critical cells of the weevil of $17\frac{1}{2}$ hours. The experimental data of Sinclair (1967) as used by Young and Fowler (1969) show that the first dose of 710 rads given to the cells would induce a mitotic delay of less than 5 hours. Thus repopulation effects should be apparent after 5 hours in the hamster cell curve but not until after $17\frac{1}{2}$ hours in the LD 50 curve for weevils.

14. 4. Estimation of τ from the split-dose curve

The precise estimation of τ (the half-life of the dose equivalent of sub-lethal damage) from a split dose recovery curve is not possible because of the difficulty in separating the effects of sub-lethal

damage repair from those of progression (Liversage, 1969a). It is however possible to determine upper and lower limits between which γ is likely to lie and the analysis of Young and Fowler (1969) is of considerable assistance in this respect. Their model showed that after a first dose of 710 rads, Chinese hamster cells display a slight increase in resistance during the first $2\frac{1}{2}$ hours (curve (a) in Fig 14b). After $2\frac{1}{2}$ hours the resistance of the cells decreases until at 10 hours they have progressed into their most sensitive phase thus producing a trough in the split-dose recovery curve.

The LD 50 split-dose curve for weevils suggests that their critical cells require a slightly longer period ($12\frac{1}{2}$ hours) to progress into a sensitive phase after receiving an initial dose of approximately 4000 rads.

Examination of curves (a) and (b) in Fig 14b shows that the first peak in the combined theoretical curve occurs when the rate of increase due to sub-lethal damage repair (curve b) becomes equal to the rate of decrease due to progression (curve a). Thus the first peak in the combined curve occurs before the sub-lethal damage repair has reached completion. In practical terms it will be assumed that the first peak occurs before sub-lethal damage repair is 99% complete. How much before will clearly depend on the shape of the progression curve. Liversage (1969a) showed that sub-lethal damage repair is 99% complete in a period of 7γ . Now the first peak for the weevil curve occurs at 7 hours and on the above reasoning 7γ must therefore be greater than 7 hours, i.e. γ must be greater than 1 hour.

Having estimated a lower limit for γ it is now necessary to estimate an upper limit.

The first peak of the weevil recovery curve is 2070 rads higher than the single dose LD 50₍₃₀₎ of 5900 rads suggesting that the total amount of recovery (expressed in rads) is 2070 rads, i.e. 35%. However, as the repair of sub-lethal damage is not complete by 7 hours and as progression is causing a fall in the LD 50 by 7 hours, the true rise due to complete repair of sub-lethal damage is likely to be greater than 2070 rads perhaps even 200 or 300 rads more, i.e. up to 40% of the single dose LD 50. The observed split-dose recovery curve rises by 1035 rads (i.e. half of 2070 rads) in 1.8 hours and by 1170 rads (i.e. half of 2340 rads) in 2.0 hours. Thus, as judged from the split-dose recovery curve, the repair of sub-lethal damage appears to be half complete in approximately 2 hours. Oliver (1964) and Liversage (1969a) have shown that because radiation effect and dose are not linearly related, the half-life, γ , of the dose equivalent of sub-lethal damage is less than the time required for the recovery to be half complete as judged from an observed split-dose recovery curve. On the above reasoning γ must be less than 2 hours.

The split-dose recovery curve for LD 50₍₃₀₎ for weevils suggests therefore that the half-life, γ , of the dose equivalent of sub-lethal damage, for the critical cells in weevils, lies between 1 and 2 hours when these are irradiated and housed at 27°C 70% R.H.

CHAPTER 15THE VARIATION OF THE LD 50 WITH THE NUMBER OF FRACTIONS N DELIVERED IN A
CONSTANT OVERALL TIME T15. 1. Iso-effect animal experiments to show the effect of varying N only

Fowler (1971) reviewed animal experiments designed to determine how iso-effect doses vary with the number of fractions and overall time. He pointed out that few experiments have been carried out in which the effects of overall time and fraction number were investigated separately.

To test the General Formula (the main objective of the present investigation) it is necessary to isolate these two effects. The present investigation demonstrates that it is not always simple to design experiments which will do this completely. The results obtained with weevils show that the increase in LD 50₍₃₀₎ with fraction number using a constant overall time is, in fact, a function of the overall time chosen.

It will be shown later (Chapter 16) that in any irradiation regime, the additional dose necessary to compensate for the repopulation occurring during the regime is a function not only of the overall irradiation time but also of the interval between fractions. Thus, irradiation regimes employing a constant overall irradiation time but different numbers of fractions require unequal additional doses to compensate for repopulation. These required additional doses are also not proportional to the iso-effect doses applicable to their respective regimes. Thus, if D_{NT} is the dose necessary to produce a given effect in N fractions in T days, $D_{N_1T_1} / D_{N_2T_1}$ will not be equal to $D_{N_1T_2} / D_{N_2T_2}$. The difference between these two ratios may be small in those systems in which repopulation has little influence. In weevils the effect can be considerable as illustrated by the following example.

15. 2. Weevil results demonstrating the dependence of $D_{N_1 T} / D_{N_2 T}$ on the value of T

The results of experiments Nos 17, 21, 24 and 26 (Table 3, 12.3.) all involving weevils from the same batch demonstrate the above effect.

If D_9 and D_4 are the LD 50₍₃₀₎ observed for regimes involving 9 and 4 fractions respectively delivered in a constant overall time of either 10 days or 28 days the following ratios are obtained. If T = 10 days

$$D_9 / D_4 = \frac{17680 \pm 3.1\%}{13920 \pm 1.4\%} = 1.270 \pm 0.044$$

If T = 28 days

$$D_9 / D_4 = \frac{25080 \pm 3.6\%}{17160 \pm 4.4\%} = 1.462 \pm 0.083$$

To calculate the 95% confidence limits of the above ratios the 95% fiducial limits of the individual LD 50's were added vectorially.

It may be concluded that in iso-effect experiments designed to study the effect of fraction number only, the influence of overall time is not necessarily eliminated simply by keeping it constant.

CHAPTER 16VARIATION IN LD 50 FOR CONSTANT N AND VARIABLE T16. 1. Attempt to fit results to an Ellis type formula

Ellis (1967), as previously referred to (5.2), claims that iso-effect doses are proportional to N^A and T^B where N is the number of fractions, T is the overall time and A and B are constants.

If the LD 50 for weevils varies with overall time T in this way, then for any constant number of fractions N , the LD 50 values plotted against overall time T on a double logarithmic scale should lie on a straight line of slope B .

With the exception of experiment No 16, the results of all the experiments involving 2, 4, 6 or 9 fractions, each of 71-79 minutes duration, together with their 95% fiducial limits are plotted in this way (Fig 16a). Experiment No 16 (6 fractions in 7 days) was excluded as it involved a 3-day gap between the 5th and 6th fraction. The LD 50 observed in this experiment is believed to be greatly influenced by the position of the gap and will be discussed more fully later (Chapter 18).

Examination of Fig 16a shows that the exponent B of time is not constant but depends on both N and T . Between 10 days and 28 days, the 9-fraction data have an average slope of 0.34 ± 0.07 whilst the 4-fraction data have an average slope of 0.205 ± 0.055 . Between 1 day and 3 days, the 4-fraction data have an even smaller slope of 0.115 ± 0.04 .

It may be concluded that the Ellis (1967) type relationship does not apply to LD 50 observations on weevils.

16. 2. Attempt to fit results to the Cohen type formula

The formula of Cohen (1968) as previously referred to (5.2) implies that keeping the number of fractions constant but increasing the overall time increases the iso-effect dose by C times the increment

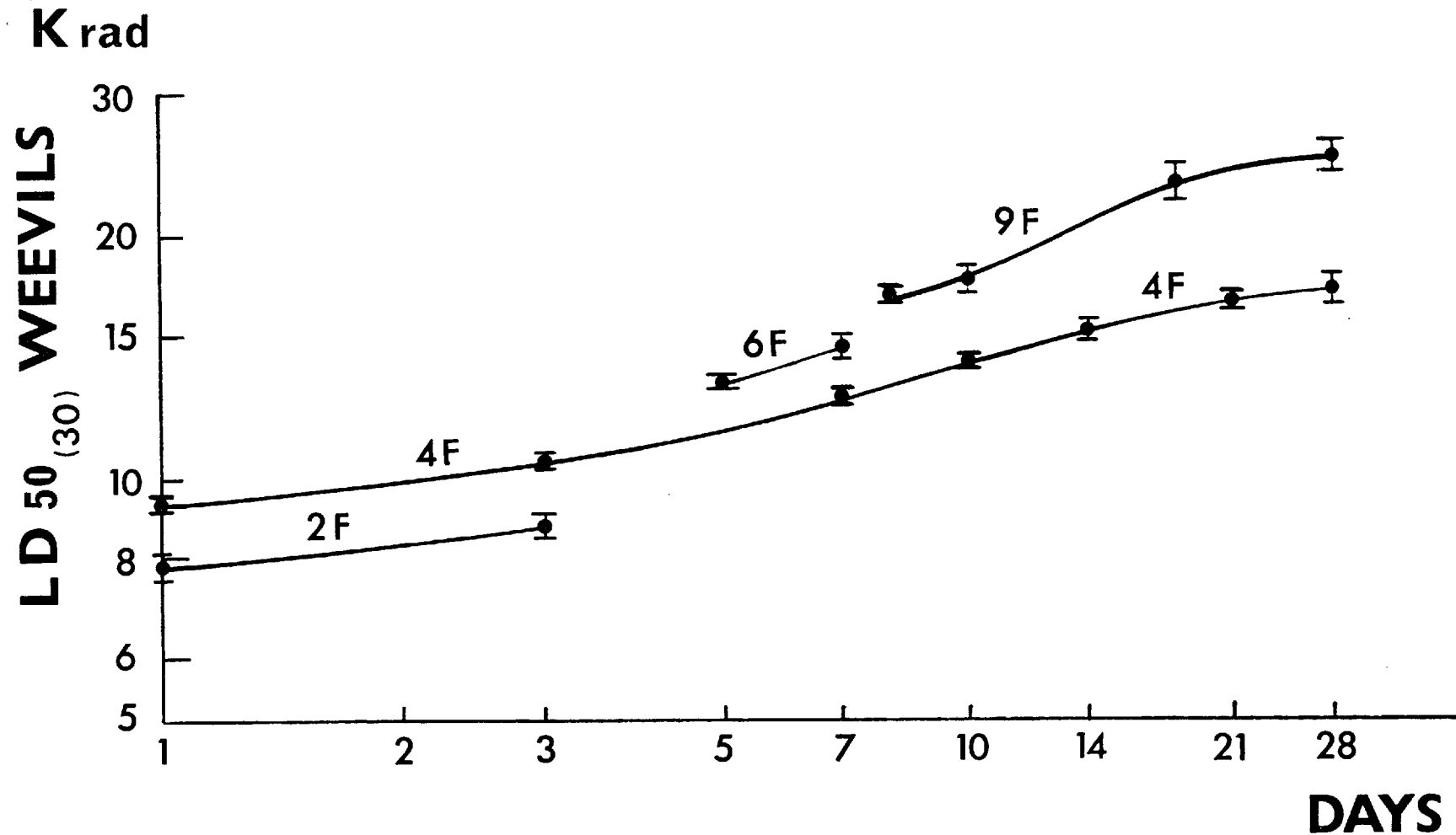


Fig. 16a The LD 50₍₃₀₎ (with 95% fiducial limits) for weevils plotted on a double logarithmic scale against overall irradiation time for fractionated regimes involving 2,4,6, or 9 fractions.

in overall time, where C is considered to be a constant.

The data plotted in Fig 16a are replotted on a linear-linear scale in Fig 16b. Examination of this diagram shows that for a constant number of fractions the LD 50 for weevils is not linearly related to overall time, e.g. from the 4-fraction data it will be seen that the LD 50 increases by 630 rads per day as the overall time is increased from one to three days but only by 77 rads per day as the overall time is increased from twenty-one to twenty-eight days. Clearly the value of C is not a constant but decreases as the overall irradiation time increases.

It may be concluded that the Cohen (1968) type relationship does not apply to LD 50 observations on weevils.

16. 3. Attempt to fit results to the Liversage type formula

As previously discussed (5.2) Liversage (1971) using published data for skin reactions suggested that for a constant number of fractions, the rate of increase in iso-effect dose would decrease as the irradiation regime was prolonged. He suggested that the rate of increase C was a variable which decreased as $(T - T_p)$ was increased and could be considered as a function of $(T - T_p)$ where T is the overall time used and T_p is the time required to give the same number of fractions at five fractions per week.

In the present experiments, data were obtained for 2, 4, 6 and 9 fractions given strictly daily and the formula of Liversage may be rewritten:

$$D_{NT} - D_{NT_D} = C (T - T_D) \dots\dots\dots (13)$$

where D_{NT} is the LD 50 for weevils irradiated with N fractions in T days and T_D is the time in days required to give N strictly daily

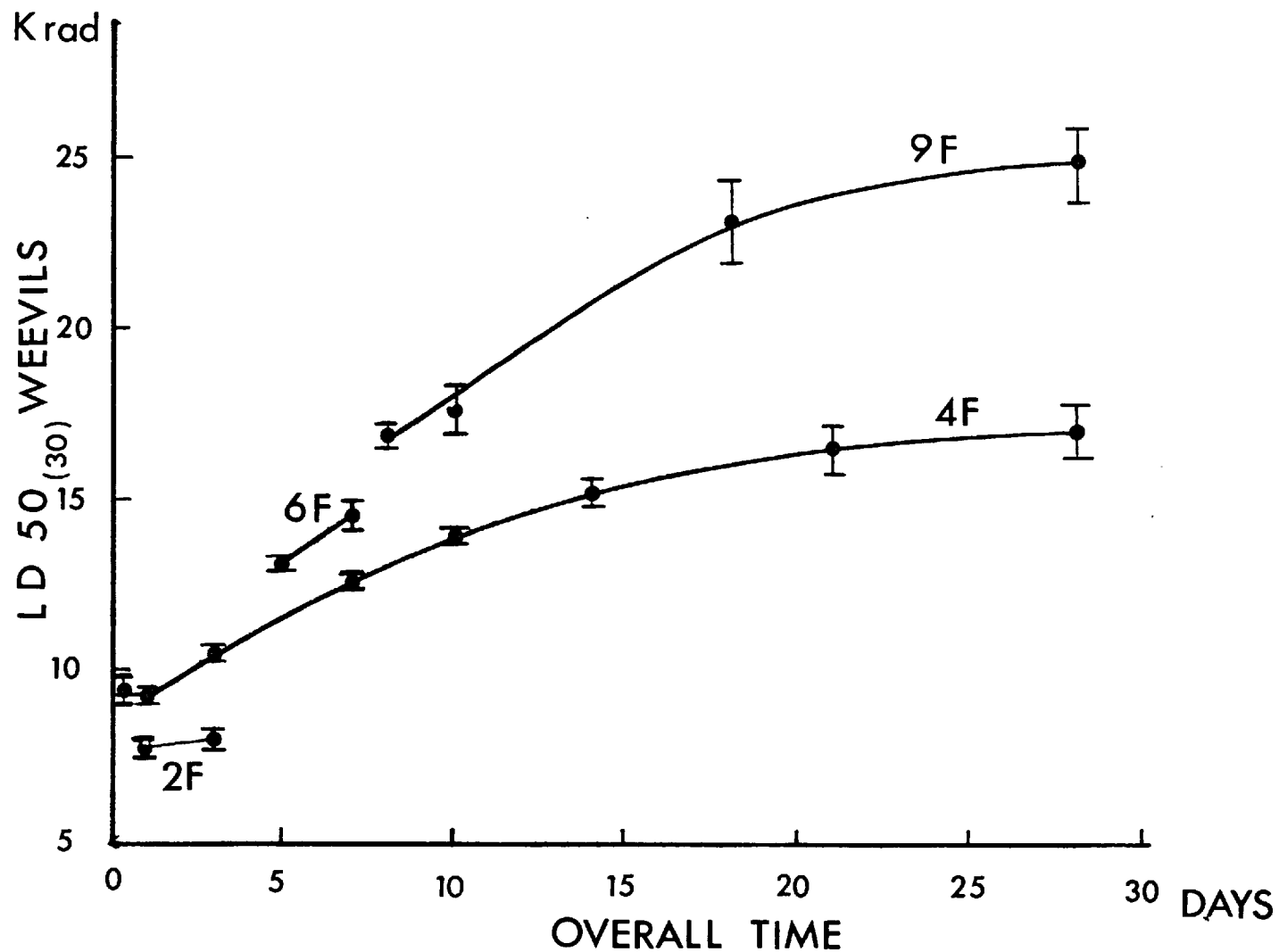


Fig. 16b The LD 50₍₃₀₎ (with 95% fiducial limits) for weevils plotted on a linear-linear scale against overall time of irradiation for fractionated regimes involving 2,4,6 or 9 fractions.

fractions (i.e. $T_D = (N-1)$ days). The parameter C should now be a variable which decreases as $(T - T_D)$ increases.

To test whether the weevil data fitted such a relationship all the data included in Figs 16a and 16b were analysed and values of $(D_{NT} - D_{NT_D})$ were plotted against $(T - T_D)$ in Fig 16c.

The results show that the value of C does decrease as $(T - T_D)$ is increased. Unfortunately it will also be observed that C is a function of the number of fractions N . For example, on changing from 9 fractions in eight days (i.e. daily fractions) to 9 fractions in 18 days, the LD 50 increases by 6360 rads i.e. 636 rads per day. On changing from 4 fractions in 3 days to 4 fractions in 13 days Fig 16c shows that the LD 50 increases by only 4500 rads i.e. only 450 rads per day even though $(T - T_D)$ is 10 days in both cases.

It may be concluded that the formula of Liversage (1971) (Equation 13) does not apply to LD 50 observations on weevils as the value of C varies not only with the time increment $(T - T_D)$ but also with the number of fractions N . The problem is further discussed below.

16. 4. The search for the true relationship

16. 4. 1. The importance of fraction interval

Examination of Fig 16c suggests that the increase in LD 50 with increase in overall time is not just a function of the increment in overall time but is also some function of fraction interval t_s . The average amount of repopulation (expressed in rads) occurring between consecutive fractions t_s days apart minus that between consecutive fractions one day apart will be $(D_{NT} - D_{NT_D}) / (N - 1)$. The numerical values of this expression are plotted against fraction interval t_s (Fig 16d) for the same experimental data as those used in the previous three diagrams.

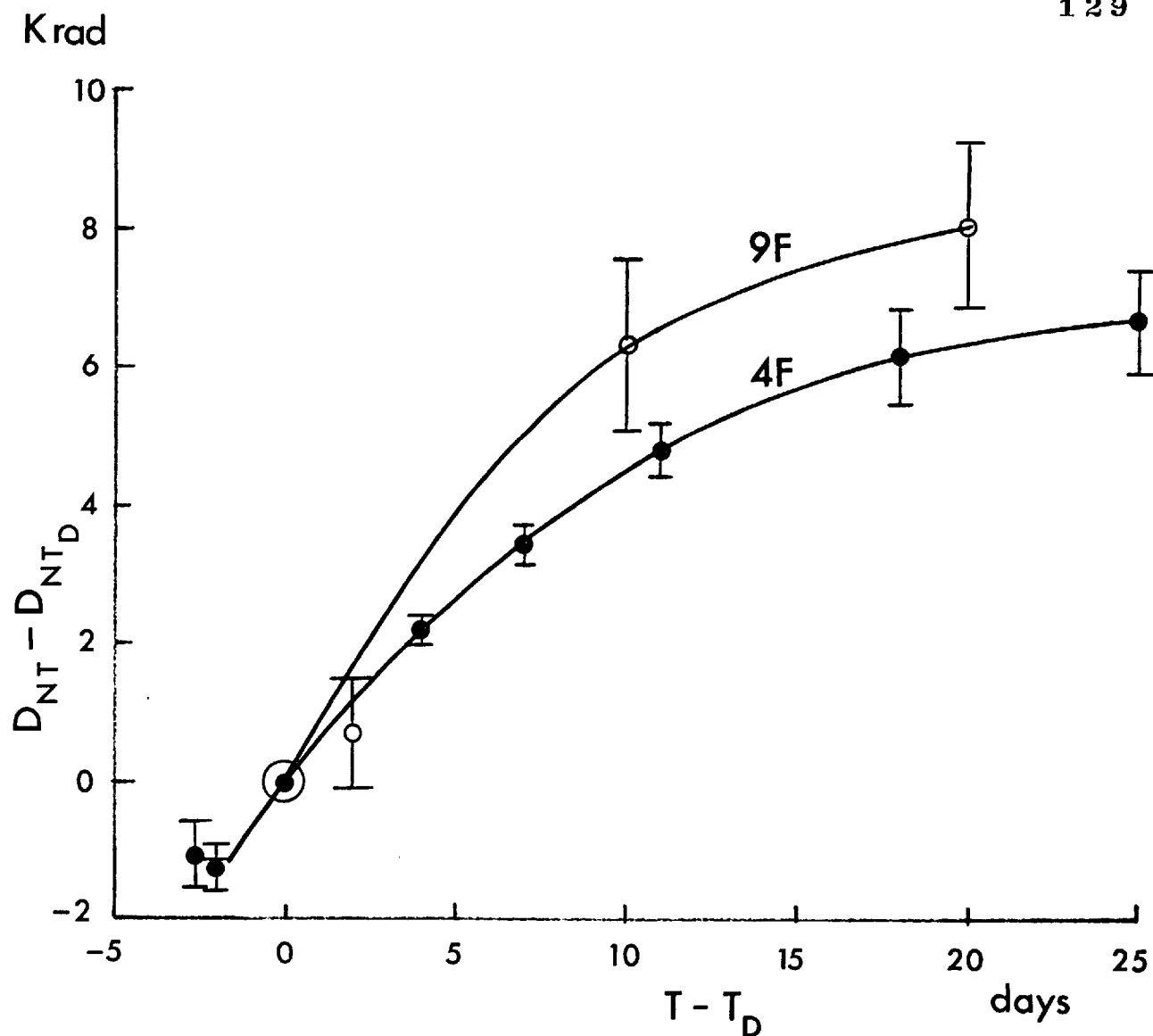


Fig. 16c The increase in $LD 50_{(30)}$ on changing from N daily fractions to N fractions in T days plotted against the corresponding increase in overall time for fractionated regimes involving 4 or 9 fractions. (D_{NT} is the LD 50 when N fractions are delivered in T days and T_D is the time required to give N daily fractions).

Irrespective of the number of fractions employed, it will be seen from Fig 16d that all the plotted data lie close to a single curve (b). The maximum and minimum limits of the relationship are represented by curves (a) and (c). It may be concluded that for any irradiation regime involving 2 to 9 fractions and killing 50% of the weevils, the average amount of repopulation per fraction interval is principally a function of that interval and is practically independent of N and T .

Before attempting to use the data in Fig 16d to estimate the effect of repopulation during irradiation regimes in general, a closer scrutiny of the data is called for.

A quantity of radiation ${}^R D_T$ has previously been defined (4.1) as that quantity of radiation necessary to compensate for the effects of repopulation occurring during a fractionated regime involving N fractions delivered in an overall time of T days. Thus ${}^R D_T$ is a measure of the amount of repopulation (expressed in rads) occurring during such a regime.

Using this terminology, the amount of repopulation per fraction interval will be ${}^R D_T / (N - 1)$ and this should be equal to the increase in $(D_{NT} - D_{NT_D}) / (N - 1)$ as the fraction interval is increased from 0 to t_g days, as shown in Fig 16d. The curves in this diagram have been drawn with a discontinuity occurring at approximately 0.5 days. The only data enabling the position of this discontinuity to be located are data obtained for regimes involving 4 fractions. For regimes involving different numbers of fractions the discontinuity could occur earlier or later than shown in Fig 16d. The discontinuity is believed to result from radiation induced mitotic delay preventing repopulation occurring

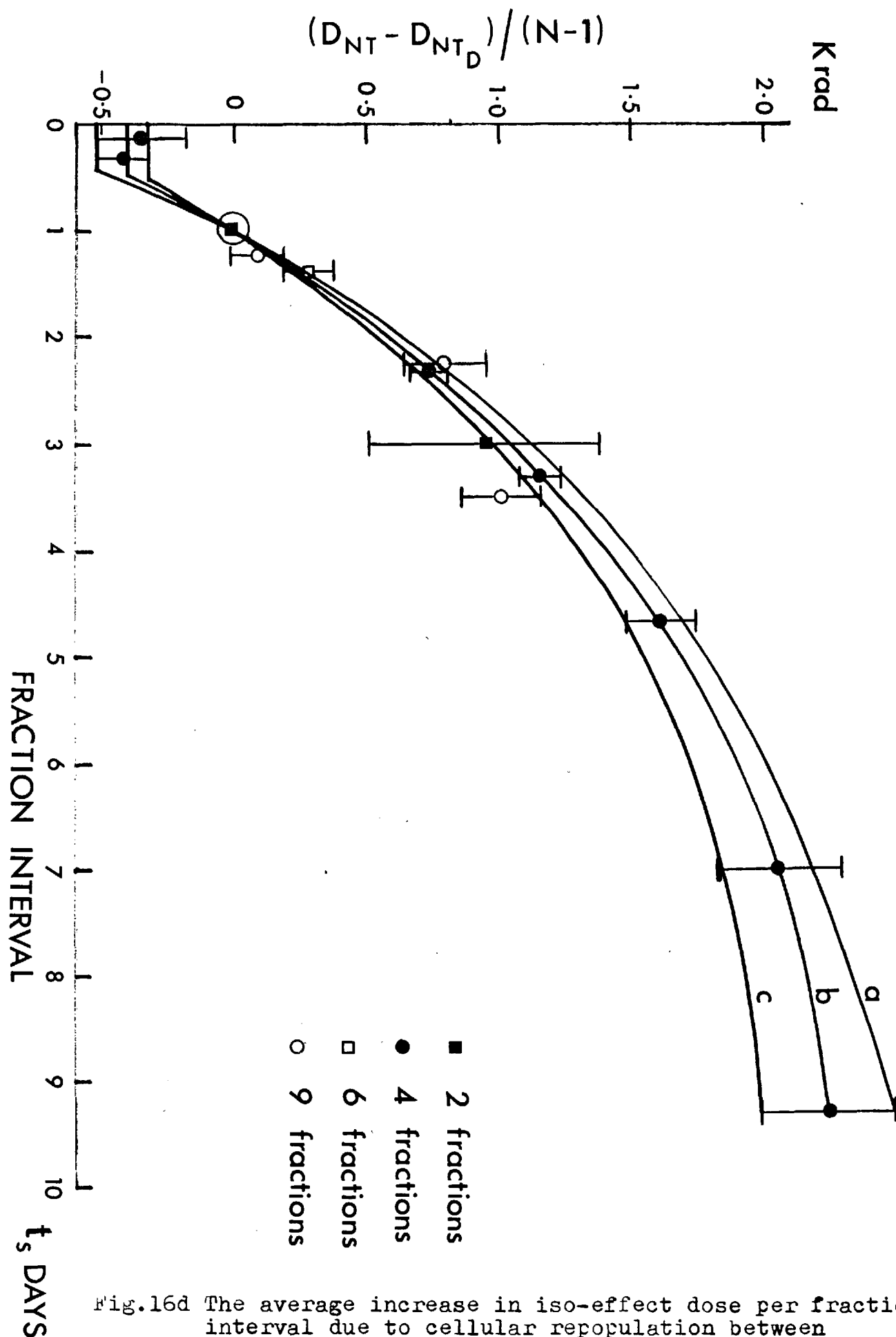


Fig.16d The average increase in iso-effect dose per fraction interval due to cellular repopulation between consecutive fractions appears to be principally a function of fraction interval t_s . The most likely relationship is represented by curve b and its upper and lower limits by curves a and c.

for a period t_m days after each fraction of radiation. Using Chinese hamster cells, Elkind, Han and Volz (1963) showed that mitotic delay was directly proportional to the dose delivered. Thus t_m is likely to vary with fraction size.

It may be concluded that if the data in Fig 16d are to be used to estimate the total repopulation occurring during any fractionated regime, due allowance must be made for the influence of mitotic delay and how this is likely to vary with fraction size. These effects are discussed below.

16. 4. 2. The effects of mitotic delay.

In an attempt to estimate the effects of mitotic delay the LD 50₍₃₀₎ was determined for 4 fractions in 3 days, 4 fractions in 1 day and 4 fractions in 9 hours; i.e. for fraction intervals of 1, 0.33 and 0.125 days (Experiments Nos 46, 45 and 51). The computed LD 50's observed were $10520 \pm 2.4\%$, $9260 \pm 2.1\%$ and $9260 \pm 2.9\%$. Thus no difference was observed in the LD 50 for intervals of 0.33 and 0.125 days. (After normalising in accordance with their respective single dose controls the LD 50 for $t_g = 0.125$ days was found to be 2% higher than that for $t_g = 0.33$ days but this difference is not significant).

The LD 50 for $t_g = 1$ day and for $t_g = 0.33$ days were determined in the same group of experiments and may therefore be compared without normalising. The difference between them is 1260 ± 330 rads i.e. 420 ± 110 rads per interval. Thus, on average, this amount of repopulation occurs after each daily fraction of 2630 rads. By extrapolation of curve (b), as drawn in Fig 16d, it will be observed that repopulation is required only for the last 0.52 days of each daily period in order to obtain 420 rads

per day. It is therefore estimated that a dose of 2630 rads produces a mitotic delay t_m of 0.48 days, i.e. 0.183 days /1000 rads.

As the mitotic delay t_m will vary with the dose delivered per fraction, the total amount of repopulation occurring during any fractionated regime is more likely to be $(N - 1)$ times some function of $(t_s - t_m)$ rather than $(N - 1)$ times some function of t_s . The experimental data presented in Fig 16d will therefore be reconsidered below.

16. 4. 3. The shape of the relationship between repopulation and $(t_s - t_m)$

The mitotic delay t_m for each of the 4-fraction regimes has been calculated assuming the delay to be 0.183 days/1000 rads. The values of $(D_{NT} - D_{NT_D}) / (N - 1)$ together with their 95% confidence limits have been replotted against $(t_s - t_m)$ in Fig 16e. It is considered that, at the 95% level of confidence, the curve relating these two factors is likely to lie between curves (a) and (c). Examination of the plotted data shows that the estimated position of the true curve would be little affected by the absence of any one point. The presence of the point therefore confirms the position of the line and thus provides a second independent estimate of its position. Now doubling the number of observations should reduce the size of the confidence limits by the square root of two. Thus curves (a) and (c) were drawn closer together, by a factor of 1.4, than the smooth curves (not shown) passing through the upper and lower limits of the individual points. Curve (b) was calculated from a

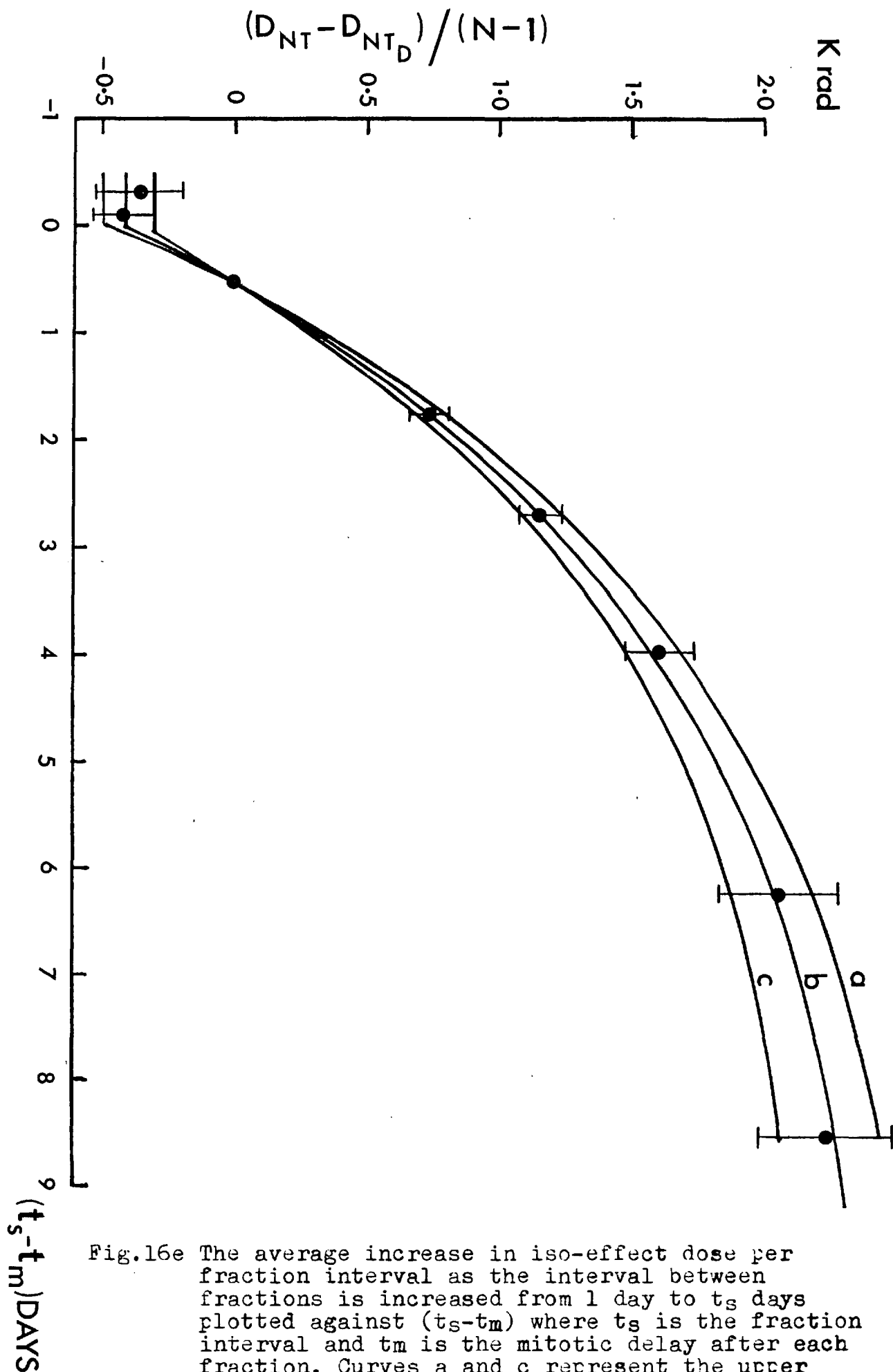


Fig.16e The average increase in iso-effect dose per fraction interval as the interval between fractions is increased from 1 day to t_s days plotted against $(t_s - t_m)$ where t_s is the fraction interval and t_m is the mitotic delay after each fraction. Curves a and c represent the upper and lower limits of the experimental data. Curve b was calculated from equation (15) and is in excellent agreement with the plotted 4-fraction experimental data.

mathematical relationship derived below.

In Fig 16e, the slope of the curve passing through the experimental points at time $(t_s - t_m)$ is equal to the average instantaneous rate of repopulation M (expressed in rads/day) occurring t_s days after each of the N fractions except the last. (N.B. It is necessary to insert the word "average" here as the rate of repopulation may vary from one fraction interval to the next).

The slopes of curves (a) and (c) in Fig 16e have been plotted against $(t_s - t_m)$ on a logarithmic - linear scale in Fig 16f to obtain upper and lower limits to the average instantaneous rates of repopulation and to show how this decreases with time. It will be observed that, to within the limits of experimental accuracy, the average instantaneous rate of repopulation M t days after the mitotic delay decreases exponentially with time and may be represented by the equation

$$M = M_0 e^{-\lambda t} \dots\dots\dots(14)$$

where M_0 is the average instantaneous rate of repopulation (expressed in rads/day) immediately following the mitotic delay period and λ is the exponential decay constant.

It follows that the total repopulation ${}^rD_T$ (expressed in rads) occurring during a fractionated regime delivering a total dose D_{FT} rads in N equally spaced fractions in an overall time of T days will be given by:

$$\begin{aligned} {}^rD_T &= (N - 1) \int_0^{(t_s - t_m)} M dt \\ &= (N - 1) \frac{M_0}{\lambda} \left[1 - e^{-\lambda(t_s - t_m)} \right] \dots\dots\dots(15) \end{aligned}$$

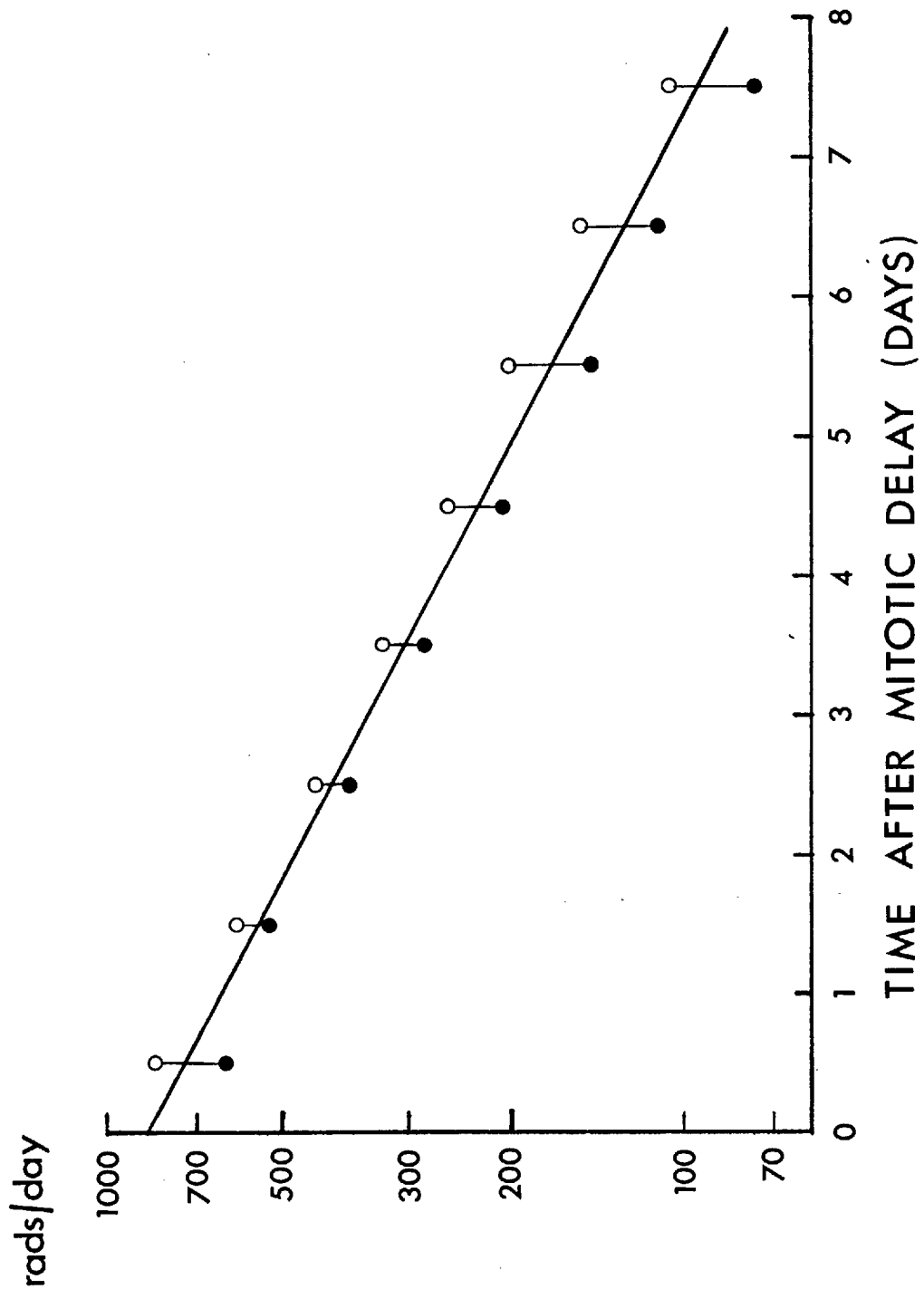


Fig.16f The average instantaneous rate of repopulation (expressed in rads/day equivalent) during the interval between fractions plotted on a logarithmic-linear scale against time after mitotic delay. The upper limits (open circles) and the lower limits (closed circles) were obtained by measuring respectively the slopes of curve a and curve c in Fig.16e at $(t_s - t_m)$ days.

where t_s is the interval between fractions (i.e. $T/(N - 1)$ days) and t_m is the mitotic delay estimated to be $0.183 D_{NT}/1000 N$ days for the critical cells of the weevil.

From Fig 16f it is estimated that $M_0 = 850$ rads/day and $\lambda = 0.291 \text{ days}^{-1}$ (The time required for the rate of repopulation to fall to half its initial value is thus 2.4 days).

In order to verify that the above relationship does in fact fit the shape of the experimental data from which it is derived, equation (15) was used to calculate how the expression $(D_{NT} - D_{NT_D})/(N - 1)$ should vary with $(t_s - t_m)$. The results of these calculations are plotted (curve b) in Fig 16e. The precise agreement at all points on the curve shows that the above mathematical relationship certainly has the correct shape. Had the best smooth curve passing through the experimental points been drawn on this diagram it would have been practically indistinguishable from the curve calculated from equation (15). The total repopulation $\bar{D}_T / (N - 1)$ per fraction interval t_s is equal to the increase in $(D_{NT} - D_{NT_D}) / (N - 1)$ as the fraction interval is increased from t_m to t_s days. For any irradiation regime this may be estimated from curve (b) in Fig 16e. The 95% confidence limits of this estimate may be obtained by considering the corresponding increases in curves (a) and (c). For convenience, the increases in these curves have been replotted against $(t_s - t_m)$ in Fig 16g which has been used to estimate the total repopulation correction $\bar{D}_T$ for each of the fractionated regimes investigated in this thesis. Values of $(D_{NT} - \bar{D}_T)$ i.e. the LD 50 for N fractions if repopulation effect is eliminated are tabulated in Table 4. The consistency

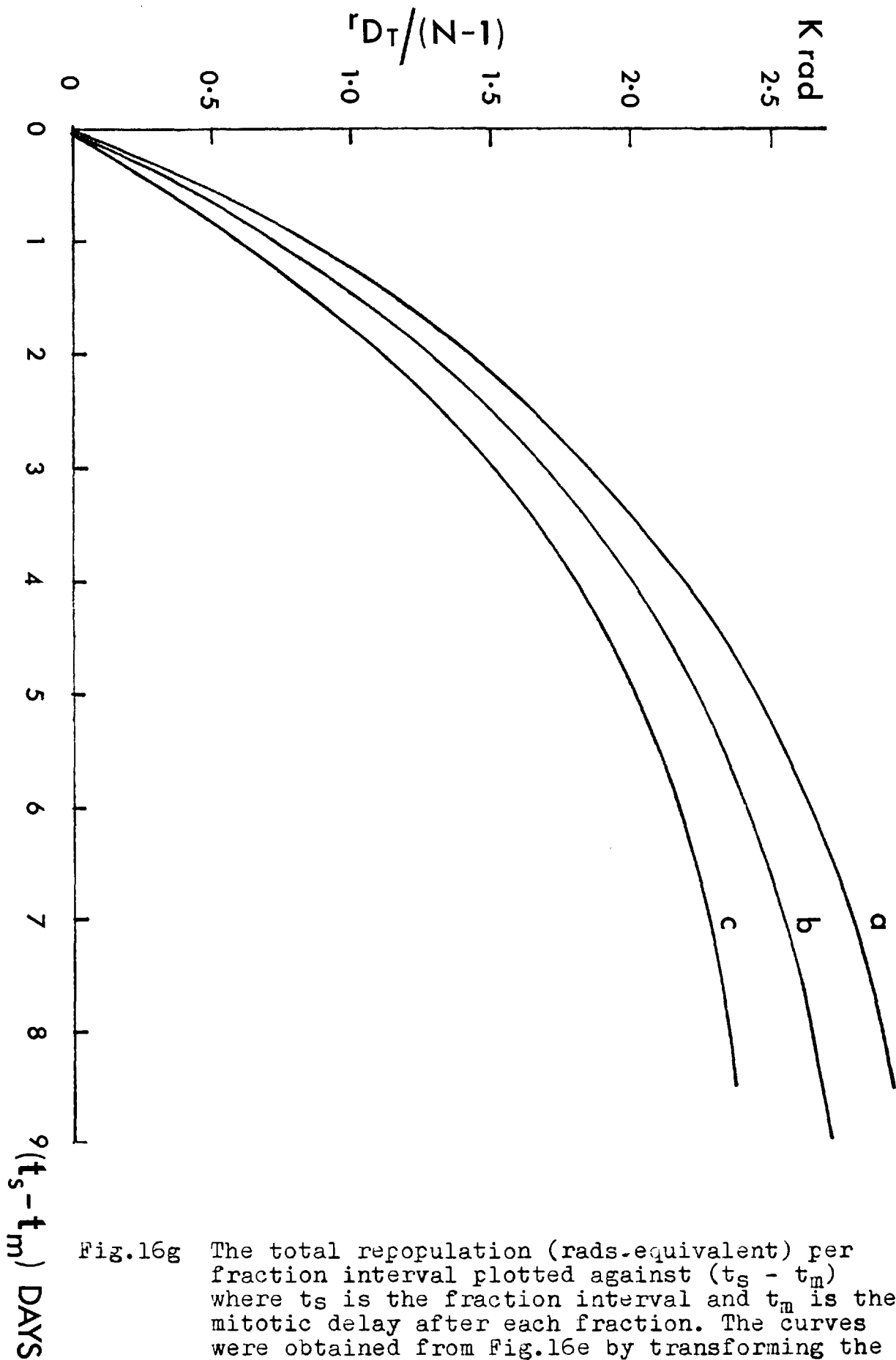


Fig.16g

The total repopulation (rads-equivalent) per fraction interval plotted against $(t_s - t_m)$ where t_s is the fraction interval and t_m is the mitotic delay after each fraction. The curves were obtained from Fig.16e by transforming the origin of the co-ordinates. Thus Curve b represents the estimated repopulation as calculated from equation (15) whilst curves a and c are the experimentally derived 95% confidence limits of this estimate.

TABLE 4

N	T DAYS	$(D_{NT} - {}^R D_T)$ rads
2	0.104	7280 $\pm$ 170
	0.208	7900 $\pm$ 240
	0.313	7970 $\pm$ 230
	0.417	7800 $\pm$ 130
	0.521	7410 $\pm$ 110
	0.625	7660 $\pm$ 110
	0.729	7340 $\pm$ 240
	0.833	7430 $\pm$ 110
	0.938	7450 $\pm$ 230
	1.042	7630 $\pm$ 310
	3.0	7350 $\pm$ 360
4	0.375	9540 $\pm$ 430
	1.0	9340 $\pm$ 250
	3.0	9370 $\pm$ 400
	3.0	9210 $\pm$ 320
	7.0	9160 $\pm$ 490
	10.0.	9160 $\pm$ 650
	14.0.	9280 $\pm$ 780
	21.0	9280 $\pm$ 1080
	28.0	9120 $\pm$ 1210
6	5.0	10790 $\pm$ 530
	7.0	11080 $\pm$ 770
9	8.0	12950 $\pm$ 800
	10.0	12350 $\pm$ 1140
	18.0	13890 $\pm$ 1680
	28.0	11500 $\pm$ 1740
14	17.0	14750 $\pm$ 1660
20	25.0	17750 $\pm$ 2570

of the values obtained for a given number of fractions but for different overall times provides supporting evidence for the validity of the derived mathematical relationship. (Equation 15).

16. 5. Discussion

It should be stressed that the above relationship was derived from experiments involving fractions of equal magnitude. In general these were as equally spaced as a five day week would allow. The most unequal spacing occurred in regimes involving 5 fractions per week as a 3-day gap occurred at each weekend. For this reason the 6-fraction and 9-fraction regimes were repeated using strictly daily fractions.

The 3-day gaps occurring in the 5-fraction per week experiments were distributed symmetrically about the mid-point of the regimes employed except for one experiment (No.16). In this experiment, involving 6 fractions in 7 days, a 3-day gap occurred between the 5th and 6th fractions and the LD $50_{(30)}$ was found to be 13410 ± 460 rads. When the experiment was repeated but with the 3-day gap between the 3rd and 4th fraction (experiment No 47) the LD $50_{(30)}$ was found to be 1200 rads higher (i.e. 14600 ± 510 rads)

In deriving the above relationship, experiment No 16 was ignored on the grounds that this result was greatly influenced by the presence of a gap so close to the end of the regime. This result will be further discussed in Chapter 18 in the light of additional experimental evidence presented in the same chapter.

Although equation (15) is more complex than any of the empirical isoeffect formulae previously referred to, it is based on the simple equation (14) which shows that during each interval the instantaneous rate of repopulation decreases exponentially with time. This experimental finding suggests that the rate of repopulation may be directly proportional to the damage present provided both entities are expressed in terms of their dose equivalents.

To satisfy such a hypothesis the repopulation occurring during an infinitely long interval must be equal to the damage present at the start of that interval. For this to be true, the numerical value of M_0/λ in equation (15) must be equal to the average damage (expressed in rads) present at the start of each fraction interval of any regime which kills 50% of the weevils. For any such regime, irrespective of the number of fractions or the overall time employed, the ultimate damage is equivalent to that produced by a single dose of 5900 rads. Thus during the whole of any such regime the average damage present will be approximately 2950 rads which is in close agreement with the value of 2920 rads found for M_0/λ ($M_0 = 850$ rads/day; $\lambda = 0.291$ days⁻¹).

The above analysis is full of approximations and is complicated by the fact that damage is not linearly related to dose. Nevertheless the agreement is encouraging and suggests that the hypothesis is worthy of further investigation and consideration. To develop the concept more fully a dose-response curve for the critical cells of the weevil is required. This is derived in Chapter 17. The hypothesis is discussed further in Chapter 18 together with the results of experiments designed to investigate the relationship between the rate of repopulation and the damage present.

16. 6. Conclusion

The LD 50₃₀ for weevils varies with overall irradiation time in a way which is not consistent with published iso-effect formulae purporting to estimate such variation for normal tissues in patients or skin reactions in animals.

The total repopulation $^R D_T$ (expressed in rads) occurring during a fractionated regime delivering a total dose D_{NT} rads in N equally spaced fractions in an overall time of T days has been found to be consistent with the following relationship:

$$r_{D_T} = (N - 1) \frac{M_0}{\lambda} \left[1 - e^{-\lambda(t_s - t_m)} \right] \dots\dots\dots(15)$$

where t_s is the interval between fractions and t_m is the mitotic delay estimated to be equal to $0.183 D_{NT} / 1000N$ days. It is estimated that $M_0 = 850$ rads/day and $\lambda = 0.291$ days.⁻¹

For any regime killing 50% of the weevils, the average repopulation occurring per fraction interval will be $r_{D_T} / (N - 1)$ and may be calculated from the above formula or may be determined, together with appropriate 95% confidence limits, from the experimentally derived curves presented in Fig 16g. Curve (b) on this diagram is the curve obtained by calculation using the above formula and is centrally situated between the upper and lower limits of the experimental data (curves a and c) thus demonstrating the excellent agreement between the formula and the experimental data.

In Chapter 4 the dose "wasted" due to intracellular recovery R_{D_N} and the dose necessary to compensate for repopulation r_{D_T} were mathematically related by the simple equation (4):

$$D_{NT} = D_1 + R_{D_N} + r_{D_T} \dots\dots\dots(4)$$

In order to test the general formula for equating protracted and acute regimes of radiation, the main objective of this thesis, it is necessary to find how the dose "wasted" due to intracellular recovery varies with fraction number. This may now be determined by applying equations (4) and (15) to the fractionated LD 50 results. The appropriate analysis is presented in Chapter 17.

The finding that the rate of repopulation decreases exponentially with time between consecutive fractions suggests that a simple relationship may exist between the rate of repopulation and the damage present. The results of experiments designed to discover whether such a relationship exists are presented and discussed in Chapter 18.

CHAPTER 17VARIATION IN LD 50 WITH FRACTION NUMBER17. 1. Daily Fractions

The LD 50 was determined for 2, 4, 6 and 9 fractions using strictly daily fractions and for 6, 9, 14 and 20 fractions delivered at the rate of five fractions per week. The results (after normalising in accordance with their single dose controls) are plotted on a linear scale against fraction number (Fig 17a). The results of all the experiments in which fractions were of 71 - 79 minutes duration are included except the result of experiment No 16. The latter was excluded because of the influence of the three-day gap between the last two fractions as previously discussed (16.5)

It will be observed from Fig 17a that from 1 to 2 daily fractions the LD 50 increases by 1900 rads (i.e. 32%) but from 2 to 20 "daily" fractions the increase per fraction is practically constant at 1350 rads per "daily" fraction for the fractions of 1600 to 3900 rads. (The term "daily" in this context includes regimes in which five fractions were given per week).

17. 2. The increase in LD 50 due to intracellular recovery between fractions

As previously discussed (16.6), equation (15) may be applied to any irradiation regime to estimate the magnitude of the repopulation component $^R D_T$. The intracellular recovery component $^R D_N$ may then be determined by substituting the estimated value of $^R D_T$ into Eqn (4):

$$D_{NT} = D_1 + ^R D_N + ^R D_T \quad \dots\dots\dots(4)$$

where D_{NT} is the LD 50₍₃₀₎ for weevils irradiated with N fractions in an overall time of T days and D_1 is the single dose LD 50₍₃₀₎.

The intracellular recovery component $^R D_N$ is a function of N but is assumed to be independent of T provided the interval between fractions is sufficiently large to allow full intracellular recovery between fractions. The split-dose recovery curve (Fig 14a) indicates that

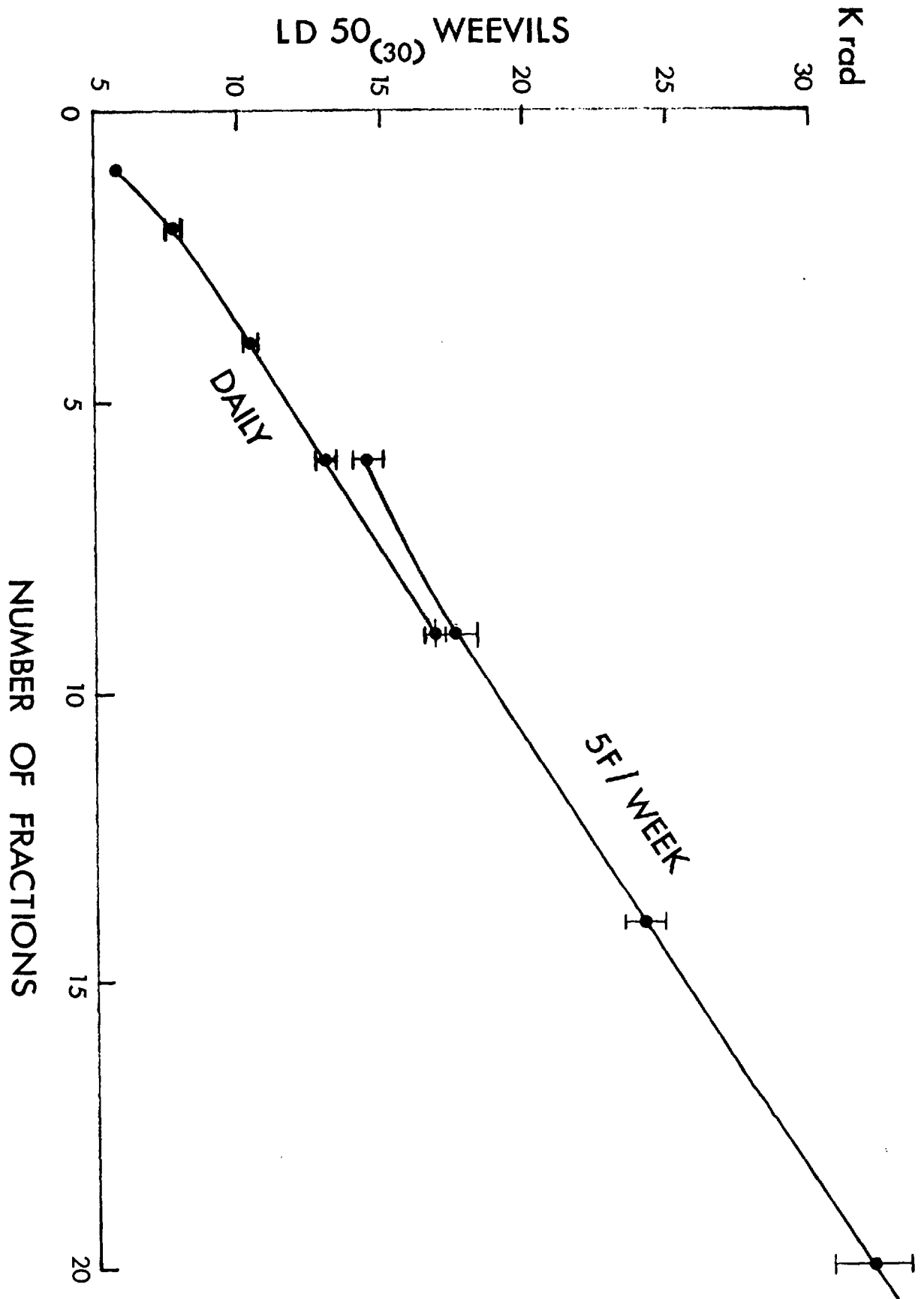


Fig.17a The LD 50₍₃₀₎ for weevils plotted against number of fractions. The lower curve is for strictly daily fractionation (e.g. 9 fractions in 8 days) whilst the upper curve is for five fractions per week (e.g. 9 fractions in 10 days)

much recovery is essentially complete in an interval of 0.3 days. The value of R_{D_N} for any regime may be estimated from equation (4) by using experimental values of D_{NT} and D_1 and estimated values of x_{D_T} calculated from equation (15) as previously discussed (16.6). The most reliable estimates of R_{D_N} are obtained when x_{D_T} is small as the 95% confidence limits on the value of x_{D_T} will then also be small (Fig 16g). Thus, to obtain reliable estimates of the effects of complete intracellular recovery between N fractions the fraction interval should be small but greater than 0.3 days. Five fractions per week data are available for all the different numbers of fractions investigated and these employ average fraction intervals of 1.4 days or less. To estimate how the LD 50 varies with fraction number it is necessary to include the five fraction per week data as these are the only data available for 14 and 20 fractions.

In view of the above considerations the estimated values of $(D_{NT} - x_{D_T})$ obtained for all regimes employing fraction intervals of between 0.3 and 1.5 days, were abstracted from Table 4 (16.4.3). For each fraction number, the average of these values is given in Table 5 and plotted against fraction number in Fig 17b. These values are in fact the same, to an accuracy of $\pm 0.5\%$, as would have been obtained using all the data presented in Table 4.

Fig 17b shows how the LD 50, corrected for repopulation, increases with the number of fractions whilst Fig 17a shows how the LD 50 uncorrected for repopulation increases with the number of daily fractions.

The increase in LD 50 with the number of daily fractions is mainly due to two effects: intracellular recovery and repopulation.

As previously discussed (3.2) synchrony may also affect the observed LD 50, especially when the number of fractions is small. However, examination of the split-dose recovery curve (Fig 14a) reveals that

TABLE 5

Number of Fractions N	LD 50 - REPOPULATION CORRECTION ($D_{NT} - x_{D_T}$) rads
1	5900
2	7590 $\pm$ 180
4	9310 $\pm$ 320
6	10940 $\pm$ 650
9	12650 $\pm$ 970
14	14750 $\pm$ 1660
20	17750 $\pm$ 2570

Each of the above fractions were delivered in a period of
71 - 79 minutes.

The derivation of this table is described in Chapter 17.2
and the repopulation correction in Chapter 16.4.3.

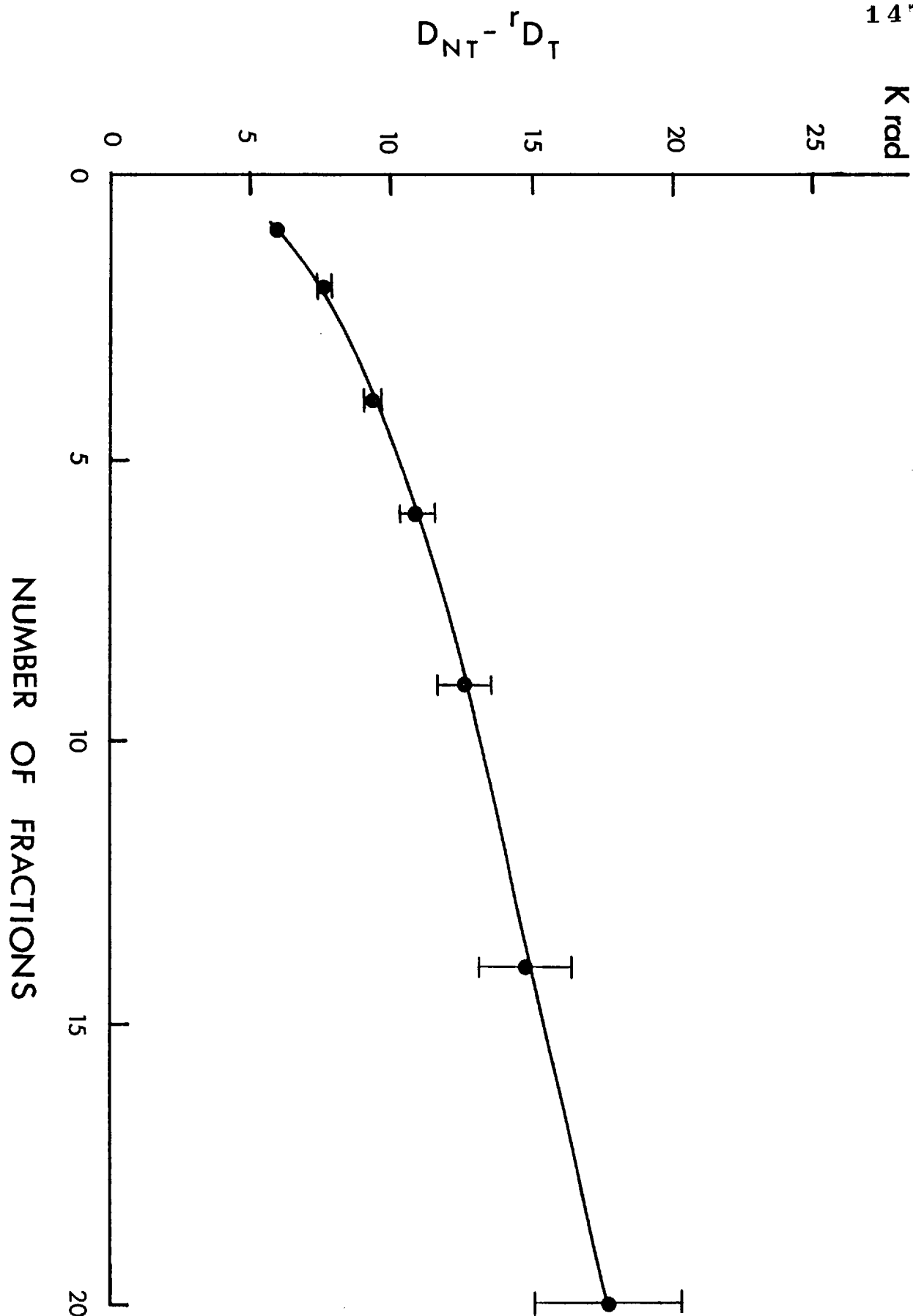


Fig. 17b The LD 50₍₃₀₎ for weevils, corrected for repopulation between fractions plotted against the number of fractions. This curve shows how the LD 50₍₃₀₎ increases with the number of fractions if complete intracellular recovery but no repopulation occurs between fractions. The plotted data are taken from Table 5.

even when only two fractions are given, synchrony only produces a variation of $\pm 4\%$ in the observed LD 50. It is believed that the effects of synchrony on the two-fraction data have been largely eliminated by averaging the eight values of $(D_{NT} - F_{D_T})$ presented in Table 4 for time intervals varying from 0.3 to 1.04 days. When more than two fractions are employed the effects of synchrony are likely to be appreciably less than $\pm 4\%$ and have been neglected in this analysis. The averaging procedure, though less effective in these cases, has probably reduced any small error due to this cause. By comparing Figs 17a and 17b, the relative importance of intracellular recovery and repopulation may be estimated. Fig 17a shows that from 2 to 6 fractions the combined effects of intracellular recovery and repopulation increase the LD 50 by an average of 1340 rads per daily fraction. From Fig 17b it will be seen that of this 1340 rads, 840 rads result from intracellular recovery and the remaining 500 rads from repopulation. From 9 to 20 fractions the total increase averages 1310 rads per daily fraction of which 460 are due to intracellular recovery and 850 rads due to repopulation. It may be concluded that in this biological system when the number of "daily" fractions is less than six, intracellular recovery has more effect than repopulation but that the relative importance of these two effects is reversed when the number of daily fractions is greater than nine.

17. 3. The effect of reducing the irradiation time per fraction

All the fractionated results analysed previously in this and earlier chapters were obtained from experiments in which each fraction of irradiation had been delivered in a period of 71 - 79 minutes.

The main objective of this thesis is to test the validity of the general formula (Liversage, 1969) for equating protracted and acute regimes of radiation discussed earlier (4.2). In deriving this formula, Liversage (1969) assumed that the acute irradiation was delivered at a sufficiently high dose-rate for its effects to be dose-rate independent, i.e. he assumed that the amount of sub-lethal damage repaired during each fraction was negligible compared with the amount produced.

It has been estimated (14.4) that the half-life of the dose equivalent of sub-lethal damage for the critical cells of the weevil is between one and two hours. Thus an irradiation time of 71 - 79 minutes would allow appreciable repair of sub-lethal damage to occur during each fraction. To test the validity of the general formula, fractionated experiments should therefore be done using shorter irradiation times. As previously mentioned (11.8) the installation of a new cobalt unit enabled the irradiation times to be shortened to between 24 and 26 minutes. The regimes listed in Table 6 were therefore repeated using these shorter irradiation times. Single dose controls using an irradiation time of 72 minutes were included in each batch of experiments and this enabled the LD 50's observed to be normalised in the usual manner.

The observed and normalised LD 50₍₃₀₎ for each regime are presented in the comprehensive list of results (Experiment Nos 67 - 77) in Table 3 (12.3). The results obtained with 24 to 26 minute exposures are compared with those obtained with 71 - 79 minute exposures in Table 6 from which it will be seen that the latter are on average 8% higher than the former. The single dose controls were irradiated at approximately 230 rads/min and 78 rads/min respectively. The results show that dose-rate effects can be appreciable at dose-rates in excess of 78 rads/min when the effect being studied is produced by a fairly

TABLE 6

REGIME	EXPERI- MENT NO.	NORMALISED LD 50 71-79 MIN IRRADIATIONS	EXPERI- MENT NO	NORMALISED LD 50 24-26 MIN IRRADIATIONS	RATIO OF LD 50's
Single Dose	67	5900	68	5320 ⁺ - 190	1.11
Single Dose	67	5900	69	5400 $\pm$ 130	1.09
Single Dose	74	5900	75	5230 $\pm$ 250	1.13
4F/3d	15	10440 $\pm$ 200	76	10070 $\pm$ 470	1.04
4F/3d	46	10600 $\pm$ 310	76	10070 $\pm$ 470	1.05
6F/7d	47	14600 $\pm$ 510	77	13530 $\pm$ 660	1.08
9F/10d	17	17680 $\pm$ 730	70	17190 $\pm$ 890	1.03
14F/17d	18	24240 $\pm$ 730	72	22400 $\pm$ 600	1.08
20F/25d	19	32130 $\pm$ 1350	73	29050 $\pm$ 1070	1.11
9F/28d	26	25080 $\pm$ 1130	71	23580 $\pm$ 920	1.06
Mean ratio					1.08

large single dose of 5000 to 6000 rads which consequently requires an irradiation time in excess of one hour.

To test the validity of the general formula referred to above, it was necessary to determine how the LD 50 corrected for repopulation varied with fraction number. Thus for each of the regimes employing 24 to 26 minute exposures it was necessary to estimate the repopulation component $^R D_T$. This was done as described previously (17.2) but because the efficiency of the radiation was 8% higher than with 71 - 79 minute irradiations, M_0 in equation (15) was assumed to be $850/1.08$; i.e. 787 rads/day. The values of $(D_{NT} - ^R D_T)$ so obtained for different numbers of fractions are tabulated in Table 7. In Chapter 19, these results are compared with predictions based on the application of the general formula to data obtained using continuous low dose-rate irradiation (Fig 19b).

17. 4. The derivation of a dose-response curve for the critical cells of the weevil

Following the method of Fowler and Stern (1963), let it be assumed that a weevil dies within 30 days of irradiation due to some unknown critical population of cells being reduced to a surviving fraction of b^{-20} , where b is some unknown but constant number.

The fraction of cells surviving a single dose of $\frac{1}{N} (D_{NT} - ^R D_T)$ should be $b^{-20/N}$ as N such doses, (delivered at intervals too small to permit any repopulation but sufficiently large to allow full intracellular recovery between fractions), would kill 50% of the weevils. The data tabulated in Table 5 may thus be used to obtain a theoretical cell survival curve (Fig 17c) for doses delivered in 71 to 79 minutes. The absolute level of survival is not obtainable from this curve as the value of b is unknown. An estimate of the

TABLE 7

NUMBER OF FRACTIONS N	LD 50 - REPOPULATION CORRECTION ($D_{NT} - {}^R D_T$) RADS
1	5320 $\pm$ 190
4	8890 $\pm$ 540
6	10150 $\pm$ 870
9	12210 $\pm$ 1250
14	13360 $\pm$ 1560
20	15490 $\pm$ 2460

Each of the above fractions were delivered in a period of
24 - 26 minutes.

The derivation of this table is described in Chapter 17.3.

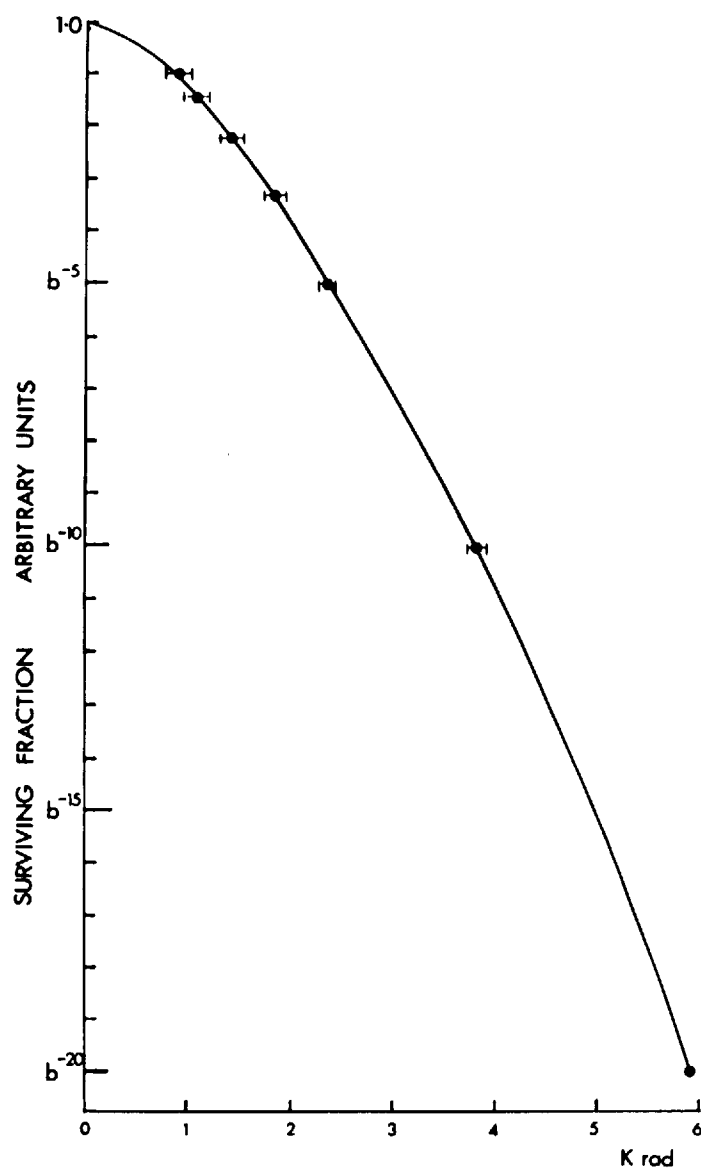


Fig.17c. Theoretical cell survival curve for the critical cells of the weevil derived as described (17.4) from the results of fractionation experiments.

surviving fraction may be obtained using the model of Lange and Gilbert (1968).

17. 5. Application of the theory of Lange and Gilbert

Lange and Gilbert (1968) described a model which related animal mortality probability to cell mortality. According to their model the slope of the animal mortality probit against dose curve is related to the slope of the appropriate cell survival curve by the following relationship

$$\frac{1}{g} = kD_0 \dots\dots\dots(16)$$

where g is the slope of the probit-dose curve

k is a constant equal to 1.2

D_0 is the dose necessary to reduce the surviving fraction of cells (at the end of the irradiation) to 37%

If G is the slope of the probit against $\log_{10}$ dose curve it follows that

$$\frac{1}{G} = \log_{10} \left[1 + \frac{kD_0}{LD_{50}} \right] \dots\dots\dots(17)$$

Using this equation it is possible to estimate the value of D_0 from a knowledge of the slope of the probit curve and the LD 50. The above theory assumes that the critical cells of all the animals in any single experiment have an equal probability of surviving a given dose of radiation. In practice, variation in the sensitivity of cells from animal to animal, due to variation in environmental conditions, will reduce the slope of the observed probit curve. The present experiments were designed to find how the LD 50 varied from one regime to another. To reduce the variations between experiments, batches of experiments were carried out simultaneously

and the specimen pots in each of the experiments were distributed in a definite pattern within the constant-temperature room, as previously described (8.3.4.) Thus any changes in sensitivity due to temperature gradients in the room would increase the spread of sensitivity within each experiment but each experiment would be affected in a similar manner. Although, as previously mentioned (12.4) the observed probit slopes in these experiments were twice as steep as those observed by Jefferies and Banham (1961), it is believed that they would have been even steeper if all the pots in any one experiment had been stored in closer proximity to one another. The 15 single dose probit against logarithm of dose curves were found to have an average slope of 22.8 with a standard deviation of 2.8. By substituting this value of G into equation (17), D_0 was estimated to be 535 rads but in view of the above reasoning this must be considered, as an overestimate of D_0 .

The theoretical cell survival curve (Fig 17c) at a dose of 5900 rads has a D_0 of $1850/\log_e b^{10}$ and by equating this to the above estimate, b^{-20} is found to be equal to 0.001. This suggests that to kill 50% of the weevils their critical cell populations have to be reduced by a factor of 1000. If D_0 is smaller than 535, as suspected, the factor would be larger than 1000.

17. 6. Variations of probit slope with regime

From equation (17) it will be observed that the smaller the value of $LD\ 50/D_0$ the smaller the slope G of the probit of mortality against the logarithm of dose curve.

The single dose $LD\ 50$ should be equal to $D_Q + \alpha D_0$ where α is a constant equal to $\log_e b^{-20}$ and D_Q is the quasi-threshold-dose defined previously (3.1) for the appropriate cell survival curve.

$$\therefore LD\ 50/D_0 = \alpha + D_Q/D_0 \dots\dots\dots(18)$$

It was shown theoretically by Lajtha and Oliver (1961) and experimentally by Berry and Cohen (1962), Bedford and Hall (1963) and Hall and Bedford (1964) that D_0 increases and D_Q decreases as the dose-rate decreases. Thus D_Q/D_0 decreases as the dose-rate decreases. The effect was most marked in the range 100 to 1 rads/minute, (Hall, 1972). The single dose LD 50 experiments employing exposure times of 71 to 79 minutes involved dose-rates of the order of 80 rads/min whilst the low dose-rate continuous irradiation involved dose-rates of 2 to 25 rads/min. Thus in the low dose-rate experiments, LD 50/ D_0 and consequently the probit slope G should be smaller than in the single dose experiments.

The mean probit slopes observed in the 12 low dose-rate and the 15 single dose experiments and their standard deviations were 18.8 ± 2.4 and 22.2 ± 2.8 respectively. This difference is significant at the 80% level of confidence and is probably due to the theoretical reasons discussed above.

The mean probit slope observed in the 35 fractionated acute experiments was 24.9 ± 4.1 which at the 75% level of confidence is significantly higher than that observed in the single dose experiments.

In order to predict how the probit slope should change with fractionation it is necessary to consider how the factor LD 50/ D_0 changes with fractionation.

From equation (4)

$$\text{LD } 50/D_0 = (D_1 + R_{D_N} + r_{D_T}) / D_0 \dots\dots\dots(19)$$

If the number of fractions is increased, R_{D_N} increases but the dose per fraction necessary to kill the weevils decreases causing an increase in the effective D_0 . Examination of Table 5 reveals that $(D_1 + R_{D_N})$ which is equal to $(\text{LD } 50 - r_{D_T})$ increases by a factor

of 3 as N is increased from 1 to 20, (i.e. the LD 50, corrected for repopulation increases from 5900 to 17750 rads). Examination of the cell survival curve (Fig 17c) reveals that D_0 also increases by a factor of 3 as the dose per fraction is reduced from 5900 rads to 17750/20 rads. Thus an increase in the number of fractions should make little difference to the slope of the probit curve provided negligible repopulation occurs between fractions.

Repopulation between fractions causes a pronounced effect. If for a constant number of fractions, the overall time is increased, T_{D_T} increases, the LD 50 increases and the dose per fraction increases. Examination of the derived cell survival curve (Fig 17c) shows that as the dose per fraction increases the effective D_0 decreases. Thus the value of LD 50/ D_0 increases appreciably as is illustrated by the 20 fraction in 25 day experiment. The LD 50 was found to be 32130 rads, T_{D_T} was estimated to be 14380 rads and the dose per fraction was 1607 rads. (The slope of the observed probit curve for this regime was 30.4 with 95% fiducial limits of ± 11.6). Thus the LD 50 is 5.4 times larger than the single dose LD 50 but from Fig 17c it may be estimated that D_0 at 1607 rads is only 2.1 times larger than D_0 at 5900 rads. Thus on changing from a single dose to a 20 fraction in 25 days regime the value of LD 50/ D_0 increases by a factor of 5.4/2.1 (i.e. by a factor of 2.6).

The above analysis indicates that there are reasons to expect the probit slopes to be steeper for fractionated regimes involving significant repopulation. The 35 fractionated regimes referred to above included regimes of short and long duration, e.g. 4 fractions in 9 hours (slope 17.7; 95% fiducial limits of ± 5.4) and 4 fractions in 28 days (slope 28.0; 95% fiducial limits of ± 12.3). Closer examination revealed no consistent relationships between probit slope and fraction number but a general tendency toward increasing slope

with increasing overall time in keeping with the above theoretical considerations. The 95% fiducial limits of the slopes observed in individual experiments were considered to be too large to justify a more detailed analysis of this effect.

17. 7. Conclusions

The LD 50₍₃₀₎ for weevils increases with the number of "daily" fractions. From 2 to 20 "daily" fractions the increase is practically constant at 1350 rads per fraction. It is estimated that from 2 to 6 fractions 63% of this increase is due to intracellular recovery and 37% to repopulation. From 9 to 20 fractions, 35% of the increase is estimated to be due to intracellular recovery and 65% to repopulation. When weevils are irradiated with 1 to 20 fractions, shortening the irradiation time per fraction from 75 minutes to 25 minutes decreases the LD 50 by an average of 8%. For single dose irradiations the average decrease observed was 11% showing that appreciable dose-rate effects can occur at dose-rates in excess of 78 rads/min, when the effect being studied requires an irradiation time in excess of one hour.

The fractionated data, corrected for repopulation have been used to derive the shape of the cell survival curve for the critical cells of the weevil (Fig 17c). By applying the theory of Lange and Gilbert (1968) it is deduced that D_0 for this curve is less than 535 rads and that to kill 50% of the weevils their critical cell populations need to be reduced by a factor in excess of 1000.

The differences in the slopes of the probit regression lines observed for different irradiation regimes may be partially explained by considering how the LD 50 and the effective slope of the appropriate cell survival curve vary with regime.

CHAPTER 18

THE RELATIONSHIP BETWEEN THE RATE OF REPOPULATION AND THE DAMAGE PRESENT

18. 1. Experiments using two doses of unequal magnitude

The relationship (Equation 15) derived previously (16.4.3) provides a means of estimating the total repopulation occurring during a fractionated regime which kills 50% of the weevils, provided the regime consists of equally spaced fractions of equal magnitude. As already discussed (16.5) the data from which this relationship was derived suggest that the rate of repopulation (expressed in rads/day) may be directly proportional to the damage present (expressed in rads). If such a simple relationship exists, it could be used to estimate the repopulation occurring during regimes involving fractions of unequal magnitude and unequal spacing. Experiments Nos 52 - 66 were designed to find whether a simple relationship existed or not. In these experiments, weevils were irradiated with an initial dose D_A and t_g days later subjected to a second dose. By keeping D_A and t_g constant and varying the second dose, the total dose ($D_A + D_B$) necessary to kill 50% of the weevils in 30 days was determined. In this way the $LD\ 50_{(30)}$ was found for five values of D_A ranging from 1040 to 5240 rads and three values of t_g namely: 1.025, 3.83 and 8.14 days. The results of these experiments are presented in the comprehensive results (Table 3, 12.3). The doses D_B (normalised in accordance with their appropriate single dose controls) are plotted against D_A (similarly normalised) in Figs 18a, 18b and 18c for each of the three time intervals. For purposes of comparison, theoretical curves predicted as described below are also shown.

18. 2. Predicted results assuming the rate of repopulation to be proportional to the damage present

The hypothesis to be tested is that at any instant of time (except during the mitotic delay) the instantaneous rate of repopulation

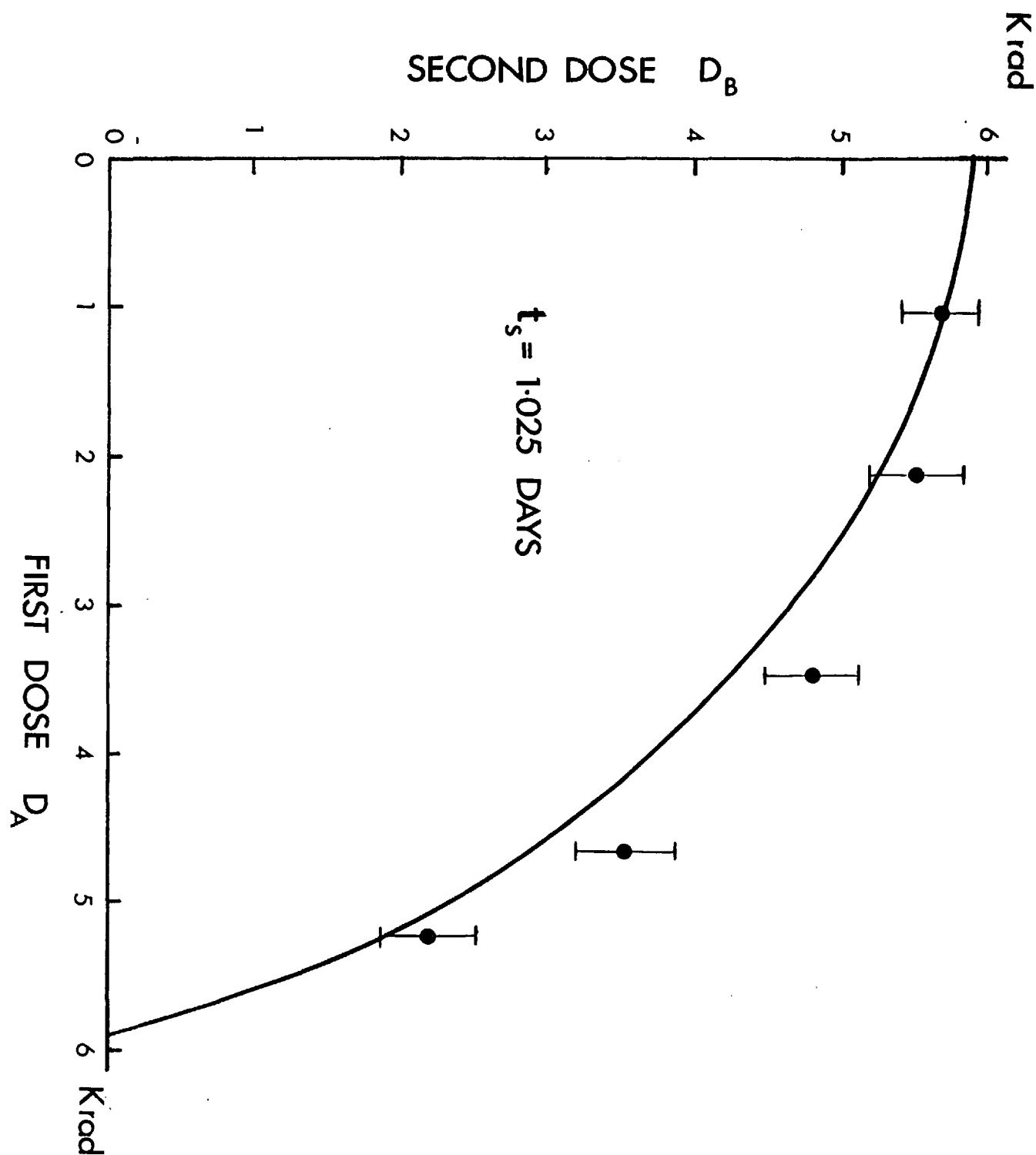


Fig.18a The dose D_B necessary to kill 50% of the weevils in 30 days if delivered 1.025 days after an initial dose D_A plotted against initial dose D_A . The observed values of D_B may be compared with the curve predicted by the hypothesis discussed in Chapter 18.2.

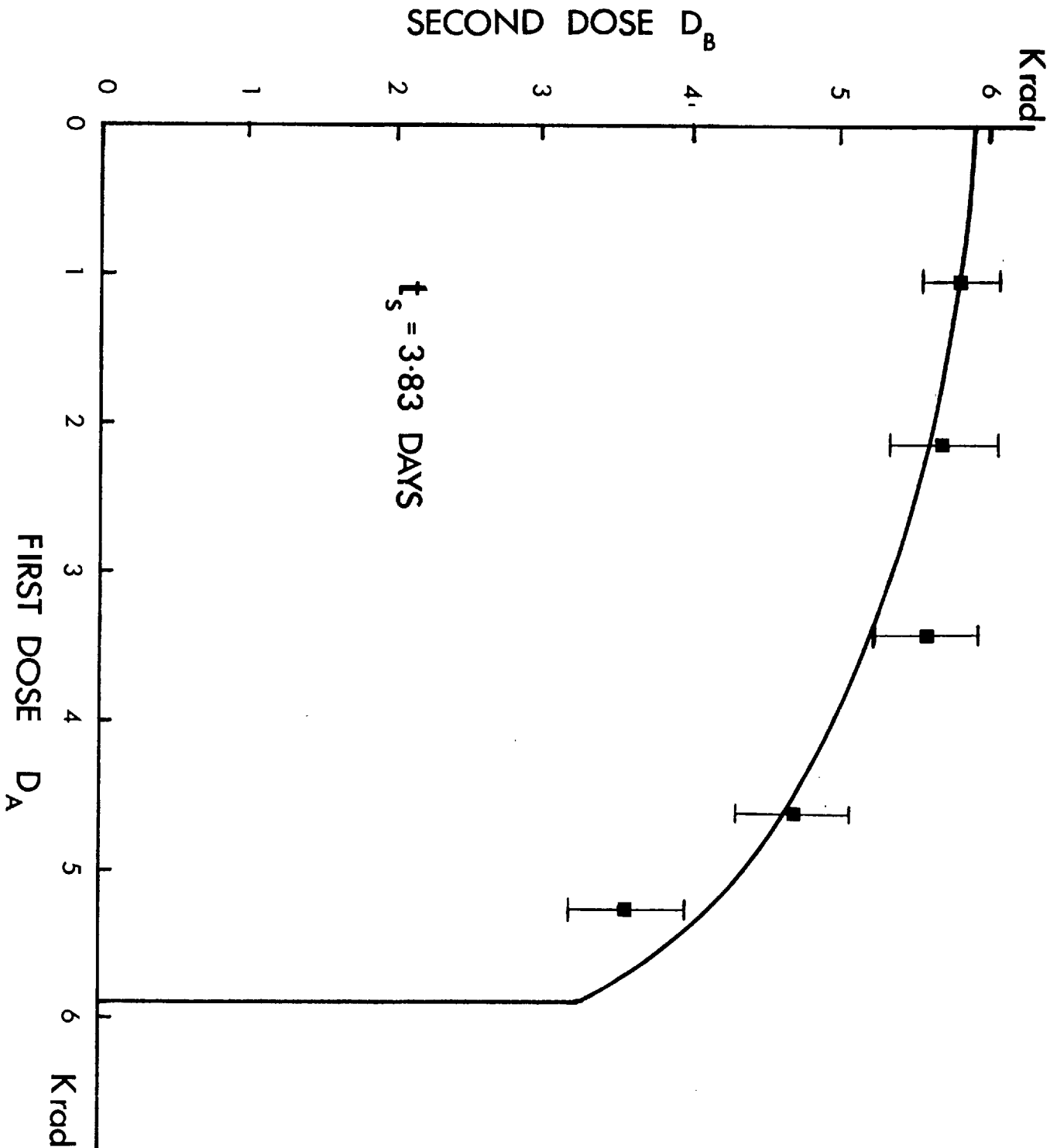


Fig. 18b The dose D_B necessary to kill 50% of the weevils in 30 days if delivered 3.83 days after an initial dose D_A plotted against initial dose D_A . The observed values of D_B may be compared with the curve predicted by the hypothesis discussed in Chapter 18.2.

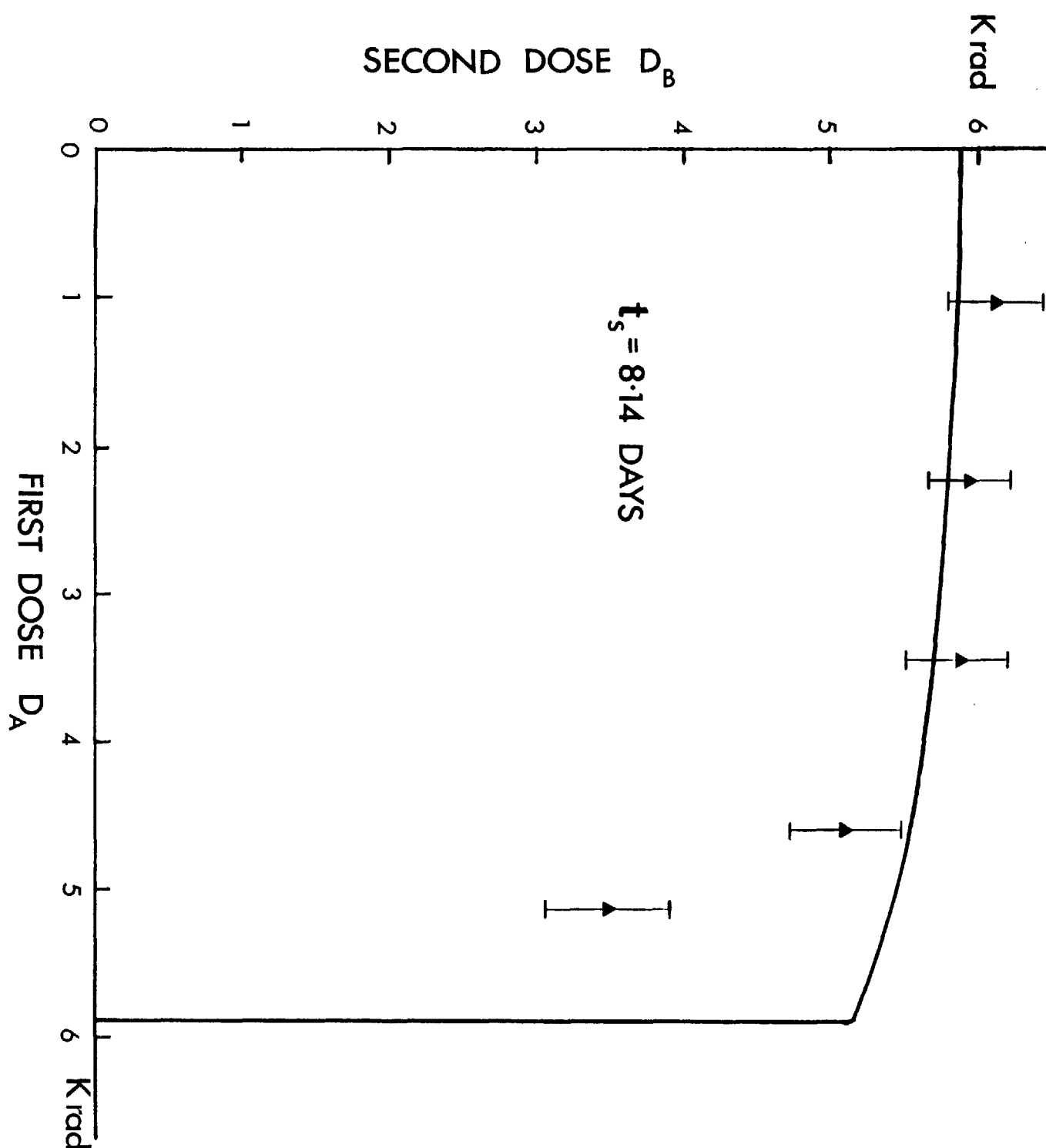


Fig. 18c The dose D_B necessary to kill 50% of the weevils in 30 days if delivered 8.14 days after an initial dose D_A plotted against initial dose D_A . The observed values of D_B may be compared with the curve predicted by the hypothesis discussed in Chapter 18.2.

(expressed in rads/day) is proportional to the damage A (expressed in rads) present at that instant of time.

The question arises as to how the dose equivalent of damage (A_0 rads) present immediately after a single dose of D_A rads should be defined. A_0 cannot be as large as D_A as part of the damage produced by D_A rads is repaired by intracellular recovery. The value of A_0 should represent the damage which remains after intracellular recovery is complete but before any repopulation has occurred. Let us therefore define the damage A_0 present immediately after a dose D_A by the following equation:

$$A_0 = D_1 - D'_B \quad \dots\dots\dots(20)$$

where D_1 is the single dose necessary to kill 50% of the unirradiated weevils and D'_B is the dose necessary to kill 50% of the weevils if delivered shortly after the dose D_A , at a time when intracellular recovery is complete but before repopulation has commenced. For any value of D_A , the value of D'_B may be determined from the theoretical cell survival curve derived previously (Fig 17c).

According to the above hypothesis, the total amount of repopulation (expressed in rads) occurring within t_s days of an initial dose D_A will be x_{D_T} where

$$x_{D_T} = A_0 \left[1 - e^{-\lambda(t_s - t_m)} \right] \quad \dots\dots\dots(21)$$

where λ is a constant previously estimated (16.4.3) to be 0.291 days^{-1} and t_m is the mitotic delay previously estimated (16.4.2) to be $0.183 \text{ days/1000 rads}$. The dose D_B necessary to kill 50% of the weevils if delivered t_s days after an initial dose D_A is therefore given by:

$$D_B = D'_B + x_{D_T} \quad \dots\dots\dots(22)$$

Values of D_B calculated from the above equation have been used to plot the theoretical curves shown in Figs 18a, 18b and 18c.

18. 3. Comparison of observed and predicted results

Fig 18a shows that for initial doses of 3000 to 5000 rads the predicted values of D_B at 1.025 days are 500 to 600 rads less than those found. The discrepancy may be partly due to experimental uncertainties (the 95% fiducial limits on the LD 50 of the irradiated controls for these experiments are unusually large at $\pm 3.5\%$) and partly due to cell synchrony as the split-dose recovery curve (Fig 14a) shows that the critical cells of the weevil are moving into a resistant phase 24 hours after an initial dose of 4000 rads.

In view of the above considerations the agreement is reasonably satisfactory. However, as the amounts of repopulation occurring in one day are predicted to be no more than the equivalent of 200 rads, this comparison is more a test of the reliability of the derived cell survival curve (Fig 17c) than the repopulation relationship.

For intervals of 3.83 days, Fig 18b shows excellent agreement between prediction and experiment except at $D_A = 3400$ rads where the prediction coincides with the lower limit of the observed result and at $D_A = 5240$ rads where the prediction is 600 rads higher than the observed result. If in these diagrams the observed results had not been normalised, they would all be 2.7% lower than plotted. Thus whilst the normalising procedure may be blamed for part of the discrepancy in Fig 18a and at $D_A = 3400$ rads in Fig 18b it is likely to have minimised the discrepancy at $D_A = 5240$ rads. Cell synchrony could still be having some effect at 3.83 days but it is doubtful whether it can account for the latter discrepancy.

For intervals of 8.14 days the suggested hypothesis predicts that the majority of the damage caused by the initial doses would be repaired by repopulation before the second dose was given. This is borne out by experiment for initial doses below 4000 rads. Above 4000 rads the prediction begins to deviate from observation and for an initial dose of 5130 rads overestimates the repopulation by as much as 1950 rads. In view of the above discrepancies it was decided to plot the relationship between the repopulation observed, the initial damage A_0 and the initial dose D_A for each of the three time intervals used (Fig 18d). The repopulation observed is assumed to be $(D_B - D'_B)$ and the initial damage is assumed to be $(D_1 - D'_B)$ as previously defined (18.2).

As previously discussed, the effects of cell synchrony are believed to have inflated the values of $(D_B - D'_B)$ observed for intervals of 1.025 days and thus the lower curve in Fig 18d is believed to overestimate the true repopulation occurring during this interval. Examination of Fig 18d shows that for all three intervals the repopulation increases as the initial dose D_A increases and at first is approximately proportional to the initial damage A_0 . As D_A is increased beyond 3500 rads the repopulation increases less rapidly and reaches a peak when D_A is approximately 4500 rads. As D_A is further increased the amount of repopulation per interval falls dramatically. It may be argued that the dramatic decrease in the value of $(D_B - D'_B)$ for high values of D_A may not be due to a decrease in repopulation but could be due to the first dose D_A killing a proportion of the weevils so that the second dose does not have to kill so many. Examination of the single dose mortality curves shows that 5200 rads kills 10% of the weevils. Thus to reduce the total surviving fraction of weevils to 50% the second dose D_B is only required to kill 45% of the 90% surviving

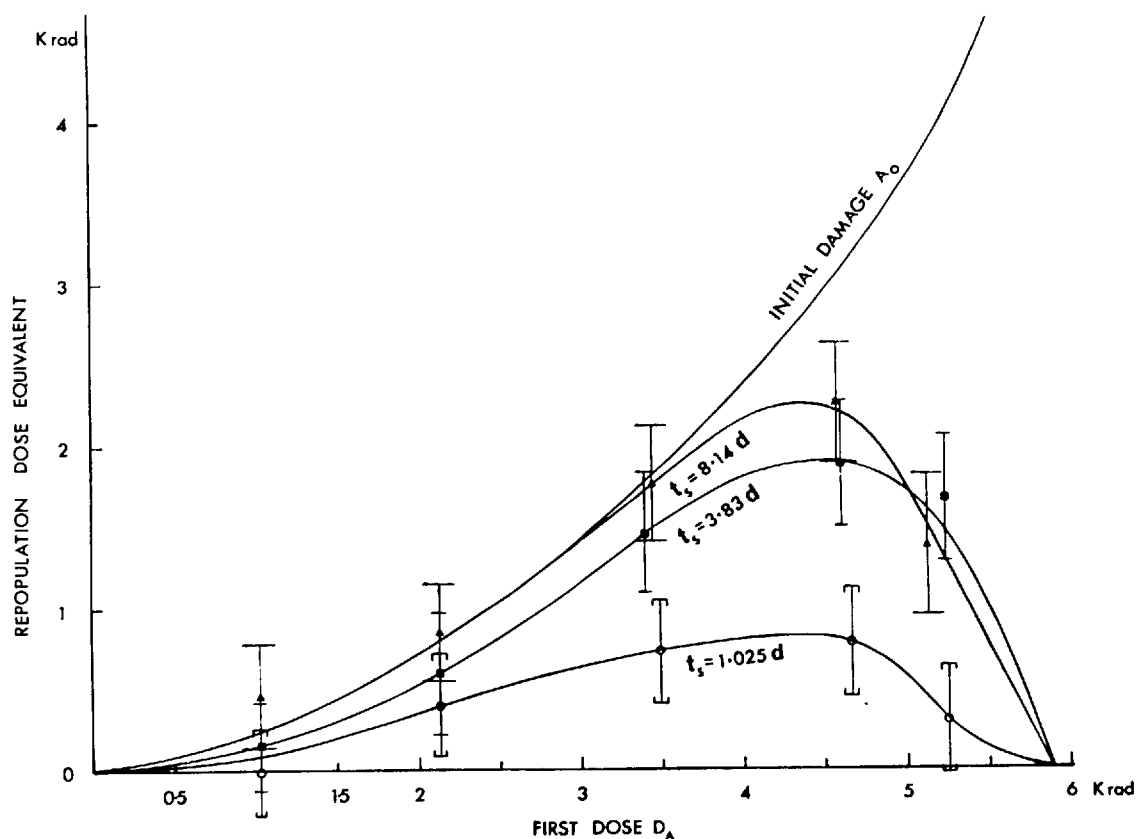


Fig.18d The amount of repopulation (rads-equivalent) occurring in a period of t_s days after an initial dose D_A , plotted against D_A . The three lower curves show the repopulation dose-equivalents found by experiment for periods of 1.025, 3.83 and 8.14 days and how these vary with initial dose. The initial damage A_0 (upper curve) is defined by equation 20 and represents the dose-equivalent of the damage present shortly after an initial dose D_A at a time when intracellular recovery is complete but before repopulation has commenced.

the first dose. The single dose mortality curves show that the dose required to kill 45% is only 70 rads less than that required to kill 50%. For intervals of 8.14 days the decrease in the value of $(D_B - D'_B)$ which occurs as D_A is increased from 4500 to 5200 rads is 860 rads and thus cannot be explained by the killing effect of the first dose.

It may be concluded that although the total repopulation is approximately proportional to the initial damage for values of D_A less than 3500 rads the subsequent rapid decrease in repopulation shows that there is no simple relationship between repopulation and damage which is applicable to the whole range considered.

The results suggest that homeostatic control mechanisms in the weevil are able to speed up the repopulation rate (rads/day) in approximate proportion to the damage present but that after doses of 4000 rads or more the reproductive capacity of the surviving cells is severely impaired and they are thus unable to respond to the homeostatic control. An alternative explanation would be that the control mechanism itself is disrupted by such large doses. (4000 rads is 68% of the single dose LD 50).

The fact that no simple relationship has been found between repopulation and damage in no way invalidates the relationship (Equation 15) established previously (16.4.3) for regimes employing equally spaced fractions of equal magnitude. The extension of this relationship to regimes involving unequal doses or unequal intervals is however, made more difficult. In certain cases, guidance may be obtained by considering the shapes of the curves relating repopulation and damage (Fig 18d) as is illustrated by considering the anomalous result discussed below.

18. 4. The anomalous result for 6 fractions in 7 days

The LD 50, corrected for repopulation, observed for 6 fractions in 7 days (Experiment No 16) was found to be 1200 rads below the smooth curve drawn through the remainder of the daily fractionation data similarly corrected. This anomaly was thought to be associated with the 3-day gap between the 5th and 6th fraction as this was the only "daily" regime in which such a gap occurred so close to the end of the regime. Experiments Nos 47 and 49 were done to test this theory and their results are compared below.

	<u>LD 50</u>
No 16, 6F/7d gap between 5th and 6th fractions	13410 $\pm$ 460r
No 47, 6F/7d gap between 3rd and 4th fractions	14600 $\pm$ 510r
No 49, 6F/5d no gap	13140 $\pm$ 280r

Comparison of experiment Nos 47 and 49 shows that extending the gap between the 3rd and 4th fractions from 1 day to 3 days allows additional repopulation, equivalent to 1460 rads, to occur.

Comparison of experiments Nos 16 and 49 shows that a similar extension between the 5th and 6th fractions allows additional repopulation equivalent to only 270 rads to occur. This difference of 1190 rads may be explained as follows.

In experiment No 16, immediately before the 3-day gap, 11,170 rads were delivered in five fractions in four days. This dose is 95% of the LD 50 for 5F/4d (Fig 17a). Thus the damage present at the start of this 3-day gap was probably similar to that caused by 95% of the single dose LD 50 (i.e. $5900 \times 0.95 = 5600$ rads). After such a single dose, Fig 18d shows that the amount of repopulation occurring in 3.83 days should be approximately 700 rads. The exact amount will depend on how the curve is drawn in this rather critical region.

In experiment No 47, on the other hand, the dose delivered immediately before the 3-day gap was 7300 rads in three fractions in 2 days which

is only 80% of the LD 50 for 3F/2d (Fig 17a). Thus the damage present at the start of the 3-day gap in this experiment was probably similar to that caused by 80% of the single dose LD 50 (i.e. $5900 \times 0.80 = 4720$ rads). After such a single dose, Fig 18d shows that the amount of repopulation occurring in 3.83 days should be close to the peak value of 1900 rads.

Thus the 3-day gap in experiment No 47 occurred at a time when it would have maximum effect whereas in experiment No 16 it occurred at a time when it would have relatively little effect.

18. 5. Conclusions

The relationship between the rate of repopulation (expressed in rads/day) and the damage present (expressed in rads) is not a simple one. When the damage present is less than that caused by a single dose of 3500 rads, the amount of repopulation occurring in a given interval is approximately proportional to the damage present at the start of that interval. The majority of the damage is completely repaired by this process in a period of eight days.

When the damage present is in excess of that caused by a single dose of 3500 rads the amount of repopulation is appreciably less than that predicted by a proportional relationship.

When the damage present is in excess of that caused by a single dose of 4500 rads the rate of repopulation decreases dramatically as the damage increases. This suggests that the critical surviving cells of the weevil may be sub-lethally damaged to such an extent that their proliferative capacity is greatly impaired or, alternatively, that the homeostatic control system of the weevil is disrupted by such high doses. If, at some stage during a fractionated regime, a rest period is introduced, the amount of repopulation occurring during that period will be very dependent on the damage present at the start of that period. In weevils, maximum repopulation occurs when the initial damage is fairly

large (equivalent to that produced by a single dose of 4500 rads).

If the rest period occurs too close to the end of the regime, the damage present may be sufficiently large to inhibit cell proliferation and little effect may be produced.

The implications of these findings and their relationship to results obtained by other workers will be discussed in Chapter 20.

CHAPTER 19TESTING THE GENERAL FORMULA19. 1. Variation in LD 50 with duration of low dose-rate continuous irradiation

The LD 50's observed with continuous low dose-rate irradiation are presented in the comprehensive table of results (Experiments Nos 31 to 43 in Table 3, 12.3). These results (after normalising in accordance with their appropriate single dose controls) are plotted against irradiation time on a linear scale in Fig 19a. It will be seen that the results obtained in January 1971 (open squares) are in close agreement with those obtained at Easter 1971 (closed squares). The LD 50 for 0.4 hours irradiation was obtained from the January and February 1972 experiments (Nos 68, 69 and 75).

It will be observed that the LD 50 increases by a factor of three as the irradiation time is increased from 0.4 to 106 hours.

The derivation of the dotted curve in Fig 19a is discussed below.

19. 2. The Correction for repopulation

To test the general formula equating protracted and acute regimes of radiation (Liversage, 1969), it is necessary to determine how $(D_t - \bar{x}D_t)$ varies with irradiation time. As previously defined (Chapter 4) D_t is the LD 50 for continuous irradiation lasting t hours and $\bar{x}D_t$ is the dose necessary to compensate for repopulation occurring during the irradiation.

Hall (1972) suggests that the dose-rate necessary to stop cell division occurring during irradiation is inversely proportional to cell cycle time and is equal to 19 rads/hour for HeLa cells having a cell cycle time of 24 hours. The large repopulation effect observed in weevils during fractionated regimes of radiation suggests that the cell cycle time for the critical cells of the weevil can be very short. The maximum rate of repopulation is estimated (16.4.3) to be 850 rads/day. The critical cells of the weevil are estimated (17.5) to have a D_0 of

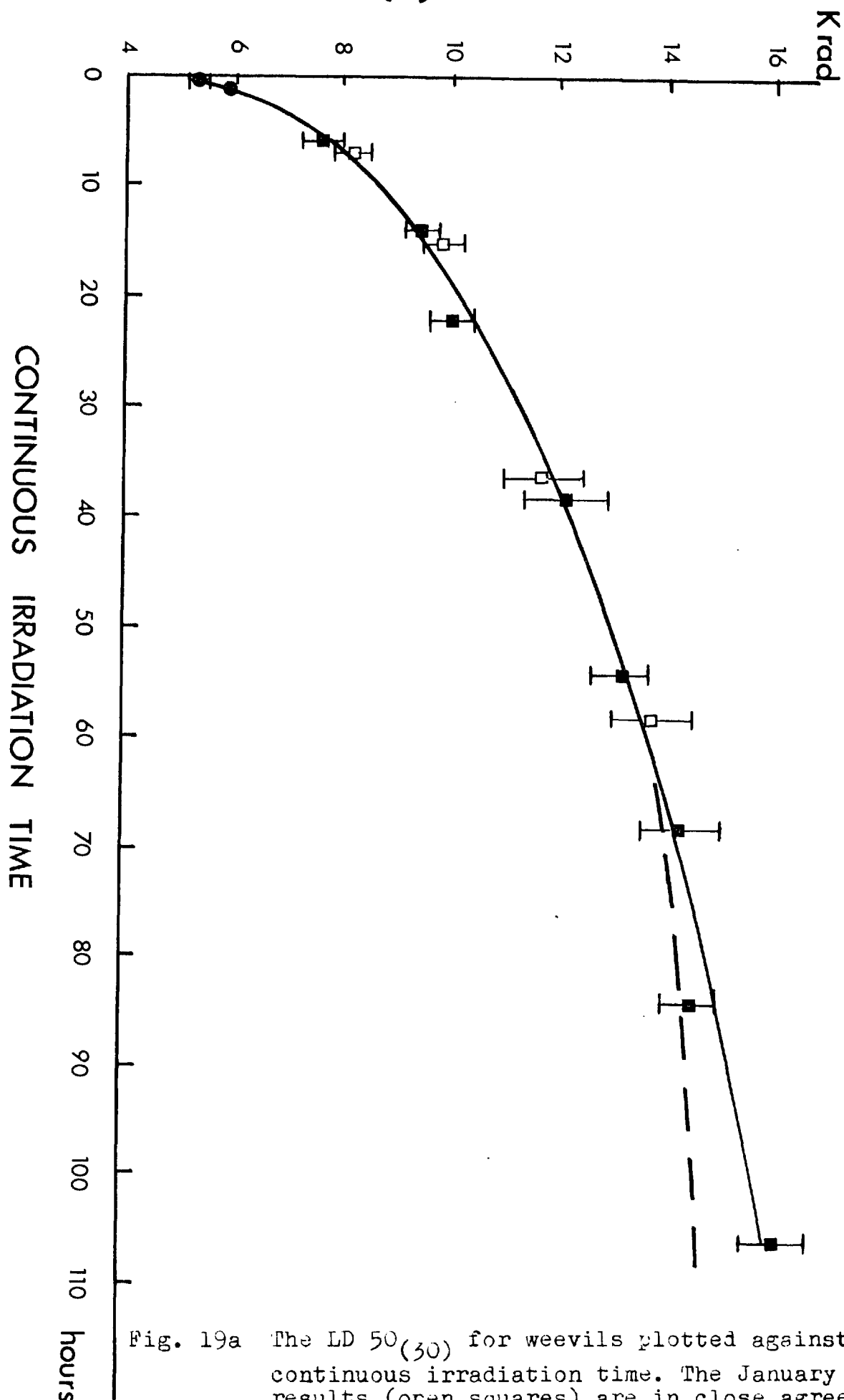


Fig. 19a The LD 50₍₃₀₎ for weevils plotted against continuous irradiation time. The January 1971 results (open squares) are in close agreement with the Easter 1971 results (closed squares). It is estimated that the LD 50 corrected for repopulation lies between the full and dotted curves, as discussed in Chapter 19.2.

535 rads. Combining these two estimates suggests that the cell cycle time for the critical cells of the weevil can be as short as 10.3 hours. It is doubtful whether it is valid to extrapolate the data of Hall (1972) to weevils but his data would suggest that a dose-rate of 44 rads/hour should be adequate to stop cell division in the weevil. The LD 50 for the most protracted continuous irradiation regime was found to be 16710 rads delivered in a period of 106 hours at a dose-rate of 158 rads/hour, (i.e. at a dose-rate 3.6 times higher than that necessary to stop cell division). It may therefore be concluded that repopulation by cell division was negligible during all the low dose-rate continuous irradiations but the extrapolation from mammalian cells to insect cells throws doubt on this conclusion.

An alternative approach is to assume that radiation induces a mitotic delay of 0.183 days/1000 rads, as found earlier (16.4.2). If the delay t_m , so calculated, exceeds the duration of the regime it is assumed that no repopulation occurs. If however the delay is less than the duration of the regime it is assumed that repopulation occurs at the maximum possible rate (equivalent to 850 rads/day) for a period of $(t - t_m)$ hours

$$\therefore R_{D_t} = \frac{850}{24} (t - t_m) \dots\dots\dots(23)$$

where t and t_m are both expressed in hours.

The dotted curve in Fig 19a shows how $(D_t - R_{D_t})$ varies with irradiation time if the amount of repopulation occurring is calculated from the above equation.

It will be observed that both methods of approach indicate that no repopulation occurs when the regime is of less than 60 hours duration. For regimes of 60 to 106 hours duration, equation (23) suggests that a small repopulation component is present whereas the original approach suggests there is no repopulation effect in this range.

It may be concluded that during the continuous irradiation regimes repopulation effects were small, if not negligible, and that the true curve showing how $(D_t - {}^x D_t)$ varies with irradiation time is likely to lie between the dotted curve and the full curve in Fig 19a.

19. 3. The Application of the General Formula to the above data

According to the general formula for equating protracted and acute regimes of radiation discussed previously (Chapter 4), $(D_{NT} - {}^x D_T)$ should be approximately equal to $(D_t - {}^x D_t)$ provided N and t are related by the following equation:

$$N = \frac{\mu t}{2 \left[1 - \frac{1}{\mu t} (1 - e^{-\mu t}) \right]} \dots\dots\dots(6)$$

where D_{NT} is the LD 50 for N acute fractions delivered in T days, ${}^x D_T$ is the dose necessary to compensate for the repopulation occurring in T days, D_t is the LD 50 for continuous irradiation given over a period of t hours, ${}^x D_t$ is the dose necessary to compensate for repopulation occurring in t hours and μ is equal to $0.693/\gamma$ where γ is the half-life of the dose equivalent of sub-lethal damage.

Fig 19a shows how $(D_t - {}^x D_t)$ varies with irradiation time t and may therefore be used in conjunction with the above relationship to predict how $(D_{NT} - {}^x D_T)$ should vary with fraction number.

Under the conditions used in these experiments, the half-life of the dose equivalent of sub-lethal damage for the critical cells of the weevil has been shown (14.4) to be greater than 1 hour and less than 2 hours. To allow for the uncertainty in the value of γ , the variation in $(D_{NT} - {}^x D_T)$ with fraction number has been predicted, as described above, using values of γ equal to 1.0, 1.5 and 2.0 hours. The resultant curves are plotted in Fig 19b. The full and dotted curves in this diagram reflect the repopulation correction uncertainty inherited from Fig 19a.

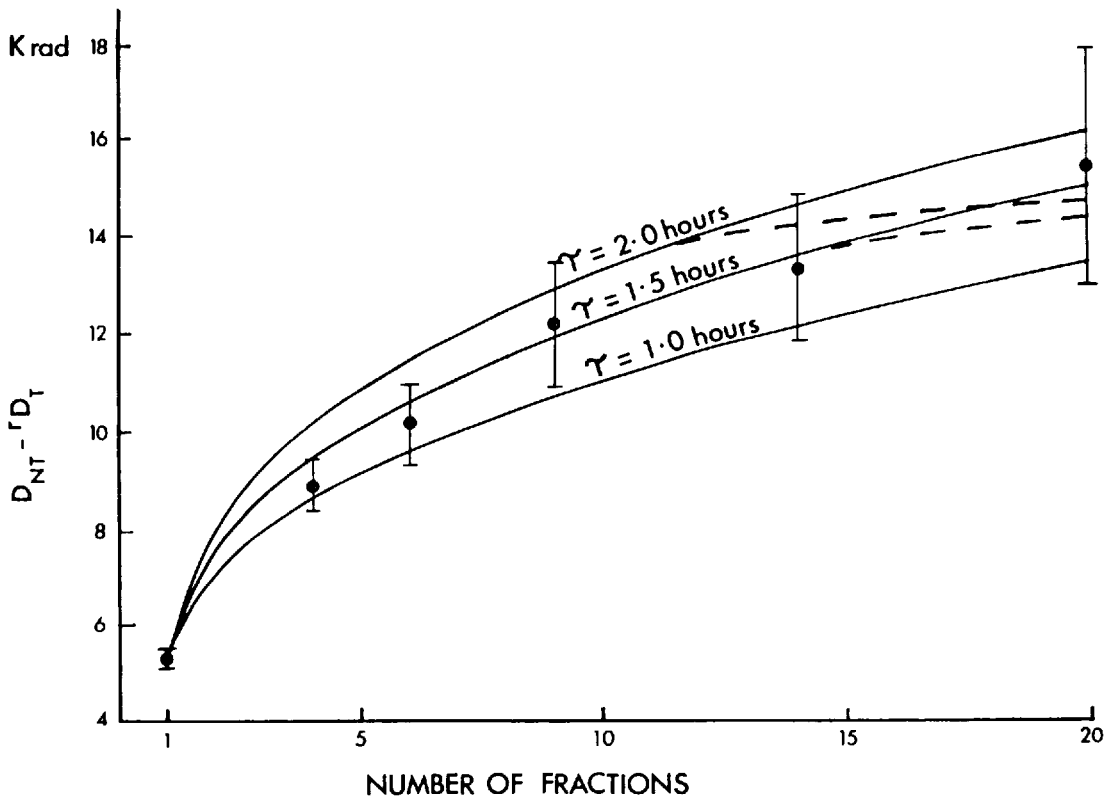


Fig. 19b The LD 50₍₅₀₎, corrected for cellular repopulation between fractions, plotted against the number of fractions. The curves show the predicted values obtained by applying the general formula (equation 6) to the low dose-rate continuous irradiation data, assuming the half-life of the dose-equivalent of sub-lethal damage to be 1.0, 1.5 or 2.0 hours. The results obtained from fractionated high dose-rate experiments, corrected for repopulation as described in Chapter 17.3, are also shown for purposes of comparison. The 95% confidence limits associated with these plotted points take into account the uncertainty in the observed LD 50's and that involved in the repopulation correction.

Values of $(D_{NT} - {}^x D_T)$, together with their 95% confidence limits, derived from the fractionation experiments (Table 7, 17.3) are also plotted in Fig 19b for comparison.

The values of 1.0 and 2.0 hours are the lower and upper limits respectively of the value of τ estimated from the split-dose recovery curve. The true value of τ is probably closer to 1.5 hours. However, any one of these three values of τ leads to predictions which are within $\pm 12\%$ of the experimental results obtained over the whole range investigated (i.e. from 1 to 20 fractions). Both the full curve and the dotted curve predicted using a value of τ equal to 1.5 hours agree with experiment to within $\pm 7\%$ over the whole range investigated and both lie within the 95% confidence limits of all the experimental points. Fig 19b suggests however that predictions using the general formula and a value of τ equal to 1.5 hours slightly overestimate the LD 50 for fractionated regimes involving small numbers of fractions (less than 6) and possibly slightly underestimates the LD 50 for regimes involving larger numbers of fractions (about 20).

19. 4. Conclusions

Liversage (1969) acknowledged that his formula for equating protracted and acute regimes of radiation was derived by making a number of assumptions and involved a number of approximations, as previously discussed (4.2). In view of this, he claimed his formula would not be strictly true but should be accurate to $\pm 10\%$.

The experimental results with weevils certainly substantiate this claim over the whole range investigated (i.e. from 1 to 20 fractions) but suggest that from 6 to 20 fractions the formula is probably accurate to $\pm 4\%$. The implications of these findings will be discussed in Chapter 20.

CHAPTER 20

Discussion of the results and their implications in radiobiology and radiotherapy

20. 1. Comparison of results with mammalian data

Qualitatively, but not quantitatively, the LD 50 for weevils varies with split-dose interval, overall irradiation time and number of fractions in a similar manner to the variation in iso-effect dose observed in normal mammalian tissues and cells. Effects have been observed in weevils which in mammalian tissues would have been attributed to each of the following phenomena:

1. Intracellular sub-lethal damage repair (14.3)
2. Progression of a partially synchronised population of surviving cells through phases of varying sensitivity (14.3)
3. Repopulation of damaged tissues by cell proliferation (Chapter 16)
4. Radiation induced mitotic delay (16.4.2)
5. Homeostatic control of repopulation (Chapter 18).

The system studied in the present investigation thus constitutes an excellent model for investigating precisely how these phenomena are likely to affect the shape of iso-effect dose relationships in normal tissues. The magnitude of the effect produced by any one of these phenomena is, of course, liable to be very different in weevils from that occurring in some other biological system.

The use of weevils has enabled a large body of accurate iso-effect data to be obtained. These iso-effect data are more comprehensive and more accurate than those obtained for any mammalian system and probably than those obtained for any other biological system to date. They have been used to test a number of hypotheses and may be used to test future hypotheses as and when these are formulated.

The findings with weevils may be compared with those obtained (perhaps in less detail or with less certainty) using systems which are more relevant to the clinical situation. If the relationships observed

in the more relevant tissues are consistent with those found in weevils the latter may be used as a guide when interpreting, interpolating or extrapolating the more relevant data. Examples of this are discussed below.

20. 2. The relationship between the rate of repopulation and the damage present

The weevil experiments involving fractions of unequal magnitude, previously analysed (Chapter 18), show that when the damage present is less than that caused by a single dose of 3500 rads, the rate of repopulation (expressed in "rads per day equivalent") is approximately proportional to the damage present (expressed in "rads equivalent"). As the damage is repaired, the rate of repopulation should decrease proportionately and should thus decrease exponentially with time. The multi-fraction experiments analysed previously (Chapter 16) show that this is true and that the time required for the repopulation rate to fall to half its initial value is 2.4 days. It is interesting to compare these findings with those obtained for mouse skin. Denekamp (1973) gave 4, 9 or 14 daily fractions of 300 rads to mouse skin followed by a series of test doses at either 0, 1, 4, 8 or 15 days later. By comparing the skin reactions she was able to estimate the rate of repopulation (rads/day) occurring after each of the fractionated regimes.

After 4 doses of 300 rads she found no significant repopulation occurred during the next 15 days. (The possibility that some repopulation was present but masked by synchrony is discussed below). After 9 doses of 300 rads, the average rates of repopulation during the first and second weeks were 58 and 22 rads/day respectively. After 14 doses of 300 rads, the corresponding rates were 130 and 18 rads/day.

These skin reaction results show:-

- a. the rate of repopulation increases with the damage present
- b. as the damage is repaired the rate of repopulation decreases
- c. the time required for the rate of repopulation to fall to half its initial value is between 2.5 and 5.0 days (assuming it falls exponentially).

These findings have important implications in split-course therapy (Scanlon, 1960; Sambrook, 1962, 1963; Holsti and Taskinen, 1964) and suggest, as Denekamp points out, that in split-course therapy the interval between courses should not be allowed to be too long or the benefit of the faster rate of repopulation may be lost.

Denekamp's results also suggest that the most beneficial time to introduce a rest period would be towards the end of treatment when the damage present (and consequently the rate of repopulation) are likely to be near their maximum values. Denekamp refrained from drawing this conclusion and the weevil results suggest that her restraint was well founded.

The weevil results show the 3 effects (a) (b) and (c) referred to above but the fall in the repopulation rate is slightly faster than in mouse skin (The time to fall to half the initial rate in weevils is 2.4 days) The weevil results demonstrate that this fall does occur exponentially (Fig 16f) but show that the rate of repopulation only increases with the damage present provided the damage present is less than that caused by a single dose of 4000 rads. As the damage present increases above this level the rate of repopulation falls dramatically (Fig 18d). Whether such a decrease occurs in mouse skin or in the normal tissues of patients is not known and requires further investigation. The weevil results, however, provide a warning of what might be expected. The experiments using 6

fractions in 7 days with a 3-day gap half-way or alternatively near the end of the irradiation regime emphasise the point, as previously discussed (18.4 and 18.5). When considering the possible implications of these results it should, however, be remembered that during the weevil experiments the whole body was irradiated whereas in radiotherapy only a small portion of the body is normally irradiated.

The results observed in mouse skin are perhaps at variance with the weevil results in one respect. After 4 doses of 300 rads, Denekamp (1973) found no effect due to repopulation during the period 1 to 15 days. The repopulation effect only became appreciable after a further week of daily irradiation. Denekamp suggested that the delay in the onset of the repopulation effect probably reflected the necessity to accumulate an appreciable amount of damage which would be both dose dependent and time dependent if the damage is only expressed at mitosis. She pointed out that the normal turnover time of the mouse epidermis (without mitotic delay) is at least 4.5 days and that the rate of expression of damage will be further slowed by radiation induced mitotic delay from each dose.

The weevil results show that the rate of repopulation is certainly dose dependent but they do not exhibit any comparable time delay. Fig 16e suggests that rapid repopulation is occurring in the critical cell population of the weevil 12 hours after a dose of 2630 rads. It has been estimated that the cell cycle time of these cells could be as short as 10.3 hours but, if allowance is made for mitotic delay, it would appear that homeostatic control has taken effect in the weevil before many cells could have suffered a mitotic death. This suggests that either interphase death is occurring in the weevil or the homeostatic control mechanism in the weevil can detect potentially lethal damage before the majority of that damage has been expressed in the form of cell death. Further evidence on this point is

required.

The data of Denekamp regarding the rate of repopulation in skin after 4 doses of 300 rads are also worthy of further consideration. The test dose necessary to produce a given skin reaction, 8 days after the 4 doses, was 450 rads more than that required zero days after the 4 doses. According to Denekamp, 195 rads of this increase was due to intracellular recovery and this suggests that 255 rads was due to repopulation and synchrony. By 8 days the effects of synchrony are likely to be small and it may therefore be deduced that, during the first 8 days, repopulation occurred at an average rate of 32 rads/day. Denekamp's estimate of no repopulation (her table actually shows - 10 rads/day) during the period 1 - 8 days may have been greatly influenced by the effects of synchrony inflating the dose necessary at one day, as she herself points out. It is thus possible that the homeostatic forces in mice are acting just as fast as those in weevils. The difficulty of separating the effects of synchrony from those of repopulation will make it difficult to obtain decisive evidence on this point.

20. 3. The relationship between iso-effect dose and overall time of a fractionated regime.

It has been shown (Chapter 16) that none of the published relationships purporting to predict how iso-effect doses for the normal tissues of patients vary with overall treatment time, are of the shape necessary to account for the variations observed in the LD 50 for weevils. The published formulae may be useful in predicting small changes in iso-effect dose for the normal tissues of patients but may be inaccurate when large changes are involved.

The form of the true relationship is more easily and accurately determined for weevils than for normal mammalian tissues, as previously

discussed (20.1). Because normal mammalian tissues and the critical cells of the weevil both exhibit the same effects (20.1) it is probable that the form of relationship (Equation 15) found for weevils will be equally applicable to normal mammalian systems. The values of the parameters involved are, of course, likely to be very different. From the work of Denekamp it appears that the repopulation rate M in the mouse skin decreases to half its initial rate in 2.5 to 5 days. If equation (14), as previously derived for weevils (16.4.3) applies to mouse skin,

$$M = M_0 e^{-\lambda t} \dots\dots\dots(14)$$

The value of λ for mouse skin will therefore lie between 0.14 and 0.28 days⁻¹.

It follows that the average rate of repopulation occurring during the first 7 days after irradiation will be equal to $M_0 (1 - e^{-7\lambda}) / 7\lambda$, where M_0 is the initial rate of repopulation. Using the above estimated value of λ , it follows that the initial rate of repopulation in mouse skin is 1.6 to 2.3 times the average rate occurring during the first 7 days.

To estimate the rate of repopulation occurring in normal tissues during fractionated regimes, it is common practice to compare the iso-effect dose for N fractions given daily with that for N fractions given, say, 7 days apart. Liversage (1969) applied this procedure to the pig skin results of Fowler, Morgan, Silvester, Bewly and Turner (1963) to obtain a repopulation rate in pig skin of 25 rads/day. The above calculation shows that the initial rate of repopulation could be twice as great as the 7-day average rate found by Liversage (1969). Both Fowler (1971) and Liversage (1971) drew attention to the fact that repopulation rates were likely to be larger during

short time intervals than those found during long time intervals but neither could accurately predict the magnitude of such increases. When daily fractions are used the rate of repopulation in pig skin, according to the above reasoning, is likely to be approximately 50 rads/day but the periods during which repopulation occurs are likely to be considerably less than the intervals between fractions due to radiation induced mitotic delay. The latter will depend upon the dose delivered per fraction and possibly on the cell cycle time of the epithelial cells at the time of irradiation (Hegazy, 1969). Thus the amount of repopulation occurring in skin during a daily fractionation regime could actually be less than that estimated on the basis of 25 rads/day. The increase in repopulation occurring on changing from a fraction interval of 1 day to a fraction interval of 2 days could, however, be twice as great as that estimated on the basis of 25 rads/day.

It may be concluded that although Equation (15) is likely to apply to mammalian tissues the values of the parameters involved are not known to a sufficient degree of accuracy at present to enable its application to be a feasible proposition

20. 4. The relative importance of intracellular recovery and repopulation

Before 1963, the importance of fraction number was not appreciated by the majority of workers in radiotherapy or radiobiology. Iso-effect doses were generally considered to be mainly determined by overall irradiation time and the number of fractions was considered to be relatively unimportant. The iso-effect data of Strandqvist (1944), Ellis (1942), Jolles and Mitchell (1947) and Cohen (1960), for example, showed how iso-effect dose varied with overall time but generally no attention was paid to the number of fractions involved.

Elkind and Sutton (1959) and others revealed the importance of the shoulder of the cell survival curve and the repair of sub-lethal damage. The crucial experiments demonstrating the importance of fraction number were undoubtedly the pig skin experiments of Fowler, Morgan, Silvester, Bewley and Turner (1963). The iso-effect doses found by these workers are given in Table 8.

<u>TABLE 8</u> <u>PIG SKIN REACTION</u>	
<u>REGIME</u>	<u>ISO-EFFECT DOSE</u>
Single dose	2000 rads
5f/4d	3600 rads
5f/28d	4200 rads
21f/28d	5500 rads

The above results showed that when 5 fractions were given, increasing the overall time from 4 days to 28 days only increased the iso-effect dose by 600 rads. On changing from 5 "daily" fractions to 21 "daily" fractions the iso-effect dose increased by 1900 rads but only 600 rads (i.e. 32% of this increase) could be attributed to the increase in overall time. The remaining 68% of the increase was therefore due to increasing the number of fractions. Increasing the overall time from 0 to 4 days is unlikely to have produced much effect in view of the increase observed in changing from 4 to 28 days. Thus practically the whole of the 1600 rads increase accompanying the change from a single dose to 5 fractions in 4 days was probably due

to the increase in the number of fractions rather than the increase in the overall time.

These results showed that the major factor which determines skin response is the size or number of fractions. This conclusion of Fowler and his co-workers has greatly influenced radio-therapy thinking during the last decade. Ellis (1968) developed the concept quantitatively by the introduction of a formula which implied that doubling the number of fractions would increase the iso-effect dose by 18% whilst doubling the overall time would only increase the iso-effect dose for normal tissue by 8%.

The constancy of these percentage variations is now being questioned. Recent work (e.g. Dutreix, Wambersie and Boumik, 1973; Denekamp, 1973; and Berry, Wiernik and Patterson, 1972) suggests that the relative importance of fraction number decreases as the number of fractions is increased and that the relative importance of overall time increases as the overall time is increased. The analysis of published experimental and clinical data by (Liversage (1971) also suggested that in order to fit the experimental data the exponent of time in the formula of Ellis (1968) would need to increase, as the overall time is increased.

Evidence is thus accumulating which suggests that whilst fraction number is the major factor responsible for the increase in iso-effect dose when the number of fractions is small, the importance of fraction number may have been over-emphasised and the importance of overall time under-emphasised, when the number of fractions is large.

The above discussion has concentrated on the relative importance of fraction number and overall time but has not yet discussed the relative importance of intracellular recovery and repopulation. The weevil results are important in this respect because they clearly

demonstrate that part of the increase due to an increase in fraction number is not due to intracellular recovery but is due to an increased rate of repopulation (at least when the latter is expressed as rads per day equivalent).

The effect of fraction number on the amount of repopulation occurring is illustrated by the weevil results presented in Table 9.

<u>TABLE 9</u>		
REGIME	LD 50	DOSE EQUIVALENT OF REPOPULATION IN 18 DAYS
9F/10d	17680 rads	7400 rads
9F/28d	25080 rads	
4F/10d	13920 rads	3240 rads
4F/28d	17160 rads	

From these results it will be observed that as the number of fractions delivered in a given overall time is increased, the amount of repopulation occurring (expressed in rads) also increases. Thus part of the increase in iso-effect dose found by Fowler et al (1963) on changing from 5 fractions in 28 days to 21 fractions in 28 days may have been due to an increase in the amount of repopulation. As previously discussed (20.3) lack of accurate data regarding mitotic delay and the rate of decrease in the rate of repopulation prevents an accurate estimation of the magnitude of the effect in the pig skin experiments.

For the LD 50 results for weevils the necessary factors have been determined and the repopulation component r_{D_T} and the intracellular recovery component R_{D_N} can be estimated with reasonable accuracy as previously described (Chapter 16).

Analogous results to those shown for pig skin in Table 8 are presented in Table 10 together with their estimated repopulation and intracellular recovery components.

TABLE 10			
LD 50 WEEVILS			
REGIME	LD 50 (rads)	r_{D_T} (rads)	R_{D_N} (rads)
Single dose	5900	0	0
4f/3d	10520	1210	3410
4f/25d	16930	7620	3410
20f/25d	32130	14380	11850

The results in Table 10 show that nearly half the increase in LD 50 on changing from 4 fractions in 25 days to 20 fractions in 25 days is due to an increase in the repopulation component r_{D_T} .

The changes in LD 50 with regime are of a similar nature to those found for skin reactions listed in Table 8 and show that fraction number is more important than overall time. On changing from 4 fractions in 3 days to 20 fractions in 25 days the LD 50 increases by

21620 rads. Using the same reasoning as that used for the pig skin results, it will be seen that 70% of this increase is due to the increase in fraction number and 30% to the increase in overall time. Examination of the repopulation and recovery components on the other hand reveals that 39% of the increase is due to intracellular recovery and 61% due to repopulation.

The above results thus confirm the conclusion of Fowler et al (1963) in that they show that, for the regimes under consideration, fraction number is more important than overall time. They do suggest, however, that repopulation may be more important than hitherto considered as part of the increase with fraction number appears to be due to repopulation and not all associated with intracellular recovery, as has been generally accepted in the past.

As previously discussed (17.2) the weevil results also show that the relative importance of repopulation increases and that of intracellular recovery decreases as the number of daily fractions is increased. This coupled with the work of Dutreix et al (1973) Denekamp, (1973) and Berry et al, (1972) together with the data presented in Table 10 suggests that repopulation effects in radiotherapy are probably more important than recently believed. More experimental work with mammalian tissues is needed to determine the magnitude of these effects.

20.5. The General Formula for equating protracted and acute regimes of radiation

The general formula for equating protracted and acute regimes of radiation was tested (Liversage, 1969) using data for skin reaction and LD 50 for mice which had been published by different workers. For both effects, the formula was shown to be true to an accuracy

equal to that of the experimental and clinical data, estimated to be $\pm 10\%$. Hall (1968) applied the formula to the in vitro cell survival data obtained for acute and protracted irradiation of HeLa cells by Hall and Bedford (1964) and by Bedford and Hall (1963). He found excellent agreement between observations and predictions based on the general formula.

As discussed earlier (Chapters 1 and 2) the derivation of the general formula was stimulated by the need to determine suitable fractionated acute regimes of radiation which could be used in clinical trials using a remotely controlled high dose-rate after loading unit for the treatment of carcinoma of the cervix.

The clinical trial initiated by O'Connell, Joslin, Howard, Ramsey and Liversage (1967) was designed to determine the acute dose, delivered in 6 fractions in 12 days, necessary to produce the same effect as a continuous low dose-rate regime, delivering 4000 rads to point A in 48 hours.

O'Connell (1972) has now established that a total dose of approximately 3300 rads to point A, in 6 fractions in 12 days produces the same vaginal epithelium reaction as that produced by 4000 rads to point A delivered continuously during 48 hours. The geometrical arrangement of the sources is the same as far as is possible in both methods of treatment and the vaginal epithelium receives a dose which is approximately 2.5 times larger than that delivered to point A.

According to the general formula, 4000 rads to point A (or 10,000 rads to the vaginal epithelium) delivered continuously in 48 hours should produce the same effect as 4000 rads to point A (or 10,000 rads to the vaginal epithelium) delivered in 12 fractions (Table 1, Chapter 4.3) in a hypothetical time of 48 hours. If the number of fractions is

reduced from 12 to 6, the data of Fowler (1965) shows that the total dose should be reduced by 16%; i.e. to 3360 rads to point A (and 8400 rads to the vaginal epithelium). In the clinical trial the six fractions were not given in 48 hours but in 12 days and therefore the doses predicted from the general formula need to be increased to allow for repopulation occurring in the vaginal epithelium in a period of 10 days. As each fraction is approximately 1400 rads, appreciable mitotic delay will reduce, if not prevent, repopulation occurring during the 2 to 3 day intervals between fractions. If and when repopulation is occurring, the rate could be as high as 50 rads/day if allowance is made for the factors previously discussed (20.3) and if vaginal epithelium is considered to repopulate at the same rate as skin. Thus the increase in overall time from the hypothetical time of 48 hours to the actual time of 12 days is liable to increase the vaginal epithelium iso-effect dose by between 0 and 500 rads (i.e. by 0 to 5%). Thus after making appropriate corrections for reducing the number of fractions from 12 to 6, and increasing the overall time to 12 days, the general formula predicts that the equivalent regime should be 3360 to 3530 rads delivered in 6 fractions in 12 days. This prediction is in good agreement with clinical observation.

The validity of the general formula has thus been tested for a number of different mammalian end-points, (skin reactions, LD 50 for mice, and HeLa cells in vitro) and is consistent with the clinical observations of O'Connell (1972) on the vaginal epithelium.

The present investigation using weevils has tested the formula against a completely different non-mammalian tissue and certainly shown it to be accurate to $\pm 12\%$ and probably to $\pm 7\%$.

Liversage (1969) claimed that, according to theory, the formula should

be approximately true for all tissues but only to an accuracy of $\pm 10\%$. The present study coupled with the above findings provides strong evidence supporting this claim.

This evidence strengthens the claim of Liversage (1969) that there is no inherent advantage in low dose-rate continuous irradiation compared with high dose-rate irradiation provided the latter is fractionated in accordance with equation (6) (Chapter 4.2). This claim is however based on the assumption that intracellular recovery is more important than repopulation and that any small effects due to repopulation on increasing the overall time from t hours to T days are, in any case, likely to increase the iso-effect doses for normal tissues to an equal if not greater extent than those for tumours. The present investigation, whilst agreeing with the general formula, has shown that repopulation effects in mammalian tissues are likely to be more important than recently considered. Thus, on changing from a low dose-rate continuous irradiation regime lasting t hours to a fractionated acute regime, in which N fractions are given in T days, the effects of intracellular recovery are likely to be the same, provided N and t are related in accordance with equation (6), but the effects of repopulation are likely to be more important than previously realised.

Whether increasing the overall time from t hours to T days is likely to benefit the normal tissues or the tumour is entirely a matter of speculation. The present position is however more optimistic than hitherto in that the present investigation (particularly when considered in conjunction with the recent results of Benekamp) has shown that the homeostatic factors which govern the rates of repopulation in normal tissues may be manipulated to obtain maximum effect, (e.g. by such

devices as selecting the optimum time for the introduction of a rest period). Ellis (1968) has, indeed, stressed that the most important difference between normal tissues and tumours is that repopulation in the former is under homeostatic control whereas, in general, in the latter this is not so.

The fact that repopulation must now be considered to be more important than recently envisaged suggests that by manipulating the homeostatic influences to increase the rates of repopulation in normal tissues these effects might be exploited to the benefit of the patient and the detriment of the tumour. However, before such benefit can be achieved in clinical practice at will, much more will need to be known about repopulation effects in both tumours and normal tissues. This suggests that more research should be directed toward this end.

SUMMARY OF CONCLUSIONS

1. The factors influencing iso-effect relationships in weevils

The manner in which the LD 50 for weevils varies with split-dose interval, overall irradiation time and number of fractions is similar to that in which iso-effect doses vary for normal mammalian tissues as explained in detail above. The LD 50 for weevils has been used as an end-point to establish iso-effect data which, because of the large number of weevils that could be used, are probably more comprehensive and accurate than those previously obtained for any tissue.

On changing from one to twenty fractions the LD 50 (corrected for repopulation) increases by a factor of 3 due to intracellular recovery (Chapter 17, Table 5). Thus the magnitude of the effect due to intracellular recovery is only slightly larger than that observed for mammalian skin reactions; Fowler (1965) found the corresponding increase for skin reactions (uncorrected for repopulation) to be a factor of 2.76. The split-dose recovery curve (Fig 14a) shows that radiation induced synchrony has relatively little effect in weevils, causing a variation in the LD 50 with split-dose interval of no more than $\pm 4\%$.

For irradiation regimes involving a constant number of fractions, the percentage increase in iso-effect dose with increase in overall irradiation time is much greater for lethality of weevils than it is for mammalian skin reactions. Under certain comparative conditions, the percentage increase is approximately three times greater as shown by the pilot experiments (10.3). Thus, when the LD 50 for weevils is the chosen end-point, repopulation effects are relatively large and therefore conducive to investigation.

The iso-effect data for weevils may be, and have been here used to investigate some of the fundamental relationships between iso-effect dose and radiation regime; relationships which are believed to be governed by intracellular recovery from sub-lethal damage and cellular

repopulation influenced by mitotic delay and homeostatic control. In view of the above findings, the LD 50 for weevils is a particularly useful end-point for studying the effects of repopulation in an organised tissue.

2. The value of τ

When irradiated and housed at 27°C and 70% R.H., the half-life τ of the dose-equivalent of sub-lethal damage for the critical cells of the weevil is between 1 and 2 hours and is therefore similar to that found for mammalian cells. (See Chapters 4.3 and 14.4)

3. The validity of the general formula

The general formula for equating protracted and acute regimes of radiation (Liversage 1969) should enable the dose necessary to produce a given effect using low dose-rate continuous irradiation to be converted to an approximately equivalent multi-fraction acute dose. Application of this formula requires a knowledge of the value of τ for the relevant cells but the equivalent multi-fraction dose so determined is only weakly dependent on the value assumed for τ , (Fig 19b).

Uncertainty in the estimation of τ for the critical cells of the weevil unfortunately leads to some uncertainty in the predicted equivalent dose. However, over the whole range investigated, (i.e. from 1 to 20 fractions) predictions based on either τ equal to 1.0 hours or τ equal to 2.0 hours agree with observation to $\pm 12\%$. The most likely value of τ is considered to be 1.5 hours and predictions based on this value agree with observation to an accuracy of $\pm 7\%$ over the whole range investigated and $\pm 4\%$ over the range 6 to 20 fractions. Considering the approximations involved in the derivation of the formula, the above agreement is considered to be satisfactory. The formula has been shown to be true to an accuracy of $\pm 10\%$ for several mammalian tissues. Now it has been tested against a very different type of tissue

it may be generally applied with a greater degree of confidence than hitherto.

4. The variation in LD 50 with overall time

For fractionated regimes, the LD 50 for weevils does not vary with overall time in a way which is consistent with published formulae purporting to estimate such variation for the normal tissues of patients, (Chapter 16).

The dose R_{D_T} necessary to compensate for cellular repopulation occurring during a fractionated regime which kills 50% of the weevils in 30 days is consistent with the following relationship:

$$R_{D_T} = (N - 1) \frac{M_0}{\lambda} \left[1 - e^{-\lambda(t_s - t_m)} \right] \dots\dots\dots(15)$$

where N is the number of fractions

M_0 is the average rate of repopulation (expressed in rads/day)
immediately after the mitotic delay t_m induced by each fraction.

t_s is the interval between fractions

and λ is an exponential decay constant.

The above relationship only applies when the irradiation regime consists of equally spaced fractions of equal magnitude. For weevils, M_0 is estimated to be 850 rads/day, λ is estimated to be 0.291 days^{-1} and t_m is estimated to be 0.183 days/1000 rads. (Chapter 16.4.3).

The above relationship probably applies to other end-points in other tissues but insufficient data are available to enable reliable estimates to be made of the appropriate values of M_0 , λ and t_m for any other tissue at present.

5. The relationship between repopulation and damage

When the damage present in the weevil is less than that caused by a single dose of 3500 rads, the dose necessary to compensate for cellular repopulation occurring in a given interval is approximately proportional

to the dose-equivalent of the damage present at the start of that interval. This suggests that the homeostatic control in the weevil does not simply switch on or off the repopulation mechanism but is capable of varying the rate of repopulation in accordance with the needs of the system. The rate of repopulation can be sufficiently high to repair in a period of 8 days the majority of the damage caused by a single dose of 3500 rads (Fig. 18c).

As the damage is increased to higher levels the rate of repopulation (expressed in rads/day) fails to increase as rapidly as would be required by a proportional relationship and, in fact, decreases dramatically when the damage present is in excess of that caused by a single dose of 4500 rads (Fig 18d). This suggests that the critical surviving cells of the weevil may be sub-lethally damaged to such an extent that their proliferative capacity is greatly impaired or, alternatively, that the homeostatic control system of the weevil is disrupted by such high whole-body doses. (N.B. The single dose LD 50 for weevils is 5900 rads).

Because the rate of repopulation varies greatly with the damage present, it follows that the beneficial effect of a rest period during a fractionated regime should be dependent on the stage at which such a rest period is introduced. The weevil experiments show that this is so. For example, the LD 50 for 6 fractions in 7 days can vary by as much as 1190 rads (i.e. 9%) depending upon the position of a 3-day rest period. These results coupled with the work of Denekamp (1973) suggest that there could be an optimum time at which such a rest period should be introduced in split-course therapy and that further experiments using local irradiation of organized mammalian tissues should be done to determine whether this is so.

6. The relative importance of repopulation and intracellular recovery

The LD 50 for weevils increases with the number of daily fractions. As for mammalian tissues, the increase in iso-effect dose per daily fraction can be explained as being partly due to intracellular recovery and partly due to cellular repopulation. For the critical cells of the weevil, the former appears to be the major factor when the number of daily fractions is less than six and the latter the major factor when the number of daily fractions is greater than nine.

Recent clinical and experimental work, discussed previously (20.4) suggests that a similar change-over in the relative importance of these factors may occur in mammalian tissues as the number of daily fractions is increased. It may be anticipated, however, that the rapidly proliferating tissues of the weevil would be likely to exhibit this change-over at a lower number of daily fractions than more slowly proliferating tissues. (It was estimated in the present work (19.2) that the cell cycle time of the critical cells of the weevil can be as short as 10.3 hours).

7. Revision of the estimated repopulation rate for skin

The weevil results show that the rate of repopulation (expressed in rads/day) decreases exponentially with time between fractions (Fig 16f). It is suggested that a similar effect occurs in the skin of mammals although the exponential decrease may occur rather more slowly with a "half-life" of between 2.5 and 5.0 days, (20.3). It follows that after the mitotic delay, the average rate of repopulation for skin reactions during short intervals could be twice as great as the seven-day average rate of 25 rads/day estimated by Liversage (1969).

8. A revised interpretation of the increase in iso-effect dose when fraction number only is varied

Because the rate of repopulation increases as the fraction interval decreases, ~~perhaps~~ the increase in iso-effect dose observed for rapidly proliferating normal tissue on changing from, say, 5 fractions in 28 days to 21 fractions in 28 days, must now be considered as being partly due to an increase in the amount of repopulation occurring and not entirely due to intracellular recovery, as is generally accepted. (20.4).

9. The possible exploitation of repopulation effects

The above conclusions suggest that cellular repopulation may be playing a much larger part in determining iso-effect relationships than has been generally accepted during the last decade, especially when the number of fractions is greater than, say, ten. Further research into the relationship between the rate of repopulation and the damage present in mammalian tissues may provide the information necessary to exploit the homeostatic mechanism believed to be present in normal tissues, but not in most tumours, to obtain a therapeutically useful differential effect.

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