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AN APPRAISAL OF *IN VIVO* ASSAYS OF EXCISED TUMOURS

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Summary.—Three *in vivo* assays of excised tumours are compared, the endpoint dilution assay, the tumour latency assay and the lung colony assay. The assay procedures are discussed in 2 phases; the preparation of the required cell suspension and the injection and growth of the tumour cells in recipients. Factors reviewed include those affecting recovery of cells during the suspension procedure, the variability of tumour response, the importance of the site of injection, the effect of heavily irradiated cells and non-lethal effects of radiation. Specific aspects of the lung colony assays are also described.

Results of an experiment to compare the 3 assay procedures with that of an *in vitro* agar colony assay are presented and indicate reasonable agreement for the KHT sarcoma except perhaps for the latency assay at low levels of survival. A list of recommendations of ways to minimize some of the potential problems and a comparison of assay procedures is presented.

THREE main *in vivo* assays will be discussed; the endpoint dilution assay introduced initially by Hewitt & Wilson (1959) as an assay for leukaemic cells but soon adapted for use with solid tumours; the growth latency assay introduced by Clifton & Draper (1963); and the lung colony assay used by a number of different workers (*e.g.* Hill & Bush, 1969). There are at least 2 other possible techniques which might have been included under the title. They are the increased lifespan method which will not be discussed because it is usually used with leukaemic cells and this paper deals largely with solid tumours, and the agar diffusion chamber technique which is essentially an *in vitro* method in which the role of host animals is that of an incubator and a provider of essential nutrients.

METHODS

The assays will be briefly described before discussing factors which may affect their performance or the results obtained. In all cases there is a control untreated group of tumours and one or more groups of tumours which have been treated in some way. The purpose of the assay procedure is to determine the effect of the treatment on the tumour cells.

For the endpoint dilution assay a suspension of single cells is produced from each group of tumours. The cells are counted and a range of dilutions prepared, mixed with a large number of heavily irradiated (HR) cells, and inoculated, usually subcutaneously or intramuscularly, into recipient mice. It is common to use more than one injection site per animal; both axillary and inguinal regions are often used for subcutaneous injections while both hind legs are used for intramuscular injections. The dilutions are chosen with the aim that tumours will grow in 0 to 100% of the injection sites over the range studied. The animals are examined regularly until no more tumours arise, then the number of tumour cells required to produce tumours in 50% of the injection sites is determined. This cell number is known as the TD_{50} and the ratio of TD_{50} values for suspensions produced from control or pretreated tumours gives a measure of the effectiveness of the treatment in terms of cell survival.

The growth latency assay requires only a crude suspension to be prepared from the tumours being studied. A range of dilutions is made from this suspension on the basis of the volume of centrifugally packed tumour material and injections are usually sub-

cutaneous or intramuscular into recipient mice. The injection sites are examined very frequently and the time at which each tumour arises is recorded. The volumes of tumour material injected are chosen so that all, or virtually all, of the injections produce tumours and the mean or median time from injection to detection (the latency period) is calculated for each different volume of tumour material injected. The latency period is then plotted as a function of the logarithm of the inoculation volume to produce a reference line for control tumours. Similar lines are produced for the treated tumours and, assuming the lines are parallel, the ratio of volumes of control suspension to treated suspension to give the same latency period will give an assessment of the surviving fraction following the treatment. An adaptation of this method allows death of the recipient animal to be the endpoint following injection of the tumour cells by a route which would make early tumour detection difficult or impossible, *e.g.* intra-cerebral, intraperitoneal or intravenous. The method then becomes essentially identical to the increased life span technique mentioned above.

The lung colony assay requires the preparation of a single cell suspension from the groups of tumours to be assayed. After counting of the cells, one or more dilutions are prepared, mixed with HR cells or plastic microspheres and injected intravenously into recipient mice. Cells lodge in the lungs and grow to form nodules or colonies. The animals are killed at a suitable time depending on the growth rate of the tumour cells involved and the colonies are counted. A cloning efficiency which is analogous to an *in vitro* plating efficiency is then calculated and the ratio of the cloning efficiencies for the treated and control tumours gives the cell survival for the treated tumours.

Comparing these descriptions demonstrates that the latency assay has an advantage over the other assays in that it can be used with tumours which are difficult to break up into single cell suspensions. Also the measurement of volume is more objective than counting cells in a haemocytometer, but it is probably less accurate. The lung colony assay has the advantage that it takes less time to get the result than with the other assays, because quite small colonies can be detected in the lung. Also in the lung colony assay each animal yields an actual assessment of the number of colony-forming cells present in the injected suspension which means that less animals are required than with the other assays.

FACTORS AFFECTING THE ASSAYS

The assay procedures can be split into 2 parts; preparation of the suspension of tumour material and injection and growth of the new tumours in the recipient animals. These 2 parts will be discussed separately.

Preparation of the suspension

One of the most important considerations is whether or not the suspension prepared is representative of the tumours being assayed. This question has been discussed by other speakers at this meeting and will not be dealt with here except for one aspect. It is well known that so-called necrotic regions in tumours contain viable cells (see *e.g.* Jirtle & Clifton, 1978) yet many workers discard "grossly haemorrhagic and necrotic tissue" from tumours before preparing suspensions for assay. They are thus starting their procedure with an unrepresentative sample.

A related question is that of the recovery of cells during the cell preparation procedure, and it is particularly important if treatments given to the tumours change this cell recovery. One treatment which can affect cell recovery over quite short periods is killing the tumour-bearing animals at some time prior to the removal of tumour, as is widely done in radiobiological experiments, to achieve anoxic conditions. This is illustrated in the lower panel of Fig. 1 which gives cell recovery and cell survival in KHT sarcomas treated with nitrogen mustard and radiation and then held for various times under anoxic conditions before the tumours were removed for assay. Cell recovery begins to decline after about 20–30 min in the dead animal but remains constant in tumours in live animals. Cell survival, determined as a ratio of cloning efficiencies using the lung colony assay, also declines quite rapidly suggesting that the fraction of cell loss is

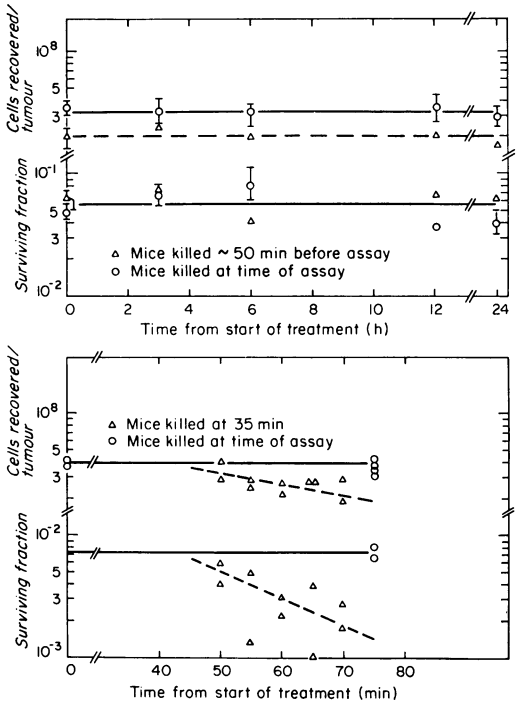

FIG. 1—. Cell recovery and surviving fraction as a function of the time from start of treatment. Lower chart: tumour-bearing animals were treated with 0·2 mg HN_2 followed immediately with 1500 rad Cs γ-rays given under air-breathing conditions. Mice were killed 35 min after the injection of the drug and tumours were removed and assayed at various times. Upper chart: tumours irradiated with 1000 rad at time zero and assayed up to 24 h later. Two groups of animals were used at each time point, in one the mice were killed just before tumour removal, in the other the mice were killed 50 min before tumour removal.

larger from the surviving cell population. This may be because the surviving cells, which were largely hypoxic in the tumour before the host animal was killed, are in a nutritionally deprived state. If a milder treatment is given then cell loss due to anoxia does not affect cell survival as is illustrated in the upper part of Fig. 1. Here groups of tumours were irradiated with 1000 rad at time zero and then assayed up to 24 h later. Some of the groups were held anoxic for about 50 min before tumour removal and this led to a reduction in cell recovery to about 60% of that from tumours kept in live animals, quite similar to the results in the lower part of Fig. 1. However, there is no consistent effect on cell survival, suggesting that the fraction of cell loss is the same in the surviving and radiation killed parts of the cell population.

Much more extensive cell loss is seen if the treatment is prolonged over many hours or days, when dead or dying cells may be removed from the tumour and new cells may be produced by cell division. The effect of such processes is illustrated in Fig. 2 which shows results of the effect of fractionated radiation doses on the KHT sarcoma. A large group of tumours of equal size at the start of the treatment was given 300 rad fractions every day either 5 or 7 days per week. Small subgroups were assayed following different numbers of dose fractions. In Fig. 2 the number of cells recovered per tumour is shown as a function of the time after the start of the treatment at which the assay was performed. The solid symbols are for tumours which were given only radiation and the open symbols for tumours irradiated at 30 min after treatment with various hypoxic cell sensitizers. Both sets of data show an initial rise in cell recovery, presumably due to cell division, followed by a rapid fall reflecting extensive loss of cells. Time appears to be a more important parameter in this cell loss, than the number of fractions given, since the results for both 5 and 7 fractions per week fall on the same line. Cell recovery can also be affected by treatment with chemotherapeutic agents as has been demonstrated by Stephens & Peacock (1978) who found reductions up to 70% in cell recovery from B16 melanomas within 20–30 h of treatment. Kal & Barendsen (1980) have reported similar findings for R1 tumours at this meeting.

Such changes in cell recovery will affect the calculated level of cell survival in any of the 3 assays and consequently the results may not indicate the true effect of the treatment. For studies of fractionated irradiation using a lung colony assay

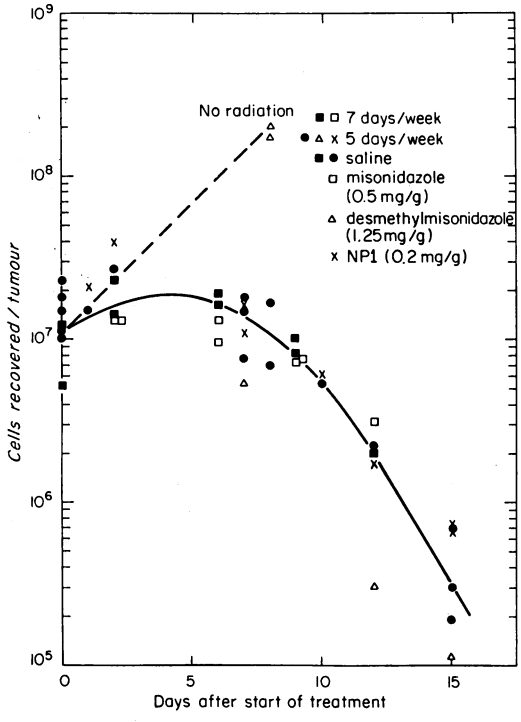

FIG. 2.—Cell recovery as a function of time for KHT sarcomas irradiated with 300 rad fractions either 5 or 7 days per week. The solid symbols are for tumours receiving 100 kVp X-rays alone while the open symbols are for treatments given 30 min after injection of one of the drugs shown. The broken line, representing a doubling time of about 2 days, shows the increase in cell recovery if no irradiation is given.

our laboratory has adopted the approach of incorporating the changes in cell recovery into the calculation of survival. The number of colony-forming cells per tumour is determined for each treatment group and compared to a similar value for control tumours measured at the start of treatment. Such an approach can also be applied to an endpoint dilution assay or the latency assay by using changes in the volume of tumour material recovered. If cell size changes as a result of treatment however, as is discussed later, changes in volume of tumour material recovered may be misleading. This approach of calculating tumour cell survival assumes that the cell loss is a result of the treatment applied and is not caused by the cell separation procedure damaging cells which would have otherwise survived.

The selection of tumours to be studied is also an important part of the experimental procedure. There is considerable variability between individual tumours and their response to treatment, even among "long transplanted" tumours growing from the same suspension of cells. Some of the variability which can be observed is illustrated in Fig. 3 where results from assaying individual tumours following radiation treatment are shown. The survival values range over a factor of about 10. A similar range of values was found for tumours treated with cyclophosphamide. Some of the variation is due to the assay procedure since plating efficiencies for individual untreated tumours range over a factor of about 3 but the rest of the difference represents

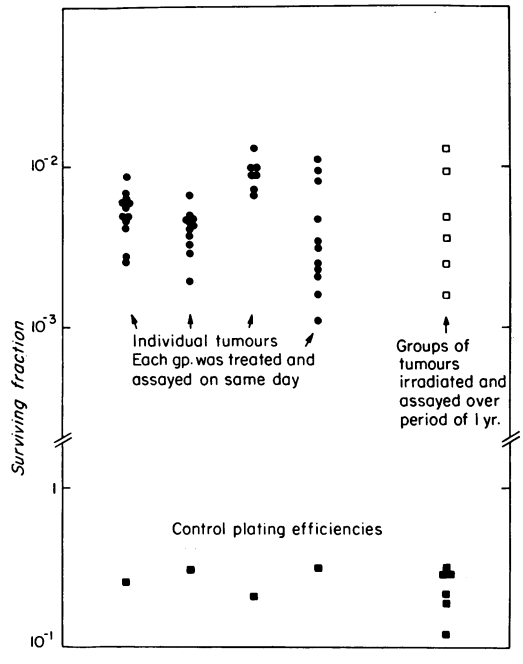

FIG. 3.—Four different groups of survival values obtained for individual KHT sarcomas treated with 1500 rad (100 kVp X-rays) are shown on the left with the control plating efficiencies (from groups of 4 tumours). Results of assays on groups of tumours spread over a period of one year are shown on the right.

intrinsic variation. Groups of tumours should be used to assess each treatment in order to reduce this variation. However, Fig. 3 also shows that even this does not prevent a considerable range of survival values being observed when similar treatments are given over a period of weeks or months. It is thus important not only to use groups of tumours but to repeat experiments a number of times in order to obtain a true assessment of the effect of the treatment being studied.

It is also important to control closely the size of tumours at treatment, since quite small changes can markedly affect response to treatment. This is illustrated in Fig. 4 where radiation dose survival curves for KHT sarcomas treated at 0·3–0·4 g or 0·6–0·7 g are shown. Mean values from a number of experiments are plotted and it is clear that there is a difference between the 2 tumour sizes which is almost certainly related to the level of hypoxia in the tumours. A further increase in the size of the tumours to about 1·0 g does not change the response any further (Pallavicini & Hill, 1979). Similar results of the effect of change in tumour size at the time of treatment are reported by Siemann (1980) for EMT6 tumours (this volume).

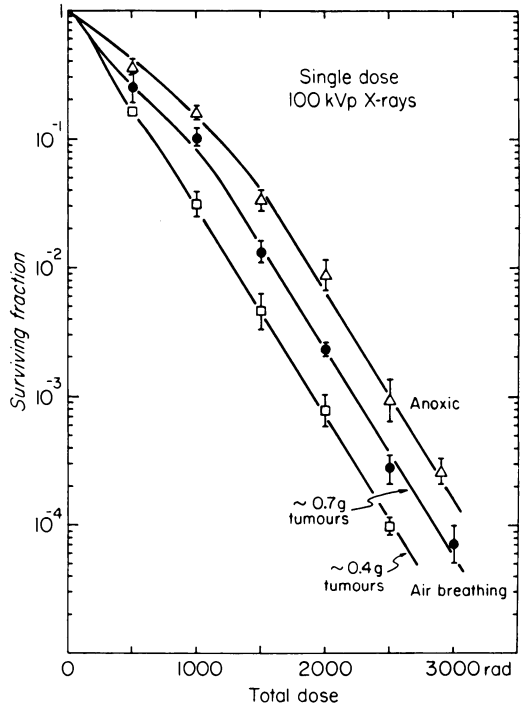

FIG. 4.—Radiation-dose survival curves for KHT sarcomas treated at 2 different sizes under air-breathing conditions. Mean values (±s.e.) from a number of repeat experiments are shown. For comparison a survival curve derived under anoxic conditions is shown. This curve is not affected by tumour size.

Injection and growth of tumours in the recipients

An important consideration in this section is the antigenicity of the tumour-host system. Ideally experiments studying treatments not involving the immune response should be done with tumour-host systems which have minimal or no detectable antigenic interaction. As has been discussed by Hewitt (1978) this is often not the case and represents an element of doubt in the interpretation of the results obtained with some systems.

When using the endpoint dilution or latency assays it is quite usual to have more than one injection site per animal which may lead to difficulties if there are differences between the sites. In general, fewer cells are required to obtain tumour growth if the cells are injected subcutaneously rather than intraperitoneally or intravenously, but it has been shown that the TD_{50} is even lower for intramuscular injection than for subcutaneous injection (Kallman *et al.*, 1967; Steel & Adams, 1975; Steel *et al.*, 1977). More important than this, however, is whether injections of the same type, *e.g.* subcutaneous, are equally effective in the inguinal and axillary areas of the body, the 2 sites most often used for endpoint dilution and latency assays. There are indications that this is not always the case, *e.g.* Auerbach *et al.* (1978) and Hewitt *et al.* (1967) have found that axillary injections may lead to tumours which appear earlier, or after a lower number of implanted cells, than inguinal injections.

Such injection site specificities could clearly have a major effect on latency assays and require that all injections be into similar sites if the results are to be comparable. Similarly for an endpoint dilution assay, if a mixture of injection sites is used they must be at least equally distributed in each treatment group. There may be some effect on the TD_{50}, but whether survival determinations would be altered is not clear. In his extensive review of *in vivo* assay systems a number of years ago, Kallman (1968) concluded that the effect of injection site was unlikely to be large, but it is probably prudent to examine the situation in each experimental system being studied. If enough animals are available the best solution is probably to use 2 injections per animal, given intramuscularly into the hind limbs.

The addition of heavily irradiated (HR) cells to the injected suspension makes a major difference to the TD_{50}, latency period and cloning efficiency. The effect of HR cells is markedly dependent on cell number (see Fig. 5), and consequently it is necessary to add a large number ($\sim 10^6$ cells) to each injection in order to achieve the maximum effect. Peters & Hewitt (1974) have suggested that HR cells stimulate local fibrin formation and thus prevent cells escaping from the injection site. A similar non-specific blocking phenomenon also seems to be the explanation for HR cell action in the lung colony assay since they can be replaced by inert plastic microspheres (Hill & Bush, 1969). Since different numbers of cells must be injected from control or treated groups of tumours, the effect of HR cells makes it imperative that all injections are corrected to the same total number of cells by the addition of HR cells.

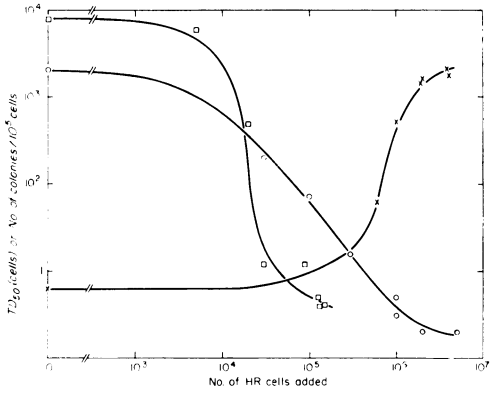

FIG. 5.—The effect of HR cells on TD_{50} values and colony efficiency. The squares and circles are for endpoint dilution experiments and represent TD_{50} values as a function of HR cells added to the injected suspension. The crosses are for lung colonies and show colonies/10^5 cells as a function of HR cells added to the suspension. In this experiment between 10^3 and 10^4 non-staining viable cells were injected depending on the number of colonies expected. □, TD_{50} carcinoma "NT" (Hewitt *et al.*, 1973); ○, TD_{50} B16 melanoma (Steel *et al.*, 1977); ×, KHT sarcoma (Hill & Bush, 1969).

Pretreatment of the injection site can also affect the transplantability of tumours and this is particularly true for cloning efficiency in the lung. Doses of radiation or a number of chemotherapeutic agents (particularly cyclophosphamide) have been shown to cause a significant increase in the cloning efficiency, an effect which can last for several weeks after the treatment (see *e.g.* van Putten *et al.*, 1975). It is not clear whether such pretreatment affects survival determinations although some workers use the technique routinely (Grdina *et al.*, 1975). It should allow a lower level of survival to be determined than might otherwise be possible.

Another problem that can affect the assays is that following treatment, particularly radiation, tumours may regrow at a slower rate than untreated tumours. This can be overcome in endpoint dilution and lung colony assays by allowing a longer period for growth for tumours from treated cells but it has important implications for the latency assay since it is delay in growth which is used in this assay to reflect cell death. The effect should be indicated by the lines, representing latency period as a function of log inoculation volume, being non-parallel for treated and non-treated groups. However, with some scatter in the data this may not be detected. The results will then tend to over-

emphasize the extent of the cell killing actually achieved. Some evidence of this effect will be seen later in a comparison of the various assays using the KHT sarcoma (see also Douple & Richmond, 1979).

An important requirement for the lung colony assay is that a good clean suspension of single cells is produced. Debris in the suspension reduces the maximum number of cells which can be injected without killing the recipient animal and clumps of cells may well have a higher cloning efficiency than single cells. Thompson (1974) found that for C_3H mammary tumours the cloning efficiency depended on the size of the cell clump injected. Cell size has also been shown to affect colony formation. In experiments with a murine fibrosarcoma (Grdina *et al.*, 1977) cloning efficiency increased by a factor of more than 3 for an increase in tumour cell size by a factor of 2. Such changes could affect calculations of cell survival since cell size has often been found to change after radiation or drug treatments (see *e.g.* Stephens & Peacock, 1978). In our studies with fractionated doses of radiation the tumours are assayed a number of days after the start of treatment and it is often noticeable during the counting procedure that there are many cells that are larger than normal. To check whether this size increase might be affecting survival calculations the lung colony assay was compared with an *in vitro* agar colony assay (Thomson & Rauth, 1974) in which cell size should be unimportant. Suspensions of cells derived from tumours treated with various fractionated radiation doses were assayed by the 2 techniques and the results are shown in Table I. There was very good overall agreement between the 2 assays suggesting that the large cells observed were incapable of forming colonies.

Grdina *et al.* (1975, 1977) have also demonstrated that cell populations can be separated from their murine fibrosarcoma which have cloning efficiencies different by factors of 2 to 3 independent of the cell size. These populations of cells were also found to have differences in their radiation dose–survival curves interpreted by the authors to be due to their cycle stage or nutritional state in the tumour. Thus, the overall response of the tumour, as measured by the lung colony technique, is a composite of surviving fraction and cloning efficiency which could lead to errors in quantitative comparisons of the effect of different treatments on the tumours.

TABLE I.—*KHT sarcoma; comparison of lung colony assay and agar colony assay done with same cell suspensions*

Survival by lung colony assay	Survival by agar colony assay	Ratio $\frac{ACA}{LCA}$
$5{\cdot}0\times10^{-1}$	$6{\cdot}7\times10^{-1}$	1·34
$4{\cdot}6\times10^{-1}$	$4{\cdot}9\times10^{-1}$	1·06
$3{\cdot}3\times10^{-1}$	$3{\cdot}9\times10^{-1}$	1·18
$3{\cdot}2\times10^{-1}$	$2{\cdot}0\times10^{-1}$	0·63
$2{\cdot}1\times10^{-1}$	$1{\cdot}9\times10^{-1}$	0·90
$1{\cdot}2\times10^{-1}$	$8{\cdot}7\times10^{-2}$	0·73
$6{\cdot}3\times10^{-2}$	$4{\cdot}0\times10^{-2}$	0·63
$6{\cdot}1\times10^{-2}$	$8{\cdot}1\times10^{-2}$	1·33
$5{\cdot}0\times10^{-2}$	$4{\cdot}2\times10^{-2}$	0·84
$1{\cdot}5\times10^{-2}$	$1{\cdot}8\times10^{-2}$	1·20
$1{\cdot}4\times10^{-2}$	$9{\cdot}7\times10^{-3}$	0·69
$8{\cdot}9\times10^{-3}$	$5{\cdot}8\times10^{-3}$	0·65
$5{\cdot}4\times10^{-3}$	$6{\cdot}9\times10^{-3}$	1·28
$3{\cdot}6\times10^{-3}$	$3{\cdot}0\times10^{-3}$	0·83
	Mean (±1 s.e.)	0·96 (±0·08)

Another source of variation in cloning efficiency is the difference which occurs from mouse to mouse. This variation would be expected, on the basis of sampling error, to be described by a Poisson distribution. However, when a group of 96 animals was injected from the same cell suspension the spread of values was much larger than expected (Hill & Bush, 1969), giving a greater than 10-fold range around a mean of about 14 colonies per lung. The standard deviation was over twice that expected. Since it becomes prohibitively expensive to use such large numbers of animals to assay each treatment group it is important to ask how much error will be introduced by using smaller groups. This question was investigated by selecting 5 animals at random from a group of about 20 mice which

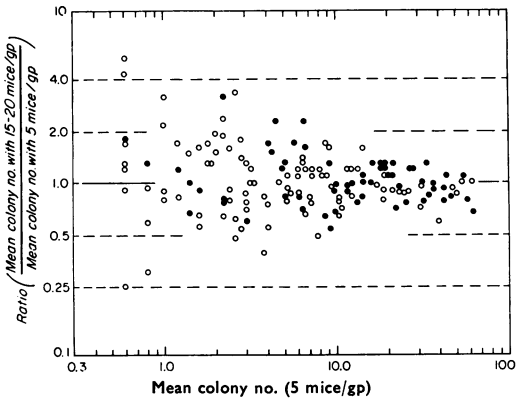

FIG. 6.—A scattergram of the ratio of mean lung colony number for the two groups plotted as a function of the mean value for the smaller group (see text). The symbols indicate mice injected with cells with (●) or without (○) admixed microspheres.

had all been injected from the same cell suspension. The mean number of colonies for the 5 animals was compared to the mean for the larger group. A total of 159 groups of mice from different experiments, which had a wide range of mean number of colonies, were studied and it was found that the 5-animal mean was always within one standard deviation of the 20-animal mean. There was considerable variation, however, if the mean colony numbers were low. Fig. 6 shows the ratio of the 2 mean values plotted as a function of the 5-animal mean. The ratio of means is often greater than 2·0 or less than 0·5 when the 5-animal mean is below about 5-colonies/animal. There is no effect on this variation of admixing microspheres with the cell suspension before injection. These results indicate that when small groups of animals are used, mean colony counts below about 5 are subject to considerable error.

COMPARISON OF RESULTS USING DIFFERENT ASSAYS

The 3 *in vivo* assay procedures were compared with one another and with an *in vitro* agar cloning assay, using groups of KHT sarcomas irradiated with 2 different doses. The results are shown in Table II as the surviving fraction with a standard error range calculated from the colony counts, the analysis of the TD_{50} or by covariance analysis for the latency assay. Two different survival values are shown for the endpoint dilution assay resulting from different methods of analysis. There is a small difference between the results but they are within the standard errors. Actually the method of Porter & Berry (1963) is not strictly applicable because the data did not conform to a Poisson distribution, but it was used because previous endpoint dilution assays with the KHT sarcoma have given a Poisson relationship. The agreement between the various assays is quite good for the 1000 rad treatment but there is a greater spread in the data for 2000 rad, with the latency assay giving a particularly low value. This may well be due to

TABLE II.—*Comparison of results from assays using KHT sarcoma*

	Survival values (± s.e.)				
	Endpoint dilution				
Treatment	S & K*	P & B†	Latency	Lung colony	Agar colony (*in vitro*)
1000 rad	$5{\cdot}5 \times 10^{-2}$ (3·1–9·6)	$4{\cdot}1 \times 10^{-2}$ (2·6–6·6)	$2{\cdot}6 \times 10^{-2}$ (1·3–5·5)	$2{\cdot}5 \times 10^{-2}$ (1·7–3·3)	$4{\cdot}4 \times 10^{-2}$ (3·8–5·0)
2000 rad	$3{\cdot}7 \times 10^{-3}$ (1·4–10·2)	$2{\cdot}6 \times 10^{-3}$ (1·6–4·1)	$1{\cdot}7 \times 10^{-4}$ (0·3–8·5)	$1{\cdot}8 \times 10^{-3}$ (0·9–2·7)	$5{\cdot}8 \times 10^{-4}$ (4·8–6·8)
Effort	12·5 h		10 h	6 h	5 h
Time for result	> 42 days		27 days	21 days	15 days
No. of mice used	120 (2 inj./mouse)		65	30	—

* Method of Spearman & Karber (see Finney, 1952).
† Method of Porter & Berry (1963).

non-lethal effects of radiation playing a part in determining the time at which the tumours arise, particularly at high dose levels. Certainly in the TD_{50} assay the tumours grew later in the treated groups than in controls. This finding of reasonable agreement between the assays is similar to that reported by Steel *et al.* (1975, 1977), who have found good agreement between endpoint dilution assays, lung colony assays and *in vitro* agar colony assays for both the Lewis lung tumour and the B16 melanoma. However, Rice *et al.* (1980) have observed some disagreements (this volume).

Also shown in Table II are the number of mice used for the assay, the time to complete the assay and an estimate of the amount of effort which was involved in doing the assay. This includes preparation and counting of the cell suspensions, preparing dilutions and injecting the recipient animals, checking the mice for tumours arising in the endpoint dilution and latency assays, and counting the colonies in the lung and agar assays. The *in vitro* and lung colony assays show up as cheaper and easier to do, but they require greater preparative background to demonstrate their feasibility, and together with the endpoint dilution assay require preparation of a single cell suspension.

CONCLUSION

A number of factors which may be important in determining the reliability and usefulness of the 3 *in vivo* assay procedures have been discussed. It is difficult, however, to assess to what extent some of the factors may influence the results obtained by the assays. A series of recommendations to minimize some of these effects is given below:

(i) Use a tumour-host system which has minimal antigenic interaction.
(ii) Add heavily-irradiated cells to all injection mixtures to raise the total number of cells injected to the same level ($\sim 10^6$ cells).
(iii) For endpoint dilution and latency assays inject intra-muscularly into both hind legs only.
(iv) For all treatment procedures use groups of tumours and control the tumour size within narrow limits.
(v) Always determine cell recovery from the suspension procedure so that corrections can be applied to the survival values if required.
(vi) Always do experiments which include control groups.

This last point has not been discussed but stems from the fact that TD_{50} values, latency periods and cloning efficiencies all vary from experiment to experiment (see *e.g.* Kallman *et al.*, 1967; Clifton *et al.*, 1966; Hill & Bush, 1969).

Other aspects of the assay procedures are not amenable to this type of recommendation and consequently an attempt has been made to rank the assays in order of preference for a number of different attributes. This ranking is shown in Table III. The most suitable assay in any given investigation will depend on the relative importance of these attributes to the series of experiments involved.

TABLE III.—*Comparison of assay procedures*

	End-point dilution	Tumour latency	Lung colony
Effect of injection site	1	3	2
Effect of tumour growth rate	1	3	2
Requirement for cell suspension	2	1	3
Effect of cell counting artifacts	2	1	2
Effort required to perform assay	3	2	1
No. of animals required	3	2	1
Sensitivity of assay	1	2	3
Potential accuracy	1	2	1

Numbers indicate order of preference under each heading.

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