Br. J. Cancer **49** (1984), Supplement VI, 1–6

Concepts, problems and the role of modifying agents in the relationship between recovery of cells' survival ability and mechanisms of repair of radiation lesions

J.S. Orr

Department of Medical Physics, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London, W12 0HS

Summary The two strands of the problem are the shapes and changes with time of cell survival curves on the one hand and the responses of cell constituents to radiation on the other. Evidence of correlations between results of studies of these two types of phenomena under the influence of a wide range of modifying agents is required to establish mechanisms. Recovery may be defined as referring to the whole cell, while repair should be regarded as a process carried out by one substance on another.

The degrees of usefulness and possible deficiencies of a multi-hit/target model and a repair model for explaining cell survival curves and cell recovery are compared in a range of circumstances. A fully satisfactory model is not yet available.

Introduction — The two strands of the problem

Cell survival curves have a fairly direct connection with successful radiotherapy treatment of cancer. Differences in the shapes of survival curves can contribute to differences between the effects of ionising radiation on tumour cells and normal cells. The possibility of modifying the shapes preferentially to increase the therapeutic ratio is very attractive.

Treatments with a number of smallish fractions, or prolonged treatments, allowing time for cellular recovery from radiation damage, give the best clinical results. The relative success of fractionating the dosage and allowing recovery time implies that attention should be focussed on the shoulders of cell survival curves, and on the processes of cell recovery, if a scientifically based approach to preferential modification is to be found.

A great deal of experiment and thoughtful analysis has been focussed on shoulders and recovery. Much insight has been obtained by interpretation of the results of experiments in which shoulders and recovery have been affected by modifying procedures, agents or conditions.

Simultaneously, a number of cell constituents have been proposed as forming part of the mechanisms whereby ionising radiation brings about the death of cells. Other related constituents have been proposed as forming part of the mechanisms whereby cells can repair radiation damage and survive. However, studies of the damage and repair of cell constituents cannot by themselves define death or survival. They must therefore be combined and integrated with survival experiments on shoulders and recovery, and interpreted together.

The demonstration of direct causal relationships between radiation induced damage to cell constituents and cell death, and between the repair of radiation damaged constituents and cell recovery, requires that the two kinds of observation on the response of cells and on the response of constituents be closely locked together under all circumstances and whatever modifying agents are used. In a similar way, images of one object viewed stereoscopically must remain superimposed whatever direction of view is used.

The ideal contribution to this field of work is therefore one in which a correlation between the response of cells and the response of constituents is sought in a wide range of circumstances. A number of the papers at this 11th Gray Conference approach this ideal. The majority of papers, however, have a bias in one direction or another. Additionally, the wide range and the complexity of the basic problems, and their relatively unsolved state, have encouraged the grouping of papers that have a relatively narrow field of study in common, so that the work described by different authors can be contrasted.

Nevertheless, it should be strongly emphasized that the effects of a wide range of modifying agents on both cell response and constituent response must all be considered together if mechanisms are to be correctly identified. It is necessary to attempt to fuse or to braid the two strands tightly together.

The concepts of recovery and repair

Recovery in this context refers to the whole cell, something that happens to the properties of the cell as an entity. It is observed as a phenomenon by

measuring properties of the cell as a whole. Such properties can be, for example, survival, death, or division time. Recovery obviously takes time and both its rate and its magnitude can be measured.

The recovery to which we are primarily referring is, of course, Elkind recovery. This is the recovery of the ability of cells to have shoulders on their survival curves. The existence of a shoulder is a demonstration that smaller doses of ionising radiation are proportionally less effective in killing cells than larger doses.

Repair, on the other hand, is a process being carried out by one substance on another substance that has been damaged or injured. The thing that is repaired is distinguishable from the process of repair, and from the means whereby such repair is carried out.

Recovery and repair are linked but not necessarily closely. A cell may recover a property that it possessed and lost by, for example, progressing through the cell cycle without there being any damage or repair. Alternatively a damaged cell constituent may be repaired without the cell recovering a lost property, such as the ability to survive.

Repair is concerned with mechanisms. Knowledge of and understanding of mechanisms open up possibilities of external manipulation. Since recovery deals with properties inherent in systems as a whole, that is, the whole cell, its behaviour cannot directly identify the detailed nature of the mechanisms of repair that may underlie it. Nonetheless, much useful work has been done on the interpretation of recovery phenomena.

Interpretation of cell survival and recovery phenomena

Douglas Lea (1938) illustrated a cell survival curve with a shoulder that represented an ability to accumulate a predetermined amount of radiation damage before lethality. He discussed the capability of the cell to recover from that damage. Lea interpreted reduced killing by low LET radiation at low dose rates as the result of such recovery. He calculated a possible recovery time from early experimental results.

The dramatic and direct demonstration of this phenomenon using the split-dose experiment by Elkind & Sutton (1959) brought recovery of the survival curve shoulder and its time course into the forefront of radiobiological thinking.

The ability of cells to accumulate radiation damage until a lethal level is reached implies that the cell must have an ability to measure the amount of injury resulting from the radiation.

The basic observations are: (1) a downward curving survival curve from a first dose of radiation plus; (2) a recovery curve created as a shoulder is recovered by the survival curve that results from the second dose of radiation. These observations by themselves can be simply and equally interpreted in two alternative ways by sequences of actions (Figure 1), conveniently referred to as the multi-hit/target model and the repair model. The possible presence of an additional and purely exponential component, common to both models, need not affect the discussion. The saturable repair mechanism of the repair model is increasingly referred to as Q-factor.

The existence of a shoulder on a survival curve shows that damage from a given amount of radiation in a low dose is less lethal than that from the same amount of radiation after a high dose. Thus some of the damage from a low dose has been described as sub-lethal damage as a descriptive way of referring to the phenomenon. The phrase sub-lethal damage has also been used to describe, as a postulated mechanism, the damage that is repaired in the last box of the multi-hit/target model in Figure 1; that is, damage where there are less than n lesions in a cell. This dual usage can lead to confusion in the logic of the argument unless the sharp distinction between the phenomenon and the postulated mechanism is kept very clearly in mind.

The time scale of a recovery curve further implies the existence of time measuring devices or clocks in any of the possible mechanisms. These must control the rate of counting and the rate of repair in the multi-hit/target model. Alternatively, they must control the rate of repair and the rate of restoration in the repair model. Time is the simplest modifying agent. Fractionated or low dose rate irradiation greatly modify the effects of later doses of radiation.

Attempts to combine the logic of the two models in Figure 1 have been unsatisfactory. This is because a compound model that suggests that limitation or saturation of repair ability from the repair model is the counting mechanism from a multi-hit/target model, i.e. where counting and repair are combined, raises a major problem that must be dealt with. The problem is that the postulated exhaustion of repair at the end of Elkind recovery would coincide with the full recovery of the shoulder on a second irradiation survival curve, and therefore the full restoration of repair in such a compound model. This apparent contradiction of the transformation of the exhaustion of repair potential into full repair potential by the act of irradiation would be a uniquely beneficial effect of radiation.

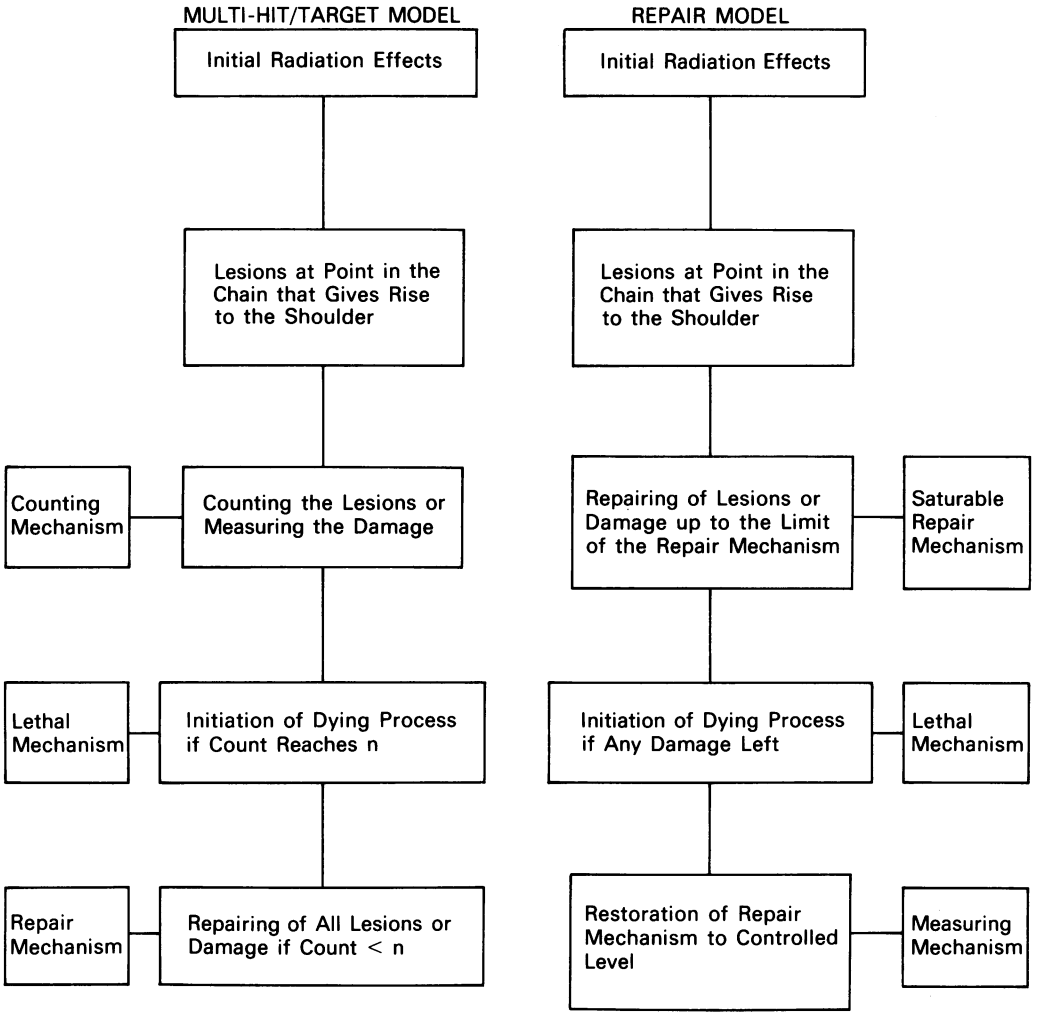

Figure 1 Flow diagrams of a multi-hit/target model and a repair model.

There are a number of difficulties with the Figure 1 models, both in their straightforward form and when tested with modifying agents. For example, on the multi-hit/target model it must be possible to count lesions several times. This is because the first count must be made to determine that the number is less than *n*, and a second irradiation before repair is complete requires a second counting that would include some lesions already counted. Similarly a third irradiation could require a third counting of the same lesion. If interaction is suggested as the counting mechanism it is necessary to explain the possibility of repeated interaction without any change in the nature of the lesion.

The responses of the models in terms of possible effects of modifying agents can be analysed. Consider a modifying agent or condition that changes the shoulder of the survival curve. On the multi-hit/target model such a change would be brought about by resetting the target number of the counting mechanism to a new value. If a geometrical relationship between lesions is suggested as the basis of counting, for example an interaction between two lesions that are close together due to the structure of the cells, it is necessary to explain how the count number could be reset by a change of the conditions in which cells are maintained.

With the repair model it is much easier to envisage mechanisms, based on balances in metabolic processes, to cope with these requirements for repeated measuring, manipulating and resetting maximum levels of repair. While there are very many observations that lend themselves to this interpretation, two illustrative experiments by colleagues (Foster *et al.*, 1971; Malone & Foster, 1972) that influenced my personal thinking can be described.

The first experiment is illustrated in Figure 2. One part shows that prolonged hypoxia prior to oxygenated irradiation has produced a survival curve with a small shoulder and that some hours of reoxygenation increases the shoulder. The second part demonstrates the time course of this increase of shoulder, between reoxygenation and irradiation, which is comparable to Elkind recovery between two doses of radiation.

The second experiment is illustrated in Figure 3. The first part shows that hypertonic shock just prior to irradiation reduces the survival curve shoulder, and that a longer interval between the shock and the irradiation tends to restore it. The second part demonstrates the time course of this restoration of shoulder between hypertonic shock and irradiation, which is comparable to Elkind recovery between two doses of radiation. The third part shows a curve of cell survival resulting from different degrees of hypertonic shock, and that prior irradiation reduces the ability of the cell to withstand such shock. This reduction is in a sense the reciprocal of the effect of shock in reducing the ability of the cell to accumulate radiation damage. The reciprocity of the two effects indicates an overlap between the mechanisms whereby the injuries are produced and repaired.

Experimental observations such as the above are more easily interpreted on a repair model. While attention has been focussed on the effects of modifying agents and modifying conditions on the survival curve shoulder, there is also a range of effects of modifying conditions on the survival curve slope that have been described as the repair of potentially lethal damage.

Early studies on what was described as repair of potentially lethal damage (e.g. Phillips & Tolmach, 1966; Whitmore & Gulyas, 1967) left an impression that this phenomenon associated with a change in modifying conditions was limited to slope changes. This suggested limitation has led to considerable confusion concerning the experimental phenomena embraced by the phrase "repair of potentially lethal damage" and has hindered clear analysis of the problem.

A repair giving rise to a slope change can be regarded as one that is not saturable, that can continue without limit. The possible relationships

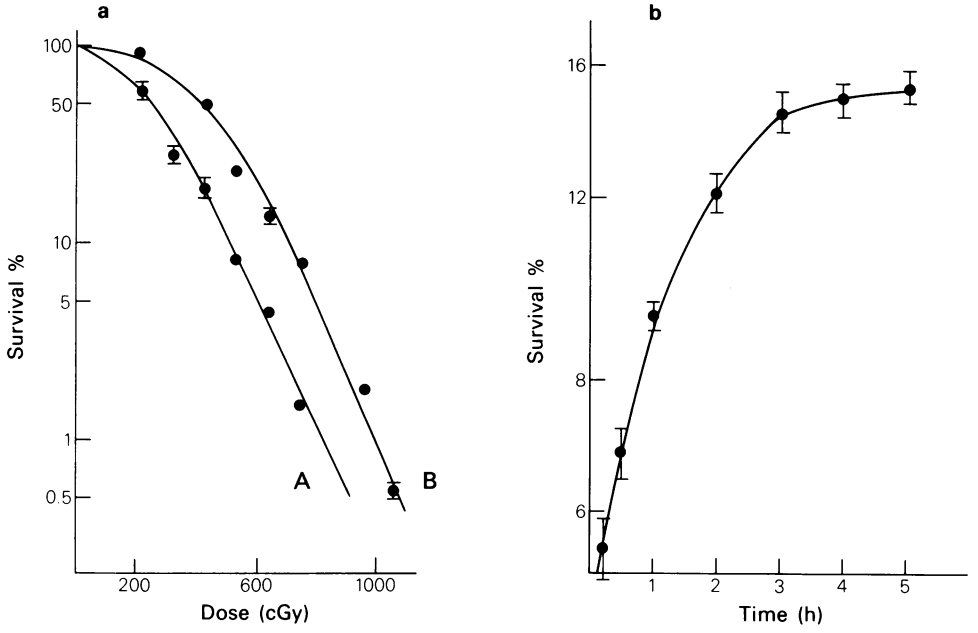

Figure 2(a) Survival curves for HeLa cells irradiated in air after 22 h of hypoxia and varying periods of reoxygenation. (A) 10 min reoxygenation. (B) 5 h reoxygenation. (b) Recovery curve for HeLa cells irradiated after varying periods of reoxygenation at room temperature following hypoxic storage.

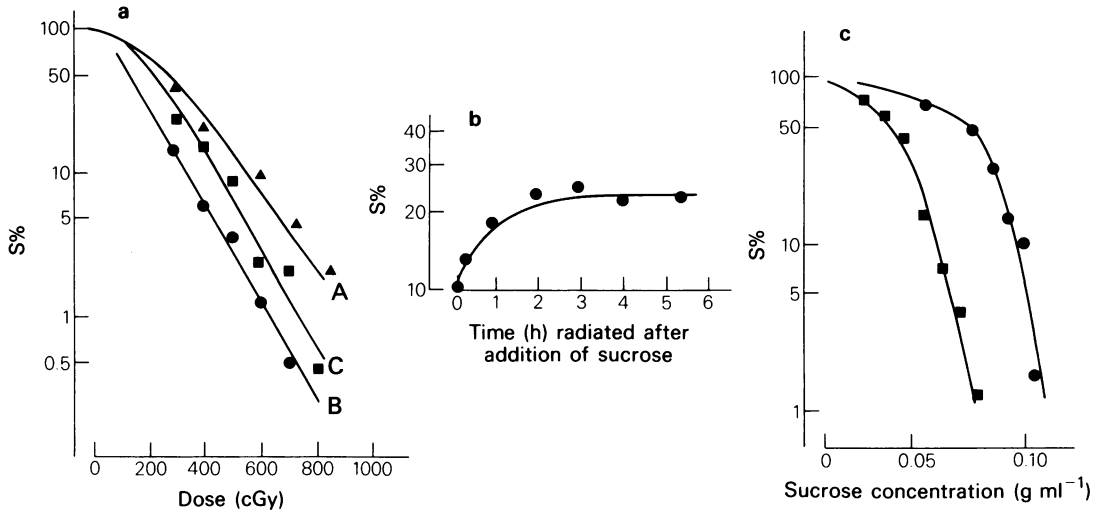

Figure 3 (a) Survival curves for HeLa S-3 cells. A; normal. B, C; cells incubated for 8 h in medium containing 0.051 g ml^{-1} sucrose, irradiated 25 min and 3 h after addition of sucrose, respectively. (b) Survival of HeLa S-3 cells given 400 cGy at various times after a hypertonic shock corresponding to a final sucrose concentration of 0.051 g m^{-1}. (c) Normalised survival of HeLa S-cells exposed to various concentrations of sucrose for 8 h with (lower curve) and without (upper curve) a 400 cGy radiation dose given 20 min before exposure to sucrose.

between a model of this effect and the repair model for the shoulder have not been examined thoroughly.

Although the theoretical and experimental considerations mentioned above seem to favour the repair model of the two in Figure 1, there are several problems associated with this model. There are also other problems that apply to both models.

One aspect of the repair model relates to the actual process of repair in the third box of Figure 1. This must be a much more rapid process than Elkind recovery, which is the lowest box on the repair model, and corresponds to the restoration or replenishment of the repair mechanism to its maximum controlled level. The difficulty is that the repair process in the third box has not been reliably observed. While it would not be expected that it could be experimentally observed solely by manipulation of doses of radiation, it should be demonstrable through the study of the timing of interactions between radiation and other agents.

Another difficulty arises from the fact that RBE increases with increasing LET. If a lesion in the second box of the repair model arises from a single energy deposition in the first box, then theory predicts that effectiveness should decrease with increasing LET. Thus since in the repair model, cell inactivation could be attributed to a single initial radiation lesion, the increase in RBE requires to be explained. A comparable problem can arise in the multi-hit/target model.

Another difficulty is common to both models and can be illustrated from the interpretation of the experimental results of Loshek *et al.* (1978); Loshek *et al.* (1981). The experiments covered the interaction of hyperthermia with high and with low LET radiation. The interpretation indicated at least four different types of damage events each with their own repair mechanism.

It is possible that the real mechanisms of repair of cellular radiation damage may contain parallel cascades of mechanisms such as those in Figure 1. Both types of model could then exist in series with each other, and the results of any one experiment could indicate only which type of mechanism had been provoked in action, and not the fundamental nature of a unique and exclusive mechanism.

Conclusion

A complex of partial understanding of results on survival and recovery has been obtained from various interpretations of the observations. However, many inconsistencies remain. In the succeeding paper by W.A. Cramp the possibilities and the problems in the response to radiation of cell constituents are presented and discussed. The needs to integrate and advance these two lines of study, and to clarify concepts, nomenclature and definitions, are the reasons for holding this 11th L.H. Gray Conference. It is hoped that the papers presented and subsequent study and discussion will help to consolidate the foundation from which the objectives may ultimately be reached.

References

ELKIND, M.M. & SUTTON, H. (1959). X-ray damage and recovery in mammalian cells in culture. *Nature*, **184**, 1293.

FOSTER, C.J., MALONE, J., ORR, J.S. & MACFARLANE, D.E. (1971). The recovery of the survival curve shoulder after protracted hypoxia. *Br. J. Radiol.*, **44**, 540.

LEA, D.E.A. (1938). A theory of the action of radiations on biological materials capable of recovery: part 1. The time-intensity factor. *Br. J. Radiol.*, **11**, 489. Also in: *The Collected Papers of Douglas Lea* (Ed. Gray), Oxford: Blackwell Scientific Publications (1966), p. 120.

LOSHEK, D.D., ORR, J.S. & SOLOMONIDIS, E. (1978). Interaction of hyperthermia and radiation: a survival model. In: *Proc. 2nd Int. Symp. on Cancer Therapy by Hyperthermia and Radiation*, Essen, 1978 (Eds Streffer *et al.*). Baltimore/Munich: Urban & Schwarzenberg, p. 211.

LOSHEK, D.D., ORR, J.S. & SOLOMONIDIS, E. (1981). Interaction of hyperthermia and radiation: radiation quality. *Br. J. Radiol.*, **54**, 40.

MALONE, J.F. & FOSTER, C.J. (1972). The effect of hypertonic shock on the radiation response of HeLa-3 cells. *Int. J. Radiat. Biol.*, **2**, 599.

PHILLIPS, R.A. & TOLMACH, L.J. (1966). Repair of potentially lethal damage in X-irradiated HeLa cells. *Radiat. Res.*, **29**, 413.

WHITMORE, G.F. & GULYAS, S. (1967). Studies on recovery processes in mouse L cells. *Natl Cancer Inst. Monogr.*, **24**, 14.