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MASTER

DNA Lesions and Mammalian Cell Killing:

Cause and Effect? *

by

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Abstract

Although indirect, studies of repair mechanisms afford a useful approach to the questions: Where and what are the sensitive sites in a cell related to division? This approach has been used with mammalian cells based on the fact that sublethal damage must be accumulated to kill a cell and the observation that cells can repair sublethal damage. Actinomycin D was found to inhibit this repair, an observation which justified a focus upon nuclear DNA as the site of damage and repair.

Sedimentation studies with alkaline gradients revealed two repair processes. Repair of single-strand breaks (also found by others) and repair to a complex of DNA, lipid, and protein. In cells sensitized to fluorescent light because of prior growth in the presence of 5-bromodeoxyuridine, the data suggest that single-strand breaks is the lethal lesion. In contrast, damage to a DNA-membrane structure appears a more likely target for X-ray cell killing.

Introduction

There is little question that nuclear DNA is a critical molecule in the affairs of cells. There is also little reason to doubt that small aberrations in DNA can have profound effects in view of the biological amplification represented by the cell's DNA → RNA → protein information storage, transfer, and expression system. For such reasons, plus the fact that radiation is known to be able to affect DNA (e.g. mutation, production and chromosome breakage), DNA has been implicated as a principal locus relative to many radiation effects although adequate supportive evidence not infrequently has been lacking.

One effect of ionizing radiation is its ability to suppress cell proliferation. This too is an end effect that often has been assumed to represent a lesion the biological expression of which starts with DNA. As concerns mammalian cell killing, supportive evidence has been developed which suggests that DNA is a principal radiation target. The purpose of this contribution is first to review some of the biological evidence which suggests that DNA is a principal target, and then to present some of the information which is available relative to physical chemical changes produced by radiation in DNA. Having described these changes, we will then inquire to what extent the evidence suggests a cause and effect relationship between DNA lesions and mammalian cell killing.

Sublethal Damage and its Repair

Cultured mammalian cells survive single doses of X- or γ -rays in accord with a damage accumulation process; that is, on a semilog plot, the survival curve starts with a threshold region followed by a region of exponential survival. An example of this, for the Chinese hamster cell line V79-1, is shown in Fig. 1 by the closed circles (1). The increasing negative slope in the region of 0-550 rad means that damage must be accumulated to kill a cell (i.e., prevent formation of a colony from a single cell), and the constant slope thereafter implies that for doses greater than ~550 rad a maximum amount of damage has been accumulated (see Chapter 2, Reference 2 for further discussion of formal survival curve theory).

The requirement for damage accumulation means that some component of the damage is sublethal and this in turn suggests the possibility that damage might be repairable. That such is the case is also shown in Fig. 1. The curves traced by

the squares show the results when an interval intervenes between a fixed first dose, 505 rad, and graded second doses (indicated along the second set of co-ordinates). In terms of the survival curve parameters n and D_0 (see legend, Fig. 1), in a 2.5 hr interval about half of the shoulder width has returned to the survival curve, and by 23 hrs the fractionation survival curve is indistinguishable from the initial, single-dose curve. Although some division of cells surviving the first dose probably had occurred by 23 hrs (although none in 2.5 hrs), because of the selective killing of sensitive cells by the first dose it is still likely that by 23 hrs cells surviving 505 rad could have completely repaired their sublethal damage.

Actinomycin D Sensitization of X-Ray Killing

To attempt to develop an understanding of the biochemical requirements of repair of sublethal damage, metabolic inhibitors of DNA, RNA, and protein synthesis were used (3). Of the inhibitors tried, only the antibiotic actinomycin D proved to be clearly effective. It was also found that this inhibition of RNA synthesis potentiated single-dose killing as illustrated in the next three figures.

The results in Figs. 2-4 were all obtained with Chinese hamster cells synchronized by exposure to millimolar concentrations of hydroxyurea (Hy) (see reference 4 for the details). The action of this drug is first, to kill those cells in the DNA synthetic phase of the cycle at the time of its addition, the S phase, and second, to accumulate the remainder of the cells at the border between the presynthetic phase, G_1 , and the S phase. Therefore, even for otherwise untreated cells, a reduction in survival occurs due to the elimination of S cells.

In Fig. 2, the single-dose survivals are compared for synchronized cells which had aged to the most resistant phase of their growth cycle; 4½ hours after the removal of the hydroxyurea from the medium which corresponds to the latter half of the S phase (8). For actinomycin D treated cells, the last 1½ hrs of this aging period was with 0.5 µg/ml of drug in the medium. While some toxicity accompanies this drug exposure alone, and although a 27% reduction in $\underline{D}_0$ results, the main effect of this preirradiation drug treatment is appreciably to reduce the shoulder width of the survival curve. This is evident from the doses shown in Fig. 2 which measure the quasi-threshold dose, 595 rad for untreated cells and 65 rad for actinomycin D preirradiation treated cells. Thus, the effect of treatment with the drug is largely to reduce the capacity cells have to accumulate sublethal damage, a result which illustrates the direct connection between repair of sublethal damage (Fig. 1), suppression of repair with actinomycin D (see references 3 and 4), and the capacity cells have for sublethal damage (Fig. 2).

One reason for the use of maximally resistant cells in Fig. 2, is that the large shoulder, which is essentially responsible for this resistance, permits reductions in it to be easily demonstrated. However, some might argue that the toxic effect due to the actinomycin D treatment by itself compromises the interpretation of such data because of the possibility that a nonspecific stress is introduced. Nonspecific stresses usually result in reductions in $\underline{D}_0$ and not in $\underline{n}$ but nevertheless, to eliminate this reservation, experiments were performed with lower, less toxic concentrations of this antibiotic. Figure 3 shows the result when the same experimental design is used as in

Fig. 2 except that the concentration of drug was decreased to 0.2 μ g/ml. The toxic effect of the drug by itself is appreciably less and the degree of reduction of the shoulder width is less as well. Figure 4 shows the shoulder reducing capacity of actinomycin D when the concentration of drug used has by itself no toxic effect at all. Cells continuously incubated in the presence of 0.002 μ g/ml actinomycin D grow somewhat more slowly but still yield a full complement of colonies. In Fig. 4, the drug was added after irradiation. This illustrates, to begin with, that actinomycin D very likely acts on lesion expression rather than on target sensitivity, a conclusion not inconsistent with the data in Figs. 2 & 3, where the drug was added before irradiation. This is true because actinomycin D binds in cells and, in fact, has a half residence time in V79-753 cells of 2.3 hrs (4). Adding the drug before irradiation insures that it is present to act immediately after irradiation producing thereby a greater reduction in shoulder width than when added after irradiation.

The main point demonstrated by the data in Figs. 2 - 4 is that graded doses of actinomycin D act as though they are equivalent to graded doses of X-irradiation since the effect of the drug is mainly to reduce the shoulder width of the survival curve to a degree which varies with the drug dose used. For example, a continuous exposure to 0.002 μ g/ml actinomycin D is equivalent to a dose of ~ 155 rad (= 615 - 460) and it is noteworthy that this is an extremely small concentration of drug, 1.6×10^{-9} M. In fact, in this concentration range it is known that the principal inhibitory effect of actinomycin D is in reference to ribosomal-RNA synthesis (7).

Relative to the question of sites of radiation damage and repair, the foregoing results are of critical importance.

It is well known that actinomycin D binds selectively to duplex DNA (8-11) probably intercalating between complementary base pairs and possibly binding to the sugar-phosphate backbone as well (12, 13). Since this drug affects both single-dose damage expression as well as sublethal damage repair, this by itself strongly implicates a DNA locus for the forward and reverse reactions of radiation effects relative to cell division. And since other metabolic inhibitors which interfere with ribosomal-RNA synthesis at a point removed from DNA do not interfere with sublethal damage repair (e.g. ^upyromycin, ref. 3), the foregoing survival results suggest that the steric rather than the metabolic properties of this drug are responsible for its potentiation of X-ray killing.

DNA Alkaline Sedimentation Studies

Having developed evidence implicating DNA, or DNA containing structures, as a principal target, a method was sought for studying damage to DNA more directly. The technique of cell lysis on top of alkaline gradients was chosen (14) because this offered the possibility of examining long stretches of DNA -- albeit single-stranded in view of the denaturation which is effected by high pH -- without the attendant shear associated with chemical extraction. In addition, the ability to examine DNA as it is released from cells offered the opportunity of looking for the effects radiation might have on DNA in association with other materials.

When cells are lysed at high pH (>12) and in high salt concentration solutions (total concentration of Na^+ = 1.0 M; see references 15, 16 for details), the sedimentation pattern depends on the time and temperature of lysis and, for a fixed time and temperature, the patterns depend on the dose the cells

received prior to lysis. The time and dose dependencies for lysis at 25°C are sketched in Fig. 5. Typically, the pattern obtained when unirradiated cells are lysed for 30 - 60 min is bimodal. With longer periods of lysis, the left peak decreases in size and moves toward the meniscus (toward the left) and this is associated with a relative increase in the amount of DNA in the right peak. Since essentially all the DNA placed on a gradient is accounted for in the 30 fractions obtained after sedimentation, the foregoing means that the size of the DNA in aggregate in the left peak initially is larger than that in the right peak.

It is useful to give these peaks names. Referring to the patterns for zero rad and 60 min lysis, the left peak has been called "the complex" and the right peak "the main peak" (15, 16). Evidence supports the inference that the main peak consists essentially of single-stranded DNA; in addition to DNA, the complex contains lipid (as we will show) as well as protein. The sketches in Fig. 5 illustrate that lysis time alone resolves main peak DNA from the complex and that small doses of radiation can effect the same result when the lysis period is relatively brief (e.g. for 60 min). Doses of about 1500 rad lead to the onset of a bulge¹ in the main peak toward the meniscus, an effect which increases with dose and is consistent with the formation of one break for each 50-60 eV in single-stranded molecules of average size 2×10^8 dalton (16, 17). Since 60-70 eV is expended per absorption event when electrons traverse condensed media (18), this suggests that about one absorption event per 2×10^8 dalton is required for a break.

Examples of the foregoing trends are shown in Fig. 6. Panel a shows the almost complete resolution of the complex when the lysis period for unirradiated cells is increased from 60 min

to 270 min (25°C). In panels b and c, the open circles show the progressive loss of the complex with dose when the lysis period is 60 min. And in panel d, also for 60 min lysis, the open circles show the strand breakage that results from large doses.

DNA Repair after Irradiation

Also contained in Fig. 6 are results which show that cells can reverse the changes produced by radiation as measured by sedimenting ability. Starting with panel d, we see that 20 min after 4330 rad under optimal growth conditions eliminates the bulge due to single-strand breaks but no complex appears. Forty minutes after 1440 rad caused a reduction of activity in the main peak and the reappearance of the complex. And after 361 rad, 20 min is long enough to effect almost as large a loss of main peak DNA to the complex. These results show that the forward reactions produced by increasing doses of radiation -- complex DNA → main peak DNA → broken main peak DNA -- are reversed by incubation for repair. Although single-strand repair is rapid, the rate of repair of the complex shows a dependence on dose as we presently illustrate more clearly.

Lipid:DNA Cosedimentation and the Speed Dependence of Sedimentation of the Complex

In Fig. 6, all of the leading edges of the main peaks are sharp; i.e. they drop from the peak maximum more rapidly than do the trailing edges. This was found to be an artefact of the speed of rotation (16). When speeds of 11,000 rpm or less were used, peak widths at half height were found to be ~60% of mode values, instead of 25-30%, presumably because of the elimination of a speed-dependent, frictional force which increases with molecular size and centrifugal force. With this awareness,

the possibility that the complex also sediments atypically was examined (16) particularly since, as we noted, its position relative to the main peak, and the fact that DNA lost from the complex appears in the main peak, is anomalous.

The cells used in Fig. 7 were dually labelled during overnight growth with ^{14}C in their DNA and ^3H in their lipid-containing structures. The sedimentation conditions at 5000 rpm were such that the main peak would only reach fractions 4-5. If the complex maintained the same relative position to the main peak at 5000 rpm that it has at 38,000 rpm, it would have appeared between the meniscus and, say, fraction 4. That this did not happen can be seen by examining first the results after 776 rad. Lysis for 60 min at 3°C after this dose is only adequate to resolve the complex since an undetectable proportion of breaks are registered in molecules of 2×10^8 dalton. Thus, the DNA profile after 776 rad locates the main peak for these spinning conditions. The DNA pattern after zero rad shows only a very small amount of material at the same position; the complex, therefore, is toward the bottom of the tube. As the dose is increased, the complex moves toward the top and ~~the size of~~ the main peak increases in size. Thus, at low speed the relative positions of the main peak and the complex are at least in the right direction to be consistent with the DNA in the complex having a larger aggregate molecular size compared to the DNA in the main peak.

Considering the choline label, we note first in Fig. 7 that in each pattern about the same amount of ^3H distributes from the meniscus over about the first 10 fractions. This activity shows essentially no particular trend as a function of dose. It is clear, therefore, that essentially no lipid-containing

material is associated with the main peak. The second point is that for doses up to about 100 rad, a major fraction of the cells' lipid cosediments with its DNA. And the third point is that after a dose of about 200 rad, the latter cosedimentation is progressively lost until by 776 rad there is clearly no cosedimentation. Thus, whatever may be the reason for the cosedimentation of lipid and DNA, it is prevented by radiation.

In contrast to the large doses needed to demonstrate single-breaks -- i.e. supralethal doses, greater than about 1500 rad and which correspond to less than 0.1% survival -- to resolve main peak DNA from the complex, and to disassociate lipid and DNA, doses within the high survival range suffice. In Fig. 7, some main peak is released by 48 rad which is about 90% survival (Fig. 1), and 194 rad -- 50-60% survival -- suffices to compromise the cosedimentation of lipid and DNA. This large disparity between single-strand breaking doses and complex resolving doses reflects, of course, the relatively small size of main peak DNA. But this point serves to emphasize that the structure in the complex which is "hit" has an aggregate size relative to single-stranded DNA inversely proportional to the dose needed to show a change in sedimentation. That is, the target in the complex must be about 10X larger than 2×10^8 dalton if a single break-type event suffices to lead to its resolution. This is, of course, an approximation for several reasons not the least being that doses having equivalent degrees of effect have not been accurately assessed (e.g. D_0 doses). However, the use of target theory notions to estimate the size of the sensitive structure comprising the complex is appropriate since other methods are subject to considerable uncertainty in view of the apparent size and chemical hetero-

genicity of the complex.

Repair of the Complex, Slow Speed Sedimentation

The greater separation afforded by slow speed sedimentation of the complex from the main peak can be used to advantage to examine repair of the complex more completely. For a fixed repair interval, 20 min, in Fig. 8 we examine repair of the complex after doses increasing by a factor of 2 starting with 722 rad. Considering first the open circles, 722 and 1440 rad essentially only eliminate the complex; the mode values of the peaks are where they should be based upon the speeds used (see legend). The two higher doses show further shifts of the modes toward the meniscus indicative of single breaks in main peak DNA.

After incubation, each of the patterns has changed but the degree of the change is dose dependent. Repair after 2890 and 5780 rad results in shifts of the modes to the right due to single break repair. Although some formation of the complex at the expense of activity in the main peak is evident after 2890 rad, very little complex is clearly evident after 5780 rad for a 20 min repair period.

In addition to larger decreases of activity in the main peaks, after the smaller doses in Fig. 8 the amount of complex that has reformed first contains more activity (inversely with dose), and second the reformed complex sediments further down the tube. Thus, it is clear that the rate of repair of the complex varies inversely with dose and further, from other data, we know that the time course of the repair (20) is similar to the time course of repair of sublethal damage (21).

Reassociation of Lipid and DNA

In addition to the reversing of the effect that

radiation has on the sedimentation of the DNA in the complex, repair also results in a reassociation of lipid and DNA. This is shown in Fig. 9 where, in addition to the features already described in Fig. 8, a return to cosedimentation of part of the lipid with the DNA in the complex is also evident. As will be reported elsewhere, we note here that the complex also contains protein which is resolved by radiation along with the DNA and which reassociates during repair.

BUdR-Fluorescent Light Cell Killing

Puck and Kao demonstrated with mammalian cells (22) that when 5-bromodeoxyuridine (BUdR) is incorporated into their DNA, they can be killed by exposure to fluorescent light (FL). This phenomenon has been studied by Yang, Hahn, and Kersen-Bax (29) and in further detail by Ben-Hur and Elkind (23, 17) particularly in respect to damage/repair questions related to cell division, and DNA damage demonstrable using sedimentation under alkaline conditions. We review here some of the latter results first because they illustrate a killing process which does not involve sublethal damage and its repair, and second because the BUdR/FL data help illuminate the radiation damage/repair picture.

When Chinese hamster cells are exposed to FL after 20 hrs of incorporation of the thymidine analog BUdR, they were observed to survive graded exposures exponentially (22, 23). [Yang and coauthors observed a small shoulder, (29).] This suggests a single hit-to-kill process and implies that no repair of sublethal damage should be demonstrable because none is accumulated as part of the killing process. Figure 10 shows that this expectation is fulfilled. The experiment is similar to that in Fig. 1 except that only the dose range corresponding to the second doses is shown (i.e., a zero min exposure corresponds to

the survival following the first dose of 12 min), and only one fractionation interval (4 hrs) was used. The data show that the same survival is obtained with and without dose fractionation, a result consistent with single-hit inactivation and which is further supported by a constant 2-dose survival when other fractionation intervals are used (23).

Since cells not containing BUdR are not killed by FL, and since BUdR is incorporated essentially only into DNA, it is reasonable to expect that FL exposure of BUdR-containing cells should lead to damage to DNA. This was studied using the McGrath and Williams technique (14) as already described in reference to the X-irradiation of Chinese hamster cells. As expected, both single and double strand breaks are produced and repair of single-strand breaks can also be demonstrated (17). A summary of the dependence of breaks on FL exposure is shown in Fig. 11. Not only is the number of breaks per 2×10^8 daltons dependent on dose and BUdR content, but to some degree the incidence of breaks can be reduced by cysteamine (MEA). The protection afforded by MEA is slightly larger relative to DNA breakage compared to cell killing (17, 23) but suggests, nevertheless, that debromination of bromuracil followed by hydrogen abstraction from an adjacent sugar is probably involved in at least a proportion of the lesions produced.

Fluorescent Light versus X-Ray Production of Single-Strand Breaks

In addition to the qualitatively different characteristics of the X-ray and FL fractionation survival curves (Figs. 1 & 10), it is of value to compare these two types of lethal irradiations relative to break production in DNA. To do this, an equal basis is needed. If dose is expressed in both cases in units of the D_0 dose, it is possible to compare the DNA

breakage effectiveness of the two radiations. However, since in the instance of FL cell killing the cells contain BUdR, it is also necessary to compare for X-irradiation the frequency of breaks produced in normal and BUdR-containing cells. Figure 12, addressed to this point, shows that at $1 \times 10^{-5} \text{M}$ BUdR, 20 hrs growth, only a small sensitization is effected relative to break formation in normal DNA. At lower drug concentrations, there is an even smaller difference if any (24).

Figure 13 shows a comparison of X-ray and FL irradiations relative to strand breakage when the dose scale is normalized to the D_0 dose to establish an equal survival basis. Considering the X-ray data (circles), the curve for BUdR-containing cells lies below that for normal cells because the reduction in D_0 more than compensates for the increased yield of breaks (Fig. 12). The data for fluorescent light show that a single curve describes all the results: i.e., for both 1×10^{-6} and $1 \times 10^{-5} \text{M}$ BUdR. This is because the enhanced breakage in cells grown in the presence of the higher concentration leads to a precisely compensating decrease D_0 . By itself, this suggests that single-strand breaks is closely related to cell killing in BUdR-containing cells exposed to fluorescent light. But the main point in the figure is the much smaller break frequencies in normal or BUdR-containing cells after X-irradiation compared to fluorescent light. For if single-strand breaks are, in fact, a class of lethal lesion in BUdR/FL treated cells, as the data suggest, this implies that single-strand breaks are not closely related to cell killing in X-irradiated cells.

DNA Lesions and Cell Killing: Cause and Effect?

Returning to our original question, schematically, we may summarize the processes involved in cell killing and DNA

damage as in Fig. 14. From an unirradiated state, a dose of X-rays can be thought of as effecting a transition into the lethal domain. (Since the biological effects of radiation are generally degradative, even though radiation absorption events lead to ionization and excited atomic and molecular states, the cell is shown displaced downward and not upward; similarly relative to DNA damage.) After the exposure, a cell might survive if repair processes are sufficient -- and rapid enough relative to damage fixation processes -- to reach at least the domain of sublethal damage. Actinomycin D, which acts as though it is equivalent to a dose of radiation (Figs. 2-4), can be thought of as: interfering with the degree of repair of damage; raising the boundary between the lethal and sublethal domains; or both.

On the other hand, our present data relative to DNA fit a somewhat different picture at least because of inherent differences in the measurements made. The DNA from cells lysing on an alkaline gradient is initially complexed with lipid and protein. Although evidence from experiments not reported here suggests that some of this complexing is nonspecific, a number of observations support the notion that chromatin and nuclear membranes have functional relationships (e.g. see 25-27). We view "DNA-membrane" in a ground state as sketched. Ionizing radiation can be expected to produce lesions in both the DNA as well as the membrane whereas fluorescent light very likely produces lesions only in the DNA in view of the requirement for BUdR incorporation. In both instances it is likely that single-strand breaks predominate over double strand breaks (see 28 for a review and ¹⁷23). Also, in both instances, cells are able to repair single-strand breaks (see reference ¹⁷23 for examples of

repair after fluorescent light exposure). However, in view of the large frequency of breaks in the BUdR/FL case -- 1-2 per 2×10^8 dalton per D_0 (Fig. 13) -- it is likely that their repair leads to "lesion fixation". An increase in sedimentation velocity is, of course, not adequate by itself to insure that a lesion has been removed and the DNA returned to a functional state.

In the instance of X-irradiation, whether or not single-break "repair" results in damage fixation is probably unimportant in connection with cell survival. Since the much smaller frequency of these breaks compared to BUdR/FL breaks implies that cells are killed mainly by some other lesion. Damage to the complex is a possibility in view of the fact that small doses produce demonstrable effects (Fig. 7 & 8) and the time course of repair of the complex is similar to that of repair of sublethal damage. Also, results with BUdR (24) and actinomycin D (16) further support an important functional role for integrity of the complex.

Whether or not sedimentation studies of the type described are proven in time to be closely relatable to questions of cell killing, they show nevertheless that cells can effect at least two types of repair of DNA. Neither teleological nor radiobiological arguments support the notion that the cells we have used (derived from lung tissue) should have DNA repair capabilities specific to radiation or BUdR/FL damage. Rather, these data probably reflect cell capabilities which come into play in normal DNA replication and segregation and do not reflect responses stimulated by irradiation.

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Fig. 1. Single-dose and fractionated-dose survival curves of initially single Chinese hamster cells, clone V79-1
Irradiation: 55kV, 722 rad/min, glass-attached cells.
Survivals are estimates based on colony formation; standard errors in surviving fractions are shown where larger than the vertical dimensions of the data points. P.Y. = plating efficiency; n = the zero-dose back extrapolate of the terminal portion of the survival curve; and D_0 is the dose which reduces survival by $1/e$ along the exponential portion of the curve. (From ref. 1.)

Fig. 2. Comparative survival curves for Chinese hamster cells synchronized by treatment with hydroxyurea (Hy) and then allowed to advance in their growth cycle to their age of maximum resistance, the latter half of the S period. The cell multiplicity at the start of the synchronization treatment is designated by $\bar{N}$ and resulted from overnight cell growth. The drop in survival of normal cells for zero dose is due to the toxic effect that the synchronizing treatment by itself has. Other details as for Fig. 1. (From ref. 5.)

Fig. 3. Potentiation of single-dose X-ray killing by actinomycin D of maximally resistant late S Chinese hamster cells. This experiment is equivalent to that in Fig. 2 in all respects except that a lower concentration of drug was used. (From ref. 5.)

Fig. 4. Potentiation of single-dose X-ray killing by actinomycin-D of maximally resistant late S Chinese hamster cells. In this experiment, the drug was added immediately after irradiation and left in the medium

until the number of colonies was assessed (From ref. 6.)

Fig. 5. Sketches of alkaline sucrose velocity sedimentation patterns of radioactively labelled DNA from Chinese hamster cells, lysis temperature 25°C. The vertical dashed lines are intended to align the right peaks from gradient-to-gradient.

Fig. 6. High speed sedimentation of H^3 -thymidine labelled material from Chinese hamster cells. Lysis in b, c, & d was for 60 min at 25°C. Sedimentation: 60 min, 38,000 rpm, $\sim 12^\circ C$, SW-39 rotor. The time intervals after irradiation indicate incubations in complete growth medium for repair. (From ref. 19.)

Fig. 7. Sedimentation of DNA and lipid-containing material from Chinese hamster cells grown overnight in ^{14}C -thymidine and 3H -choline chloride. Lysis was for 60 min, $3^\circ C$. Sedimentation: 16.25 hrs, 5,000 rpm, $0^\circ C$, SW-50.1 rotor; $0^\circ C$ versus $12^\circ C$ requires a 50% increase in sedimentation time other things being equal. "P.Y.'s" stand for the percent of radioactivity loaded on a gradient which was recovered in 30 fractions. (From ref. 20.)

Fig. 8. Slow speed sedimentation of DNA from Chinese hamster cells with (closed circles) and without (open circles) incubation for repair. The results for 722 and 5780 rad were obtained at 6000 rpm, and for 1440 and 2890 rad at 5000 rpm. Consequently, the mode value for main peak DNA is at fraction 6 for 6000 rpm compared to fractions 4-5 for 5000 rpm. Other details as in Fig. 7. (From ref. 20.)

- Fig. 9. Demonstration of the radiation dissociation of DNA (^{14}C) and lipid (^3H) from dually labelled Chinese hamster cells and their reassociation with incubation for repair. Sedimentation was at 6000 rpm; other conditions as for Fig. 7. (From ref. 20.)
- Fig. 10. Fluorescent light (FL) inactivation of Chinese hamster cells grown for 20 hrs in the presence of $1 \times 10^{-5}\text{M}$ BUdR. The dose scale shows only the range of second doses; zero dose corresponds to the survival after the first dose only which was 12 min. Other details as for Fig. 1. (From ref. 23.)
- Fig. 11. Single-break production in the DNA from Chinese hamster cells grown for 20 hrs in the presence of 5-bromodeoxyuridine (BUdR). Breaks are expressed per 2×10^8 dalton and MEA stands for cysteamine.
- Fig. 12. Single-break production in the DNA from Chinese hamster cells grown for 20 hrs with (or without) 5-bromodeoxyuridine (BUdR). Single breaks are expressed per 2×10^8 dalton. (From ref. 17.)
- Fig. 13. A comparison between single-strand break production, per 2×10^8 dalton, in Chinese hamster cells by fluorescent light (FL) and X-rays. BUdR-containing cells were grown in the presence of this analog for 20 hrs. (From ref. 17.)
- Fig. 14. Schematic representations cell killing related processes (left) and DNA damage related processes (right).

Fig. 1. Elkind and Ben-Hur

Fig. 2. Elkind and Ben-Hur.

Fig. 3. Elkind and Ben-Hur.

Fig. 4. Elkind and Ben-Hur.

Fig. 5. Elkind & Ben-Hur.

Fig. 6. Elkind & Ben-Hur.

Fig. 7. Elkind and Ben-Hur.

Fig. 8. Elkind & Ben-Hur.

Fig. 9. Elkind & Ben-Hur.

Fig. 10. Elkind & Ben-Hur.

Fig. 11. Elkind & Ben-Hur.

Fig. 12. Elkind & Ben-Hur.

Fig. 13. Elkind & Ben-Hur.

Fig. 14. Elkind & Ben-Hur.