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THE RELEVANCE OF PHOTOBIOLOGICAL REPAIR[1,2]

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INTRODUCTION

The study of photochemistry and photobiology, so well exemplified by the papers in this symposium, is relevant to a wide range of human endeavors. These fields have supplied many of the crucial ideas and approaches to problems as diverse as the origin of life and the impact of a fleet of supersonic transports on skin cancer in man. I describe briefly three basic research areas in which photochemistry and photobiology have made contributions to practical problems -- problems that were not envisioned at the time the original research was done. These areas are:

(1) The effects of ionizing radiation on DNA and the measurement of base damage. This work has been carried out in collaboration with William L. Carrier (Setlow and Carrier, 1973) and Malcolm C. Paterson (Paterson and Setlow, 1972).

(2) The damage to DNA and the repair of DNA resulting from the action of chemical carcinogens on human cells. This work has been done in collaboration with James D. Regan (Regan and Setlow, 1973).

(3) The relation of photoproducts in DNA to uv-induced skin cancer. This work has been carried out in collaboration with Ronald W. Hart (Hart and Setlow, 1973, 1974).

1. The Relevance of Photobiology to Ionizing Radiation Damage

Many biological systems are able effectively to repair ultraviolet damage to their DNAs. One of the repair schemes -- discussed by Grossman in this volume -- involves the removal of uv-induced pyrimidine dimers from irradiated DNAs. This process, called excision, was discovered as a result of attempts to explain the varied responses of different bacterial mutants to uv irradiation (Setlow and Carrier, 1964; Boyce and Howard-Flanders,

1964). (There are other ultraviolet repair mechanisms, such as those described by Bridges, Devoret, Howard-Flanders and Witkin in this symposium, but the molecular mechanisms involved in these schemes are not as well understood as in the excision process.) The first step in the excision mechanism is the attack by uv-endonuclease on uv-irradiated DNA. The nuclease makes a single-strand break near a pyrimidine dimer. Its action *in vitro* may be detected by the accumulation of such breaks in uv-irradiated DNAs. We have used this concept to search for endonucleases that attack γ-irradiated DNAs. Since ionizing radiation itself makes numerous strand breaks, we (Setlow and Carrier, 1973) measure the ability of a crude endonuclease preparation to make additional breaks in such DNAs. The procedure is outlined in Table 1 and typical T-1
data are presented in Fig. 1. Figure 1a shows that the enzyme extract F-1
has no effect on unirradiated DNA but as indicated in Fig. 1b, enzyme treatment of irradiated DNA results in a marked decrease in its sedimentation constant. The decrease is equivalent to a 5-fold change in molecular weight. These data are clear evidence for the enzymic recognition of damage other than strand breaks in γ-irradiated DNAs. The relative numbers of these endonuclease-sensitive sites depend on the irradiation conditions. Figure 2 indicates that the number of such sites is much larger for DNAs F-2
irradiated anoxically than irradiated oxically and that under protected conditions (in the presence of yeast extract) the number of endonuclease-sensitive sites per chain break is small. It is clear from a comparison of Figs. 2a and b that the endonuclease makes single-strand breaks and does not produce double-strand scissions.

The type of experiment illustrated in Figs. 1 and 2 has opened up a new way of looking at DNA damaged by ionizing radiation. Such new ways

of investigating ionizing radiation damage are important since many investigators feel that the more obvious measures of damage -- single-strand breaks -- are not usually associated with lethal or mutagenic events because in most biological systems the strand breaks are repaired very efficiently (Setlow and Setlow, 1972).

2. The Repair of Chemically-Induced Damage to DNA

The concepts of DNA repair were derived from studies on microbial systems. Two photobiological experiments have extended these concepts to human cells. The first was the finding that normal human cells are able to excise appreciable numbers of pyrimidine dimers from their DNA (Regan et al., 1968). Such cells can repair this type of uv damage and, by analogy with bacteria, should also be able to repair various chemical insults to their DNAs. The second finding was the determination that cells derived from individuals with the disease xeroderma pigmentosum are defective in DNA repair (Cleaver, 1968) and are unable to excise dimers from their DNA (Setlow, et al., 1969). (There are some exceptions to this rule.) The disease is genetically determined and is characterized by a very high incidence of light-induced skin cancer. These data indicate that such individuals probably get skin cancer because of their inability to repair uv damage to their DNA.

We have developed an esoteric but practical and informative photochemical technique for detecting repair of damage to cellular DNA (Regan, et al., 1971). The technique is easily extended (Setlow and Regan, 1972; Regan and Setlow, 1973) to measurements of repair of chemical damage since it does not require a knowledge of the nature of the alteration induced in DNA by the deleterious agent. A schematic diagram of it is shown in Fig. 3. It makes use of the following basic

photochemical and photobiological findings. If altered cells carry out excision repair in the presence of BrdUrd, they will remove lesions from their DNA and will incorporate the analogue into the parental DNA strands. If they repair in thymidine, they incorporate thymidine into the repaired strands of DNA. DNAs that contain BrdUrd are much more sensitive to uv radiation than thymine-containing ones and the increased sensitivity is manifest by a high degree of alkaline lability in the irradiated BrdUrd DNA. Thus irradiation of repaired DNA results selectively in breaks in the BrdUrd-containing regions of the DNA. The selectivity for BrdUrd photolysis can be accentuated by using a wavelength, such as 313 nm, that is poorly absorbed by normal DNA but appreciably absorbed by the substituted polymer. The breaks resulting from the photochemical treatment with 313 nm can be assessed by sedimenting these DNAs in alkaline gradients. On such gradients DNAs of molecular weights over 10^8 are easily handled and therefore this technique is capable of measuring small numbers of breaks in 10^8 daltons of DNA. The ability of 313 nm radiation to break the repaired DNA depends upon two parameters: (a) The number of repaired regions and (b) the number of BrdUrd residues per repaired region and by measuring the kinetics of breakage we are able to estimate both these parameters. Although I do not give the analytical details, it is obvious that the larger the size of the region the less light needed to break it. Despite the fact that we do not know the molecular details of most physical and chemical lesions (although we do know the details of the uv-induced ones), we have been able to analyze the DNA of human cells treated <u>in vivo</u> with these agents so as to estimate the sizes of the repaired regions. Table 2 indicates that T-2 we can separate a large number of chemical treatments into two classes (Regan and Setlow, 1975). One class is similar to ultraviolet damage in

that it results in the production of large repaired regions in normal human cells and a negligible amount of repair in cells from individuals with xeroderma pigmentosum. The second class is found in both types of cells and is exemplified by treatments such as γ irradiation and ethyl methanesulfonate treatment. Thus xeroderma pigmentosum cells are not only unable to repair uv-induced pyrimidine dimers but also are incapable of repairing damage resulting from a number of chemical agents. It is a reasonable inference that agents that result in DNA repair in normal cells but not in xeroderma pigmentosum ones are potentially deleterious. It is also reasonable to suppose that unrepaired lesions may result in death, mutation or neoplastic transformation.

It is worth re-emphasizing that our knowledge of the repair of chemical damage in cells is based almost completely on photobiological techniques and concepts.

3. UV-Induced Skin Cancer: Dimers or Other Macromolecular Change?

Excessive uv irradiation may result in skin cancer in mouse and in man (Blum, 1959; Epstein, 1970), and the high incidence of skin cancer in individuals that are not able to excise pyrimidine dimers in their DNA implies that damage to the DNA of cells is the causative agent, although the mechanisms by which such damage is translated into a neoplastic transformation are not known. An adequate assessment of the danger to man of an increased uv fluence -- such as might arise from a decrease in stratospheric O_3 as a result of the flights of a large fleet of supersonic transports -- requires an understanding of the photochemistry and photobiology of the process. The need for basic knowledge is especially important because existing epidemiological data may not be adequate to evaluate quantitatively the hazard of an increase in uv level at the earth's

surface. Although we have good clues that damage to DNA is the causative agent, we do not know whether the important photoproducts are pyrimidine dimers or one of the many other types of alterations induced in DNA by irradiation. The absence of dimer excision in xeroderma pigmentosum cells is not strong evidence in favor of dimers as the important causative agent because other changes may also not be excised. However, such other changes have not been looked for quantitatively in irradiated mammalian cells.

Enzymic photoreactivation -- a process described in this symposium in detail by Rupert and by Sutherland -- is specific for pyrimidine dimers (Cook, 1970; J. K. Setlow, 1972). Enzymic photoreactivation reverses only one photoproduct -- pyrimidine dimers. The specificity is illustrated in Fig. 4 where I emphasize the notion that uv irradiation may make many products in DNA and that enzymic photoreactivation only monomerizes dimers and leaves the other products untouched. If uv-induced tumors were photoreactivable, this finding would be evidence for cyclobutane pyrimidine dimers as a lesion in the DNA that results in neoplastic transformation. Such an experiment cannot be done on placental mammals since they do not contain the enzyme (Cook, 1970). Fish, however, do. John S. Cook, James D. Regan and myself conceived that an excellent system for such an experiment would be the gynogenetic (nonsexually reproducing) fish, Poecelia formosa. The offspring of the fish are identical, and therefore there are no immunological barriers to transplanting cells from one member of the clone to another. The experiment actually carried out by Ronald W. Hart and myself is shown in Figure 5. Tissue from a number of fish are homogenized so as to yield clumps of cells. These clumps are treated as indicated in the Figure, injected into isogenic recipients and the recipients scored for tumors a number of months later. The experiment is almost completed (Hart

F-4

F-5

and Setlow, 1973, 1974). (We only have preliminary results on the last line of Fig. 5.

The effect of uv exposure in producing tumors in the fish into which irradiated cells are injected is shown in Fig. 6. There is a reasonable production of tumors and the production rate depends on both dose and on the tissue that has been irradiated. Figure 7 shows the effects of photoreactivating illumination on cells that had first been irradiated with an exposure of 1000 ergs/mm^2 of 254 nm radiation. The photoreactivating illumination reduces the tumor incidence with a dose reduction factor of about 0.8. Since these fish contain photoreactivating enzyme, these data are strong evidence implicating pyrimidine dimers in DNA as the initial change that results in neoplastic transformation. Many additional experiments need to be done. For example, the effect of injected cell number, the direct correlation of photoreactivation of tumor incidence with photomonomerization of dimers, the wavelength dependence of the various phenomena and, most importantly, the biological characteristics of the transformed cells. From such experiments we should be able to obtain the probability of a neoplastic transformation per pyrimidine dimer per 10^8 daltons of DNA. With this point as a calibration mark we then are in a position to assess -- using the data on repair of chemical damage described in the previous section -- tumor incidence per chemical lesion per 10^8 daltons of DNA. Thus photochemistry and photobiology have not only identified the first molecular lesion -- a lesion in DNA -- that is responsible for the neoplastic transformation, but give us the opportunity of quantitating chemical changes in DNA with the probability of a transformation.

F-6

F-7

CONCLUSION

It is clear from the papers in this symposium that photobiological and photochemical researches are some of our most important endeavors in matching ourselves to our environment. They are important not only for our well being and for their beauty and the insight they give us into molecular and cellular processes, but also for the solution to a number of important practical biological questions outside of the photobiological field.

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Table 1. The procedure used to detect endonuclease-sensitive sites in γ-irradiated DNA using an endonuclease preparation.

1. DNA $\xrightarrow{\gamma\text{-irradiation}}$ altered DNA (chain breaks / "base damage")

2. altered DNA $\rightarrow$ sediment in alkali
   altered DNA $\rightarrow$ enzyme treatment $\rightarrow$ sediment in alkali

3. calculate, from sedimentation profiles in (2), the number of breaks per 10^6 daltons introduced by endonuclease treatment.

Table 2. The numbers of BrUra residues in the repaired regions of the DNA of human cells treated with the indicated agents. (The BrUra-photolysis technique was used for these measurements.) Data from Regan and Setlow (1973).

Agent	Normal cells	XP cells
UV	25	little repair
N-acetoxy-AAF	40	" "
ICR-170	10	" "
γ-rays	~ 1	~ 1
ethylmethane sulfonate	~ 1	~ 1

Figure Legends

Figure 1. (26182-1) - Sedimentation patterns of ^{3}H-labeled E. coli DNA in alkaline sucrose gradients. Before sedimentation the DNA was either treated or not treated with an extract from Micrococcus luteus. (a) Unirradiated DNA, (b) DNA irradiated anoxically with 20 krads. Data from Setlow and Carrier (1973).

Figure 2. (26183) - Plots of the reciprocal of the weight average molecular weight (a measure of the number of breaks per molecule) vs. dose for various irradiation conditions. O —— DNA treated with endonuclease; ● --- DNA not treated with endonuclease. Sedimentation in alkali so as to measure single-strand breaks except for (b). (a) Irradiated in air, (b) irradiated in air and sedimentation in neutral sucrose so as to measure double-strand breaks, (c) irradiated anoxically, (d) irradiated in the presence of 10 mg/ml of yeast extract so as to approximate the direct action of radiation. Data from Setlow and Carrier (1973).

Figure 3. (24240) - An outline of the procedure for the detection of repaired regions in parental DNA by the photolysis of incorporated BrdUrd (Regan, Setlow and Ley, 1971). In most of our experiments we have used a double-label procedure in which the cells to be incubated in BrdUrd were labeled with ^{3}H and those to be incubated in dThd were labeled with ^{32}P. The difference in the number of breaks between these two labels measures the net effect of photolysis of BrdUrd.

Figure 4. (27996) - A schematic diagram indicating some of the possible uv-induced photoproducts in DNA involving pyrimidines (T*, U, $\hat{TT}$, $\hat{CT}$, $\hat{CC}$), purines (A*) and DNA protein links and the fact that enzymic photoreactivation reverses only one class of product -- cyclobutane pyrimidine dimers ($\hat{TT}$, $\hat{CT}$, $\hat{CC}$).

Figure 5. (27286-1) - An outline of an experiment to determine if pyrimidine dimers in DNA result in neoplastic transformation. If they do, then there should be very few tumors in those fish injected with cells treated with uv plus PR compared to uv alone or compared to PR plus uv. The no-treatment and PR samples are controls.

Figure 6. (27812) - A dose response curve for tumor induction. The average fluence through the samples is approximately 0.1 of the indicated exposure. Data from Hart and Setlow (1973, 1974).

Figure 7. (27813-1) - UV-irradiated or unirradiated cells were subject to photoreactivating illumination. The photoreactivating illumination by itself had little effect on tumor induction, but if it followed the uv irradiation it resulted in a very marked decrease in tumors. Data from Hart and Setlow (1973, 1974).

26182-1

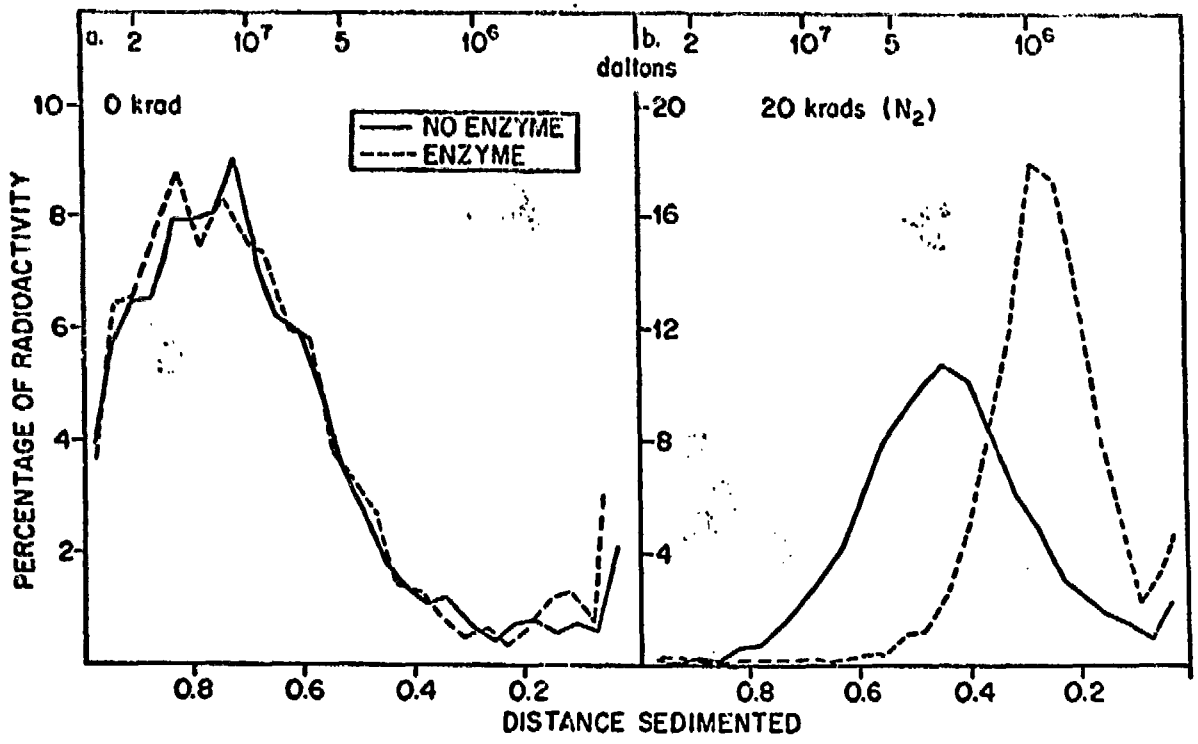

a. 2
10^7
5
10^6
b. 2
10^7
5
10^6
daltons
0 krad
20 krads (N2)
NO ENZYME
ENZYME
PERCENTAGE OF RADIOACTIVITY
10
8
6
4
2
20
16
12
8
4
0.8
0.6
0.4
0.2
DISTANCE SEDIMENTED

Fig. 1

26183

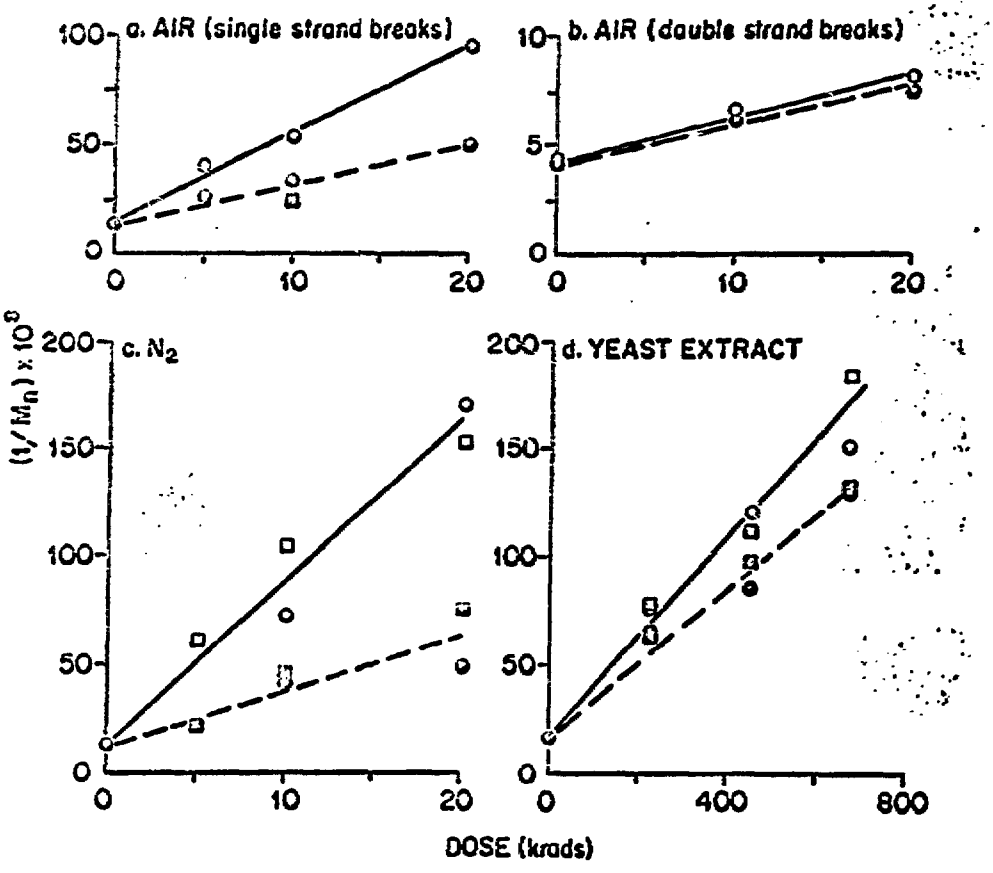

a. AIR (single strand breaks)
b. AIR (double strand breaks)
c. N_2
d. YEAST EXTRACT
$(1/M_n) \times 10^6$
DOSE (krads)

Fig. #2

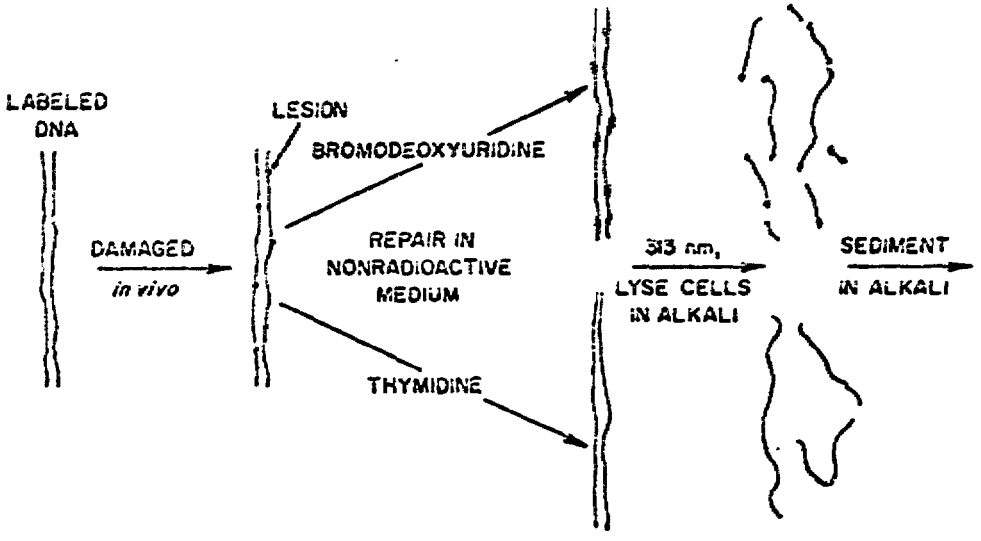
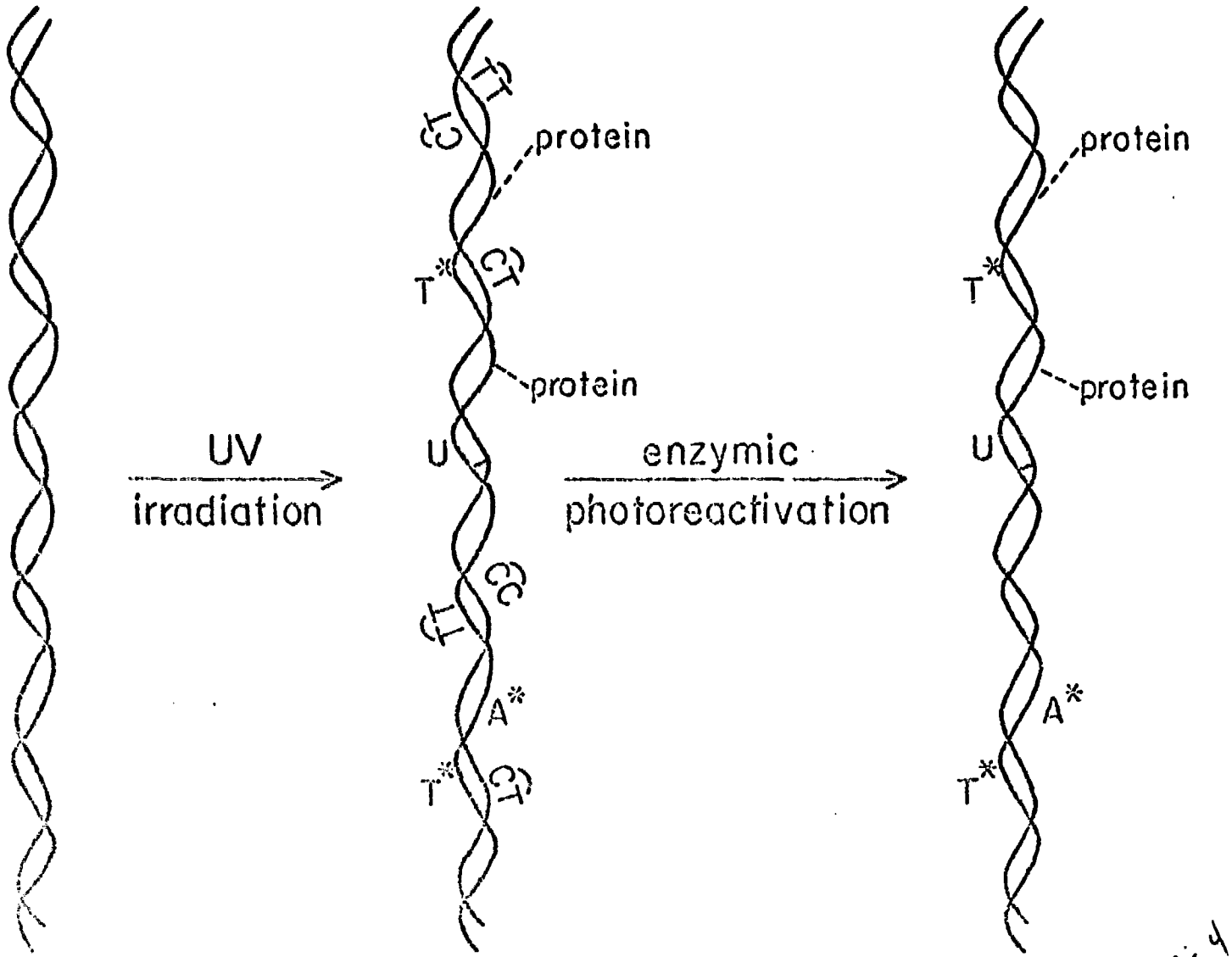

LABELED
DNA
DAMAGED
in vivo
LESION
BROMODEOXYURIDINE
REPAIR IN
NONRADIOACTIVE
MEDIUM
THYMIDINE
313 nm,
LYSE CELLS
IN ALKALI
SEDIMENT
IN ALKALI

Fig 3

PHOTOREACTIVATION MONOMERIZES PYRIMIDINE DIMERS



DO PYRIMIDINE DIMERS IN DNA RESULT IN TUMORS?

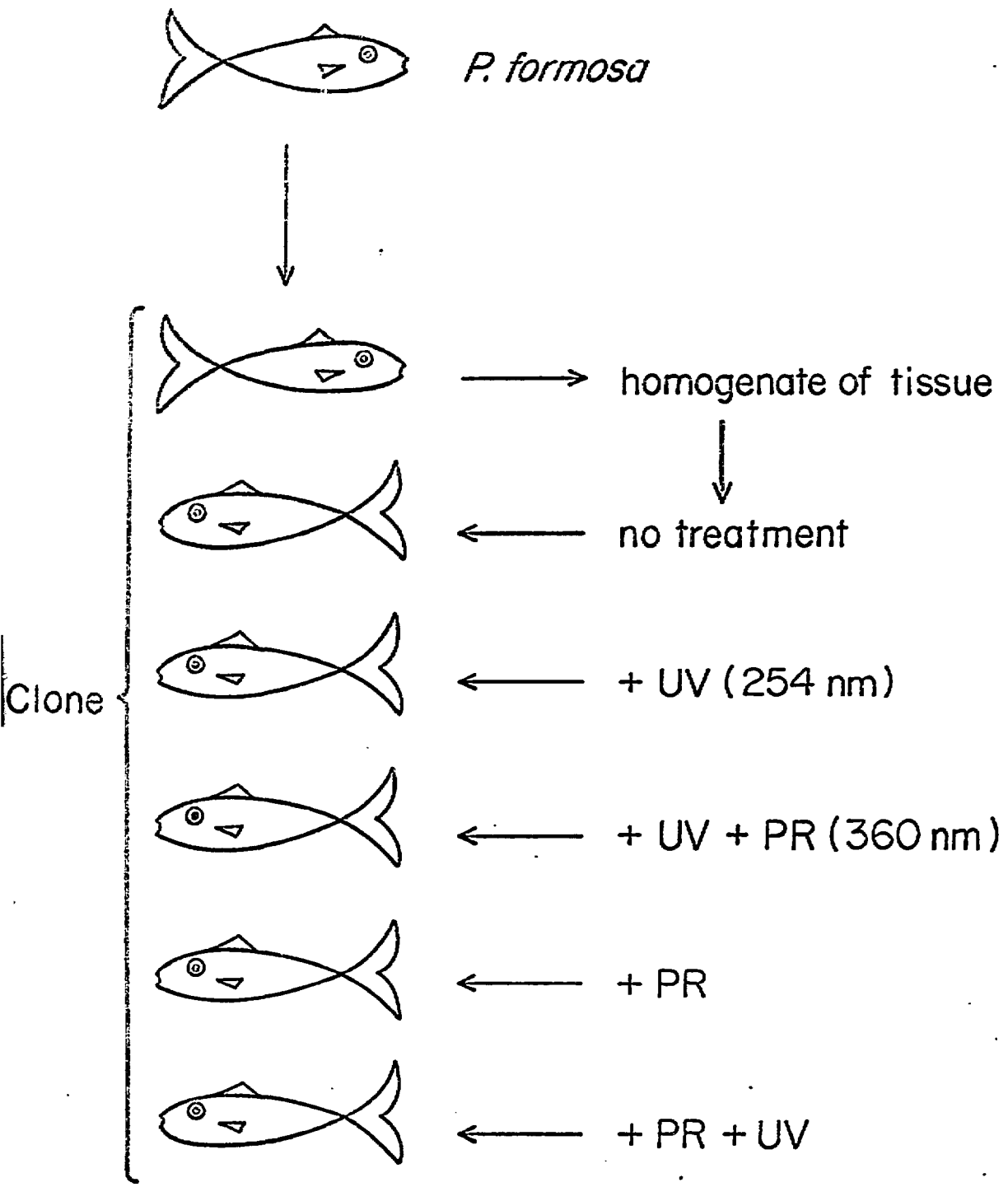
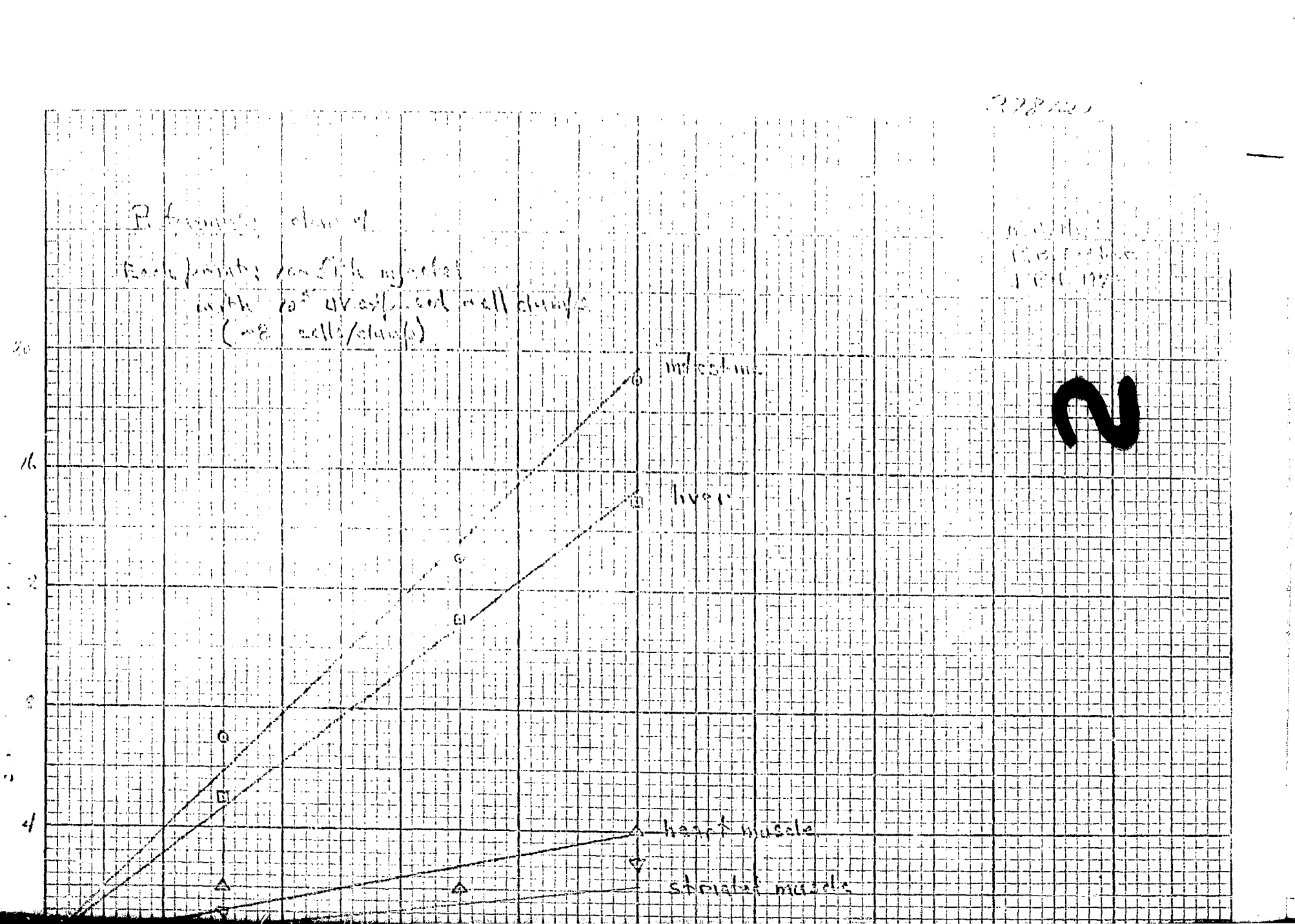


Fig 5

10 X 10 TO THE INCH 46 0732
7 X 10 INCHES
KEUFFEL & ESSER CO.
MADE IN U.S.A.


intestine
liver
heart muscle
straight muscle

27.9.13.

2

Performance chart

Each point = 200 Sicily island cell elements
10^{-5} liver cell elements (Sorrell/Elliott)
exposed to 0 or 10 energy J/mm^2 of KrF/nm
followed by photoreactivating illumination

16
12
8
4

[b]
[a]

change