

THE EFFECT OF HETEROLOGOUS DNA AND DNA  
PRECURSORS ON IRRADIATED L-CELLS

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## ABSTRACT

Strain L mouse cells at exponential phase of multiplication were diluted with growth medium to the concentration of 10-20 cells per ml. and seeded in 16 plastic flasks with 5 ml. of cell suspension in each. The flasks were divided into four groups, C, T, R, and RT, with each group containing four flasks.

After incubation in an incubator at 37°C. for 10-15 hours with air containing 5% CO<sub>2</sub> and 98% H<sub>2</sub>O, the cells of R and RT were irradiated with 500 r of gamma-radiation from a <sup>60</sup>Co source. Immediately following the irradiation the medium in all four groups was renewed. The testing materials were added to the cultures of T and RT and all cultures were returned to the incubator for further incubation. Testing materials included equimolar mixtures of four deoxyribonucleoside monophosphates (dAMP, dCMP, dGMP and TMP), four deoxyribonucleoside triphosphates (dATP, dCTP, dGTP and TTP), and calf thymus DNA. They were dissolved in pure growth medium. Final concentrations of the deoxyribonucleoside monophosphates, triphosphates, and DNA in the cultures were 10<sup>-4</sup> M, 10<sup>-5</sup> M, and 0.05 mg. per ml., respectively.

Seven days after the initial inoculation, media in the four groups were replaced with fresh growth medium. On the 12th day the medium was removed. The cells were fixed and stained with hematoxylin. The number of colonies in each flask was counted, and plating efficiency of each group was computed. Differences between the plating efficiencies of C and T, or R and RT were analyzed statistically.

Data showed that all testing materials significantly increased the viability of the irradiated cells, especially deoxyribonucleoside triphosphates and DNA. It is believed that the testing materials are utilized by cells in restoring the radiation damaged DNA at the nucleotide level.

THE EFFECT OF HETEROLOGOUS DNA AND DNA PRECURSORS  
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## INTRODUCTION

Previous experiments have shown that ionizing radiation can change the physicochemical properties of DNA and its precursors in vitro. Barron, et al. (1954) observed that with exposure to 20,000 r of X-radiation the absorption spectrum of purines and pyrimidines was changed. Chemical changes of de-aerated solutions of cytosine such as the formation of uracil, 5 and 6-hydroxycytosine, and 5,6-dihydroxycytosine due to the effect of  $10^{19}$  to  $10^{22}$  ev. per ml. of solution of gamma-radiation were reported by Khattak and Green (1966a, b). Formation of ammonia and hydroxyperoxides by irradiating nucleic acid solutions with  $2 \times 10^6$  r was noted by Scholes, et al. (1949, 1956). In vitro experiments also have demonstrated that  $10^5$  rads of ionizing radiation can decrease the viscosity of DNA and depolymerize it (Cox, et al., 1955; Cook, et al., 1966), as well as change its reactivity with other substances such as  $Mg^{++}$ ,  $Ca^{++}$ , and  $O_2$  (Cavalieri, et al., 1955; Alexander and Lett, 1960). Decreased viscosity and rupture of the structure of nucleoprotein by doses ranging from 850 r to 20,000 r have been found by Sparrow and Rosenfeld (1946), Bernstein (1954), and Cole and Ellis (1955b, 1956a).

When single cells or organisms are irradiated with

ionizing radiation, changes of the physicochemical states and biological functions of DNA and chromosomes of the cells occur. Limpero (1951) observed that DNA isolated from rats irradiated with 250 to 1,000 r was completely depolymerized 24 hours after the exposure. Deoxyribonucleoprotein isolated from mice exposed to 850 r of X-radiation splits more readily in a salt solution than that from unirradiated ones (Cole and Ellis, 1956b). A portion of saline-extractable polydeoxyribonucleotide from the lymph tissue of irradiated rats had a 3'-hydroxyl terminate which was regarded as evidence of radiation damage to nucleic acids (Swingle and Cole, 1967). DNA damage was also suggested by chromosomal aberrations such as fragmentation, and formation of bridges and rings (Amand, 1956; Puck, 1958; Neary and Savage, 1966).

It is well known that to damage DNA or chromosomes in vivo requires a lower dose of radiation than in vitro. Even a small dose when delivered to a living cell may activate radicals which eventually damage the chromosome although it is never directly hit by the irradiation (Kuzin, 1964). Since a cell is a living unit and there are intimate connections between cellular structures as well as metabolism, it is not sufficient to attribute the damage to genetic apparatus by radiation as the only factor resulting in the arrest of cell division or death of the cell. The question as to which constituent part of a cell

is most sensitive to ionizing radiation has been the subject of long and intensive debate. It has been shown that any part of a cell may be affected by radiation (Spear, 1958). For example, radiation damage on cell membranes, whether it is a result of direct hit, or the action of radicals such as OH, HO<sub>2</sub>, and H, has been demonstrated by the electron-microscopic study of Lessler (1959), and Lessler, et al. (1962) with doses of 50 r or greater of X-radiation. Physicochemical changes of cell membranes occurring as a result of radiation damage would soon cause the alterations in permeability (Creasey, 1960) and enzyme adsorption resulting in an imbalance of metabolism and destruction of the already ionized vulnerable structures such as chromosomes by the released enzymes (Bacq and Aleander, 1961). Following irradiation, increase of the activity of DNase and ATPase in the irradiated tissue has been observed with a dose as low as 25 r or greater (Kowlessar, et al., 1953; Fellas, et al., 1954; Douglas and Day, 1955; Kurnick, et al., 1959; Dale, 1952; Ashwell and Hickman, 1952). Presumably, the enzymes are released from lysosomes and mitochondria whose membranes have been damaged by radiation (Kuzin, 1964). DNase would then excise the damaged base of a DNA molecule (Howard-Flanders and Boyce, 1966). Injury to DNA, either direct or indirect, is well documented as one of the radiation effects which cause visible mutation, arrest of cell

division and cell death.

Following this line, investigators have studied the possibility of radiation recovery by supplying DNA or its precursors to exposed organisms or cells. Cole and Ellis (1955a) reported that administration of deoxyribonucleoprotein extracted from mouse spleen to mice which had been exposed to an X-ray dose of 750 r resulted in increased survival. Kanazir, et al. (1958), Savkovic (1963), and Wilczok and Mendecki (1965) found that injection of homologous DNA into lethally X-irradiated rats also increased survival. A recovery effect of homologous DNA on rat's adrenal glands, digestive organs, spleen and bone marrow which had been damaged by X-rays was histologically demonstrated by the works of Pantic, et al. (1962a, b, c). In addition, Savkovic (1964) noted that young rats exposed to 600 r of X-rays followed by the injection of homologous DNA have higher reproductive ability than those uninjected when both are mature.

Contradictory results regarding the effect of heterologous DNA on irradiated animals have been reported. Kanazir, et al. (1958) observed that heterologous DNA has no effect on lethally X-irradiated rats, while homologous DNA significantly increased their survival. However, studies by Wilczok and Mendecki (1965), Savkovic, et al. (1966), and Juraskova and Drasil (1966) show that heterologous DNA also has recovery effect on irradiated animals.

Administration of either homologous or heterologous DNA to lethally irradiated mammalian cells in culture increases survival (Djordjevic, et al., 1962; Miletic, et al., 1963; Horikawa, et al., 1964; Miletic, et al., 1964; Smets, 1965; Petrovic, et al., 1966).

Although the mechanisms of the recovery effect of DNA on irradiated organisms and cells are still unknown, three possibilities have been considered. Based on the in vivo experimental results which indicate that larger molecules of DNA have greater recovery effect than smaller ones, Savkovic, et al. (1966) proposed that DNA which has been damaged during irradiation can be replaced or restored by injected homologous or heterologous DNA. Another postulate offered by Petrovic, et al. (1966) without detailed explanation is that the administered DNA somehow influences the metabolic pathways involved in the biosynthesis of nucleic acids in the irradiated cell. A third possible mechanism is that the added DNA was degraded by DNase of the recipient cell to small molecular substances which are then utilized by the cell to synthesize its own DNA and thus restore the damaged DNA (Horikawa, et al., 1964). If this is true, one would expect that heterologous DNA as well as DNA precursors such as dAMP, dCMP, dGMP and TMP, or dATP, dCTP, dGTP and TTP, might enable irradiated cells to recover. It would also appear that there should not be significant differences between the efficiency of DNA

and its precursors in promoting recovery in cells damaged by irradiation.

In the present study, experiments were designed to obtain three objectives: (1) to test whether heterologous DNA enables mammalian cells to recover from radiation effects; (2) to study the possibility of the recovery effect of deoxyribonucleoside mono- and triphosphates; and (3) to compare the restorative efficiency of the DNA and deoxyribonucleotides on irradiated cells.

## MATERIALS AND METHODS

Earle's L-929 strain mouse fibroblasts were grown in Eagle's growth medium (Eagle, 1955) supplemented with 10% calf serum, 100 units per ml. of penicillin, and 100 mcg. per ml. of streptomycin (Grand Island Biological Co., Inc., N. Y.). Deoxyribonucleoside monophosphates, deoxyribonucleoside triphosphates and calf thymus DNA obtained from General Biochemical Inc., Ohio, were prepared in  $10^{-3}$  M,  $10^{-4}$  M, and 0.05% concentration, respectively. All solutions were made in growth medium, stored at  $-18^{\circ}\text{C}$ . and warmed to  $37^{\circ}\text{C}$ . prior to use. All experimental cells were grown in Falcon 30 ml. plastic flasks and maintained in a Wedco incubator (Model 2-17SS; Wedco Inc., Silver Spring, Md.) with a  $\text{CO}_2$  concentration of 5% and a relative humidity of 98%.

In each experiment, 16 flasks were inoculated with 5 ml. of medium containing 50-100 cells obtained from a stock culture in the log phase of multiplication as recommended by Merchant, *et al.* (1964). After 10-15 hours of incubation when the cells had attached to the surface of the flasks (Puck and Marcus, 1956; Petrovic, *et al.*, 1966), the caps were tightened, and the flasks were divided into four groups, C, T, R, and RT, with each group containing 4 flasks. Then the flasks of R and RT groups

were exposed to a  $^{60}\text{Co}$  source at room temperature. A total dose of approximately 500 r was administered at a dose rate of 52.6 r per minute for 9.5 minutes. Dose calibration was made with a Radicon Model 575 (Victoreen Instruments, Inc., Oak Lawn, Ill.). C and T cultures were left at room temperature for the same period as the irradiated cultures.

In order to reduce the possibility of effects due to radiation products in the medium, immediately following irradiation, the medium in all four groups was removed. Five ml. of pure growth medium were added to each flask of the C and R groups. Five ml. of the following solution were added to the flasks of the T and RT groups: (1) the mixture of four deoxyribonucleoside monophosphates (dAMP, dCMP, dGMP and TMP), (2) the mixture of four deoxyribonucleoside triphosphates (dATP, dCTP, dGTP and TTP), or (3) calf thymus DNA. The final concentrations of each deoxyribonucleoside mono- and triphosphates in cultures were  $10^{-4}$  M and  $10^{-5}$  M, respectively, and that of DNA was 0.05 mg. per ml.. Cultures were then returned to the incubator and caps loosened for a 12-day incubation. Media in all groups were replaced with pure growth medium on 7th day.

Concentrations of the DNA and nucleotides were chosen according to the results of Miletic, et al. (1964) and Petrovic, et al. (1966). These authors reported that 0.0005 mg. to 0.05 mg. per ml. of homologous DNA had

restorative effect on irradiated L-cells. The effective concentration of the mixture of four deoxyribonucleoside monophosphates ranged from  $10^{-7}$  M to  $10^{-4}$  M. A single deoxyribonucleoside monophosphate had no restorative effect on the irradiated cells even when the concentration was as high as  $10^{-4}$  M.

After 12 days of incubation, plating efficiency was determined as described by Puck, et al. (1956), and Fisher and Puck (1956). Cells were fixed with Carnoy's fixative (3 ethanol : 1 glacial acetic acid) for 30 minutes and stained with hematoxylin for 10 minutes. Puck and Marcus (1956) found that a mammalian cell in culture when damaged by 500 r of radiation would die or form a colony of less than 50 cells if continuously cultivated to 17 days, and that the colony number of an irradiated culture at the 11th day was the same as that at the 17th day. Petrovic, et al. (1966) utilized colonies with 100 or more cells in their work and this criterion was used in the present study.

## RESULTS

Table I shows the results of the experiment utilizing deoxyribonucleoside monophosphates in which the mean value of colony counts, or plating efficiencies, and the standard error are shown for four duplicated flasks. As previously stated, cells of group C were unirradiated and untreated with the nucleotides; cells of group T were unirradiated but treated with the mixture of dAMP, dCMP, dGMP and TMP at equimolar concentration ( $10^{-4}$  M); cells of group R were irradiated with 500 r of gamma-radiation but untreated with the nucleotides and; cells of group RT were irradiated with 500 r and followed by the treatment with the mixture of four monophosphates ( $10^{-4}$  M). Plating efficiencies of the cultures in the C, T, R, and RT groups are 60-68%, 58-70%, 10-20% and 19-24%, respectively. The values of C and T groups are very close, but those of R and RT are rather different. These data are further analyzed in Table IV.

Table II presents the results of the experiment utilizing deoxyribonucleoside triphosphates in which the experimental design was identical to that of the experiment of deoxyribonucleoside monophosphates, with an equimolar ( $10^{-5}$  M) mixture of dATP, dCTP, dGTP and TTP substituted for the monophosphates. Plating efficiencies

of the cultures in the C, T, R, and RT groups are 58-64%, 56-68%, 8-15%, and 16-25%, respectively. The values of C and T groups are very similar. A conspicuous difference between the plating efficiencies of the R and RT groups is noted (Table IV).

Table III illustrates the results of the experiment with DNA in which 0.05 mg. per ml. of calf thymus DNA was added to the cultures of T and RT. Plating efficiencies of C, T, R, and RT are 52-61%, 54-64%, 6-22%, and 16-37%, respectively. Close plating efficiencies between C and T groups are clear. A further analysis of these data is shown in Table IV.

In order to determine the effect of the testing materials on the viability of irradiated and unirradiated cells, a t-test, corrected for small sample size, was employed. Each figure in Table IV is the mean value of plating efficiencies of four experiments with its standard error. The t-ratio and probability of the mean difference by chance between each C and T, or R and RT pair are also presented. Ratio of t between C and T for the three tested materials are 1.16, 1.63, and 0.42, respectively. The results indicate that the addition of the testing materials has no effect on the unirradiated cells. The treatment of irradiated cells with the mixture of four monophosphates increases the plating efficiency of the cells and is significant at the 5 per cent level. Both the mixture of four triphos-

phates, and calf thymus DNA greatly increase plating efficiency of the irradiated cells and are significant at the 1 per cent level.

Reduction of colony number and size in the irradiated cultures as compared to those of unirradiated are photographically shown in Fig. II through Fig. IV. These figures also illustrate that the addition of the testing materials increases the colony number of the irradiated cells, but does not affect the unirradiated cells.

Table I. Mean Number of Colonies and Plating Efficiency of the Experiment Utilizing Deoxyribonucleoside Monophosphates.

Treatment	No. of Cells Seeded/Flask	No. of Colonies	Plating Efficiency (%)
C	50	32 ± 3	64 ± 5
	50	34 ± 5	68 ± 9
	70	46 ± 3	66 ± 4
	70	42 ± 8	60 ± 12
T	50	33 ± 1	66 ± 2
	50	35 ± 5	70 ± 9
	70	46 ± 7	65 ± 10
	70	41 ± 5	58 ± 7
R	50	8 ± 1	15 ± 2
	50	5 ± 1	10 ± 3
	70	10 ± 2	14 ± 3
	70	14 ± 2	20 ± 3
RT	50	12 ± 3	24 ± 6
	50	10 ± 1	21 ± 1
	70	13 ± 3	19 ± 4
	70	17 ± 4	24 ± 6

Table II. Mean Number of Colonies and Plating Efficiency of the Experiment Utilizing Deoxyribonucleoside Triphosphates.

Treatment	No. of Cells Seeded/Flask	No. of Colonies	Plating Efficiency (%)
C	50	29 ± 1	58 ± 3
	100	61 ± 1	61 ± 1
	50	32 ± 3	64 ± 6
	60	38 ± 4	63 ± 6
T	50	28 ± 2	56 ± 3
	100	63 ± 3	63 ± 3
	50	34 ± 2	68 ± 3
	60	39 ± 4	65 ± 7
R	50	6 ± 2	12 ± 2
	100	15 ± 1	15 ± 1
	60	4 ± 1	8 ± 2
	50	6 ± 1	10 ± 1
RT	50	12 ± 1	24 ± 1
	100	25 ± 3	25 ± 3
	60	8 ± 2	16 ± 3
	50	14 ± 1	23 ± 1

Table III. Mean Number of Colonies and Plating Efficiency  
of the Experiment Utilizing Calf Thymus DNA.

Treatment	No. of Cells Seeded/Flask	No. of Colonies	Plating Efficiency (%)
C	90	47 ± 2	52 ± 5
	100	58 ± 5	58 ± 5
	50	30 ± 1	60 ± 1
	100	61 ± 4	61 ± 4
T	90	49 ± 7	54 ± 7
	100	57 ± 5	57 ± 5
	50	32 ± 4	64 ± 9
	100	58 ± 6	58 ± 6
R	90	20 ± 2	22 ± 3
	100	22 ± 3	22 ± 3
	50	3 ± 1	6 ± 1
	100	12 ± 3	12 ± 3
RT	90	28 ± 4	31 ± 4
	100	37 ± 1	37 ± 1
	50	8 ± 1	16 ± 2
	100	22 ± 5	22 ± 5

Table IV. Comparison of Mean Plating Efficiency of Irradiated and Unirradiated L-cells when Treated with Monophosphates, Triphosphates, and Calf Thymus DNA.

Testing Material	C	T	R	RT
dNMP	65 $\pm$ 2%	65 $\pm$ 3%	15 $\pm$ 2%	22 $\pm$ 1%
	t = 1.16		t = 4.89	
	p < 0.4		p < 0.05	
dNTP	61 $\pm$ 1%	63 $\pm$ 2%	11 $\pm$ 2%	22 $\pm$ 2%
	t = 1.63		t = 9.04	
	p < 0.2		p < 0.01	
DNA	58 $\pm$ 2%	58 $\pm$ 2%	15 $\pm$ 3%	27 $\pm$ 4%
	t = 0.42		t = 9.29	
	p < 0.6		p < 0.01	

Fig. I. Comparison of the effect of monophosphates, triphosphates, and calf thymus DNA on irradiated and unirradiated L-cells. The small bars represent standard error. C: unirradiated and untreated; T: unirradiated but treated with one of the testing materials; R: irradiated but untreated; RT: irradiated and treated with one of the testing materials. All testing materials do not affect the unirradiated cells, but significantly increase the plating efficiency of the irradiated cells.

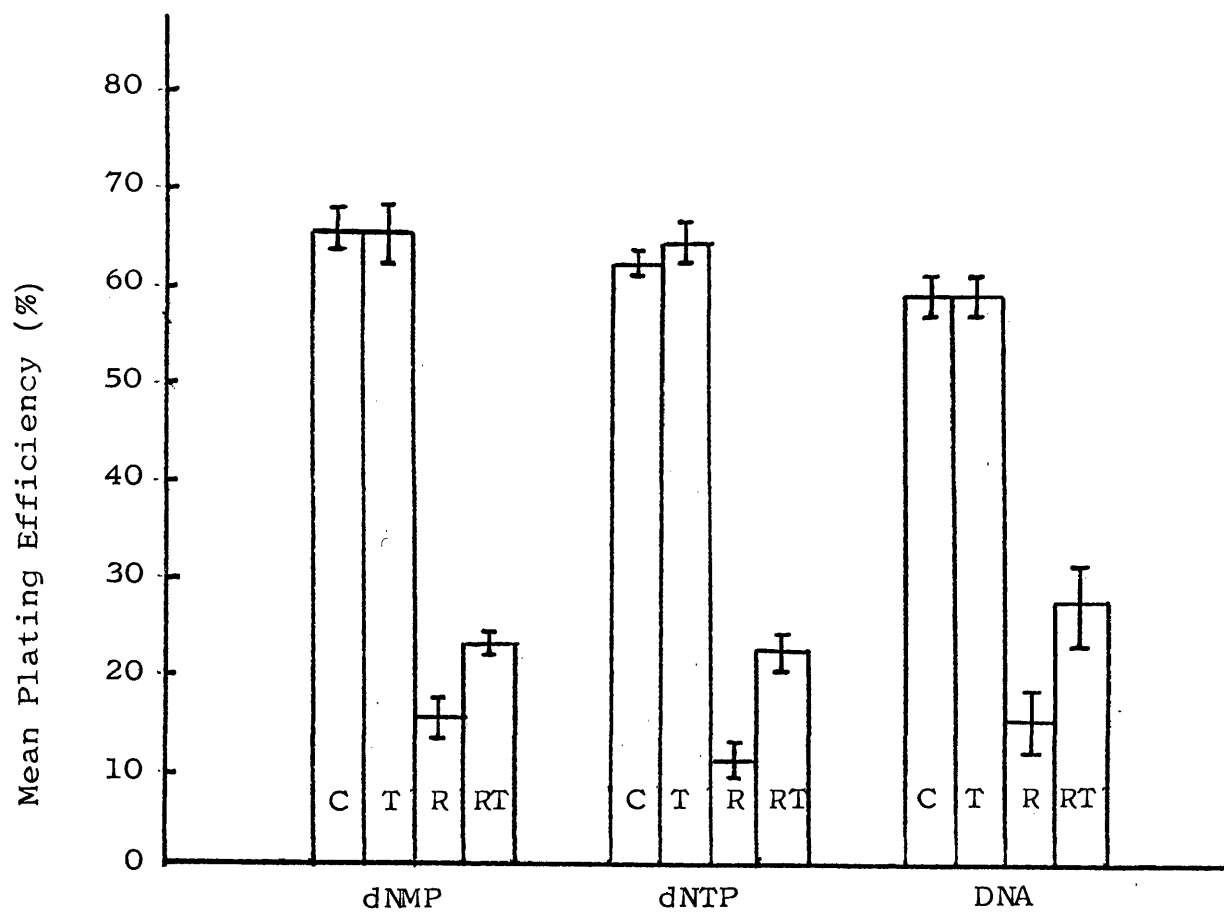


Fig. 11. Effect of adding a  $10^{-4}$  M mixture of four deoxyribonucleoside monophosphates on the colony formation of unirradiated and irradiated L-cells. Each flask was initially seeded with 70 cells. The upper 2 flasks were unirradiated, and the lower 2 flasks were irradiated with 500 r. The mixture was added to the right 2 flasks immediately following the irradiation; the left 2 flasks were untreated.

C



T



R



RT



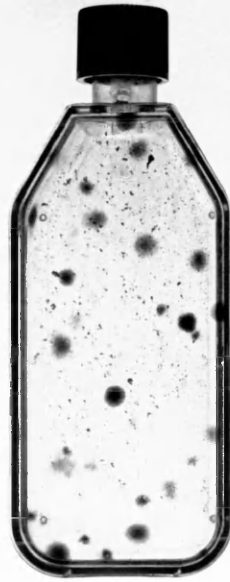
dNMP

Fig. III. Effect of adding a  $10^{-5}$  M mixture of four deoxyribonucleoside triphosphates on the colony formation of unirradiated and irradiated L-cells. Each flask was initially seeded with 50 cells. The upper 2 flasks were unirradiated, and the lower 2 flasks were irradiated with 500 r. The mixture was added to the right 2 flasks immediately following the irradiation; the left 2 flasks were untreated.

C



T



R



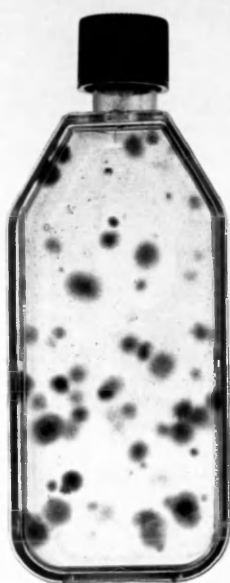
RT



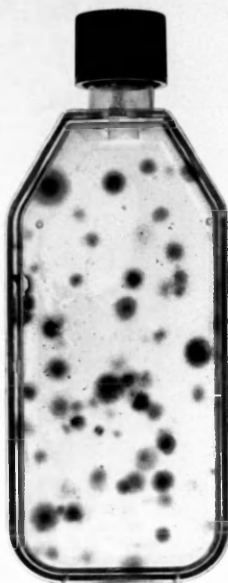
dNTP

Fig. IV. Effect of adding calf thymus DNA (0.05 mg. per ml.) on the colony formation of unirradiated and irradiated L-cells. The upper 2 flasks were unirradiated, and the lower 2 flasks were irradiated with 500 r. The DNA was added to the right 2 flasks immediately following the irradiation; the left 2 flasks were untreated.

C



T



R



RT



DNA

## DISCUSSION

Savkovic, et al. (1966) postulated that administered DNA can replace or restore the DNA damaged by radiation. This hypothesis may gain its support from the experiment of heritable transformation and DNA hybridization which indicate that the transformation of homologous DNA is possible (Gartler, 1959; Benoit, et al., 1960; Sorieul and Ephrussi, 1961; Kay, 1961; Szybalska and Szybalski, 1962). However, this is very unlikely, since the experiments conducted in this study indicate that not only DNA, but also the mixtures of deoxyribonucleotides have a restorative effect. Furthermore, the mixture of deoxyribonucleoside triphosphates has the same restorative efficiency as DNA. In addition, the untransformability of DNA between different species and the action of interferon against foreign nucleic acids in higher animals (Issacs, 1963) make it difficult to explain the replacement of a portion of damaged DNA by heterologous calf thymus DNA.

Since the treatment of irradiated L-cells with the mixture of four deoxyribonucleoside mono- or triphosphates significantly increased survival, the results included here appear to support the theory of Horikawa, et al. (1963, 1964) who stated that the administered DNA was depolymerized by DNase in the cytoplasm of the recipient cell and then utilized by the cell in repairing the injured DNA.

Evidences which show that a donor DNA is degraded before used by the irradiated cell also come from the increase of DNase activity immediately after irradiation (Kowlessar, et al., 1953; Fellas, et al., 1954; Douglas and Day, 1955; Kurnick, et al., 1959). By labelling the donor DNA with radioactive isotopes, Horikawa, et al. (1963, 1964) and Hudnik-Pelvnik, et al. (1959) were able to show that the added DNA was degraded to tetranucleotides and smaller units. The fact that addition of deoxyribonucleoside mono- and triphosphates can increase the viability of the irradiated cells as does DNA, would tend to support further this hypothesis. The lower restorative efficiency of deoxyribonucleoside monophosphates as compared to those of triphosphates and DNA perhaps may be due to the impairment of the biochemical processes for phosphorylating them. Doses ranging from 25 to 640 r cause an increase of ATPase in irradiated animal tissue (Dale, 1952; Ashwell and Hickman, 1952). ATP in the tissues would then be destroyed and thus the energy source available for the biosynthesis of DNA precursors as well as other metabolites is decreased. Even with a small dose of 50 r, suppression of biochemical reactions requiring energy, such as the incorporation of phosphate, orotic acid, sodium formate, ureidosuccinic acid, and thymine into DNA have been found (Harrington and Lavik, 1953; Logan, et al., 1959; Nygaard and Potter, 1959; van Lancker, 1959; Yen, et al., 1959). Phosphorylation in

nuclei, a process of energy flow, is also prevented by exposure to 100 r (Stocken, 1959). The fact that a shortage of energy results in the impairment of the synthesis of phosphorylated DNA precursors seems clear. However, it would appear doubtful that the incorporation of nucleoside triphosphates into DNA is also inhibited. Kornberg (1961) has found that in the mixture of four deoxyribonucleoside triphosphates, polymerase,  $Mg^{++}$  and a DNA template, synthesis of DNA occurs. The system does not require ATP. Although the nucleotides themselves are high energy compounds, there is no enzyme involved in using their energy in that system.

Beatty and Beatty (1967) using Tradescantia have postulated that nucleotides furnish the energy for chromosomal repair. However, their results indicate that adenosine, adenosine 2'-, 3'-, and 5'-monophosphates, deoxyadenosine-5'-mono and 5'-triphosphate have no restorative effect on irradiated chromosomes. These compounds possess very much energy and should be effective in chromosome repair if their theory is correct. In addition, one can not neglect the importance of energy in the biochemical synthesis of DNA precursors, or to claim that without it the added deoxyribonucleoside triphosphates can not insert into the radiation damaged DNA. Apart from this, the destruction of ATP and other energy-carrying compounds is a quantitative problem. It is difficult to say that the energy in an irradi-

ated cell is so reduced as to be the key factor in radiation repair mechanisms. After exposure, the synthesis of DNA is still possible, but the normal synthetic pathways force the cell to use the damaged precursors, the radiation-products, in restoring its DNA. The result would be the production of incorrectly coded enzymes and metabolites which then cause the arrest of cell division, formation of giant cell, or cell death (Kuzin, 1964). The lower restorative efficiency of deoxyribonucleoside monophosphate as compared to those of deoxyribonucleoside triphosphate and DNA in the present study may reflect a shortage of energy in the irradiated cell. But the fact that monophosphates can accomplish some repair also indicates that there is still some energy available for phosphorylation. Therefore, it may tentatively be concluded that the addition of deoxyribonucleotides or DNA supplies undamaged DNA precursors directly or through degradation of DNA to the irradiated cells, and that the materials are utilized by the cells at the level of deoxyribonucleoside triphosphates or tetranucleotides in repairing the damaged DNA.

The process of restoration has been postulated by Howard-Flanders, et al. (1966). When the double-strand DNA is injured either by a radiation-induced chain break or by the enzymic excision of damaged bases, nucleotides are released, presumably through the action of an enzyme on the free single-strand ends. The DNA twin helix is reconstruct-

ed by a DNA repair polymerase that inserts complementary nucleotides into the gap, adding them onto one of the single-strand ends. Thus, the end of a single strand serves as initiator, while the intact strand opposite serves as a template. The repair is completed by joining the phosphodiester backbone when the last nucleotide is inserted into the gap.

Although we lack experimental evidence, the administered nucleotides or DNA may accomplish repair through other biochemical mechanisms as postulated by Petrovic (1966), and Beatty and Beatty (1967).

## SUMMARY AND CONCLUSIONS

Following irradiation with 500 r of gamma-radiation, treatment of the exposed strain L mouse cells in culture with an equimolar mixture of four deoxyribonucleoside monophosphates dAMP, dCMP, dGMP and TMP at the concentration of  $10^{-4}$  M slightly increases the viability of the cells in term of plating efficiency. The mixture of four deoxyribonucleoside triphosphates dATP, dCTP, dGTP and TTP at the equimolar concentration of  $10^{-5}$  M, or a calf thymus DNA solution at the concentration of 0.05 mg. per ml., very significantly increase the plating efficiency of the irradiated cells.

Several mechanisms by which the testing materials function in restoring the irradiated cells have been discussed. The present study supports the theory of Horikawa, et al. (1964) that the administered DNA is depolymerized to smaller units through the action of DNase and that the testing materials are all utilized by the irradiated cells at the nucleotides level in repairing the damaged DNA. The lower restorative efficiency of the deoxyribonucleoside monophosphates as compared to those of the deoxyribonucleoside triphosphates and DNA is attributed to the impairment of phosphorylation by the irradiation. However, other possible restorative mechanisms are not excluded.

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