culture flasks were incubated at a fixed temperature of 25° ± 1°C for 25 days. The controls, which were devoid of 8-azaguanine, were simultaneously maintained in respect of each isolate.

Results and discussion. The results of a representative experiment are presented in the Table, and the morphogenetic variations of interest are depicted in Figures 1 and 2.

After 15 days of growth on the medium reinforced with 8-azaguanine, the colour of the strains was completely altered from the normal Rainette green (Ridgway Pl. XXXIX) to Sayal brown (Ridgway Pl. XXIX) on the upper side, and Pale Chalcedony yellow (Ridgway Pl. XVII) on the lower side[3].

The other induced morphological variations are enumerated below: Proliferation of the sterigmata into secondary stalks (only in heads bearing biseriate sterigmata); stalks septate, 6 to 14 in number, terminating into miniature fertile heads with uniseriate sterigmata, bearing chains of globose or sub-globose conidia in basipetal succession. Stalks varying in length from 65 to 150 µm, and breadth 5 to 9 µm. Secondary miniature vesicles 5 to 8 µm long and 6 to 8 µm broad; Conidia 5 to 7.5 µm in diameter.

These morphogenetic variations have been repeatedly observed in diverse strains of *A. oryzae*, as we repeated our experiments to confirm the observations. The frequency of the abnormal heads obtained in diverse strains of *A. oryzae* is depicted in the Table. The controls, which were devoid of 8-azaguanine, remained unaffected and no variations were detected in them. The diverse strains produced 52% to 73% abnormal heads under the impact of 8-azaguanine. Similar aberrant development of the phialids was also induced in the present strains of *A. oryzae* by 6-azauracil (80 mg/l) and 2-amino-pyrimidine (110 mg/l). The earlier reports of proliferation in *Aspergilli* are those of THIELKE[4], MILLER and ANDERSON[5] and TURIAN[6].

During repeated transfers from 8-azaguanine-reinforced medium to 8-azaguanine-reinforced medium, the strains persisted in producing a high percentage of abnormal heads, but on transfer to the medium devoid of 8-azaguanine, the strains reverted to normal within 72 h, and the abnormal traits quickly disappeared. This clearly proves that the morphogenetic variations encountered in *A. oryzae* strains were in response to the programmed environmental signal of 8-azaguanine.

In view of the foregoing observations the authors are tempted to hazard the explanation that the synthesis of abnormal m-RNA's, following the incorporation of 8-azaguanine, invariably leads to the production of altered but stable catalytic proteins with novel potentialities, which may be responsible for inducing the recognizable morphogenetic variations in *A. oryzae*[7].

Résumé. Proliférations de l'appareil sporifère d'*Aspergillus oryzae* traité à l'azaguanine.

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Frequency of proliferating biseriate abnormal heads produced after 15 days of growth of *A. oryzae* cultures on complete modified Czapek's medium to which 85 mg/l of 8-azaguanine had been added, as compared to controls to which no 8-azaguanine had been added

Aspergillus oryzae cultures		
No.	Without 8-azaguanine (%)	With 8-azaguanine (%)
136	0.0	63.0
151	0.0	66.0
159	0.0	52.0
168	0.0	73.0
190	0.0	69.0

[3] R. RIDGWAY, *Color Standards and Color Nomenclature* (Washington, USA 1912).
[4] C. THIELKE, Arch. Mikrobiol. *34*, 65 (1959).
[5] C. W. MILLER and N. A. ANDERSON, Mycologia *53*, 433 (1961).
[6] G. TURIAN, in *The Aspergilli* (The Williams and Wilkins Co., Baltimore, Maryland 1965) and *The Fungi* (Eds. G. C. AINSWORTH and A. S. SUSSMAN; Academic Press, New York 1966), vol. 2.
[7] The authors are deeply indebted to Dr. O. ISLER of F. Hoffmann-La Roche, Basel (Switzerland) for his generous gift of 8-azaguanine and 6-azauracil. They are also grateful to the University Grants Commission, at New Delhi, which financed this research programme by grant-in-aid No. F-8/5-63 (G), to R.K.K.

DNA Repair Synthesis after X-Irradiation in *E. coli*

Ionizing radiation disrupts DNA, and the killing effects of such radiation on cells are to a large extent, the result of damage to DNA[1]. Although the precise nature of this damage is unknown, X-rays are known to produce discontinuities in the sugar-phosphate backbone of the molecule[2]. The damage is probably not limited to the production of hydrolyzed phosphadiester bonds but likely involves the ribose moiety[3] and the bases as well[4]. Evidence for enzymatic repair of radiation-induced damage to DNA has been found in many systems and has resulted largely from the study of bacterial mutants having altered radiation sensitivity[1], and from measurement of changes in the molecular weight of DNA following irradiation of living cells.

In *E. coli*, the time course of strand rejoining, presumably the final step in the repair process, is similar in UV- and X-ray induced damage, being essentially complete in 1 h[2,5]. The early steps in the latter case are completely unknown. A serious difficulty in the search for early enzymatic steps in X-ray repair is the lack of a well defined lesion in the DNA molecule, such as pyrimidine dimers following UV, of which the reversal or removal could be followed.

[1] P. HOWARD-FLANDERS, A. Rev. Biochem. *37*, 175 (1968).
[2] R. A. MCGRATH and R. W. WILLIAMS, Nature, Lond. *212*, 534 (1966).
[3] D. S. KAPP and K. C. SMITH, Radiat. Res. *42*, 34 (1970).
[4] J. J. WEISS, Prog. Nucl. Ac. Res. molec. Biol. *3*, 103 (1964).
[5] L. KANNER and P. HANAWALT, Biochem biophys. Res. Commun. *39*, 149 (1970).

Repair synthesis, the replacement of altered or excised nucleotides from a damaged region of DNA, by radioactive nucleosides, such as bromodeoxyuridine, has been shown most convincingly in the UV model by a technique employing isopycnic centrifugation, by which previously synthesized and newly-replicating DNA can be efficiently separated[6,7]. The uptake of radioactive nucleosides, such as bromodeoxyuridine into 'old' DNA induced by UV or other agents is evidence for a patching process. Repair synthesis has not been demonstrated by this method for X-ray at low or moderate dosage[8], although PAINTER and CLEAVER[9] were able to detect non-semiconservative synthesis in HeLa cells after exceedingly large (100 kr) X-ray doses. The authors stressed, however, that no relationship was established between this synthesis and closure of strand breaks.

Two recent developments have offered the possibility of shedding new light on repair synthesis. The first was the isolation of mutants of *E. coli* with defective or absent Kornberg polymerase, the presumptive enzyme for repair synthesis[10,13]. The second was the technique devised by REGAN, SETLOW and LEY[11] for detecting repaired regions into which nucleotides have been incorporated by substituting bromodeoxyuridine (BUdR) for thymidine in the medium in which prelabeled cells are repairing, and exploiting the susceptibility of repaired BUdR-containing DNA to breakage by long-wavelenth UV light (313 nm). We have performed experiments with the first of the Cairns mutants (W3110, *pol* A1, here to be referred to as *pol*-[10]) and its parental strain (*pol* +) for ability to join strand breaks after X-ray by following sedimentation of prelabeled DNA in alkaline sucrose gradients. We have

[6] D. PETTIJOHN and P. HANAWALT, Biochim. biophys. Acta. *72*, 127 (1963).
[7] P. C. HANAWALT and R. H. HAYNES, Biochem. biophys. Res. Commun. *19*, 462 (1965).
[8] D. BILLEN, R. R. HEWITT, T. LAPTHISOPHON and P. M. ACHEY, J. Bact. *94*, 1538 (1967).
[9] R. B. PAINTER and J. E. CLEAVER, Nature, Lond. *216*, 369 (1967).
[10] P. DELUCIA and J. CAIRNS, Nature, Lond. *224*, 1164 (1969).
[11] J. D. REGAN, R. B. SETLOW and R. D. LEY, Proc. natn. Acad. Sci., USA *68*, 708 (1971).
[12] C. D. TOWN, K. C. SMITH and H. S. KAPLAN, Science *172*, 851 (1971).
[13] W. S. KELLEY and H. J. WHITFIELD, Nature, Lond. *230*, 33 (1971).

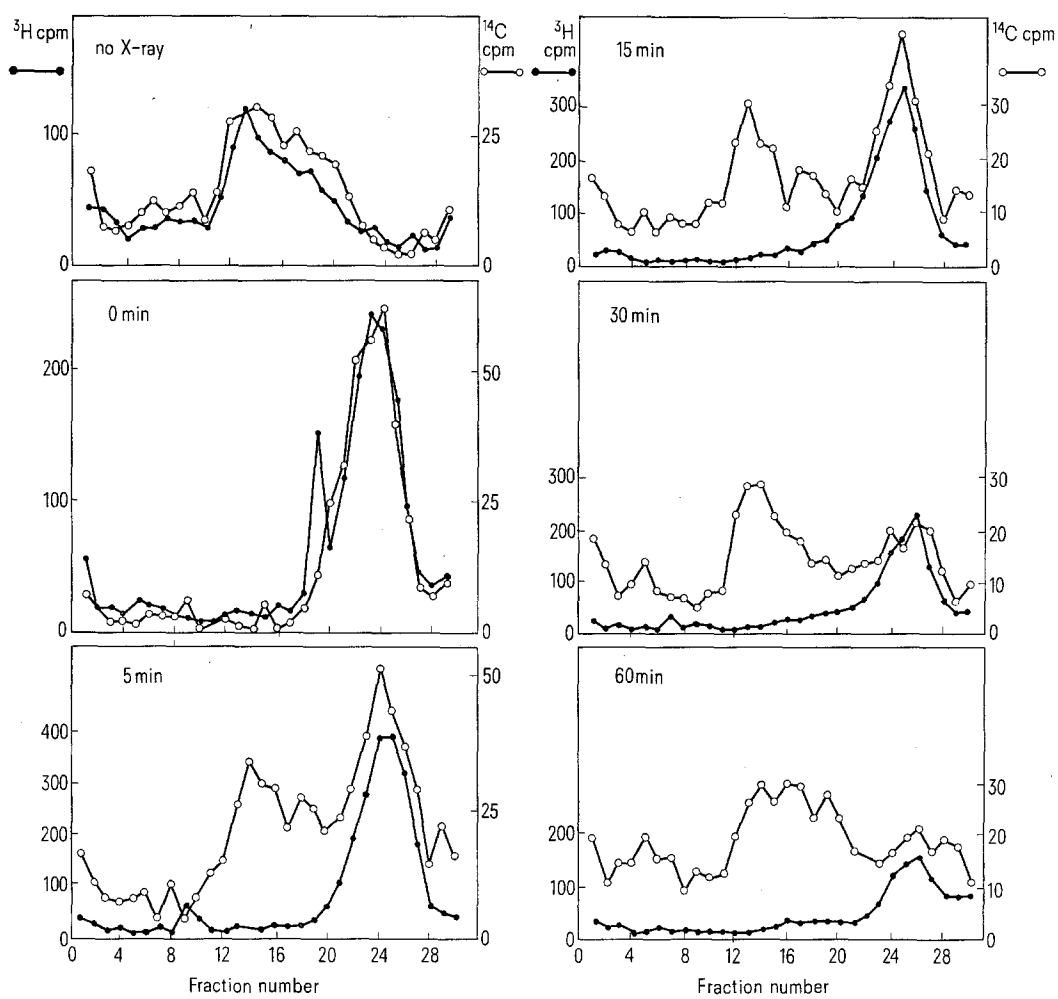

Fig. 1. Alkaline sucrose sedimentation of γ-irradiated *E. coli*. Bacteria were grown in M-9 medium supplemented with thymine. *Pol*+ and *pol*— were grown at 37 °C for several generations in medium containing ^{14}C-thymidine (44 mCi/mmole, 2 μCi/ml) and ^{3}H-thymidine (18 Ci/mmole, 5 μCi/ml), respectively. The cell suspensions were then combined. Cells received 20 kr of γ-irradiation from a ^{60}Co source at 0 °C under air. Samples were removed at the indicated times, 0.01 *M* KCN added and chilled to 0 °C. Approximately 5×10^6 cells were layered onto a 5–20% alkaline sucrose gradient[2]. Samples were centrifuged in a 'Spinco' 56 Ti rotor at 42,500 rpm for 1 h. Eight-drop fractions were collected on filter paper and counted in toluene + PPO + POPOP. ○—○, *Pol*+; ●—●, *Pol-E. coli*.

also sought evidence for regions of repair synthesis in DNA from *pol* + and from an X-ray resistant strain, B/r, by allowing the cells to recover from X-ray damage in the presence of BUdR and measuring its susceptibility to photolysis by 313-nm irradiation.

Town et al.[12] reported an increase in susceptibility of the *pol* mutant to X-rays, as measured by colony-forming ability. We have confirmed this observation (unpublished data) and have also followed changes in molecular weight after X-ray by sedimentation in alkaline sucrose gradients. Figure 1 shows the results of an experiment in which *pol* + and *pol* — cells were grown in ^{14}C- and ^{3}H-thymidine, respectively, then resuspended in non-radioactive medium and combined prior to γ-irradiation (20 kr). After only 5 min incubation at 37 °C the *pol* + cells have begun to show reappearance of DNA with high molecular weight typical of unirradiated *E. coli*. After 60 min, there is still no appreciable return in the *pol*— cells. These gradients suggest that the mutant (which has an amber mutation in what is very likely the structural gene coding for Kornberg polymerase[13, 14]) is unable to close the strand breaks caused by X-ray at this dose, presumably because polymerization is a required step in the repair of these lesions.

On the basis of this observation suggesting that repair synthesis may be required in the X-ray model, we have

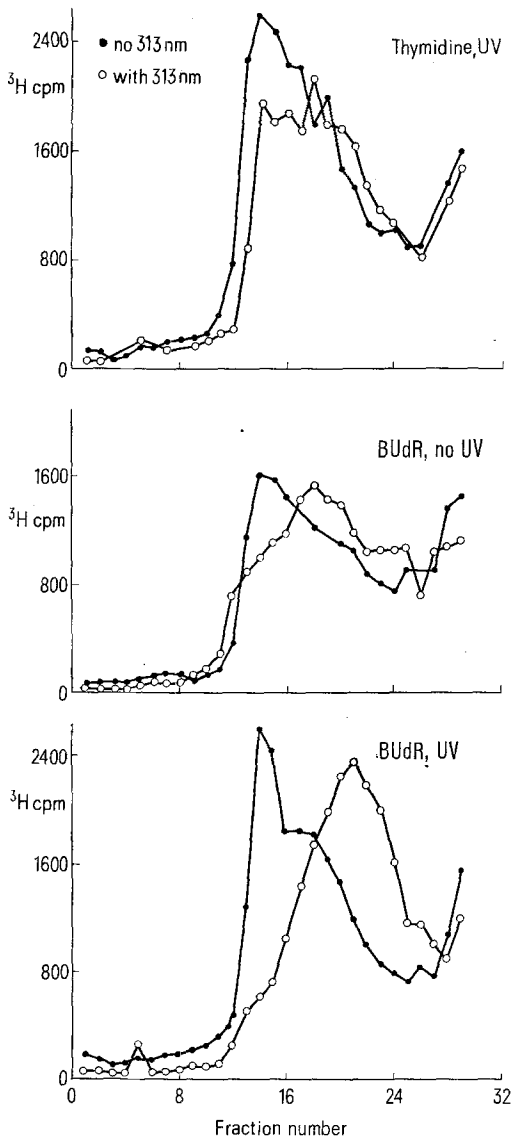

Fig. 2. Breakage of UV-repaired, BUdR-containing DNA after 313 nm irradiation. *Pol*+ cells were grown in M-9 medium supplemented with thymine, containing ^{3}H-thymidine, a 6.7 μCi/ml to a concentration of 10^{9} cells/ml, resuspended in non-radioactive medium without thymine and divided into 3 tubes of 10^{9} cells each, to 2 of which were added BUdR and to the third thymidine, all at 0.06 m*M*. Cells were incubated for 5 min at 37 °C, then resuspended in water, and exposed to UV from a germicidal lamp (350 erg mm^{-2}). They were then diluted 5-fold into the appropriate medium and shaken in the dark at 37 °C for 1 h. They were again resuspended in medium containing thymidine, 0.06 m*M*, then further incubated for 1 h. Each aliquot was resuspended in 0.5 *M Tris*, pH 7.9 + 0.01 *M* KCN and converted to protoplasts by treatment with lysozyme. The protoplasts were then exposed to monochromatic light of wavelength 313 nm (1.0×10^{5} erg mm^{-2}) and layered on alkaline sucrose gradients as in Figure 1.

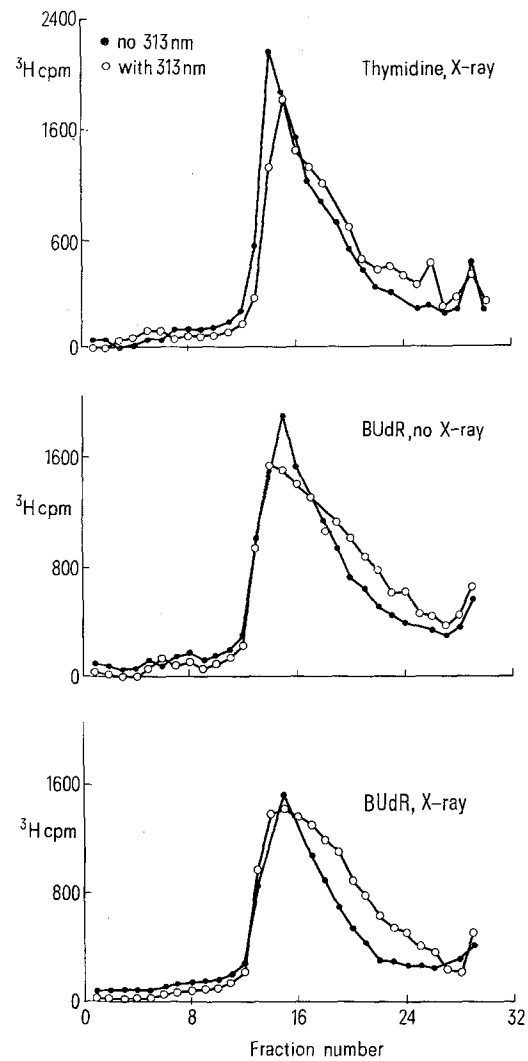

Fig. 3. Effect of 313 nm light on DNA from cells repaired from *x*-irradiation in BUdR. *Pol*+ cells were grown and treated exactly as in Figure 2 except that resuspension in water was omitted, and instead of UV, cells were irradiated in medium under air at 0 °C (15 kr) with a Siemens 250 kV *ortho*voltage radiation therapy unit (15 mA, 4 mm Al filter) at 1.0 kr per min.

[14] J. Gross and M. Gross, Nature, Lond. *224*, 1166 (1969).

attempted to demonstrate repair replication using the method of REGAN et al.[11]. Figure 2 shows the result of a preliminary experiment using UV-light. *Pol* + bacteria were grown in medium containing ^{3}H-thymidine for several generations to label the DNA uniformly. The cells were removed from radioactive medium and resuspended in medium containing BUdR or thymidine, where they were incubated for 5 min at 37 °C, then chilled, centrifuged, resuspended in water, and irradiated with UV with bulk of radiation at 254 nm. They were then returned in darkness to the appropriate medium, where they were allowed to repair for 1 hour to incorporate BUdR into stretches of previously labeled DNA. Isopycnic centrifugation of this DNA at this stage confirmed that the BUdR molecules were actually incorporated into the cell during the recovery phase. Earlier experiments had also suggested that a semiconservatively synthesized BUdR-containing daughter strand (in the unirradiated control) hydrogen bonded to the labeled strand rendered the parent strand more susceptible to breakage by 313-nm irradiation. Therefore, all cells were grown for an additional generation (1.0 h in this medium) before photolysis. The cells were converted to protoplasts, then exposed to 313-nm irradiation (1.0×10^5 erg mm^{-2}). The shift in single strand molecular weight was most pronounced in the DNA from cells repaired in the presence of BUdR, although some breakage also occurred both in cells repairing in thymidine and in unirradiated cells incubated in BUdR. The weight average molecular weights from this and the following experiment, summarized in the Table, are calculated from summation of the equation $d = aM^x$ for all fractions, where d = fractional distance sedimented and M = molecular weight[15]. The values of a and x (0.0145 and 0.316, respectively) were derived from sedimentation data from DNA samples of known molecular weight. When a similar experiment was carried out using X-irradiation (Figure 3), no such decrease in the average molecular weight was observed. Since this dose of X-ray in our system results in breakage of DNA down to at most half of the unirradiated weight average molecular weight, if the fraction of repaired regions photolysed were 0.33 to 0.5, as calculated by REGAN et al.[11] in the case of UV, this should have yielded a readily apparent change in the sedimentation pattern. We have also used the radiation-resistant mutant B/r and a larger but acceptable X-ray dose (30 kr) and again failed to detect appreciable photolysis in cells repaired in BUdR. The dose of 313-nm irradiation was varied in several experiments between 0.23×10^5 and 2.3×10^5 erg mm^{-2} without enhanced resolution.

A reasonable interpretation of these data is that DNA repair synthesis is a necessary step in the closure of strand breaks caused by X-ray in *E. coli*, but that it involves much shorter segments of DNA than in UV excisional repair. This explanation is also consistent with the failure by others, except at the highest doses, to detect repair synthesis by isopycnic centrifugation in CsCl gradients[16].

Change in average molecular weight caused by 313-nm irradiation (1.0×10^5 erg mm^{-2})

Condition	Pre. 313 nm M.W. ($\times 10^{-6}$)	Post 313 nm M.W.	Reduction of M.W. (%)
UV, thymidine	85.1	72.7	14.5
no UV, BUdR	73.2	62.6	14.4
UV, BUdR	93.6	49.2	47.4
X-ray, thymidine	97.2	83.2	14.4
no *X*-ray, BUdR	99.3	80.1	19.3
X-ray BUdR	104.9	84.0	19.9

Zusammenfassung. Nachweis, dass bei einer *E. coli*-Mutante mit fehlender DNS-Polymerase eine DNS-«Repair»-Synthese möglicherweise ein notwendiger Schritt vor dem Schliessen der von Röntgenstrahlen verursachten Chromosomenbrüche ist.

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[15] P. DOTY, B. MCGILL and S. RICE, Proc. natn. Acad. Sci., USA *44*, 432 (1958).

[16] This investigation was supported by USPHS research grant No. 5T01-GM00516-11, VA Hospital research grant and AEC contract No. AT (40-1) 3631.

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Nuclear DNA Amounts in Polychaete Annelids

Recent work on evolutionary trends in the specific amount of nuclear DNA has been limited mainly to vertebrate groups[1-3]. Much less is known about invertebrate genome sizes[4]. We report here the results of measurements made on 36 polychaete species. These worms constitute an invertebrate group of particular evolutionary interest. Morphologically some polychaetes are very close to the postulated ancestral body plan of the segmented coelomates. This basic pattern has been modified in various ways by adaptation to diverse marine habitats. There are free-swimming, crawling, burrowing, and temporarily or permanently sessile species, ranging from generalized to very specialized morphology.

Nuclear DNA amounts were determined by either of 2 methods. Data on some species are based on microdensitometric determinations of Feulgen dye content of individual somatic nuclei, others have been obtained by fluorometric quantitation of the DNA of a known number

[1] R. HINEGARDNER, Am. Nat. *102*, 517 (1968); Am. Nat., in press (1972).

[2] S. OHNO, *Evolution by Gene Duplication* (Springer, Berlin 1970).

[3] K. BACHMANN, O. B. GOIN and C. J. GOIN, Brookhaven Symp. *23*, in press (1972).

[4] A. H. SPARROW, H. J. PRICE, and A. G. UNDERBRINCK, Brookaven Symp. *23*. in press (1972).