DNA Turnover in Growing Yeast after X-Irradiation

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Growing yeast cells incorporate radioactivity from labeled nucleotides, deoxynucleotides, deoxyadenosine, and deoxycytidine into acid-insoluble material. Most of these compounds are incorporated predominantly into RNA, only dTMP and dTTP serve as effective and selective DNA precursors.

When yeast is prelabeled with dTMP, "specific" radioactivity of DNA decreases in the growing cells due to DNA augmentation. In X-irradiated yeast, "specific" radioactivity of DNA again decreases remarkably, though DNA augmentation of the growing giant cells in delayed. Simultaneously, radioactivity is rendered acid-soluble. *Vice versa*, X-irradiated unlabeled yeast incorporates radioactivity from labeled precursors into DNA, even if the DNA content of the cells does not increase.

When growing yeast cells are treated with cycloheximide, protein synthesis and DNA augmentation are inhibited completely with $5 \cdot 10^{-6}$ M cycloheximide. The decrease of "specific" radioactivity of DNA in X-irradiated cells, however, proves independent on an intact protein synthesis, even with $1 \cdot 10^{-4}$ M cycloheximide.

The data provide clear evidence on DNA turnover in growing yeast after X-irradiation.

Introduction

Repair of UV-induced pyrimidine dimers in DNA by a cut-and-patch mechanism is a well-studied fundamental event in cell recovery [1, 2]. Biochemical mechanisms of cell survival after X-irradiation, on the contrary, are less clear, especially when one considers the manifold molecular lesions following ionizing radiation, which comprise different modes of DNA strand breaks, DNA base alterations, etc. Various studies with bacteria and animal cell systems [3–16] demonstrate, that DNA repair doubtless plays an essential role in cell survival also after X-irradiation. But diverging data on repair mechanisms were presented, *e. g.* simple rejoining of single strand breaks as well as repair replication were observed with HeLa cells [17–20]. The biological function of repair replication after X-radiation recently was questioned again [2, 21].

Irradiation of baker's yeast *(Saccharomyces cerevisiae)* with 50 kr of X-rays destroys the proliferation ability without inhibition of cell growth [22]. Giant cells are formed, which show a pronounced lag only of DNA augmentation. This delayed DNA replication can be explained neither by a general radiation effect on DNA polymerase activity [23], nor by insufficient supply of the irradiated growing yeast cells with DNA precursors [24]. So we were stimulated to study the behaviour of radioactively labeled DNA in growing yeast after X-irradiation. An essential prerequisite for these investigations was the development of a sensitive and specific system for an analysis of radioactively labeled DNA from growing yeast, since this organism does not incorporate thymidine. Some of our results were reported in a preliminary form [25].

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Results and Discussion

Radioactive labeling of DNA in growing yeast

Labeled thymidine and thymine are commonly used precursors in studies on DNA synthesis *in vivo*. Incorporation of these compounds into DNA in growing yeast cells is nearly impossible, however, since both precursors scarcely enter the cells, and yeast apparently lacks thymidine kinase [26–28]. When growing yeast cells were incubated with [^{3}H]deoxyadenosine, [^{3}H]deoxycytidine, or with methyl[^{3}H]dTMP, however, the acid-insoluble cell fraction is labeled effectively (Fig. 1). Radioactivity from [2-^{14}C]dTMP likewise can be incorporated into acid-insoluble material and DNA from yeast [29].

Comparing the incorporation kinetics of these precursors (Fig. 1), deoxynucleosides seem more

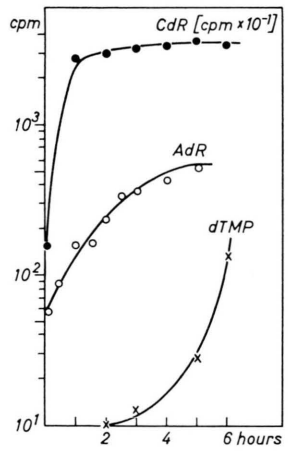

Fig. 1. Incorporation of various DNA precursors into acid-insoluble cell constituents by growing yeast. $2.5 \cdot 10^8$ cells from an asynchronous culture (cell clone derived from baker's yeast, *Saccharomyces cerevisiae*, "Germania-Hefe", Deutsche Hefewerke Hamburg) were grown at 25 °C in 100 ml growth medium (*cf.* 18) with 100 μCi each of [^{3}H]dTMP (methyl [^{3}H]dTMP, 11.4 Ci/mmole, Amersham-Buchler), or [^{3}H]CdR (deoxycytidine[5-^{3}H], 8.75 Ci/mmole, NEN). In another experiment, $3.38 \cdot 10^8$ cells/100 ml were grown with 2 μCi [^{3}H] AdR (deoxyadenosine[^{3}H], G, 19.2 Ci/mmole, NEN). The cells from 10 ml samples were spun down at +2 °C, washed with 10 ml of icecold water, and extracted with 3.0 ml 5% TCA (30 min 25 °C). The insoluble residue was washed twice with 5.0 ml icecold 5% TCA, and twice with 5.0 ml ethanol. Nucleic acids were hydrolyzed (*cf.* Table I b), and radioactivity was estimated from 0.5 ml of the clear supernatant in a Packard Liquid Scintillation Spectrometer. DNA was estimated from 0.5 ml supernatant according to Burton [30].

suitable than thymidine deoxynucleotides, which are incorporated to a remarkably less degree and with a lag of about one generation time. Analysis of the labeled acid-insoluble cell constituents by alkaline and acid hydrolysis, and by enzymic degradation, resp., clearly shows, however, that only a small part of radioactivity, which is incorporated from deoxynucleosides, belongs to DNA (Table I). Label incorporated from thymidine deoxynucleotides, on the other hand, proves alkali-resistant, but is degraded by acid hydrolysis, and enzymatically only by treatment with DN'ase, thus indicating an incorporation of these precursors solely into DNA.

The suitability of thymidine deoxynucleotides for labeling exclusively DNA also follows from the "specific" radioactivity of DNA as estimated after acid and enzymic degradation. As demonstrated in Table II, nearly the same values are obtained, when the acid-insoluble residues from methyl[^{3}H]dTMP- or [2-^{14}C]dTMP-labeled cells are treated with acid, or with DN'ase.

As can be seen from Table III, the acid-insoluble material is labeled best at $1 \cdot 10^{-6}$ M dTMP, when methyl[^{3}H]dTMP was taken for a precursor. Incorporation of exogenous dTMP is calculated to increase nearly linearily, however, with dTMP concentrations up to $1 \cdot 10^{-3}$ M. Neither cell growth, nor DNA augmentation appear disturbed, even with

Table I. Incorporation of various radioactive precursors into RNA and DNA by growing yeast cells. Yeast from an asynchronous culture was grown 14 hours at 25 °C with [^{3}H]AdR (deoxyadenosine[^{3}H], G, 19.2 Ci/mmole, NEN, 10 μCi/$56.8 \cdot 10^6$ cells in 50 ml), or with [^{3}H]CdR (deoxycytidine[5-^{3}H], 8.75 Ci/mmole, NEN, 10 μCi/$34.6 \cdot 10^6$ cells in 10 ml), or with [2-^{14}C]dTMP (48.8 mCi/mmole, NEN, 10 μCi/$190 \cdot 10^6$ cells in 100 ml), or with methyl[^{3}H]dTMP (Amersham-Buchler, 11.4 Ci/mmole, 10 μCi/$34.6 \cdot 10^6$ cells in 10 ml), or with methyl[^{3}H]dTTP (Amersham-Buchler, 23.9 Ci/mmole, 10 μCi/$14.6 \cdot 10^6$ cells in 10 ml). The cells were collected by centrifugation, and soluble cell constituents were removed as described in Fig. 1. The insoluble residues were treated as follows: a. nucleic acids were hydrolyzed with 2.5 ml 10% TCA (30 min, 100 °C), the insoluble material was centrifuged off, and radioactivity was estimated from the clear supernatants. b. RNA was separated by hydrolysis with 2.0 ml 1 N KOH (18 hours, 37 °C), DNA was reprecipitated with HCl and TCA (*cf.* 22), washed with 2.5 ml 5% TCA, and hydrolyzed with 2.5 ml 10% TCA. Radioactivity was estimated from the clear supernatant. c. The residue was resuspended in 2.0 ml 0.1 M tris-HCl-buffer + 0.01 M $MgCl_2$ at pH 7.6, RNA and DNA were degraded by incubation of 0.5 ml aliquots with 1 mg RN'ase A (pancreatic RN'ase, Boehringer Mannheim, 10 min heated at 85 °C), and with 0.1 mg DN'ase I (pancreatic DN'ase, 2000 units/mg, SERVA Heidelberg) resp., 60 min at 37 °C. Control assays were incubated without enzyme, they served as references. Insoluble material was precipitated with 1.0 ml 10% TCA, and radioactivity was estimated from the clear supernatants.

Experiment	Precursor	cpm/TCA-extract	cpm acid-insoluble	After KOH-digestion cpm/RNA-fraction	After KOH-digestion cpm/DNA-fraction	After RN'ase treatment cpm solubilized/suspension	After DN'ase treatment cpm solubilized/suspension
1	[^{3}H]AdR	10 950		37 000	1 360	27 560	4 640
2	[^{3}H]CdR	7 300		7 450	675	370	170
3	methyl[^{3}H]dTMP	9 900	10 890				
4	methyl[^{3}H]dTMP	3 540		15	350	0	114
5	methy[^{3}H]dTTP	1 200	1 875				
6	[2-^{14}C]dTMP	6 120		0	1 600	0	132

Table II. "Specific" radioactivity of DNA, as estimated by acid hydrolysis, and by enzymic digestion, resp. 10 ml of an asynchronous yeast culture ($25.6 \cdot 10^6$ cells) were grown 14.75 hours at 25 °C with 20 μCi methyl[^{3}H]dTMP (25.6 μCi/nmole) + 5 nmoles dTMP. $10.9 \cdot 10^6$ cells/10 ml were grown with 16 μCi [2-^{14}C]dTMP (48.8 μCi/μmole). The acid-insoluble cell material was washed with TCA, and with ethanol (*cf.* Fig. 1), and dried in a vacuum. The residue was resuspended with 2.0 ml 0.1 M tris-HCl-buffer + 0.01 M $MgCl_2$ at pH 7.6, 0.5 ml-aliquots were incubated with 50 μg DN'ase I (60 min, 37 °C). The reaction was stopped with 0.5 ml 20% TCA, solubilized radioactivity and DNA were estimated in the clear supernatant. Control assays were incubated without enzyme, they served as references. In a parallel series, DNA and radioactivity were estimated from the acid-insoluble material as described in Table I a. DNA was estimated according to Burton [30].

DNA digestion by	DNA rendered soluble/assay [μg]	methyl-[^{3}H]dTMP cpm/μg DNA	[2-^{14}C]dTMP cpm/μg DNA
DN'ase I	19.8	143.4	181.5
acid hydrolys.	22.7	153.8	190.7

$1 \cdot 10^{-3}$ M dTMP. With every concentration examined, only about one thousandth of the supplied DNA precursor is incorporated, *e. g.* 13.5 nmoles with $1 \cdot 10^{-3}$ M dTMP. This is, on the basis of 110 μg DNA/10^9 cells corresponding to 330 nmoles deoxynucleotides, about $1/6 - 1/7$ of the DNA synthetized by the cells during the incubation time.

As demonstrated in Table III, some radioactivity remains in the acid-soluble cell fraction even after long labeling times. This label is removed completely, when the labeled cells are grown for three doubling times in non-radioactive growth medium. During this incubation a great part of the label apparently is incorporated into DNA. By this means advantageous conditions are obtained for an investigation of DNA during cell growth.

Radioactively labeled DNA in growing yeast after X-irradiation

Resulting from constant amounts of total radioactivity and increasing amounts of DNA, "specific" radioactivity of DNA from prelabeled asynchronously growing yeast decreases steadily, corresponding to the augmentation of DNA (Fig. 2). At to be expected, no radioactivity is rendered soluble, as judged from control investigations including acid-soluble cell fraction and growth medium.

Irradiation of prelabeled yeast with 70 kr of X-rays induces a pronounced delay of DNA augmentation. "Specific" radioactivity of DNA, however, decreases again, even when the DNA content in the irradiated cells remains constant (Fig. 2). Simultaneously, radioactivity appears in the acid-soluble cell fraction, and is in part eliminated from the cells into the growth medium. Analysis of the acid-soluble cell constituents by thin-layer chromatography exhibits labeled thymidine, and increasing amounts of labeled dTMP (Fig. 2).

As evident from Fig. 2, the DNA label decreases in the X-irradiated cells by about 40% (65 cpm/1 μg DNA) during the first 90 min lag of DNA augmentation. This includes a remarkable loss of total activity from DNA. At the same time the total label in the unirradiated control cells remains constant, only "specific" radioactivity of DNA decreases, by about 50% due to DNA augmentation. When the amount of radioactivity, which is lost from DNA after X-irradiation of the cells, is compared with the amount of label found soluble, only about 20% of

Table III. Asynchronously growing yeast: Dependence of methyl[^{3}H]dTMP incorporation on dTMP concentration, and removal of label from the DNA precursor pool by cell growth in unlabeled medium. 10 ml of an asynchronous culture ($20 \cdot 10^6$ cells) were grown 13.5 hours at 25 °C with 10 μCi methyl[^{3}H]dTMP (11.4 mCi/μmole). The labeled cells were spun down, washed with icecold water, and divided into two parts: b. was reincubated 22 hours at 25 °C with fresh growth medium ($5 \cdot 10^6$ cells/ml), the cells were then analyzed for incorporated radioactivity. a. was extracted immediately with TCA, the insoluble nucleic acids were hydrolyzed, and radioactivity was estimated from the clear supernatants. For further details see Table II.

μM dTMP	cpm acid-soluble after		cpm acid-insoluble after		nmoles dTMP incorporated
	a. 13.5 hours	b. 13.5+22 hours	a. 13.5 hours	b. 13.5+22 hours	
0.0877	4 920	48	5 440	9 400	$1.55 \cdot 10^{-3}$
1.088	5 640	24	8 310	10 500	$17.3 \cdot 10^{-3}$
10.088	4 440	54	4 600	7 500	$123 \cdot 10^{-3}$
100.09	5 580	18	3 550	6 000	1.08
1 000	6 840	24	3 315	8 200	13.5

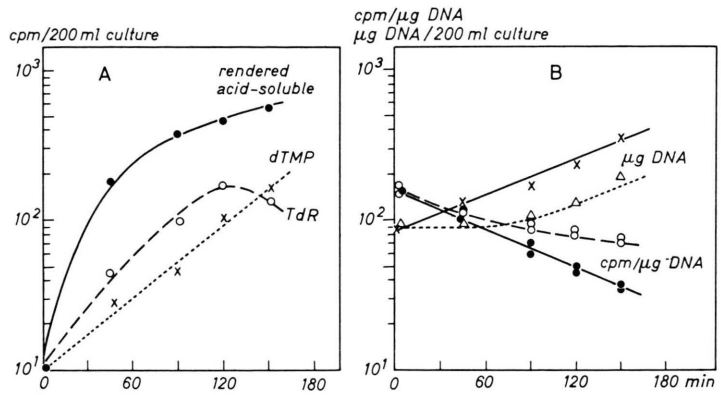

Fig. 2. [^{3}H]dTMP-labeled DNA in asynchronously growing yeast after X-irradiation. 10 ml of an asynchronous culture ($3.54 \cdot 10^6$ cells/ml) were grown 15 hours (5 generation times) at 25 °C with 50 μCi methyl[^{3}H]dTMP (25.6 μCi/nmole, Amersham-Buchler) + 3.8 nmoles dTMP. The cells were spun down, washed, and grown in 400 ml unlabeled growth medium (8 hours, 31 °C). The yeast was then collected by centrifugation, washed twice with icecold water, resuspended in water ($1.4 \cdot 10^8$ cells/ml), and X-irradiated. Irradiation with 70 kr of X-rays, and post-irradiation incubation were performed as described earlier [24]. The cells were analyzed as described in Table II. Additionally, acid-soluble deoxynucleosides and deoxynucleotides were examined as follows: TCA was removed from the extracts with ether, and the aqueous phase was evaporated to dryness in a rotary vacuum evaporator at 30—35 °C. The residue was dissolved with 0.5 ml H_2O, and analyzed by thin-layer chromatography on PEI cellulose as described in l. c. [24]. Nucleosides were separated by ascending chromatography with water, deoxynucleoside monophosphates were separated two-dimensionally with 1 M LiCl, and with 0.215 M H_3BO_3 in 0.67 M LiCl. For further details see [24].

the released label proves soluble material. The rest probably is reincorporated into acid-insoluble cell constituents, by re-utilization of the labeled methyl group. It remains open, to which degree again DNA is included into this process.

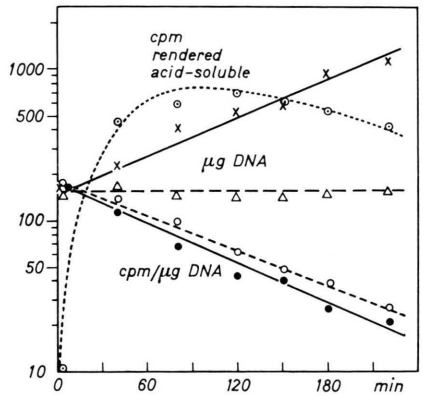

Fig. 3. [^{14}C]dTMP labeled DNA in asynchronously growing yeast after X-irradiation. Asynchronously growing yeast cells (10 ml culture, $0.77 \cdot 10^6$ cells/ml) were labeled with [2-^{14}C] dTMP (16 μCi, 0.327 μmoles, NEN), irradiated with 79 kr of X-rays, and incubated, as described in Fig. 2. DNA was analyzed from the growing cells as described in Table II, the following variation excepted: The acid-insoluble material was resuspended with 3.0 ml buffer, 1.0 ml were added immediately to 1.0 ml 10% TCA, and the resulting residue taken for DNA estimation. From the remaining 2.0 ml suspension, DNA was degraded enzymatically, as described in Table II. Closed symbols: Control. Open symbols and broken lines: X-irradiated. Dotted line: cpm/200 ml culture rendered acid-soluble.

When similar experiments were performed with yeast prelabeled with [2-^{14}C]dTMP, radioactive label again is rendered acid-soluble after X-irradiation (Fig. 3). No low-molecular label can be detected in extracts from the unirradiated control. "Specific" radioactivity of DNA from irradiated cells decreases again during the DNA lag, thus indicating a corresponding behaviour of methyl groups and ring moieties of thymine bases. During the first 90 min of incubation, the DNA label is diminished by about 50% (100 cpm/μg DNA), which value is similar to that obtained with methyl [^{3}H]dTMP-labeled cells.

The decrease of "specific" radioactivity of DNA during the radiation-induced lag of DNA augmentation is not impaired, when the protein synthesis completely is inhibited in the X-irradiated cells by $1 \cdot 10^{-4}$ M cycloheximide (Fig. 4). DNA augmentation, on the other hand, is not at all resumed under these conditions, the values remain constant also after long incubation times. It should be noted, that DNA augmentation in unirradiated yeast [31] is inhibited completely even with $5 \cdot 10^{-6}$ M cycloheximide. These results point to a distinct independency on an intact protein synthesis of those processes, which result in a decreasing DNA label after X-irradiation.

Taken together, the above experiments exhibit clear evidence for removal of parts of the parent

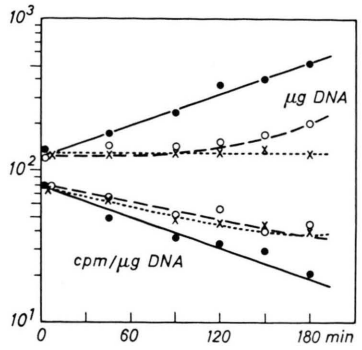

Fig. 4. Effects of cycloheximide on DNA augmentation and on radiation-induced decrease of "specific" radioactivity of DNA. Asynchronously growing yeast was prelabeled with [^{3}H]dTTP (100 µCi/13.84·10^6 cells in 10 ml, methyl[^{3}H] dTTP, 28 Ci/mmole, Amersham-Buchler), irradiated with 70 kr of X-rays, and grown at 31 °C with $1 \cdot 10^{-4}$ M cycloheximide (Boehringer Mannheim). Cells from 50 ml samples were analyzed for DNA content and radioactivity, as described in Table II. Closed symbols: Unirradiated control. Open symbols and broken lines: X-irradiated. Dotted lines: X-irradiated, + $1 \cdot 10^{-4}$ M cycloheximide.

DNA after X-irradiation of yeast. This process must be strongly combined with repair replication, because the DNA content of the irradiated yeast does not decline. We therefore started another series of experiments, on incorporation of labeled precursors into DNA after X-irradiation. For technical reasons these investigations were performed with [^{14}C]ATP,

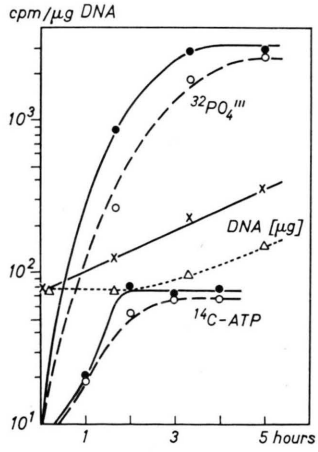

Fig. 5. Incorporation of radioactivity into DNA by asynchronously growing yeast after X-irradiation. Yeast from an asynchronous culture was irradiated with 70 kr of X-rays (*cf.* Fig. 2, and l. c. [24]), and grown at 31 °C: a. with 250 µCi $^{32}PO_4'''$ (disodium-hydrogen-orthophosphate[^{32}P], Buchler, Braunschweig) in 500 ml culture (containing 5 mmoles PO_4''', 5·10^6 cells/ml). b. with 50 µCi ^{14}C-ATP (u., 0.05 mCi/0.031 mg, NEN) + 250 µmoles ATP in 500 ml culture. Cells from 100 ml samples were analyzed as described in Table II. Closed symbols and -x-: Unirradiated control. Open symbols, broken and dotted lines: X-irradiated.

and with $^{32}PO_4'''$, resp. As shown in Fig. 5, X-irradiated growing yeast incorporates radioactivity from both precursors into acid-insoluble material, parts of the label can be rendered soluble by digestion with DN'ase. Such DN'ase-sensitive material is labeled, even when DNA augmentation is delayed. At the end of the lag phase (100 min of cell growth), the "specific" radioactivity of DNA amounts to about 30% of the control with ^{32}P, and 50% with [^{14}C]ATP.

A slight incorporation of DNA precursors into DNA already was observed previously with yeast and other cell systems, if colony forming ability was destroyed by X-irradiation. This was explained by an incomplete synthesis of short DNA fragments retained in the irradiated cells [29, 32]. The facts, however, that synthesis of many essential cell constituents, such as RNA, protein, glycolytic enzymes, and coenzymes continue to a normal degree in X-irradiated yeast [22, 33], exclude an essential role of such a mode of DNA replication. Taken together, our results constrain another conclusion. The above data can be interpreted only by exchange of parts of DNA, by means of a "cut-and-patch" mechanism. This "repair replication" [1, 32, 34–37] seems to affect a great part of the genetic material. Since cycloheximide has no effect on it one must assume, that the enzyme system belonging to is present in the irradiated yeast just at the beginning of cell incubation. An essential effect of the drug on de novo synthesis of DNA precursors seems unlikely – though it cannot be excluded exactly –, since previous studies showed an increase of purine deoxyribose derivatives in X-irradiated yeast even under cycloheximide [38]. The lacking recovery of DNA augmentation under cycloheximide thus probably is based on a distinct sensitivity against cycloheximide of the enpyme system responsible for semiconservative DNA replication.

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[1] P. Howard-Flanders, Annu. Rev. Biochem. **37**, 175 [1968].
[2] M. Fox and B. W. Fox, DNA repair mechanisms. Symp. Schloß Reinhartshausen, 1971, p. 17, F. K. Schattauer, Stuttgart 1972.
[3] H. Harrington, Biochim. biophysica Acta [Amsterdam] **41**, 461 [1961].
[4] H. S. Kaplan, Proc. nat. Acad. Sci. USA **55**, 1442 [1966].
[5] R. A. McGrath and R. W. Williams, Nature [London] **212**, 534 [1966].
[6] W. K. Sinclair, Proc. nat. Acad. Sci. USA **58**, 115 [1967].
[7] S. Nakai and S. Matsumoto, Mutation Res. **4**, 129 [1967].
[8] R. G. Evans and A. Norman, Nature [London] **217**, 455 [1968].
[9] J. F. Scaife and H. Brohée, Canad. J. Biochem. **47**, 237 [1969].
[10] S. Nakai and E. Yamaguchi, Jap. J. Genetics **44**, 355 [1969].
[11] P. Alexander, C. J. Dean, A. R. Lehman, M. G. Ormerod, P. Feldschreiber, and R. W. Serianni, Radiat. Protect. and Sensitization, ed. H. L. Moroson and M. Quintiliani, p. 15 —34, Barnes & Noble, New York 1970.
[12] S. Kondo, H. Ichikawa, K. Iwo, and T. Kato, Genetics **66**, 187 [1970].
[13] Ch. D. Town, K. C. Smith, and H. S. Kaplan, Science [Washington] **172**, 851 [1971].
[14] E. Cabela, E. Wawra, and H. Altmann, Biophysik **8**, 88 [1972].
[15] H. Matsudaira and I. Furuno, Biochim. biophysica Acta [Amsterdam] **272**, 202 [1972].
[16] Ch. D. Town, K. C. Smith, and H. S. Kaplan, Radiat. Res. **52**, 99 [1972].
[17] R. B. Painter and J. E. Cleaver, Nature [London] **216**, 369 [1967].
[18] P. H. M. Lohman, Mutat. Res. **6**, 449 [1968].
[19] W. J. Kleijer, P. H. M. Lohman, M. P. Mulder, and D. Bootsman, Mutat. Res. **9**, 517 [1970].
[20] T. P. Brent and G. A. Wheatley, Int. J. Radiat. Biol. **19**, 339 [1971].
[21] R. B. Painter, DNA repair mechanisms. Symp. Schloß Reinhartshausen, 1971, F. K. Schattauer, Stuttgart 1972.
[22] H. Hilz and H. Eckstein, Biochem. Z. **340**, 351 [1964].
[23] H. Eckstein, V. Paduch, and H. Hilz, Eur. J. Biochem. **3**, 224 [1967].
[24] H. Eckstein, S. Ahnefeld, and K. Albietz, in preparation.
[25] H. Eckstein, U. Koll, and S. Ahnefeld, Abstr. Commun. Meet. Fed. Europ. Biochem. Soc. **8**, 1017 [1972].
[26] E.-R. Lochmann, Naturwissenschaften **52**, 498 [1965].
[27] A. R. Grivell and J. F. Jackson, J. Gen. Microbiol. **54**, 304 [1968].
[28] L. G. Williams and H. K. Mitchell, J. Bacteriol. **100**, 383 [1969].
[29] S. Jannsen, E.-R. Lochmann, and W. Laskowski, Z. Naturforsch. **23 b**, 1500 [1968].
[30] K. Burton, Biochem. J. **62**, 315 [1956].
[31] H. Eckstein, unpublished results.
[32] R. B. Painter and R. E. Rasmussen, Nature [London] **201**, 162 [1964].
[33] H. Eckstein, V. Paduch, and H. Hilz, Biochem. Z. **344**, 435 [1966].
[34] D. E. Pettijohn and P. C. Hanawalt, J. molecular Biol. **9**, 395 [1964].
[35] R. E. Rasmussen and R. B. Painter, J. Cell Biol. **29**, 11 [1966].
[36] D. Billen, R. R. Hewitt, T. Lapthisophon, and P. M. Achey, J. Bacteriol. **94**, 1538 [1967].
[37] R. E. Rasmussen, B. L. Reisner, and R. B. Painter, Int. J. Radiat. Biol. **17**, 285 [1970].
[38] H. Eckstein, Z. Naturforsch. **26 b**, 1266 [1971].
[39] O. Th. Schönherr and F. Wanka, Biochim. biophysica Acta [Amsterdam] **232**, 83 [1971].
[40] J. R. Gautschi, B. R. Young, and J. E. Cleaver, Exp. Cell Res. **76**, 87 [1973].

Note added in proof: The interpretation given above agrees well with findings by Wanka and coworkers [39], who showed a marked sensitivity of DNA polymerase activity in synchronized growing *Chlorella* against 3×10^{-5} M cycloheximide. During preparation of this paper, Cleaver and coworkers [40] reported on experiments with X-irradiated HeLa S-3 cells, showing similarly an independency on concomitant protein synthesis of DNA repair replication, by use of 0.76 M cycloheximide.