DNA Synthesis by Mammalian Cells Inhibited in Culture by 5-Iodo-2′-deoxyuridine

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SUMMARY

In the presence of 5-iodo-2′-deoxyuridine (IUdR; $1–2 \times 10^{-4}$ M) P815Y murine mast tumor cells in culture complete only one doubling of their cell number and one doubling of their DNA. Even though DNA does not increase beyond this one doubling, DNA synthesis continues in IUdR-inhibited cultures. ^{3}H-Thymidine is incorporated into DNA, and the pattern of the incorporation as seen with CsCl density gradient centrifugation agrees with the pattern that would be expected from replication of hybrid DNA containing IUdR in one strand (IU-THY DNA) in medium without IUdR. A new band appears at the density of native DNA, and both the new band and the IU-THY band are radioactive. Because in all experiments the specific radioactivity of the IU-THY band was about one-half that of the native DNA band, it is suggested that only one-half of the IU-THY DNA replicates.

The failure of the amount of DNA in the culture to increase despite the continued synthesis of DNA suggested that breakdown of DNA must occur in IUdR-inhibited cultures. IU-THY DNA was labeled with radioactivity in either the IUdR-containing strand or in the thymidine-containing strand. Both strands were found to break down at about the same rate, and the rate of breakdown appeared to be sufficient to account for the failure of DNA to accumulate in the inhibited culture. This experiment also indicated that the breakdown of DNA was not a consequence of DNA "repair," since the distinguishing feature of "repair" is selective removal of abnormal DNA.

INTRODUCTION

5-Iodo-2′-deoxyuridine (IUdR)[1] and 5-bromo-2′-deoxyuridine (BUdR) have been studied extensively in bacterial and mammalian cell systems. These thymidine analogs have been shown to be potentially lethal to both bacterial and mammalian cells and to be incorporated into the DNA of these cells in place of thymidine (1–12). Under certain conditions, they also have been shown to cause biochemical and morphological changes that closely resemble the "unbalanced" type of growth that is seen in "thymineless death" (1–5).

In bacteria, in the presence of the analogs, DNA synthesis continues for several generations; however, the bacterial cells become filamentous and the viable count drops rapidly (1). In mammalian cells, with *low concentrations* of the drugs, cell division and DNA synthesis continue appar-

[1] Abbreviations: IUdR is 5-iodo-2′-deoxyuridine. BUdR is 5-bromo-2′-deoxyuridine. IU-THY DNA signifies hybrid DNA in which part or all of the thymine in one strand has been replaced with iodouracil. THY-THY DNA is normal thymine-containing native DNA. An asterisk is used to indicate radioactive isotope in a particular strand. For example, IU*-THY DNA is radioactive in the iodouracil-containing strand.

ently unimpaired, even though a significant fraction of the DNA-thymine may be replaced by the analogs (11). In the presence of sufficiently *high concentrations* of IUdR or BUdR, or in the presence of lower concentrations, when *de novo* synthesis of thymidylate is prevented with FUdR or methotrexate, mammalian cells undergo a single doubling of the cell number and of the DNA (6–10). There is no apparent increase in DNA after this doubling, even if the cells are transferred to fresh medium without IUdR. Protein synthesis, and presumably RNA synthesis, is not inhibited under these conditions (6).

The apparent failure of DNA synthesis in mammalian cells in the presence of BUdR or IUdR suggests that the replacement of thymidine in the DNA with the analog may prevent the DNA from functioning properly as a template for DNA polymerase. Only one direct investigation of this possibility in a mammalian system has been reported (13). A comparison of normal and IUdR-substituted DNA showed that both were good primers for crude DNA polymerase from rat liver; however, no attempt was made to demonstrate rigorously that the IUdR-containing DNA was not contaminated with normal DNA. In bacteria, the presence of IUdR or BUdR in DNA seems to have no appreciable effect upon the fundamental ability of the DNA to act as primer for *E. coli* polymerase, although errors in replication occur (14). The ability of this DNA to function as transforming principle is unimpaired (5).

The present study was designed to evaluate in detail the effect of IUdR upon DNA synthesis in mammalian cells. The data presented in this paper demonstrate that in P815Y mouse tumor cells inhibited in culture with IUdR, the observed inhibition of DNA synthesis is apparent rather than real. Although there is no accumulation of DNA after one generation of growth with IUdR, DNA synthesis can be shown to continue, but at only about one-half the normal rate. A concomitant loss of DNA sufficient in magnitude to account for the absence of any net DNA synthesis has also been demonstrated.

METHODS

The method of Dische (15) was used for the analysis of DNA. The culture medium (16), cell line (murine neoplastic mast cells, P815Y) (17), and general techniques (18) (Figs. 1–3, Tables 1 and 2) have been described previously, with the exception that the Coulter cell counter was used for determinations of the number of cells. Cultures (87,500 cells/ml) in logarithmic growth were either treated or not treated with IUdR (1×10^{-4} M); and the increase in cell number and the DNA content were determined at intervals (Fig. 1). After it was determined that the cell number of the drug-treated cultures had doubled and stopped increasing, the cells in appropriate aliquots of the cultures were sedimented by centrifugation, freed of medium, and quickly resuspended in fresh, warm medium containing ^{3}H-thymidine (Schwarz, 25 μC per μmole, 1×10^{-5} M final medium concentration). An aliquot of the drug-treated cells was retained in the drug-containing medium without further treatment for the determination of cell number. The cell number, the DNA content, and the incorporation of radioactive thymidine by the control cells and by the drug-pretreated cells were followed at intervals as before. The radioactive thymidine incorporated into the acid-insoluble cell fraction was determined as follows: duplicate 50-ml aliquots of cells were chilled in ice and harvested by centrifugation. Calf thymus nucleoprotein (14 mg dry weight) was added and the pellet was washed 4 times with 1 M perchloric acid and hydrolyzed at 90° for 30 min with 0.5 ml of this acid. The sample was centrifuged and the radioactivity in the supernatant was counted in 20 ml of POPOP:PPO:toluene:ethanol liquid scintillant in the Model 3002 Packard Tri-Carb Scintillation Counter.

DNA was prepared from 50-ml samples (200,000 cells/ml) of the cultures by a modification of the method of Kirby (19) (Fig. 2). The pellet (30–50 mg) was suspended in 5 ml of cold 6% *p*-aminosalicylic acid and rapidly transferred to a beaker containing 5 ml cold 80% phenol. The mixture was stirred for 1 hour at room tem-

perature and then centrifuged at 12,800 *g* in the Lourdes refrigerated centrifuge. The aqueous upper layer containing the DNA was deproteinized with 5 ml of chloroform:isoamyl alcohol (24:1) until no protein was apparent at the interface. The collected chloroform–isoamyl alcohol and phenol interfaces were reextracted once with 5 ml of dilute saline citrate (0.015 M sodium chloride, 0.0015 M trisodium citrate, pH 7.0), and this material was combined with the deproteinized aqueous phase. The combined aqueous material was dialyzed against 1 liter of dilute saline citrate for 1 hr and then incubated with 5 μl/ml of a 5.5 mg/ml solution of RNase (Worthington; preheated to 80° for 20 minutes to destroy traces of DNase) for 30 minutes at 37°. The material was then deproteinized once more with 5 ml of chloroform–isoamyl alcohol and dialyzed against dilute saline citrate overnight. If necessary the volume was reduced by putting the dialysis bag in finely powdered polyethylene glycol in the cold for 1–2 hr before the overnight dialysis.

For density gradient centrifugations, 4.305 g of optical grade CsCl (Harshaw) was dissolved in a plastic Spinco tube containing 3.5 ml of DNA solution with N. F. paraffin oil layered on top. The tubes were centrifuged at 20° for 60 hr at 35,000 rpm in the SW 39 rotor of the Spinco Model L preparative centrifuge. The centrifuge was decelerated without braking, and approximately sixty fractions of 10 drops each were collected from each tube. Densities of the samples were determined directly by weighing appropriate 50-μl samples in a precalibrated micropipette. Dilute saline citrate (0.5 ml) was added to each sample, and the optical densities of the samples were determined at 260 mμ, 280 mμ, and 310 mμ. For determination of radioactivity in samples containing ^{3}H-thymidine, 50-μl samples were hydrolyzed with 50 μl of 1 M PCA at 90° for 30 min and transferred quantitatively to plastic counting vials containing 10 ml of POPOP–PPO–toluene–ethanol liquid scintillant. Quenching did not occur under these conditions. Radioactivity in samples containing ^{32}P was determined by drying 50-μl samples with 1-ml portions of water on planchets and counting in a gas-flow Geiger-Müller counter.

For preparation of samples for recentrifugation in alkaline CsCl, the fractions to be recentrifuged were pooled and dialyzed against 100 volumes of dilute saline citrate to remove CsCl, followed by dialysis for 2–4 hr against 20 volumes of 0.1 M sodium bicarbonate adjusted to pH 11.6 with sodium hydroxide. The volume of the sample was adjusted to 3.5 ml with this buffer and 4.305 g of CsCl was added. The sample was centrifuged and the fractions were collected and analyzed as above.

The following methods were used to study the loss of DNA from cells after inhibition with IUdR (Fig. 3). Tritiated thymidine (Schwarz, 1.5 μC/μmole, 1×10^{-5} M final medium concentration) was added to two-thirds of a culture (13,000 cells/ml) in logarithmic growth; to the remaining one-third, nonradioactive thymidine only was added. After three cycles of division, the cells were sedimented by centrifugation, washed free of residual medium, and resuspended rapidly in the same respective volumes of fresh, warm medium. IUdR (1×10^{-4} M, final medium concentration) was added to one-half of the culture treated with radioactive thymidine, as well as to the nonradioactive thymidine-treated culture not pregrown with radioactive thymidine; in the latter case, ^{3}H-IUdR (0.05 μC/μmole, 2×10^{-4} M final concentration) was also added. After it was determined that the cell number of the IUdR-treated cultures had doubled and stopped increasing, the cells of all three cultures were again sedimented by centrifugation and quickly resuspended in fresh, warm medium containing thymidine (1×10^{-5} M final concentration). Aliquots (100 ml) were taken at the indicated times for determination of the mitotic index (Table 2) and of the cellular acid-insoluble radioactivity. For the determination of mitotic figures, 5-ml aliquots of cells were centrifuged, and the cell pellets were resuspended in 3 drops of aceto-orcein (2% orcein in 65% acetic acid). One drop of suspended cells was placed on a slide, covered with a coverslip, and squashed. One

thousand cells were counted per time point. Cells with tightly coiled chromosomes were scored as mitoses.

RESULTS

Mast cells (P815Y) were grown in culture in the presence of sufficient IUdR ($1–2 \times 10^{-4}$ M) to limit their growth to one cycle of division and to introduce IUdR into the newly synthesized strand of the DNA helix. The effect of this treatment upon cell division and DNA synthesis is shown in Fig. 1A and B. During the first

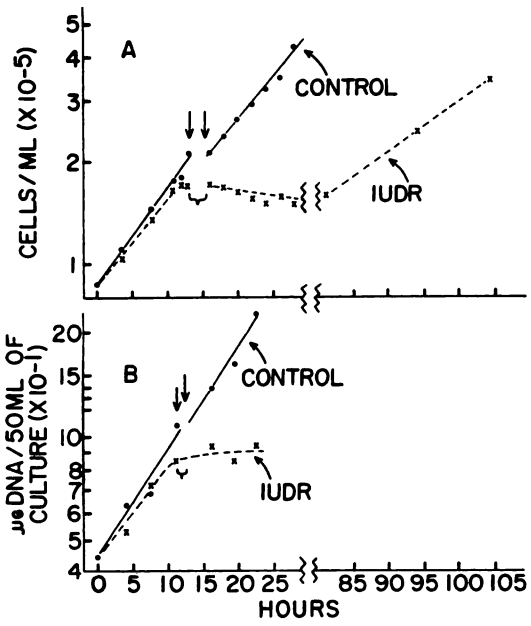

FIG. 1. *Effect of IUdR upon (A) the rate of increase in the number of P815Y cells in culture, (B) the rate of net increase in DNA as measured by the diphenylamine reaction*

Arrows indicate the time at which cells were removed from IUdR-containing medium and were resuspended in fresh medium containing thymidine (10^{-5} M). Cells kept continuously in IUdR-containing medium failed to show any outgrowth, even after 65 hours.

10–12 hr of growth in the presence of this concentration of IUdR, the cell number and the DNA content of the culture continued to increase at the normal rate; however, after this, no further increase in either of these parameters occurred in the IUdR-treated cells. Resuspension of the cells in drug-free medium failed to result in any further increase in the cell number or in the DNA content of the culture for 65 hr, a period of time equivalent to 5 or 6 normal division times; however, after 65 hr the cell number did begin to rise at a rate nearly equal to the normal rate of increase. Cultures kept continuously in the presence of IUdR failed to grow out after 65 hr; therefore, the outgrowth apparently was not caused by the appearance of resistant cells. There was no further increase in the DNA content of the culture after one generation in the presence of IUdR (Fig. 1B); nevertheless, ^{3}H-thymidine was incorporated into the acid-insoluble fraction of the cells, presumably DNA, at a rate between one-half and three-fourths the rate of incorporation into the uninhibited control cells (Table 1).

After one cell doubling in the presence of IUdR, more than 90% of the DNA banded in a CsCl density equilibrium gradient at a density[2] (1.79 g/cm^3) consistent with the formation of IU-THY DNA (Fig. 2B). DNA, extracted from the cells 12 hr after IUdR was removed and ^{3}H-thymidine added, formed two bands in the gradient, one at the density of IU-THY DNA, the other at the density of native DNA[2] (1.71 g/cm^3) (Fig. 2C). Radioactivity was present in both bands, but the specific radioactivity of the IU-THY DNA was only 49–65% that of the THY-THY DNA (Table 2). The introduction of ^{3}H-thymidine during the last 6 hr only, of the 12-hr period after removal of IUdR, resulted in the incorporation of only one-half as much radioactivity into the DNA as in the previous experiment, but the distribution of radioactivity was the same (Table 2). Thus, DNA synthesis continued at a constant rate throughout the 12-hr period. In a separate experiment, ^{32}P-phosphate, instead of ^{3}H-thymidine, was added to the IUdR-pretreated cells and to the controls with the same results (Table 2). The DNA of the hybrid band from one of the experiments with ^{3}H-thymidine was

[2] Average of five determinations.

TABLE 1

Decreased rate of ^{3}H-thymidine incorporation into DNA by P815Y cells inhibited by IUdR

Time after IUdR removed (hr)	IUdR pretreatment for one generation	Acid-insoluble radioactivity (cpm/cell × 10^7)[a]		
		Expt. 1	Expt. 2	Expt. 3
0.75–1.00	+	—	175 (0.76)	396 (0.80)
	−	—	229	492
1.75–2.00	+	—	330 (0.48)	720 (0.73)
	−	—	695	960
2.75–3.25	+	11080 (0.47)	773 (0.61)	1266 (0.81)
	−	23440	1265	1560
4.00	+	—	886 (0.61)	1707 (0.82)
	−	—	1455	2083
5.00	+	—	—	1830 (0.64)
	−	—	—	2861
6.00–6.50	+	22040 (0.57)	2010 (0.53)	2380 (0.73)
	−	38530	3777	3277
9.50	+	28300 (0.38)	2940	—
	−	74350	—	—

[a] The concentration of ^{3}H-thymidine in the medium in Expt. 1 was 1×10^{-5} M (25 μC/μmole); in Expts. 2 and 3 it was 1×10^{-4} M (2.5 μC/μmole). The numbers in parentheses are the ratios of IUdR-treated:control expressed as cpm/cell.

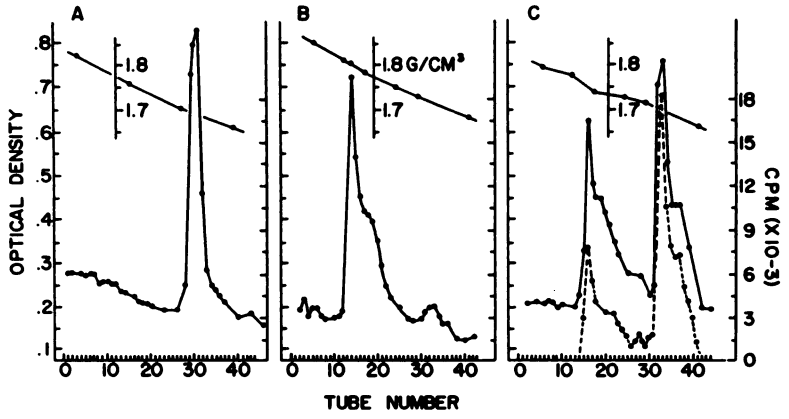

FIG. 2. *CsCl density equilibrium centrifugation*

Centrifugation of (A) DNA extracted from control P815Y cells, (B) DNA extracted from P815Y cells after one replication in IUdR-containing medium, and (C) DNA extracted after one replication in IUdR followed by incubation for 12 hours in a medium containing ^{3}H-thymidine, but no IUdR. ●—●, Optical density at 260 mμ; ○—○, radioactivity. Densities (in g/cm^3) are indicated in the upper portion of each figure.

recentrifuged in CsCl at pH 11.6. Two bands were formed, one at about 1.85 g/cm^3, which was devoid of radioactivity, and the other at 1.72 g/cm^3, which was radioactive. Therefore, all the radioactivity in the IU-THY DNA was apparently located in the newly synthesized light strand. The DNA was then hydrolyzed with 60% perchloric acid and the radioactivity was demonstrated to be in DNA-thymine by cochromatography on paper with authentic thymine.

TABLE 2

Comparison of the specific radioactivities of IU-THY DNA and THY-THY DNA extracted from P815Y cells after one replication in IUdR followed by incubation in ^{3}H-thymidine or in ^{32}P-phosphate for 12 hr[a]

Isotope	Period of uptake (hr)	DNA fraction	DNA per peak[b] (μg) Expt. 1	DNA per peak[b] (μg) Expt. 2	Specific activity of DNA[c] (cpm/μg) Expt. 1	Specific activity of DNA[c] (cpm/μg) Expt. 2	Ratio of DNA specific activity IU-THY:THY-THY Expt. 1	Ratio of DNA specific activity IU-THY:THY-THY Expt. 2
^{3}H-Thymidine	0–12	IU-THY	54	27	427	267	0.65	0.58
		THY-THY	57	19	651	461		
	6–12	IU-THY	48	42	233	85	0.62	0.49
		THY-THY	44	28	378	176		
			Expt. 3	Expt. 4	Expt. 3	Expt. 4	Expt. 3	Expt. 4
^{32}P-Phosphate	0–12	IU-THY	—	70	20	66	0.55	0.49
		THY-THY	—	40	36	135		

[a] The experimental techniques are described under Methods.

[b] Calculated from the optical density of the DNA at 260 mμ, assuming that 0.02 optical density units per milliliter represents 1 μg of DNA per milliliter.

[c] Based on simultaneous determinations of radioactivity and of diphenylamine color on pooled material from the CsCl bands.

The fact that there was no increase in the amount of DNA in the IUdR-treated culture despite the continued synthesis of DNA suggested that a breakdown of DNA, equivalent to the amount of DNA synthesized, occurred. The following general technique was used to look for the disappearance of IU-THY DNA from the culture. Cells were incubated with ^{3}H-IUdR for one generation to label the heavy strand of the IU-THY DNA. To label the opposing light strand of the hybrid helix, the cells were incubated with ^{3}H-thymidine for three generations preceding the addition of nonradioactive IUdR. In both experiments, after one generation in the presence of IUdR, the cells were transferred to fresh isotope-free medium, and the amount of radioactivity in acid-insoluble cellular material was determined at intervals throughout the next 18 hr. Radioactivity in the acid-insoluble fraction disappeared throughout the 18-hr period at a rate of about 35% per 12 hr, a time period equivalent to one generation (Fig. 3). The acid-insoluble ^{3}H-thymidine was lost from the cells at about the same rate as the ^{3}H-IUdR, even

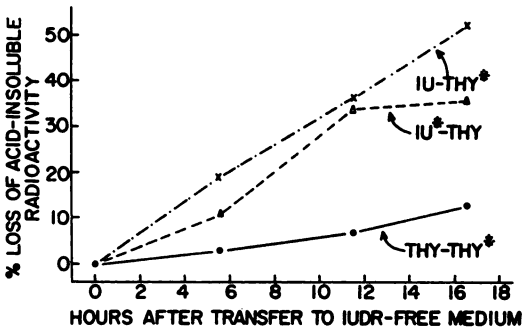

FIG. 3. *Loss of acid-insoluble ^{3}H-thymidine or ^{3}IUdR from P815Y cells treated with IUdR*

●—●, Controls labeled with ^{3}H-thymidine in one strand (THY-THY*). △—△, IUdR-treated, labeled with ^{3}H-IUdR in the heavy strand (IU*-THY). x–x, IUdR-treated, previously labeled with ^{3}H-thymidine in the light strand (IU-THY*). The strand containing radioactivity is indicated with an asterisk.

though the ^{3}H-thymidine was in the opposite strand; thus, it appears that both strands of the DNA are degraded at the same rate.

Mitotic indices were obtained during the course of one experiment to obtain a rough

measure of cell division under conditions of IUdR inhibition (Table 3). Even though

TABLE 3
Effect of IUdR on mitotic index

Hours of incubation with IUdR	% Mitoses	
	IUdR-treated	Control
6	1.6	2.2
10	3.1	3.3
13	1.5[a]	2.7
19	1.5[a]	1.7
25	1.5[a]	2.2
30	1.7[a]	1.7

[a] After 13 hours with IUdR, growth stopped (see Fig. 1) and the drug was removed.

there was no significant increase in cell number after one generation in the presence of IUdR, the mitotic index did not drop significantly for 18 hr, but remained essentially the same as in the control population.

DISCUSSION

The inhibition of reproduction of murine mast tumor cells caused by IUdR is similar to the inhibition produced by IUdR and BUdR in other mammalian cell lines in culture (6–12). In the presence of a high concentration of IUdR (about $1–2 \times 10^{-4}$ M) these cells undergo one doubling of their DNA and of their cell number. After one doubling more than 90% of the DNA bands as IU-THY hybrid in the CsCl gradient. Even if the cells are transferred into medium containing thymidine in place of IUdR after one generation, neither DNA nor cell number undergoes any further increase.

Even though the amount of DNA in the culture does not increase after one cell division with IUdR, DNA synthesis continues, as evidenced by the continued incorporation of ^{3}H-thymidine into DNA during the subsequent period of 12 hr. DNA from these cells can be separated into an IU-THY band and a new THY-THY band, both of which contain ^{3}H-thymidine. The appearance of the new THY-THY band and the presence of ^{3}H-thymidine in the light strand of the IU-THY band indicate that the original IU-THY DNA from the cells incubated with IUdR for one generation is able to replicate.

It seems clear, however, that not all the IU-THY DNA can be utilized for the synthesis of new DNA. Two facts support this conclusion. First, in two of three experiments given in Table 1, the rate of incorporation of ^{3}H-thymidine by IUdR-pretreated cells was only about one-half of the rate of the controls. In a third experiment, the rate was three-fourths of the normal rate. Second, in the CsCl density gradients the specific radioactivity of the IU-THY band was always about one-half (50–65%) that of the THY-THY band (Table 2). The specific radioactivity of the IU-THY DNA was lower than that of the normal DNA even when ^{32}P-phosphate was used in place of ^{3}H-thymidine. Therefore, the lower specific activity of the IU-THY band was not the result of a replication error, for example, that a deoxyribonucleoside triphosphate other than ^{3}H-thymidine triphosphate was incorporated into a part of the newly synthesized DNA. The difference in specific activity of the two DNA bands could conceivably be the result of different rates of replication of IU-THY and THY-THY DNA. Replication of IU-THY DNA in ^{3}H-thymidine would first result in the formation of equal amounts of IU-THY* and THY-THY* DNA. By this model the THY-THY* DNA would then replicate more rapidly than the IU-THY* DNA to produce an excess of THY*-THY* DNA. Several facts, however, would seem to negate this argument. Even if the THY-THY* DNA replicates more rapidly than the IU-THY* DNA, it is unlikely that the THY-THY* DNA can undergo a second replication during the period of exposure to ^{3}H-thymidine, since this was equivalent to only one generation time. The above model also demands that at the end of the experiment there should be more THY-THY DNA than IU-THY DNA. Analysis of the cellular content of the two types of DNA by CsCl density equilibrium centrifugation showed that the amount of THY-THY DNA in the cells

was at most equal to, and usually less than, the amount of IU-THY DNA (Table 2).

An alternative explanation for the lower specific activity of the IU-THY DNA is that only about one-half of the original IU-THY DNA in the culture replicates during the 12-hr incubation with ^{3}H-thymidine. The newly synthesized IU-THY* DNA would thus be diluted with an equivalent amount of residual nonradioactive IU-THY DNA; therefore, the specific radioactivity of the IU-THY DNA would be about one-half that of the newly synthesized THY-THY DNA. The advantage of this explanation is that it requires only the normal orderly replication of the portion of the DNA that does replicate, and it is consistent with the observed analytical data from the CsCl ultracentrifugations.

Direct evidence supporting the idea that only one-half of the IU-THY DNA replicates has recently been obtained from radioautographic analysis of the cell population containing IU-THY DNA. Only about half of the cells label with ^{3}H-thymidine during the 12-hr postincubation period; therefore, it can now be stated with certainty that only about one-half of the IU-THY DNA of these cultures replicates. These studies are in progress and will be reported in detail elsewhere.

A substantial turnover of DNA in cells inhibited with IUdR is indicated by the incorporation of ^{3}H-thymidine into DNA in the absence of any increase in total DNA. At first it seemed likely that this turnover might reflect "repair" (20, 21) of the IU-THY DNA. However, both strands were found to break down at about the same rate, 35% per generation (Fig. 3). The rate of degradation is sufficient to explain the failure of DNA to accumulate in the inhibited cultures. These results do not support "repair" as an explanation for the loss of DNA, since the most characteristic feature of DNA "repair" (20, 21) is selective removal of abnormal DNA.

The CsCl density gradient experiments provide additional evidence that the incorporation of ^{3}H-thymidine into DNA does not represent the synthetic phase of a "repair" process. For, if the new radioactive THY-THY band were the result of excision of IUdR from DNA and its replacement with ^{3}H-thymidine, then only the THY-THY DNA, but not the residual IU-THY DNA, would be radioactive.

The cells double once in the presence of IUdR and after this doubling most of the cells are nonviable, since, when they are transferred to fresh medium lacking IUdR, the cell number does not increase for about 65 hr. In contrast, when P815Y cells are made "thymineless" for one generation with either FUdR or methotrexate, they resume growth almost immediately in fresh medium lacking the drug (J. W. Cramer, unpublished observations). The eventual increase in cell number of the IUdR-treated cultures appears to represent outgrowth of a small fraction of the population not killed by IUdR.

The continuation of mitotic activity in IUdR-inhibited cultures after 12 hr (Table 3) suggests that although cell division continues, it is balanced by cell lysis, just as DNA synthesis continues, but is balanced by a loss of DNA. An alternative explanation could be that coiling of the chromosomes (i.e., formation of the mitotic figure) in the IUdR-inhibited culture is not followed by cell division. Some cell divisions were directly observed in the IUdR-inhibited cultures, but the frequency of cell division under these conditions has not yet been determined.

There is an apparent correlation between cell death and replacement of the cellular DNA-thymidine with IUdR, but a causal relationship between the two has never been unequivocally demonstrated (22, 23); nor have we shown in this paper any causal relationship between the incorporation of IUdR into DNA and the failure of cell growth after one division. If incorporation of IUdR into DNA is causally related to the lethal effect, it is clear from our data that incorporation into only one of the strands of the DNA double helix suffices to injure the cells. With synchronized HeLa cells, a major fraction of the cell population can be killed by BUdR during an incubation so short that only a small amount of BUdR is incorporated into

the DNA (12). Our results together with those reported in the literature (9, 12) suggest that some specific small fraction of the cellular DNA is sensitive to substitution in one strand with IUdR or BUdR.

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