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SPONTANEOUS UNSCHEDULED DNA
SYNTHESIS IN HUMAN LYMPHOCYTES*

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SPONTANEOUS UNSCHEDULED DNA SYNTHESIS IN HUMAN LYMPHOCYTES*

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ABSTRACT

FORELL, BRUCE, MYERS, L.S., JR., and NORMAN, Amos. Spontaneous Unscheduled DNA Synthesis in Human Lymphocytes. Radiat. Res.

We have estimated the rate of spontaneous unscheduled DNA synthesis in human lymphocytes from measurements of tritiated thymidine incorporation into double-stranded DNA (ds-DNA) during incubation of cells in vitro. The contribution of scheduled DNA synthesis to the observed incorporation was reduced by inhibiting replication with hydroxyurea and by separating freshly replicated single-stranded DNA (ss-DNA) from repaired ds-DNA by chromatography on benzolyated naphthoylated diethylaminoethyl (BND) cellulose columns. The residual contribution of scheduled DNA synthesis was estimated by observing effects on thymidine incorporation of a) increasing the rate of production of apurinic sites by increasing the temperature, and alternatively, b) increasing the number of cells in S-phase by incubation with phytohemagglutinin. Corrections based on estimates of endogenous pool size were also made. The rate of spontaneous unscheduled DNA synthesis is estimated to be 490 ± 120 thymidine molecules incorporated per cell per hour. These results compare favorably with estimates made from rates of depurination and depyrimidination of DNA, measured in molecular systems by other workers, if we assume thymidine is incorporated by a short patch mechanism which incorporates an average of four bases per lesion.

Spontaneous UDS

Lymphocytes

Hydroxyurea

BND- cellulose UDS - Spontaneous

INTRODUCTION

This paper addresses the idea that *in vivo* DNA is continuously damaged and repaired. In it, we report on an attempt to measure the extent of these reactions in human lymphocytes *in vitro* using measurements of thymidine incorporation by unscheduled DNA synthesis (UDS) as an index. Determination of this quantity is complicated by incorporation due to scheduled DNA synthesis (SDS). Attempts were made to reduce the contribution of SDS to our measurements by (a) using human peripheral blood lymphocytes (HPBL), which have only a small proportion of S-phase cells in the population (5) and (b) using hydroxyurea (HU) to suppress SDS. We also use BND-cellulose to separate ds-DNA from DNA containing single-stranded regions to further reduce SDS contribution to our UDS measurements. Nevertheless, there may be some contribution of SDS to the rate of thymidine uptake (UR). We have varied the ratio of SDS to UDS to estimate contributions of SDS to UR by (a) increasing the incubation temperature to 42°C, which increases UDS significantly while only slightly perturbing SDS (9) and (b) by stimulating the cells to proliferate, which increases the proportion of cells in SDS in the population. Our results suggest that the rate of spontaneous UDS is 490 ± 120 thymidine molecules incorporated per cell per hour at 37°C. If we assume the incorporation is due to a short patch repair mechanism (an average of 4 bases incorporated per lesion), then these results compare favorably with the results of Lindahl and Nyberg (1) and Lindahl and Karlstrom (2) for rates of depurinization and depyrimidinzation of extracted E. coli DNA in solution. A brief mention of our work has been published (3).

MATERIALS AND METHODS

A. Lymphocyte Isolation

Lymphocytes and monocytes were obtained from freshly drawn heparinized human blood from normal donors or from chronic lymphocytic leukemia (CLL)

patients undergoing leukophoresis. Lymphocytes and monocytes were isolated by a ficoll-hypaque separation procedure (4). Cells were washed twice with NaCl-EDTA (0.8% NaCl and 0.05% EDTA) and resuspended in RPMI 1640 with HEPES buffer and 10% fetal calf serum (media; both from Grand Island Biological Company, New York) with or without HU (Sigma Chemical Company, St. Louis).

B. Cell Culture and Viability Testing

Cells (10^8) were cultured by suspension in 100 ml of media containing 0.45 mg/ml streptomycin sulfate, 450 units/ml potassium salt of Penicillin G (both from Pfizer Labs., New York) and Phytohemagglutinin (PHA; 0.8 ml of stock solution in 100 ml media, Burroughs Wellcome Co., North Carolina). They were incubated at a concentration of 10^6 cells/ml at 37°C for 48, 72, or 80 hours in Falcon Tissue Flasks (VWR Scientific, Inc., Los Angeles, Ca.). They were then concentrated by centrifuging for 10 minutes at 730 g and resuspended in media without PHA.

Viability of cells was determined by Trypan Blue (Grand Island Biological Company, New York) dye exclusion.

C. DNA Synthesis

DNA synthesis was measured at a cell concentration of 5 X 10^6 cells/ml. Cells were preincubated in media containing 10 mM HU at 37°C for 30 minutes with constant motion in a water bath. (Methyl-^{3}H) Thymidine (^{3}HTdR; 49.8, 55.2, or 50.0 Ci/mM, New England Nuclear, Mass.) was then added to give an activity of 10 μCi/ml and the incubation was continued for various times at 37°C. Synthesis was terminated by adding an equal volume of media at 4°C containing 60 μg/ml Thymidine (TdR) to the samples and centrifuging for 10 minutes at 730 g.

D. Irradiation of Cells

Cells were prepared for irradiation by incubation in media with or without HU for 30 minutes. ^{3}HTdR was added to the cell suspension

resulting in an activity of 10 μCi/ml and the suspension was irradiated by a ^{60}Co source at a dose rate of 50 rads/minute for various times. The cells were then incubated at 37°C for 1 hour and synthesis was terminated as mentioned previously.

E. BND-Cellulose Chromatography

The separation of DNA from cell lysates, and ds-DNA from ss-DNA, was accomplished using BND-cellulose column chromatography. Cells were (a) lysed with 0.2% sodium dodecyl sulfate and 3 cycles of rapid freezing and thawing, (b) RNA was digested with 0.05 ml of 1 mg/ml Pancreatic Ribonuclease and 0.02 ml of 2500 units/ml Ribonuclease T_1 (both from Calbiochem, La Jolla, Ca) at 37°C for 1 hour, and (c) protein was digested by incubation with 0.15 ml of 10 mg/ml Pronase (Calbiochem, La Jolla, Ca) for 2 hours more. The lysates were cooled to 4°C and the DNA was sheared by passing the samples through a 20 gauge needle 5 times (5). The samples were made 0.3 M in NaCl by addition of 0.4 M buffer (400 mM NaCl, 0.1 mM EDTA, 10 mM Tris HCl; pH 7.5).

Procedures of Scudiero, et al. (6) were followed for preparing and using BND-cellulose columns.

The concentration of DNA was calculated from measurements of optical absorbance taking the value of the molar extinction coefficient per phosphate as 6000 liters per mole-cm. Tritium was assayed by liquid scintillation counting; a 3 ml aliquot was added to 15 ml of Phase Combining Scintillant (both from Amersham/Searle, Arlington Heights, Illinois) in LSC vials, shaken, dark adapted for 8 hours and counted. The activity was converted to number of TdR molecules incorporated per pg DNA per hour.

F. Estimation of Thymine Precursor Pool Size

It was necessary to estimate the thymine precursor pool size to correct for dilution of exogenous ^{3}HTdR incorporation by normal routes of thymine

incorporation. The "effective" pool size was estimated by measuring ^{3}HTdR uptake in the presence of different exogenous TdR concentrations (6,7).

RESULTS AND DISCUSSION

A. Estimation of Effective Thymine Precursor Pool Size

While TdR is not directly involved in the normal sequence of reactions leading to thymine incorporation in DNA, other pathways in the cell compete with ^{3}HTdR incorporation. We have estimated the contribution of these pathways as giving an "effective" pool size equivalent to 0.42 μM TdR. This estimate compares well with previous estimates using the same (6) and different (8) methods.

Conner and Norman (8)	0.35 μM
Scudiero, et al. (6)	0.38 μM
This work	0.42 μM

If we assume these values are from a normally distributed population, the average and standard deviation are 0.38 ± 0.04 μM. In analyzing results given below we have used this value as our estimate of effective pool size. We have worked with ^{3}HTdR at a concentration of approximately 0.19 ± 0.01 μM. Thus, for each labeled thymidine incorporated, approximately two competing thymines are incorporated. Our observed values of incorporation of ^{3}HTdR have been multiplied by a factor of 3.00 ± 0.26 to correct for dilution of label by endogenous pools.

B. Excision-Repair Patch Size Induced by Ionizing Radiation

We have accepted as an estimate of patch size, measurements reported previously (9-13) for the number of nucleotides inserted per lesion by ionizing radiation. We have re-interpreted the work of Spiegler and Norman (9,10) and now estimate that the data are consistent with 2-6 bases incorporated per

lesion. Other estimates include 3 bases/lesion by Painter and Young (11) and 3-4 bases/lesion by Regan and Setlow (12,13). Different methods were used for these estimates but the results agree well and suggest an average patch size of approximately 4 bases per lesion with an uncertainty of about 1 base per lesion.

C. Upper limit of Spontaneous UDS

Table 1 shows the thymidine uptake per pg DNA in ss-DNA, ds-DNA and total DNA when cells were incubated with ^{3}HTdR and 10 mM HU for 1,2 or 4 hours. BND-cellulose was used to remove non-incorporated ^{3}HTdR from all samples and the DNA was eluted at one time (total DNA) or fractions of DNA were sequentially eluted (ds-DNA or ss-DNA). Corrections were not made for possible SDS contributions to ds-DNA measurements. These ds-DNA measurements (Table 1) allow us to estimate 1098 ± 215 T/C/H due to spontaneous UDS (Table 3-1). Prior to these experiments we had confirmed that 10 mM HU resulted in maximum reduction in SDS for lymphocytes.

Spontaneous UDS could be the result of continuous background level insults suffered and repaired by the cells, damage induced while handling the cell prior to incubation with ^{3}HTdR, or decay of tritium continuously damaging the DNA during the incubation with the cells that is being repaired over the time span we are investigating. If the measured UDS was a result of induced damage prior to incubation with ^{3}HTdR, the initial rate of UDS would decrease and level off after 1 hour (10). Table 1 indicates that incorporation of thymidine is relatively linear over the time span investigated, suggesting that measured UDS is due to continuous damage and repair. This linearity of incorporation as a function of time could be explained either by a lesion limited or enzyme limited repair system. If enzymes are limiting, then increased rate of repair should not occur with increased number of lesions.

Figure 1 shows the resultant TdR incorporation per pg DNA as a function of dose of ^{60}Co gamma rays (Curve B). The increased rate of incorporation observed with increased damage suggests the lesions are rate limiting if the repair enzymes and patch sizes for AP-sites and ^{60}Co induced damage are the same. The decay of tritium continuously damaging the DNA during the incubation of cells with ^{3}HTdR was considered but our calculations indicate less than 1 rad per hour was deposited in the media by decay of tritium for our conditions (10 μCi/ml 3HTdR; 49.8 - 55.2 Ci/mM). Djordjevic, et al. (7), calculated the dose delivered in a 2 hour incubation to be less than 1 rad for their conditions (10 μCi/ml 3THdR; 15.9 Ci/mM). This small amount of damage can not explain the amount of incorporation observed in Table 1.

D. Expected Spontaneous UDS Due to AP sites

An estimation of spontaneous UDS can be made using the rate constants calculated by other investigators for production of AP-sites in DNA in aqueous solutions at 37°C and pH 7.4 (1,2). If we assume (a) 6 pg DNA per lymphocyte, (b) the rate of production of AP-sites equals the rate of repair of these lesions at pH 7.4 and 37°C, and (c) each AP site is repaired by the short patch excision repair mechanism, then we calculate 490 T/C/H due to spontaneous UDS (Table 3-2).

E. Estimation of Spontaneous UDS by Varying Temperatues

We investigated UR in ds-DNA as a function of temperature. The effect of increasing the incubation temperature from 37°C to 42°C results in reducing SDS to approximately 80% the rate of 37°C (14). The rate of production of AP sites by hydrolysis in neutral solution is temperature dependent (1,2), and the effect of increasing the temperature from 37°C to 42°C has been calculated (Table 2-a). The rate of UDS induced by ionizing radiation is relatively constant between 37°C and 42°C (14). Experiments

performed at different temperatures vary the ratio of UDS to SDS in ds-DNA and allow estimates of UDS and SDS contributions to ds-DNA. Table 2 shows the expected effect of temperature upon (a) the rate of AP lesions induced, (b) rate of SDS, and (c) our measurements of UR. If we assume (a) the repair rate equals the lesion production rate, (b) the rate of SDS at 42°C is reduced to 80% the rate of 37°C (14), (c) the reports of constant UDS rates when induced by ionizing radiation for these two temperatures (14), (d) there is not a temperature dependent increase in patch size, and (e) there is not a decrease in thymine precursor pool size due to increased temperature, we obtain an estimate of UDS at 37°C for these data of 430 ± 100 T/C/H (Table 3-3).

F. <u>Estimation of Spontaneous UDS by Varying SDS Contribution to UR in ds-DNA</u>

We investigated UR as a function of proportion of S-phase cells in the cell population. Incubation of HPBL with the mitogen PHA results in a variation in the proportion of S-phase cells in the cell population compared to non-stimulated cells. To quantitate the amount of SDS + UDS in either non-stimulated or PHA-stimulated cells, the total DNA (ds-DNA + ss-DNA) was eluted from BND-cellulose and the incorporation of ^{3}HTdR into the DNA measured. The ratio of these measurements results in a rough estimate of the amount of increased SDS in PHA-stimulated samples relative to SDS in non-stimulated cell populations. From replicate samples, we eluted only the ds-DNA loaded onto BND-cellulose, and measured the UR. The assumptions that (a) in the PHA-stimulated population the total spontaneous UDS over 1 hour is the same as spontaneous UDS in non-stimulated cells and (b) the amount of SDS in the population is reflected by the incorporation of ^{3}HTdR into ss-DNA, allows us to estimate the UDS in these cell populations from our measurements (appendix). These calculations suggest 90 ± 11 TdR incorporated per pg DNA per hour or 540 ± 70 T/C/H due to spontaneous UDS (Table 3-4).

The results of Scudiero, et al. (5), with the human lymphoblastoid cell line RAJI, indicate approximately 27 times as much UR in RAJI controls as in HPBL or CLL controls. The difference in induced UR between these cell types was suggested to be a function of the capacity of the RAJI cells for normal DNA synthesis. The rate of induced UDS may be a function of the concentration of similar enzymes used in SDS and UDS but the rate of spontaneous UDS in the controls should be constant due to it being lesion limited. Their controls and our assay measure non-induced incorporation which might be a result of either SDS incorporation or spontaneous UDS. A difference in uptake rate due to repair of spontaneous AP-sites is not expected because spontaneous damage should be dependent only on the quantity of DNA per cell which should be relatively constant for these types of lymphocytes. On the other hand, if the ratio of S-phase cells in the RAJI population to S-phase cells in CLL or HPBL population were 27:1, then the UR measurements of ds-DNA might reflect SDS incorporation only. We determined the contribution of SDS to our spontaneous UR measurements in ds-DNA by varying the number of cells in S-phase and measuring the effect on spontaneous thymidine incorporation in ds-DNA (appendix). Our results suggest significant contributions to background UR in ds-DNA due to S-phase incorporation of 3HTdR and this might explain the differences observed between the human lymphoblastoid cell line RAJI and HPBL or CLL (15).

G. Best Estimate for Spontaneous UDS

We averaged the two estimates for spontaneous UDS where SDS contributions have been corrected for (Table 3-3 and 3-4) and obtained an average estimate of 490 ± 120 T/C/H (Table 3-5). This estimate is surprisingly close to that based on the experiments of Lindahl, et al. (1,2) on the hydrolysis of DNA in neutral solution (Table 3-2). This suggests that the complex chromatin

structure and nuclear environment in which the DNA is embedded does not on the average change the rate of hydrolysis from that observed in neutral solution at the same temperature. The estimate of TdR pool size contributes significantly to these two estimates of UDS but is not involved in the estimate of UDS calculated from the rate of AP sites (Table 3-2). This suggests the calculation of TdR pool size is correct.

The doubling dose, the dose which yields twice the spontaneous UR per hour, can be estimated from Figure 1. We see that the calculated or measured doubling dose is a function of both slope and Y-intercept (zero-dose UR value). The UR per rad is about the same for data generated by Speigler and Norman (9; Curve A) and our results (Curve B). The estimated doubling dose for Spiegler and Norman's data is approximately 540 rads. Our data, not corrected for SDS contributions to UR, is 140 rads. One explanation for this difference is the contribution of SDS to UR is different. We have two independent estimates that suggest 60% of the UR in our experiments is due to spontaneous UDS. We generate Curve C by correcting Curve B for SDS contributions by subtracting 40% of our zero-dose measurement from all the values of Curve B. The doubling dose for this corrected data is approximately 90 rads. This is a value similar to the D_0 found for many mammalian cells experimentally.

Table 1

Newly Inserted Thymidines Incorporated Per pg DNA for Non-Fractionated and BND-Cellulose Fractionated DNA Versus Time

^{3}HTdR Incubation Time (Hours)	Total DNA (TdR Molecules Inserted per pg DNA)	ss-DNA (TdR Molecules Inserted Per pg DNA)	ds-DNA (TdR Molecules Inserted Per pg DNA)
1	396 ± 34	1359 ± 118	117 ± 15
2	892 ± 77	2252 ± 195	424 ± 37
4			876 ± 76

1.5 X 10^7 cells/samples were incubated in media with 10 mM HU for 30 minutes, media with ^{3}HTdR (49.8 Ci/mM; final activity 10 µCi/ml) was added and incubation continued for 1, 2 or 4 hours. The cells were washed 3 times in NaCl-EDTA with 60 µg/ml TdR, lysed, and chromatographed on BND-cellulose to remove non-incorporated ^{3}HTdR from the DNA. The DNA was either eluted all at once (total DNA) or first the ds-DNA and then the ss-DNA was eluted. After exhaustive dialysis, the activity was measured and TdR incorporated per pg DNA calculated.

Table 2

Effect of Temperature on Thymidine Uptake

	Temp 37°C	Temp 42°C	Ratio
a. Calculated Lesion Production Rate (1)	99 AP-Sites/pg DNA/H	222 AP-Sites/pg DNA/H	2.24
b. Expected SDS Rate (9)	100 %	80%	0.80
c. Measured UR	120 ± 28 TdR Incor/pg DNA/H	199 ± 42 TdR Incor/pg DNA/H	1.66

Equations:

1. at 37°C: 1.0 SDS + 1.0 UDS = 120 TdR Incor/pg DNA/H
2. at 42°C: 0.8 SDS + 2.24 UDS = 199 TdR Incor/pg DNA/H

Solving for UDS: UDS = 72 ± 17 TdR Incor/pg DNA/H

We incubated HPBL in media with 10 mM HU for 30 minutes at 37°C. Media with 10 mM HU and ^{3}HTdR (10 μCi/ml; 60 Ci/mM) was added and samples incubated at either 37°C or 42°C for 1 hour longer. After BND-cellulose column chromatography to separate the ds-DNA from non-incorporated ^{3}HTdR and ss-DNA, we measured the incorporation of ^{3}HTdR in ds-DNA and calculated the number of TdR's incorporated per pg DNA per hour.

Table 3

Estimates of Spontaneous UDS

1.	From Table 1, for ds-DNA,	1098 ± 215
2.	From Lindahl, et al., (1,2)	490 (a)
3.	From temperature Expts., Table 2	430 ± 100 (b)
4.	From PHA Expts., appendix	540 ± 70 (c)
5.	Best Estimate, Avg. 3 and 4 above,	490 ± 120

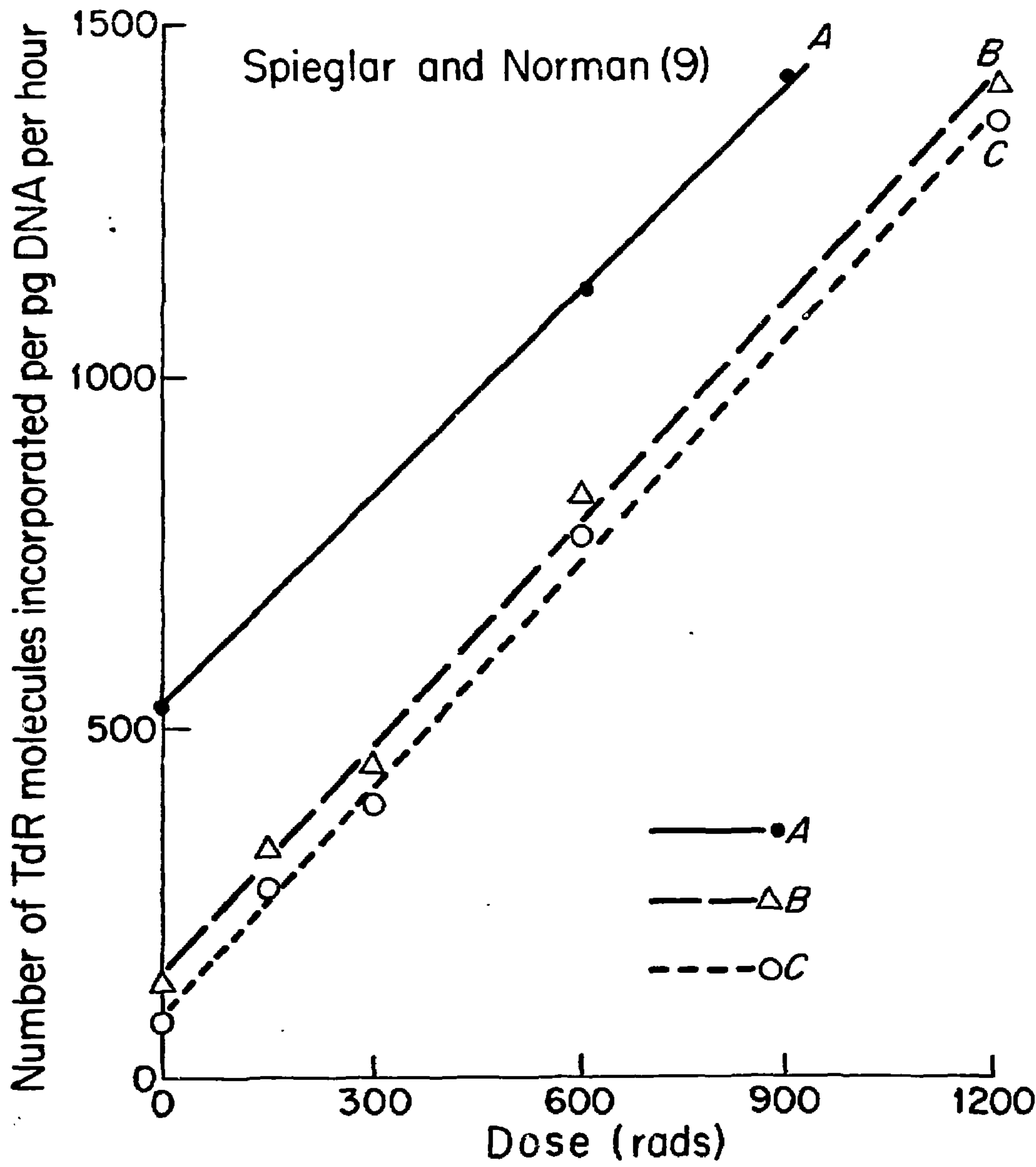
Assumptions:

a) Rate of production of AP-sites equals rate of repair at pH 7.4 and 37°C. each AP-site is repaired by short-patch excision repair so on the average, 0.77 TdR is incorporated for each lesion repaired, and there is 6 pg DNA per lymphocyte.

b) Repair rate equals lesion induction rate, patch size remains constant with temperature, effective TdR pool size remains constant with temperature.

3) UDS is constant in non-stimulated and PHA stimulated cells and repair rate is lesion limited.

Fig. 1. Uptake of thymidine by irradiated lymphocytes. 4 X 10^7 cells/sample were incubated in 10 mM HU for 30 minutes, then media with 10 mM HU and ^{3}HTdR (50 Ci/mM; final activity 10 μCi/ml) was added and the cell suspensions were irradiated with ^{60}Co gamma-rays (see materials and methods) to final doses of 150, 300, 600, or 1200 rads. The cells were incubated for 1 hour at 37°C, washed 5 times with NaCl-EDTA with 60 μg/ml TdR, and the ds-DNA was separated from non-incorporated ^{3}HTdR, and ss-DNA, by BND-cellulose chromatography. The activity per μg of the ds-DNA was measured and the thymidine incorporation per μg DNA was calculated. (A) Data from Spiegler and Norman (9); (B) Data from this work, not corrected for SDS contribution to UR; (C) Data from this work, corrected for SDS.


Spieglar and Norman (9)
Number of TdR molecules incorporated per pg DNA per hour
1500
1000
500
0
0
300
600
900
1200
Dose (rads)
A
B
C
A
B
C

Appendix: Results of PHA Experiments

1×10^8 HPBL were suspended in 100 ml of media with 0.45 mg/ml streptomycin sulfate, 450 units/ml potassium salt of Penicillin G and 0.8 ml of stock solution of PHA and incubated at 37°C for 48, 72, or 80 hours. After incubation, the cells were removed from the flasks and an aliquot was taken for viability determinations.

The stimulated lymphocytes were resuspended in media with 10 mM HU and at the same time, HPBL, freshly isolated from whole blood, were resuspended in media with 10 mM HU. The cell suspension contained 17×10^6, 20×10^6, and 7.5×10^6 cells per sample for 48, 72, and 80 hour PHA stimulated cultures, respectively. After preincubation for 30 minutes at 37°C in media with 10 mM HU, ^{3}HTdR (49.8 Ci/mM, New England Nuclear, Mass.) was added resulting in a final activity of 10 μCi/ml and a cell concentration between $2-5 \times 10^6$ cells/ml. The cell suspensions were incubated 1 or 2 hours at 37°C. The cells were washed 3 times in NaCl-EDTA with 60 μg/ml TdR.

To estimate the relative increase in SDS in PHA-stimulated cultures we used BND-cellulose column chromatography to separate ds-DNA from total DNA (SDS + UDS incorporation) for both PHA-stimulated and non-stimulated cultures. When all the DNA is eluted at one time using 50% formamide (Gallard-Schlesinger Chem. Manufacturing Corp., Carle Place, New York) - 1.0 M buffer (1.0 M NaCl, 0.1 mM EDTA, 10 mM Tris HCL; pH 7.5), the measured incorporation is due to both SDS and UDS. When only the ds-DNA is eluted using 1.0 M buffer, the measured incorporation should be due to only UDS (2). By subtracting the value for DS-DNA (UDS) from the value for total DNA (SDS + UDS), we obtain a value for incorporation due to SDS not effected by HU. The ratio of the residual SDS in PHA-stimulated cultures to residual SDS in non-stimulated cultures gives us a relative value of increased SDS to to PHA stimulation.

Appendix: Results of PHA Experiment

Assumptions in PHA stimulated cells

1. Total UDS over 1 hour remains equal to that in unstimulated cells
2. SDS and SS-DNA increase by the same factor

UDS = *incorporation due to spontaneous damage and repair*
SDS = incorporation due to semiconservative replication in DS-DNA
SS = amount of SDS in SS-DNA removed by BND-cellulose from total DNA
a = factor for increased number of S-phase cells due to PHA stimulation
UR = measured uptake rate

Equations:

1. $(UR)_1$ = UDS + SDS = Δ TdR incor/pg DNA/H in DS-DNA eluted off column
2. $(UR)_2$ = UDS + SDS + SS = Δ TdR incor/pg DNA/H in total DNA (SS- and DS-DNA eluted off together)
3. $(UR)_3$ = UDS + a(SDS) = Δ TdR incor/pg DNA/H in DS-DNA eluted off column after PHA stimulation of the cells
4. $(UR)_4$ = UDS + a(SDS + SS) = Δ TdR incor/pg DNA/H in total DNA (SS- and DS-DNA eluted off together) after PHA stimulation of the cells.

From these four measurements an estimate of UDS, SDS, a and SS can be obtained.

	80 hrs	48 hrs	72 hrs
1. $(UR)_1$	195	141	141
2. $(UR)_2$	421	333	333
3. $(UR)_3$	2472	3380	2430
4. $(UR)_4$	8013	16911	9490
rearrange 3	UDS = 2472 - a(SDS)		
sub. into 4	2472 -a(SDS)+a(SDS)+a(SS)=8013		
solve for a (SS)	a(SS) = 5541	13531	7060
Eq. 2	UDS + SDS + SS = 421		
- Eq. 1	-(UDS + SDS = 195)		
solve for SS	SS = 226	192	192
sub. into a(SS)	a (226) = 5541		
solve for a	a = 24.52	70.47	36.77
sub. into Eq. 3	UDS + (24.52)SDS = 2472		
subtract 1	-(UDS + SDS = 195)		
	23.52 SDS = 2277		
solve for SDS	SDS = 97	47	64
sub. into Eq. 1	UDS + 97 = 195		
Δ TdR incor/pg DNA/H due to UDS	= 98	94	77

average Δ TdR incor/pg DNA/H due to UDS = 90 ± 11

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