Proc. Nat. Acad. Sci. USA
Vol. 69, No. 7, pp. 1685–1689, July 1972

Excretion of Deoxyribonucleic Acid by Lymphocytes Stimulated with Phytohemagglutinin or Antigen

(mitogenesis/pulse labeling/density gradient centrifugation/DNase)

JOHN C. ROGERS,* DAVID BOLDT,† STUART KORNFELD,† SISTER ANN SKINNER,† AND C. ROBERT VALERI*

* Naval Blood Research Laboratory, Chelsea, Massachusetts 02150; and † Departments of Medicine and Biochemistry, Washington University School of Medicine, St. Louis, Missouri 63110

Communicated by Carl V. Moore, May 1, 1972

ABSTRACT When human lymphocytes are cultured in the presence of phytomitogens, 70–90% of the cells undergo blast transformation and synthesize DNA. However, less than 40% of these lymphocytes actually undergo mitosis while 35–90% of the newly synthesized DNA is excreted into the media. The release of DNA by the cells is selective since experiments with [^{14}C]uridine indicate that RNA is not lost into the culture media. DNA excretion occurs under many culture conditions. The excreted DNA has an estimated molecular weight of 3 to 12 $\times$ 10^6 as determined by gel filtration on Sepharose 2B. It forms a single sharp peak at a density of 1.055 g/cm^3 when examined by sucrose density gradient centrifugation, suggesting that the DNA is complexed to protein or lipid.

Although phytohemagglutinin (PHA) is known to be a mitogen, the number of mitoses actually counted after addition of PHA to lymphocyte cultures is relatively small. For example, Nowell found only 10% after 18 hr of incubation with colchicine at the peak of DNA synthesis despite morphologic transformation of up to 90% of these cells (1). Such PHA-transformed lymphocytes are known to be synthesizing DNA, since they are labeled when pulsed with radioactive thymidine and studied by autoradiography. The discrepancy between many cells making DNA and few caught dividing has been attributed to asynchrony of the cultured cells (2).

In PHA-stimulated lymphocyte cultures, one might expect to find a significant increase in cell number resulting from mitosis, as well as a concomitant increase in the total DNA content of the culture. The reports dealing with this question do not consistently support this expectation. Schellekens and Eijsvoogels noted that total cell number declined 17% the first day, while DNA content dropped 25%; by day 3, the cell count was still only 89% of the initial values, and the DNA content was 96% (3). Hirschhorn *et al.* reported a decline in DNA content during a 48-hr culture of PHA-stimulated lymphocytes to 75% of the initial value (4). Clearly, if most PHA-transformed lymphocytes in these experiments underwent mitosis, a large number of daughter cells must have died and lysed. In contrast, Loeb and Agarwal measured a PHA-induced increase in DNA to nearly twice that of unstimulated cultures by 72 hr (5). Their results could be explained if each stimulated lymphocyte underwent a single mitosis and suggest that lymphocyte cultures reported in the former papers were complicated for some reason by excessive cell lysis. However, this simple explanation is confounded by the data of Polgar and Kibrick (6). These authors report experiments in which greater than 90% of lymphocytes stimulated for 24 hr with PHA ultimately became blasts, as judged morphologically; when grown continuously in the presence of [^{3}H]thymidine, a maximum of 63% of the total number of lymphocytes present were labeled, as revealed by autoradiography on day 5, while on day 10 only 6% of the lymphocytes were labeled, yet the number of viable cells on day 10 was 57% of the original number. Cumulative in-

Abbreviations: PHA, phytohemagglutinin—E-PHA, erythroagglutinating and L-PHA, leukoagglutinating phytohemagglutinin, respectively.

TABLE 1. *DNA content of PHA-stimulated cells related to [^{3}H]thymidine uptake and loss*

Day	DNA (μg per culture)	[^{3}H]Thymidine acid-precipitable cpm in cells	[^{3}H]-Thymidine acid-precipitable cpm in medium
0	26.9	0	0
3	22.0 (23.5)	23,600 (305)	0
4	23.1 (26.6)	15,600 (246)	11,000
5	21.8 (22.7)	11,100 (240)	13,000
6	23.5 (21.3)	5,300 (175)	26,000

Quadruplicate cultures of 3.3 $\times$ 10^6 lymphocytes in 2 ml of medium were stimulated with 24 μg of E-PHA. On day 3, 3 μCi [^{3}H]thymidine (6.7 Ci/mmol) was added; at the end of a 4-hr pulse, 8 ml of minimal essential medium was added, the cultures were centrifuged at 500 $\times$ *g* for 5 min, the cell pellets were suspended in 2 ml of medium, and grown for up to 3 more days. On the days noted above, duplicate tubes were harvested. Culture tubes were centrifuged at 500 $\times$ *g* for 10 min. On one set, DNA content was determined by the diphenylamine reaction (11). Acid-precipitable [^{3}H]thymidine cpm were determined on the other set: 0.2 ml of medium was precipitated with 2.8 ml of ice-cold 5% Cl_3CCOOH. The remainder of the medium was removed and the cells were washed with 10 ml of ice-cold 0.9% NaCl. The cell pellet was precipitated with 3 ml of 5% Cl_3CCOOH and sonicated for 15 sec with a Biosonix IV sonicator at low intensity, 30% maximum. Cl_3CCOOH precipitates were centrifuged at 27,000 $\times$ *g*, 4°, for 20 min, washed with 2 ml of 5% Cl_3CCOOH, digested in 0.5 ml of Nuclear Chicago Solubilizer, and counted in 10 ml of Bray's solution. Values obtained for identical cultures not stimulated with PHA are in parentheses.

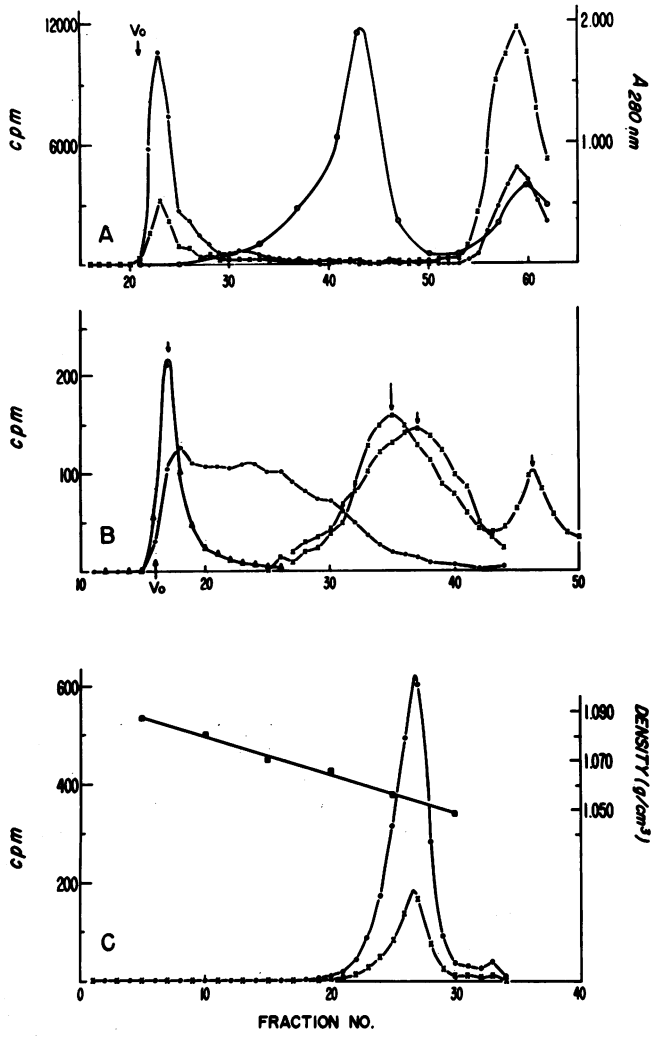

FIG. 1. (*A*) Sepharose 6B gel filtration chromatography. Three cultures containing 2 × 10^6 lymphocytes per culture were stimulated with E-PHA, pulsed with [^{3}H]thymidine and [^{14}C]-uridine on day 3, and harvested on day 6 as in Table 3; the cells in these cultures lost 85% of their ^{3}H-labeled DNA between day 3 and day 6, 80% of which was recovered in acid-precipitable form in the culture medium. The 6 ml of medium was made 0.1% with sodium dodecyl sulfate, applied to a Sepharose 6B column, 2.2 × 55 cm, and eluted at room temperature with Tris–NaCl–EDTA buffer [0.2 M NaCl–0.01 M Tris·HCl (pH 7.4)–0.001 M EDTA]. 3-ml Fractions were collected. All acid-precipitable ^{3}H and ^{14}C that had been applied eluted in the front peak. ^{3}H and ^{14}C eluted in fractions 55–62 were not acid-precipitable and represent traces of the thymidine and uridine pulse that were not washed out on day 3. Fractions 22–24 were pooled. ^{3}H cpm (●——●); ^{14}C cpm (×——×); $A_{280\ nm}$ (○——○). *Arrow: V_0.*

(*B*) Sepharose 2B gel filtration chromatography. A 0.9 × 60-cm column of Sepharose 2B equilibrated with Tris–NaCl–EDTA–0.1% sodium dodecyl sulfate was calibrated at 23–24° with ^{3}H-labeled *E. coli* DNA (gift of Dr. W. Sly) to determine the void volume and with ^{14}C-labeled HeLa cell rRNA, 32S (molecular weight 2.1 × 10^6), 28S (1.7 × 10^6), and 18S (0.7 × 10^6) (15) (gifts of Dr. A. Kumar) as molecular weight markers. 0.5 ml of the pooled fractions (22–24) made 0.1% with sodium dodecyl sulfate was chromatographed and collected in 0.9-ml fractions. Symbols are as in (*A*). *Arrows* (*left* to *right*) designate *E. coli* DNA, 32S RNA, 28S RNA, and 18S RNA.

(*C*) Sucrose density gradient equilibrium centrifugation. 0.5 ml of the pooled fraction (1*A*) made 0.1% with sodium dodecyl sulfate was layered on 17 ml of a 5–20% linear gradient of sucrose in Tris–NaCl–EDTA–sodium dodecyl sulfate and centrifuged at 4° in a SW27.1 rotor at 27,000 rpm for 16 hr. 0.5-ml Fractions were collected from the bottom of the tube. Density, g/cm^3 (■——■); other symbols are as in (*A*).

corporation of the [^{3}H]thymidine into the cells in acid-precipitable form also peaked on day 5, but then declined so that on day 11 the cumulative counts approached those of unstimulated cells. The authors' interpretation of this work was that many PHA-transformed cells do not synthesize DNA and do not divide, and that most daughter cells produced by mitosis (those cells labeled with [^{3}H]thymidine) die.

These confusing data motivated the following experiments in an attempt to clarify the fate of DNA synthesized by lymphocytes stimulated with phytomitogens and antigens.

MATERIALS AND METHODS

All experiments described were performed on human peripheral-blood lymphocytes that were isolated as described (7) and cultured in Eagle's minimal essential medium (pH 7.4) supplemented with 20% autologous serum, 4 mM glutamine, 50 units/ml of penicillin, and 50 μg/ml of streptomycin. The cultures were maintained at 37° in a 5% CO_2 atmosphere. Erythroagglutinating (E-) and leukoagglutinating hemagglutinin (L-PHA) from *Phaseolus vulgaris* were prepared from Difco PHA-P according to the method of Weber *et al.* (8). Pokeweed mitogen was obtained from Grand Island Biological Co. and reconstituted according to the supplier's instructions. Concanavalin-A was purchased from Calbiochem. RNase was obtained from Sigma Chemical Co. and DNase (RNase free) from Worthington Biochemicals. [*methyl*-^{3}H]Thymidine and [^{14}C]uridine were purchased from New England Nuclear Co. ^{3}H-labeled *Escherichia coli* DNA was a gift of Dr. W. Sly. The mitotic index was obtained by exposure of the cultures for 4 hr to 0.02 μg/ml colcemid at 37°. Metaphase chromosomes were prepared by the method of Mendelsohn *et al.* (9). To obtain accurate cell counts of lymphocytes cultured in the presence of E-PHA, the aggregated cells were dispersed by the addition of fetuin (5 mg/ml), a potent haptene inhibitor of the binding of E-PHA to lymphocytes (10).

RESULTS AND DISCUSSION

Loss of labeled DNA

When lymphocytes were incubated with E-PHA over a 6-day culture period, the number of viable cells remained within ±20% of the initial count. At least 95% of the cells counted on any day were viable as judged by trypan blue exclusion; by day 3, about 80% of the lymphocytes were transformed into blasts. The amount of DNA per culture, as measured by the diphenylamine reaction (11), also remained constant. However, cells pulse labeled with [^{3}H]thymidine on day 3 (the peak time of thymidine incorporation), washed, and cultured again for 3 more days lost 35–90% of the acid-precipitable [^{3}H]thymidine incorporated on day 3 (Table 1). The acid-precipitable [^{3}H]thymidine lost from the cells can be accounted for in the culture medium. The mitotic index was determined at daily intervals on these cultures; an increase in the number of mitoses occurred with the maximum being 3% after 4 hr of exposure to colcemid on day 3.

Lymphocytes stimulated with Concanavalin-A, *Phaseolus vulgaris* L-PHA, and mumps antigen (data in Table 2) as well as with pokeweed mitogen, *Lens culinaris* phytohemag-

glutinin (12), and PPD (purified protein derivative) antigen (data not presented) similarly excreted DNA into the culture medium.‡ When lymphocyte cultures are maintained for 3 days or more in the absence of phytomitogens or antigens, a small percentage of the cells spontaneously begin to synthesize DNA. Much of the [^{3}H]thymidine incorporated into DNA by these lymphocytes is also excreted into the medium (Table 2).

TABLE 2. *Effect of different mitogens on DNA excretion*

Mitogen	Acid-precipitable cpm: Day of pulse 3	Day of pulse 5	Day experiment completed 6	Day experiment completed 8
E-PHA (36 μg):				
Cells	52,600	—	24,300	—
Medium	0	—	16,200	—
L-PHA (30 μg):				
Cells	34,200	—	16,700	—
Medium	0	—	9,570	—
Con-A (20 μg):				
Cells	115,000	—	39,100	—
Medium	215	—	52,700	—
Mumps antigen (0.005 ml):				
Cells	—	45,900	—	14,000
Medium	—	0	—	22,700
None:				
Cells	3,040	—	713	—
Medium	0	—	302	—
None:				
Cells	—	6,950	—	1,770
Medium	—	0	—	7,630

8.5 × 10^6 lymphocytes in 2 ml of medium were cultured as described in Table 1. The cells were pulsed with 3 μCi of [^{3}H]-thymidine (6.7 Ci/mmol) for 4 hr on the days indicated. After the cells were washed and resuspended in fresh medium containing the appropriate mitogen, the cultures were incubated for 3 more days. Acid-precipitable counts in cells and medium were determined as in Table 1.

Selective DNA excretion

To confirm that intracellular, acid-precipitable [^{3}H]thymidine was in DNA, the DNA from cells pulsed and harvested on day 3 was extracted by a slight modification of the method outlined by Gottlieb *et al.* (13). Nucleic acid precipitated by cold ethanol after one phenol extraction was treated with alkali (1 N NaOH, 22°, and 18 hr) and then precipitated with Cl_3CCOOH. More than 90% of the radioactivity initially present was recovered in the precipitate, suggesting that the [^{3}H]thymidine was in DNA. The selective nature of DNA excretion was determined by experiments in which stimulated cells were pulsed on day 3 with both [^{3}H]thymidine and [^{14}C]uridine. The results of five consecutive experiments performed on lymphocytes from different individuals are outlined in Table 3. In each instance the cultures were pulsed on day 3 and then followed for 3 more days to compare the fate of the two isotopes. About 10–15% of the acid-precipitable ^{3}H was lost from the cells during the first 24 hr after the pulse label (experiments 3–5). In contrast, during this same 24-hr period, the amount of intracellular acid-precipitable ^{14}C increased in each instance. The explanation for this finding is that there is a large intracellular pool of ^{14}C-labeled acid-soluble nucleotides present after the 4-hr pulse on day 3 (data not presented); by day 4 the radioactive nucleotides in this pool have been used by the cells and converted to an acid-precipitable form. No such pool of ^{3}H is found. The acid-precipitable ^{14}C counts shown in Table 3 are expressed as being either alkali labile (presumably RNA) or alkali stable (presumably DNA). While most of the acid-precipitable ^{14}C on day 3 was in RNA, and while the number of counts in RNA increased by day 4, much of the ^{14}C incorporated into acid-precipitable form during the 24 hr after the pulse was alkali stable, and presumably DNA. The apparent conversion by lymphocytes of [^{14}C]uridine to [^{14}C]-thymidine and its incorporation into DNA has been noted (14).

Between days 4 and 6, the ^{14}C counts in cellular DNA declined, while the total ^{14}C counts in DNA (cells + media) increased, indicating the continued conversion of RNA degradation products into DNA. During this time a significant amount of ^{14}C-labeled DNA appears in the culture media while essentially no [^{14}C]RNA is released. These data suggest that the loss of isotope from RNA is due to normal turnover by cells, which must be viable in order to convert the RNA degradation products into DNA. Further, if loss of DNA were due to cell lysis an equal amount of RNA should be released into the medium. As shown in Table 3, essentially no acid-precipitable RNA was released by the cells. These observations are confirmed by the data in Table 4. Treatment for 2 hr with DNase solubilized 70% of both ^{3}H and ^{14}C radioactivity from the medium, while RNase and alkaline hydrolysis were without effect.

In an attempt to exclude the possibility that DNA excretion occurred because the culture medium was deficient in some factor necessary for complete DNA synthesis, other experiments were performed in medium TC-199, TC-199 buffered with 0.01 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid; pH 7.6), or TC-199 supplemented with thymine (5 μg/ml); all gave results similar to those experiments with minimal essential medium. Use of [^{3}H]deoxycytidine rather than [^{3}H]thymidine does not alter the pattern of DNA excretion. When mouse L-cells growing in log phase in minimal essential medium under conditions similar to those of lymphocyte cultures were pulsed for 2 hr with [^{3}H]thymidine, washed, and resuspended in fresh medium, none of the radioactivity incorporated during the pulse was lost from the cells during the following 72 hr.

Characterization of excreted DNA

As demonstrated in Fig. 1*A*, all acid-precipitable ^{3}H and ^{14}C radioactivity present in the culture medium eluted in a peak at the void volume on Sepharose 6B gel filtration chromatography. The medium applied to this column was taken

‡ While we did not determine the percent blast transformation in the cultures stimulated with PPD and mumps antigen, others have found that in such cultures about 2% of the lymphocytes are blast-like at 48 hr, while up to 50% of the cells present at day 6 are transformed (16). This increase was thought to represent multiple-cell divisions by a small number of antigen-responsive cells, although recruitment of other populations of lymphocytes could not be excluded (16).

TABLE 3. *Preferential loss of DNA from lymphocytes pulsed with [^3H]thymidine and [^{14}C]uridine*

Exp.	Day 3			Day 4			Day 6		
		^{14}C			^{14}C			^{14}C	
	^3H	Alkali stable	Alkali labile	^3H	Alkali stable	Alkali labile	^3H	Alkali stable	Alkali labile
1. Cells	23,200	782	5,090		—	—	13,800	7080	7190
Medium	0	0	0		—	—	8,320	1310	161
2. Cells	28,800	791	7,200		—	—	6,340	3380	4830
Medium	0	0	0		—	—	20,600	3050	160
3. Cells	23,700	2230	8,760	20,400	6560	12,200	9,910	7340	9600
Medium	0	0	0	1,380	325	0	11,200	3400	21
4. Cells	55,400	2750	10,400	46,500	7340	14,000	24,100	7410	7570
Medium	0	0	0	3,650	285	249	20,800	2780	484
5. Cells	104,000	3700	17,800	84,500	9660	21,600	17,500	7130	7650
Medium	0	0	0	12,500	950	558	67,000	7000	514

2 to 8 × 10^6 lymphocytes in 2 ml of medium were cultured with 36 μg of E-PHA (Exp. 1, 3, and 4) or 20 μg of concanavalin A (Exp. 2 and 5), pulsed for 4 hr on day 3 with 3 μCi of [^3H]thymidine (6.7 Ci/mmol) and 1 μCi of [^{14}C]uridine (59.8 mCi/mmol), and washed and resuspended as in Table 1. On appropriate days duplicate tubes were harvested. Cl_3CCOOH precipitates were dissolved in 1 ml of 1 N NaOH and incubated at room temperature (23°). At the end of 18 hr, the tubes were neutralized with HCl, precipitated with Cl_3CCOOH, and centrifuged at 37,000 × *g* for 20 min. 1 ml of the supernatant (alkali labile radioactivity) was counted in 15 ml of Bray's solution. The pellet (alkali-stable radioactivity) was dissolved and counted as in Table 1; cpm are corrected for quenching and channel crossover.

from 3 cultures containing a total of 6 × 10^6 cells. These cells lost 85% of their ^3H-labeled DNA between days 3 and 6 (data not shown). Since 1 × 10^6 lymphocytes contain roughly 8 μg of DNA (4), lysis of 85% of the cells in these cultures would have released about 40 μg of DNA. A DNA concentration of 3 μg/ml present in the 12 ml encompassing the front peak in Fig. 1*A* would have been detected by measurement of the absorption at 260 nm; no significant A_{260} or A_{280} accompanied this peak, even though it contained 80% of the labeled DNA lost from the cells. This observation is added evidence that cell lysis alone cannot explain the loss of DNA into the culture medium. The amount of DNA actually lost must be fairly small compared to the total amount of DNA in the cells, which would explain the apparent paradox noted in Table 1; namely, much of the acid-precipitable [^3H]-thymidine left the cells, but the total DNA in the cells measured colorimetrically remained constant.

TABLE 4. *Treatment of acid-precipitable ^3H and ^{14}C radioactivity in the culture medium with DNase, RNase, and alkaline hydrolysis*

	Acid-soluble cpm		Acid-precipitable cpm	
	^3H	^{14}C	^3H	^{14}C
$^3H/^{14}C$ alone	7	7	1670	528
$^3H/^{14}C$ + RNase	5	5	1560	516
$^3H/^{14}C$ + DNase	7	5	1680	580
$^3H/^{14}C$ + Mg^{++} + DNase	1260	357	551	187
$^3H/^{14}C$ alkaline hydrolysis	21	15	1560	526
E. coli DNA alone	32	—	3140	—
E. coli DNA + RNase	22	—	3070	—
E. coli DNA + Mg^{++} + DNase	1950	—	1130	—

For enzyme incubations, 1 ml of acid-precipitable $^3H/^{14}C$ radioactivity from the Sepharose 6B front peak (Fig. 1*A*), or 5 μl of ^3H-labeled *E. coli* DNA (7.5 μg/ml) in 1 ml of Tris–NaCl–EDTA buffer, were incubated at 37° for 2 hr alone, with 5 μg of RNase, or with 5 μg of DNase in 5 mM $MgCl_2$. For alkaline hydrolysis, 1 ml of $^3H/^{14}C$ plus 0.2 ml of 5 N NaOH were incubated at 22° for 18 hr, then the solution was neutralized with 5 N HCl. At the end of the incubation period, 0.1 ml of bovine serum albumin, 50 mg/ml, and 0.9 ml of 12% Cl_3CCOOH were added. Acid precipitate and supernatant were prepared and counted as in Table 3.

Gel filtration chromatography on Sepharose 2B was used to estimate the molecular weight of DNA found in the culture medium (Fig. 1*B*). The column was calibrated with ^{14}C-labeled HeLa cell rRNA; elution volume was determined with ^3H-labeled *E. coli* DNA. DNA from lymphocyte-culture media eluted in a broad heterogeneous peak with a molecular weight ranging between 3 and 12 × 10^6. This heterogeneity may result from partial hydrolysis of the excreted DNA by DNase activity in the culture media.§ When this excreted DNA was examined by sucrose density gradient centrifugation (Fig. 1*C*) it formed a single sharp peak at a density of 1.055 g/cm^3. A single peak formed at this density even when centrifugation conditions were varied as follows: (*A*) 18 hr in the same system as in Fig. 1*C* except at 20° rather than 4°; (*B*) 3.5 hr in the SW50.1 rotor, 49,000 rpm, at 4° on a 5–20% linear gradient of sucrose in 0.1M NaCl–0.01 M Tris (pH 7.4)–0.001 M EDTA–0.1% sodium dodecyl sulfate; (*C*) 3.5 hr in the same system as in (*B*), except on a linear 5–10% sucrose gradient. We therefore conclude that this position represents density equilibrium. This type of behavior by such a large particle would be consistent only if DNA were complexed to protein or lipid. Further characterization of this particle is in progress.

§ Evidence that the medium contains DNase activity was provided by demonstrating that when *E. coli* DNA was added to the cultures it was rapidly degraded to an acid-soluble form. The reason why the excreted DNA was not degraded to an acid-soluble form in the medium may be that it is complexed to protein or lipid.

These data can most logically be interpreted as follows: Some lymphocytes in a culture stimulated with PHA synthesize DNA and undergo mitosis; if up to 3% of the cells divide every 4 hr during a 60-hr period, the number may reach 30–40% of the total cells present. However, since some cells may undergo more than one division during this time (16), the percentage of the original cells actually undergoing mitosis is probably less than 40%. Some cells die, although not necessarily those synthesizing DNA. Since these two variables are not in balance one would expect measurement of cell number and DNA content to give inconstant results, as has been true in the literature. Up to 70% of the cells become blast-like in appearance and synthesize DNA, but these cells excrete the DNA without necessarily undergoing mitosis. The quantity of DNA they make must be small in relation to total DNA in the culture.

While the explanation for the excretion of DNA by these cells is obscure, a number of possibilities can be considered: These could be cells prevented from successfully completing the steps necessary for mitosis by some deficiency in the culture medium. Thus, frustrated in their mitotic attempts, they might excrete the unnecessary DNA and return to a resting state. Alternatively, these cells could have a purpose for DNA synthesis other than mitosis (17). The DNA might be used within the cell to amplify a yet undefined function and then excreted when no longer useful. The observation that lymphoblasts in PHA-stimulated cultures revert to small, resting lymphocytes after 6–9 days (6) is consistent with such an explanation. Gene amplification has been demonstrated to occur during oocyte development in amphibia (18), and it may occur in antibody-synthesizing plasma cells (19). Recently, Lerner *et al.* have found that about 0.5% of the DNA in a long-term lymphocyte cell line is associated with the cytoplasmic membrane (20). Whether this species of DNA is related to the DNA excreted by the PHA-stimulated lymphocytes is unknown. Finally, it is possible that the DNA is excreted as a message directed to an unknown recipient. Since lymphocytes that undergo spontaneous transformation excrete DNA in a manner similar to lymphocytes stimulated with PHA and antigen, it is likely that this phenomenon is an intrinsic property of the lymphocyte rather than a special function induced by phytohemagglutinin or antigens.

While this manuscript was in preparation, an abstract appeared by Sarma and Rutman describing findings similar to ours (21). These investigators found that PHA-stimulated lymphocytes incorporated labeled thymidine into small fragments that were then converted to high molecular weight material. Subsequently, the labeled thymidine reappeared in light fragments that were then released into the medium in an acid-precipitable form.

We thank Drs. Boyce Burge and Philip W. Robbins for allowing the use of their Beckman L2-65 centrifuge, Dr. A. Kumar for generously providing [^{14}C]rRNA, Drs. Howard Green and Burge for most helpfully discussing the manuscript, and Dr. David Botstein for his valuable advice. This work was supported in part by the U.S. Navy and in part by grants from the United States Public Health Service (HE-00022, 5 T01 AM 05089, and R01-CA-08769) and the American Cancer Society (PRA-31). The opinions or assertions contained herein are those of the authors, and are not to be construed as official or reflecting the views of the Navy Department or Naval Service at large. S. K. is the recipient of a Research Career Development Award from the National Institutes of Health.

1. Nowell, P. C. (1960) *Cancer Res.* **20**, 462–466.
2. Hirschhorn, K. & Ripps, C. S. (1965) in *Isoantigens and Cell Interactions*, ed. Palm, J. (Wistar Institute Press, Philadelphia), pp. 57–63.
3. Schellekens, P. Th. A. & Eijsvoogel, V. P. (1968). *Clin. Exp. Immunol.* **3**, 571–584.
4. Hirschhorn, R., Grossman, J., Troll, W. & Weissman, G. (1971) *J. Clin. Invest.* **50**, 1206–1217.
5. Loeb, L. A. & Agarwal, S. S. (1971) *Exp. Cell Res.* **66**, 299–304.
6. Polgar, P. R. & Kibrick, S. (1970) *Nature* **225**, 857–858.
7. Mendelsohn, J., Skinner, S. A. & Kornfeld, S. (1971) *J. Clin. Invest.* **50**, 818–826.
8. Weber, T., Nordman, C. T. & Gräsbeck, R. (1967) *Scand J. Haematol.* **4**, 77–80.
9. Mendelsohn, J., Moore, D. E. & Salzman, N. P. (1968) *J. Mol. Biol.* **32**, 101–112.
10. Kornfeld, R. & Kornfeld, S. (1970) *J. Biol. Chem.* **245**, 2536–2545.
11. Tedesco, T. A. & Mellman, W. J. (1967) *Exp. Cell Res.* **45**, 230–232.
12. Howard, I. K. & Sage, H. J. (1969) *Biochemistry* **8**, 2436–2441.
13. Gottlieb, A. A., Taylor, L. & Seinsheiner, F. (1970) *Biochemistry* **9**, 4322–4328.
14. Donahue, H. A., Moore, V. G. & Little, J. R. (1970) *J. Immunol.* **105**, 592–603.
15. Kumar, A. & Warner, J. R. (1972) *J. Mol. Biol.* **63**, 233–246.
16. Marshall, W. H., Valentine, F. T. & Lawrence, H. S. (1969) *J. Exp. Med.* **130**, 327–343.
17. Pelc, S. R. (1968) *Nature* **219**, 162–163.
18. Brown, D. D. & Blackler, A. W. (1972) *J. Mol. Biol.* **63**, 75–83.
19. Little, J. R. & Donahue, H. A. (1970) *Proc. Nat. Acad. Sci. USA* **67**, 1299–1306.
20. Lerner, R. A., Meinke, W. & Goldstein, D. A. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 1212–1216.
21. Sarma, D. S. R. & Rutman, J. (1972) *Fed. Proc.* **31**, 607.