In contrast to nuclei containing a single Barr body, those containing two Barr bodies tend to be very large. This is particularly evident in cells with tetraploid, or 4*C*, DNA values. According to Atkin[9], in normal female cells with tetraploid, or 4*C*, DNA values, nuclei with two Barr bodies have arisen from tetraploid mitoses, whereas nuclei with single Barr bodies have arisen from diploid mitoses and have subsequently undergone DNA duplication. In accordance with this view, my data suggest that nuclei which have recently arisen from new polyploid mitoses tend to be very large indeed. It is interesting that the increase in size of nuclei with two Barr bodies is much less in cells containing the hexaploid, or 6*C*, DNA value (Table 3). The most likely explanation for this is that most of these cells did not arise from hexaploid mitoses but from triploid mitoses with subsequent DNA duplication. Triploid cells with three *X* chromosomes may form interphase nuclei containing two Barr bodies, although usually this only occurs in a small proportion[2,10,11] of cells. The fact that no cells with two Barr bodies were found among the triploid, 3*C*, class, can be correlated with the relatively low incidence (41 per cent) of single Barr bodies in this class. On the other hand, in the cells which had duplicated their DNA and were thus in the 6*C* class, the incidence of single Barr bodies was 62 per cent. A small proportion of such cells are likely to form two Barr bodies.

With regard to the size of polyploid cells, the available evidence suggests strongly that triploid mammalian cells are on an average larger than the corresponding diploid ones. In certain plants and invertebrates, polyploidy brings about large cell size without affecting the number of cells, so that the polyploid organisms are larger than normal ones[12]. In polyploid amphibia, an increase in cell size is accompanied by a reduction in cell number, so that the resulting organism is of about normal size[1]. In contrast to all these groups, polyploidy in mammals is highly lethal. The reason for this is not obvious. Bomsel-Helmreich[3] found that the only constant abnormality to be observed in triploid embryos of the rabbit was a retardation of development, that is, in spite of an increase in cell size the embryos were smaller than diploid ones of the same age. It is possible that the triploid condition also causes delayed development in human embryos. The birth weights of the three diploid/triploid mosaic patients described so far were below normal; that of the patient[4] from whom the present cell culture was derived was 5 pounds 13 ounces, or 2,640 g, the other two being 2,190 g (ref. 13) and 1,850 g (ref. 14) respectively. A fourth patient, who had multiple sex chromosomes as well as diploid triploid mosaicism, weighed 1,900 g at birth[15]. Low birth weights are also a feature of chromosomal abnormalities other than triploidy. Naye[16] has recently shown that the small size at birth of patients with trisomy 13/15, trisomy 18 and with mongolism (trisomy 21) is due to a subnormal number of cells in many organs. The possibility is therefore suggested that the addition to the normal mammalian karyotype of certain chromosomes or entire chromosomal sets may slow down the normal rate of cell proliferation[17].

While this possibility is still merely conjectural, there is every indication that studies of cell size and cell kinetics are likely to provide valuable leads in the search for the part played by different chromosome constitutions in mammalian development.

I thank Dr J. R. Ellis and Dr K. P. Lele for the cell cultures, Professor C. A. B. Smith for his assistance with the computations and Dr I. C. S. Normand for his co-operation. The integrating microdensitometer was provided by the Central Research Fund, University of London.

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Received May 29; revised June 26, 1968.

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Lymphocyte *in vitro* Cytotoxicity: Lymphotoxins of Several Mammalian Species

MOUSE lymphocytes can be induced *in vitro* by treatment with phytohaemagglutinin (PHA), or with rabbit anti-mouse serum, and by the mixed lymphocyte reaction, to produce material(s) which is toxic to *L* cells[1]. The term lymphotoxins (previously termed lymphocyte cytotoxic factor) is applied here to the toxic factor(s). We report that lymphocytes from several animal species, including man, can be stimulated with phytohaemagglutinin *in vitro* to release lymphotoxin-like materials.

The tissue culture medium used in all experiments was Eagle's minimal essential medium (MEM), supplemented with 5 per cent foetal calf serum, 0·2 μg/ml. glutamine, 100 U/ml. of penicillin, 100 μg/ml. of streptomycin, and 50 μg/ml. of mycostatin. Suspensions of small lymphocytes were prepared from the spleens of the *C57Bl/6* mouse, hamster and cat[2]. Human adenoid tissue was obtained immediately after surgical removal from healthy children. The whole tissue was rinsed three times with chilled MEM and was minced, after which repeated pipetting released the cells from the tissue fragments. Suspensions of 99 per cent viable small lymphocytes were obtained after differential centrifugation[3]. Human peripheral blood lymphocytes were prepared as follows: 10 ml. of heparinized whole blood was mixed with an equal volume of 6 per cent citrated bovine fibrinogen in 0·15 M NaCl, and placed upright in a water bath at 37° C. After 20 min the cells in the supernatant fluid were collected and washed twice by centrifugation (for 3 min at 300*g*) and were then resuspended in MEM. After removal of the final supernatant, the cell pellet was suspended in 0·75 ml. MEM and poured onto a 0·9 × 10 cm column filled with glass homogenizing beads which had been washed with 0·01 M *tris* buffer, *p*H 7·2. The column was allowed to stand at room temperature for 20 min. The non-adhering cells were eluted with 5·0 ml. of MEM, concentrated by centrifugation (3 min at 300*g*) and finally resuspended in 1·0 ml. MEM. A total viable cell count was made in a Neubauer chamber in 0·1 per cent eosin *Y* and a differential count was made on smears which were stained by the May–Grünwald–Giemsa method. (This is a modification of the technique personally communicated by W. Hildemann.) The cell preparations routinely consisted of 50–80 per cent of small and medium lymphocytes and the remainder of red blood cells.

Ten million lymphocytes in 2·0 ml. of MEM were established in separate tube cultures. Thirty μg of phytohaemagglutinin-*P* (Difco) was added to experimental tubes,

while control tubes received either no phytohaemagglutin or phytohaemagglutinin and no lymphocytes. These cultures were incubated at 37° C for 48 h in an atmosphere of 5 per cent CO_2 and 95 per cent air. The cells and cell debris were then removed by centrifugation at 1,000*g* for 20 min, and the culture fluid was passed through 'Millipore' filters of pore size 0·45μ. This fluid was placed on fresh individual tube cultures of mouse *L* cells, Moden bovine kidney (MBK) cells, and human (HeLa) cells, all of which were established 24 h before use at a density of 200,000 cells/tube. After 48 h incubation, these cultures were examined microscopically and cell viability was assayed by their ability to incorporate ^{14}C amino-acids into protein[1,2].

Table 1 shows the results obtained with lymphocytes from human peripheral blood, human adenoid tissue, mouse, hamster and cat spleen. It can be seen that phytohaemagglutinin stimulated the release of a toxic factor(s) from the lymphocytes of the various animal species, whereas no toxicity was caused by the medium from control, unstimulated lymphocytes or was caused by phytohaemagglutinin itself. It should be mentioned that of the nine peripheral blood lymphocyte samples which were obtained from different patients, seven responded in the same way as that reported in the table, while the other two demonstrated minimal toxicity. Human adenoid tissues from five different patients were tested and all responded in the manner reported. The data for mouse, hamster and cat spleen tissues are representative of many separate experiments.

The specificity of the lymphotoxins obtained from stimulated human and mouse lymphocytes was tested next. The experimental methods were as before, except that the medium was tested simultaneously on three cell lines: MBK, *L*, and HeLa. The results of these experiments (Table 2) show that all the cell lines were susceptible to the toxic action. Lymphocytes from several different mammalian species appear to release lymphotoxin-like materials when stimulated with phytohaemagglutinin, and these materials can act in various types of cells. Some of the chemical characteristics of mouse lymphotoxin have been described[3] and we are investigating the similarities and immunological distinctions between mouse and human lymphotoxin (unpublished results of Granger, Kolb and Williams).

Table 1. CYTOTOXIC EFFECT OF LYMPHOTOXINS FROM VARIOUS ANIMAL SPECIES ON MOUSE *L* CELLS

Source of lymphocytes	Phyto-haemagglutinin	Counts per min incorporated into *L* cell protein
Human peripheral blood		
Lymphs	+	5,640
Lymphs	−	21,328
None	+	19,980
Human adenoid tissue		
Patient *A*		
Lymphs	+	7,173
Lymphs	−	19,275
Patient *B*		
Lymphs	+	7,158
Lymphs	−	20,025
None	+	19,317
Mouse spleen		
Lymphs	+	1,082
Lymphs	−	19,742
None	+	17,941
Cat spleen		
Lymphs	+	5,025
Lymphs	−	19,913
None	+	21,105
Hamster spleen		
Lymphs	+	6,496
Lymphs	−	21,777
None	+	20,978

After 48 h incubation in the presence of the lymphotoxin medium, the cells were pulse labelled for 15 min with 2·0 ml. of a ^{14}C amino-acid hydrolysate, 0·30 μCi/ml.

Table 2. CYTOTOXIC EFFECT OF HUMAN AND MOUSE LYMPHOTOXIN ON SEVERAL MAMMALIAN CELL LINES

Source of lymphocytes ⟶			C.p.m. incorporated into cellular protein: Human peripheral blood	Mouse spleen
Cell line				
L cell	Lymphs	+ PHA	907	2,001
	Lymphs	− PHA	8,497	7,543
	None	+ PHA	8,141	7,601
MBK	Lymphs	+ PHA	2,325	2,136
	Lymphs	− PHA	6,471	4,206
	None	+ PHA	6,082	4,012
HeLa	Lymphs	+ PHA	1,487	2,541
	Lymphs	− PHA	9,743	9,243
	None	+ PHA	9,884	9,091

After 48 h incubation in the presence of lymphotoxin medium, the cells were pulse labelled for 8 min with 2·0 ml. of a ^{14}C amino-acid hydrolysate, 0·30 μCi/ml.

G. G. was supported by a grant from the US National Institutes of Health and a grant from the Cancer Research Coordinating Committee of the University of California. T. W. was supported by a grant from the National Institutes of Health, US Public Health Service.

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Received June 11; revised July 9, 1968.

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Relationship between Cell Size and Time of Initiation of DNA Replication

ROUNDS of DNA replication are initiated in *Escherichia coli* at different stages in the cell cycle of bacteria growing at different rates[1]. It is possible to calculate that the initiation of a round of DNA replication always takes place at a time when the cell mass/chromosome origin reaches a particular critical value. In other words, the mass at which initiation takes place is always an integral multiple of a particular mass. This constancy in turn provides an explanation for the increase in size of cells with increase in the rate of growth.

Schaechter, Maaløe and Kjeldgaard[2] showed that the size of cells of *Salmonella typhimurium* depended on the medium in which they were growing. The size of cells increases exponentially with the rate of growth in populations growing in different media. Thus log (cell mass) = *k* (growth rate).

Cooper and Helmstetter[1] have used synchronous populations of *Escherichia coli B/r* to show that the time of initiation of rounds of DNA synthesis relative to cell division varied with the growth rate of the cells. The time of initiation of DNA replication could be predicted very precisely by assuming that there was a constant interval of time between the initiation of a round of DNA replication and a subsequent division of the cell. In their experiments this time interval was close to 60 min for growth rates between one and three doublings/h. Because the time taken to replicate one chromosome was constant (40 min) at all rates of growth, rounds of DNA replication overlapped in cells growing with a generation time of less than 40 min. Thus in fast growing cells a new round of replication begins before the previous one has finished. In slowly growing cells with generation times greater than 40 min, there is a gap between the end of a round of replication and cell division.

These empirical rules are based on the observation that the time of initiation of DNA replication varies relative to the previous division of cells growing at different rates. No explanation was suggested as to why replication of DNA was initiated at a particular time. A combination of these observations with those of Schaechter *et al.*, on the average size of cells growing at different rates, however, reveals a remarkable constancy of cell mass at the time of initiation of replication. This is shown graphically in Fig. 1. This constancy in turn suggests possible mechanisms whereby the time of DNA initiation is determined.