ABNORMAL CYCLE PHASE DURATIONS IN POLYPLOID CELLS OR CELLS WITH IRREGULAR MITOSIS IN A HELA CELL POPULATION

H. FIRKET and A. F. HOPPER. From the Laboratoire d'Anatomie Pathologique, Université de Liège, Liège, Belgique. Dr. Hopper is on leave from the Department of Zoology, Rutgers University, The State University of New Jersey, New Brunswick, New Jersey 08903

As a preliminary to an attempt to establish a computer model population which might be used to analyze modifications induced by physical or pharmacological agents (1), we have had to remeasure the mean durations of G_2, S, and G_1, and especially their variability for a HeLa strain (wild type) by running several Quastler-Sherman curves (2). Duplicate cultures maintained on coverslips in Leighton tubes (Leighton Labs, Glen Ridge, N. J.) were given a 15 min pulse of tritiated thymidine (1 μCi/ml), washed repeatedly with prewarmed medium, and fixed at intervals; the coverslips were processed for radioautography according to the technique of Basrur et al. (3). 100 mitotic figures were scored on each slide by a standardized scanning. Two cultures were used for each interval of time. The obtained curves are similar to previous ones for the same strain (4, 5). A typical one is given in Fig. 1.

While reading the slides, we became aware that the labeling of polyploid or abnormal mitoses was different from that of the majority of the population of normal cells. We questioned whether these "abnormal" cells might contribute significantly to the spread of values for the cell cycle phase durations.

We considered as "abnormal," in this experiment, very large mitotic figures having at least twice the average number of chromosomes characteristic of the strain, and cells in which the chromosomes separated abnormally into three or more groups (tri- and tetrapolar mitoses). Usually, these cells were also abnormally large. Pyknotic mitoses were not included in the counts. Doubtful figures were always ascribed to the "normal" groups. About 11–16% of all mitotic figures scored belong to the "abnormal" subpopulation, but they constitute a variable fraction of the labeled or unlabeled compartments (Fig. 2). These abnormal figures become an increasing fraction of the un-

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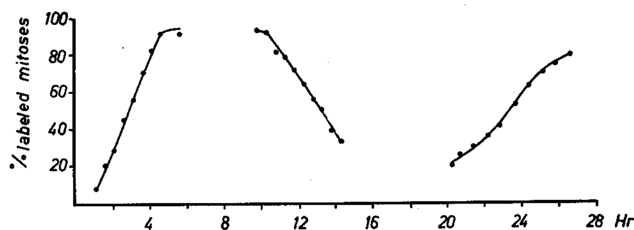

FIGURE 1 Quastler-Sherman curve (after a 15 min thymidine-^{3}H pulse) for total population.

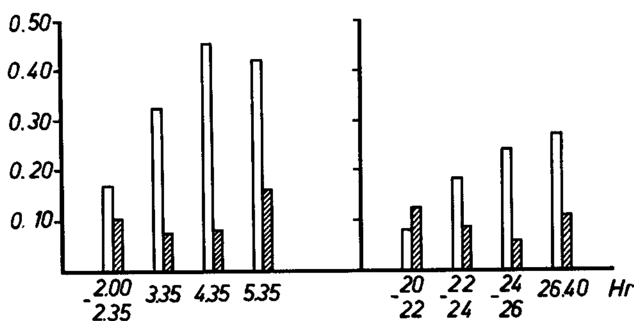

FIGURE 2 Ratio of "abnormal" mitoses among labeled (stippled columns) or unlabeled (white columns) mitoses at various periods after the pulse.

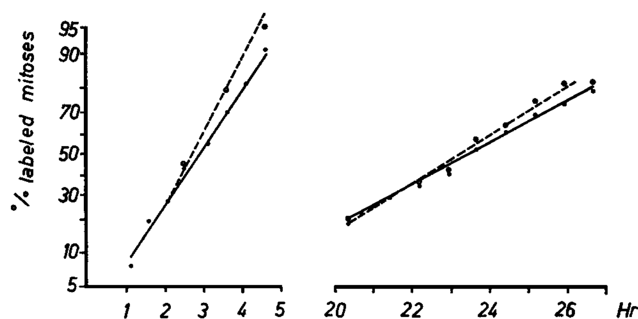

FIGURE 3 Per cent labeled mitoses plotted on probability paper for "total" (full line and points) or "normal" (broken line and circles) populations.

labeled mitoses compartment during the first rise in per cent of labeled mitoses after the pulse. This means that they have a longer $G_2 + \frac{1}{2}$ M than the "normal" subpopulation. Similarly, during the second rise in per cent of labeled mitoses, they become again a larger fraction of the unlabeled compartment, denoting a delay in entering the second cycle after the pulse. When the labeled mitoses curve is on its descending part (10 to 15th hr), a reverse effect is observed. The fraction of "abnormals" rises from 13% at 11.20 hr to 25% at 14.20 hr, among labeled mitoses, whereas it decreases to very low values among the unlabeled mitoses. Thus large or abnormal mitotic figures remain labeled longer, on the average, than "normal" ones. However, we concentrated our study on the two rising parts of the curve.

Abnormal mitoses are not numerous enough on the slides to count them separately and draw a precise Quastler-Sherman curve for this subpopulation, but they can be subtracted from the total population so that the percentage of labeled mitoses can be determined exclusively on "normal" cells. The durations measured by these counts have been plotted on probability graph paper. Their distribution is reasonably close to a gaussian distribution so that a best fitting straight line could be calculated in each case (Fig. 3). The mean durations (abscissa of the point where the straight line reaches 50% of labeled mitoses) and standard deviations can be read directly on the graph or, better, calculated from the equations of the straight lines. These values are given in Table I.

The mean durations are not considerably shifted because the abnormal subpopulation is only a small part of the total. For the two rises in the curve, the 50% labeled mitoses mark is reached about 7 and 25 min earlier. These differences are

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rather small. But, in each case, the lower part of the rise is hardly modified whereas in the upper part the differences become larger. The "normal" subpopulation curves reach higher values (up to 95% in the first rise; up to 82% in the second) and reach them earlier. This tends to decrease the spread of values both for G_2 and for the whole cycle. From these results and the relative frequency of the two subpopulations, a simple calculation shows that the "abnormal" cells increase their labeled mitotic index later than the "normal" population with a delay of 1 to 3 hr and a much wider scatter. The limited amount of data that can be plotted separately tends to confirm this.

DISCUSSION

The fact that there are at least two populations with different cell cycle parameters in our cultures is not due to the use of the wild type HeLa strain instead of one of the cloned strains. Painter and Drew (4) already saw a wider scatter of values (especially for G_2) in the HeLa S_3 line than in the wild type line. Recloning experiments in our laboratory have also led to higher heterogeneity. It is difficult to ascribe the lag specifically to one phase of the cell cycle. It is probable that a longer mitosis is responsible for at least a part of the observed difference. In a recent cinematographic record of the duration of mitosis in HeLa cells, Rao and Engelberg (6) do not mention any special characteristics of the cells displaying a long ($\pm$ 100 min) mitotic duration. However, in their older study, Moorhead and Hsu (7) have shown that the spread of values for the duration of mitosis is exceptionally large in "aberrant" mitoses (mainly multipolar). They found the mean duration of mitosis to be 96 min longer for "aberrants" than for "non aberrants." Oftebro and Wolf (8) studied mitosis in bi- and multinucleated cells, which often give rise to multipolar mitoses, and they noted also longer mitotic durations for these cells (average delay: 43′).

It is possible that in several other cases the result that the slopes of Quastler-Sherman curves are extended or even impossible to separate properly, is due to the fact that several morphologically indistinguishable populations (but with different cell cycle parameters) are mixed. Perry and Swartz (9) have brought evidence for the presence of such a population with a greatly extended G_2 in normal adult liver.

To include or not such aberrant populations in the measurements of cell cycle parameters depends on the use to be made of the obtained values. In our case, it is unlikely that the "abnormal" group represents a subpopulation genetically isolated from the "normal" cells. After a tri- or tetrapolar mitosis, daughter cells usually do not divide further. No stable strain with high ploidy has ever been isolated from the HeLa line, but giant polyploid cells reappear soon after cloning. So it is likely that this "abnormal" group loses cells regularly, and that cells from the "normal" stock replenish it continuously.

TABLE I

Mean Durations

	First rise	First fall	Second rise
		hr (decimalized)	
Total population	2.85 ± 1.31	13.57 ± 1.77	23.58 ± 3.81
Normal population	2.73 ± 1.11	13.30 ± 1.62	23.13 ± 3.16

SUMMARY

Large polyploid mitoses and multipolar mitoses show a delay in labeling [in comparison with] "normal" HeLa cells, when Quastler-Sherman curves are run. This delay does not affect considerably the mean values for the durations of G_2, S, or total cycle time, but tends to increase the spread of values. This effect is probably due in part to the fact that mitosis lasts longer in these "abnormal" cells.

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REFERENCES

1. TIBAUX, G., H. FIRKET, and A. F. HOPPER. 1969. Cell Tissue Kinetics. 2:333.
2. QUASTLER, H., and F. G. SHERMAN. 1959. *Exp. Cell Res.* **17**:420.
3. BASRUR, P. K., V. R. BASRUR, and J. W. GILMAN. 1966. *Nature (London)*. **212**:424.

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4. PAINTER, R. B., and R. M. DREW. 1959. *Lab. Invest.* **8**:278.
5. TERASIMA, T., and L. J. TOLMACH. 1963. *Exp. Cell Res.* **30**:344.
6. RAO, P. N., and J. ENGELBERG. 1968. *Exp. Cell Res.* **32**:198.
7. MOORHEAD, P. J., and T. C. HSU. 1956. *J. Nat. Cancer Inst.* **16**:1047.
8. OFTEBRO, R., and I. WOLF. 1967. *Exp. Cell Res.* **48**:39.
9. PERRY, L. D., and F. J. SWARTZ. 1967. *Exp. Cell Res.* **48**:155.

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