The Incidence of Abnormal Mitoses in Cultured Chick Embryo Fibroblasts

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Mitotic abnormalities, particularly those involving the chromosome number, have been reported for human tissues (1) as well as for the mouse, rat, guinea pig, and dog (2), and are frequently encountered in neoplastice tissues (3). Such aberrations have recently been observed in cells of human origin that had been grown in tissue culture (4) and these same strains were subsequently shown to give rise to neoplastic growths when injected subcutaneously into human subjects. It would appear that the exposure of these cells to the artificial environment of the tissue culture preparation induced cytological changes which gave rise to multinucleated cells, multipolar mitoses, and aberrant chromosome numbers and also helped to complete the transition from a normal to a neoplastic state.

Aberrant mitoses (see figs.) involving pyknosis, polyploidy, multipolar spindles, as well as displaced, lagging, sticky, and fragmented chromosomes are invariably encountered in cells of tissues that have been experimentally exposed to any of a great variety of physical and chemical factors that may be radiomimetic in nature or that may exert a mitotic poisoning action on the spindle apparatus. However, a relatively high frequency of abnormal mitotic figures may also be encountered in untreated cells (5) and these should not be ignored in the study of the control groups of experiments involving the use of agents which affect the mitotic process.

In the work reported here, mitotic counts were made on groups of ten separate experiments employing fibroblasts grown from 8 to 10 day old embryonic chick heart tissue cultured for 48 hours at 37.5°C. in a hanging drop culture using Tyrode-embryo extract mixture as medium. In each of ten groups, all cultures were prepared from a single embryonic heart between the hours of 2:00 and 6:00 P. M. and they were given fresh media at 24 hours. At 48 hours they were fixed and stained in Aceto-Orcein solution. In the counting process all abnormal divisions were noted and their percentage was calculated.

In Table I may be seen the percentage of abnormal mitoses found in each of the ten groups of cultures.

The most common abnormality encountered was a lagging or displaced chromosome. Broken and fragmented chromosomes were next most frequently found.

A separate study was made to determine any possible diurnal effect. A number of cultures were prepared and then fixed and stained as before at intervals of from 24 to 48 hours after the time of their preparation. Table II presents the findings in this study.

While the mean of 18.2% was higher than that given in Table I, it was within the range of deviation. There was first a slight increase followed by a decrease in the percentage of abnormalities. The value at 48 hours of 13.3% approximates very nearly the mean value obtained at 48 hours for the groups in Table I.

Whether these abnormalities are inherent qualities of these tissues or whether they are induced by stagnation or by the abnormal environment of the medium cannot be ascertained at this time. Since in each group all the cultures had been prepared from a single embryonic heart, it appears that the tendency for the development of abnormal mitoses may vary from one animal to the next.

REFERENCES CITED

1 S. Timonen and E. Therman. Variations of the somatic chromosome number in man. Nature *166*: 995-996, 1950.

2. T. C. Hsu and C. M. Pomerat. Aneuploidy in mammalian cells. J. Morph. *93*: 301-330, 1953.

3. S. Timonen. Mitosis in normal endometrium and genital cancer. Acta Obst. Gyn. Scand. *31*: Suppl. 2: 1-50, 1950.

4 A. E. Moore, C. M. Southam, and S. B. Sternberg. Neoplastic changes developing in epithelial cell lines derived from normal persons. Science *124*: 127-129, 1956.

5. A. A. Katzberg. The mechanism of action of steroidal compounds on the process of mitosis. Doctoral dissertation (University of Oklahoma), 1956.

TABLE I

FREQUENCY OF ABNORMAL MITOSES IN FIBROBLASTS OF EMBRYONIC CHICK HEART

Group Number	1	2	3	4	5	6	7	8	9	10
Percentage of Abnormal Mitosis	7.9	10.2	10.4	11.3	12.1	12.2	12.9	14.4	15.8	18.5

Mean=12.57

TABLE II

DIURNAL EFFECT ON THE PERCENTAGE OF ABNORMAL MITOTIC FIGURES

Time	24 hrs.	26 hrs.	28 hrs.	31 hrs.	48 hrs.
Percentage of Abnormal Mitosis	18.5	20.5	20.0	18.9	13.3

Mean=18.2

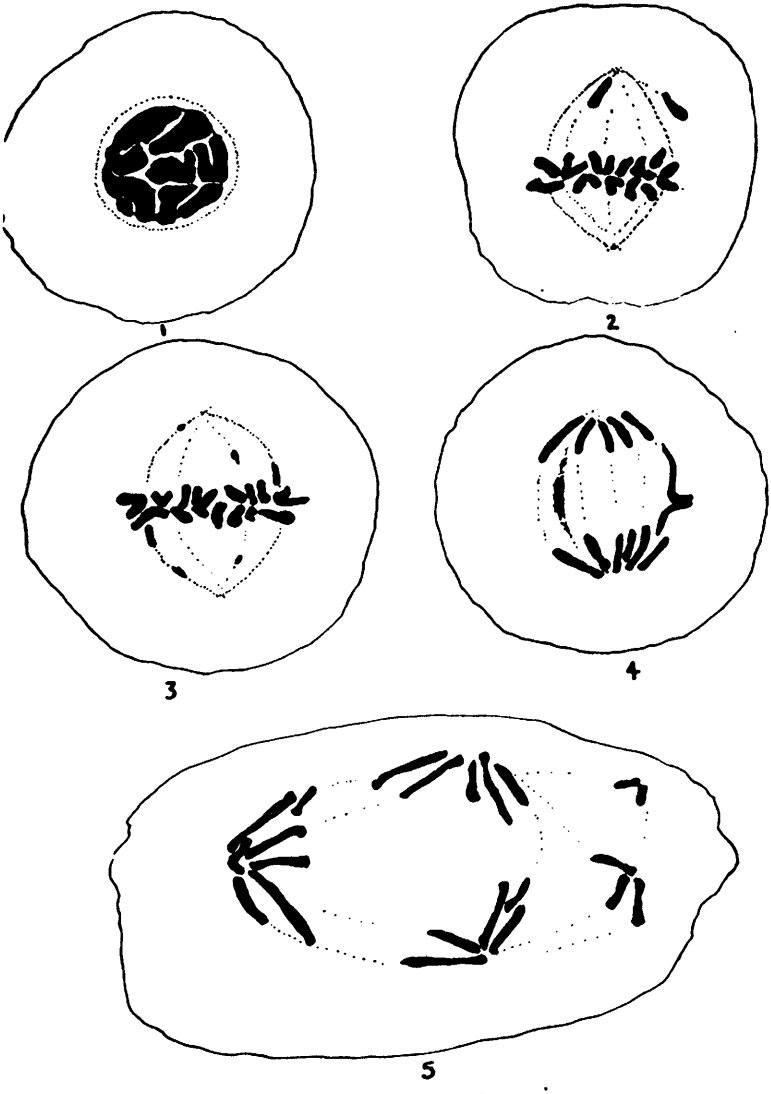

FIG. 1. pyknosis—confluence of chromatin
FIG. 2. metaphase with lagging and displaced chromosomes
FIG. 3. metaphase with broken and fragmented chromosomes
FIG. 4. anaphase with lagging chromosome and sticking chromosomes forming a bridge
FIG. 5. anaphase with multipolar spindle