

EFFECT OF CONTAMINATION BY MYCOPLASMAS AND DECONTAMINATION OF CELL LINES ON PHASE RELATIONSHIPS IN MITOSIS

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Relative percentages of the phases of mitosis were studied for 3 years in cell lines contaminated with mycoplasmas and after decontamination. Microorganisms of the family Mycoplasmataceae were shown to affect the phase distribution of mitosis.

The duration of mitosis and of its individual stages is constant for cells of each type [6, 9]. Each species of cell thus has its own characteristic ratio between the phases of mitosis. This index is so stable that the results obtained by different investigators working with the same object at different times and in different laboratories coincide [2].

The object of the present investigation was to determine whether the mitotic phase ratio differs in cell cultures depending on whether they are infected or not by microorganisms of the Mycoplasmataceae family.

EXPERIMENTAL METHOD

The distribution of mitosis by phases was studied in cell lines MĖD-14 and MĖD-15 obtained as described previously [4] and grown on nutrient medium consisting of two parts Eagle's medium and one part of 0.5% lactalbumin hydrolyzate solution in 10% bovine serum without antibiotics.

Microorganisms of the Mycoplasmataceae family were found in 1966 in lines MĖD-14 and MĖD-15, just as in the overwhelming majority of cultivated cell lines.

At the beginning of 1967 some strains of the MĖD lines were decontaminated from mycoplasmas by V. V. Neustroeva by means of antibiotics using a method devised by herself [7]. Since that time cell lines MĖD-14 and MĖD-15 have cultivated in two parallel variants: contaminated with microorganisms of the Mycoplasmataceae family and decontaminated.

To study the distribution of mitosis by phases the cells of both variants were grown simultaneously in penicillin flasks with half coverslips, the rate of seeding being 30,000 cells to 1 ml medium. The cells were fixed in 1:3 acetic alcohol 20, 28, 36, 44, and 52 h after the last subculture and stained with aceto-orcein. The indices of the phases of mitosis were determined in percentages of the total number of mitotically dividing cells. Altogether 500 mitotic figures were analyzed in each preparation. In the course of 3 years 16,500 mitoses were analyzed for each variant of MĖD-14 and 15,000 for each of the two variants of MĖD-15.

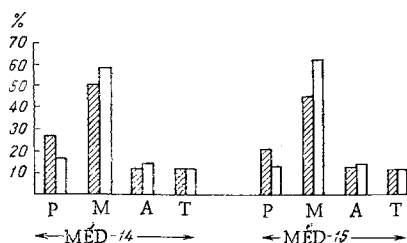


Fig. 1. Relative percentages of phases of mitosis in cell lines MĖD-14 and MĖD-15: P) pro-phases; M) metaphases; A) anaphases; T) telophases; unshaded columns denote decontaminated, shaded columns contaminated variants.

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EXPERIMENTAL RESULTS

The distribution of mitosis by phases in the variants of lines MÉD-14 and MÉD-15 are given in Fig. 1. The indices of the late phases of mitosis (anaphase and telophase) were the same in both contaminated and decontaminated variants of lines MÉD-14 and MÉD-15, whereas the indices of the early phases of mitosis differed in the contaminated and decontaminated variants. The difference was particularly marked in the prophase indices. The prophase index of the contaminated MÉD-14 variant was 58% higher than the prophase index of the decontaminated variant of the same line. The prophase index of the contaminated MÉD-15 variant was 61% higher than the corresponding index for the decontaminated variant.

Higher values of the metaphase indices were obtained in the decontaminated variants of both MÉD lines than in the contaminated variants (Fig. 1). The same ratio between the phases of mitosis as is indicated in Fig. 1 was obtained at all times during the 3 years of observations and it was independent of the value of the mitotic index of the cultivated variants of the MÉD lines. It is not yet known how the species of mycoplasma present in lines MÉD-14 and MÉD-15 influences the duration of the mitotic cycle, of mitosis as a whole, and of its individual phases. According to statistical laws, the probability of finding a particular phenomenon in a particular state is directly proportional to the time during which it can be found in that state [10]. Accordingly it can be taken that prophase occupies a longer time in contaminated variants of lines MÉD-14 and MÉD-15 than in decontaminated variants.

Changes in the ratio between the phases of mitosis have often been studied previously under different experimental conditions on cell cultures without regard to the presence or absence of mycoplasmas [1, 8].

The results now obtained indicate that when such experiments are carried out on cell cultures the presence of mycoplasmas must be allowed for and steps must be taken to ascertain whether infection by mycoplasmas has taken place if the cell cultures were sterile at the beginning of the experiments, for spontaneous infection by mycoplasmas may itself contribute to a change in the ratio between the phases of mitosis quite apart from the action of any additional factors on the cells.

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