MITOTIC CYCLE OF CIRCULATING LEUKOCYTES OF THE MONKEY Macaca mulatta

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A population of cultivated circulating lymphocytes of these monkeys is heterogeneous with respect to duration of the mitotic cycle; different types of cells begin the first mitosis asynchronously. The mitotic cycle in the period of blastogenesis lasts for between 40 and 72 h. Cultures fixed after growth for 72 h consist of a mixture of the first and second cell generations. The duration of individual stages of the mitotic cycle is established for cells starting the first mitosis after growth for 50 and 72 h.

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Cultures of human and other mammalian leukocytes are extensively used in cytogenetic research. Leukocytes of monkeys, animals closest to man in their anatomical and physiological properties, are of special interest. No data concerning the duration of the mitotic cycle of monkeys' leukocytes when cultivated in vitro could be found in the accessible literature.

In this investigation the kinetics of incorporation of thymidine-H^3 into chromosomes of leukocytes of the monkey Macaca mulatta and variations in the duration of the mitotic cycle were studied.

EXPERIMENTAL METHOD

Experiments were carried out on circulating leukocytes of male monkeys of species M. mulatta aged 8-11 years. The leukocytes were cultivated in pencillin flasks without addition of antibiotics by the method of Moorehead and co-workers [3] with certain modifications. To stimulate mitotic division of the leukocytes, phytohemagglutinin (Wellcome) was used. DNA synthesis was studied by autoradiography with thymidine-H^3 (specific activity 1200 μCi/mmole) in a concentration of 1 μCi/ml. The label was introduced for 25 min at 38°, after which the leukocytes were washed twice in medium No. 199, using centrifugation. In the

TABLE 1. Incorporation of Thymidine-H^3 into Circulating Leukocytes of Monkeys M. mulatta (cultures fixed 50 h after seeding)

Age of culture at moment of labeling (in h)	Number of metaphases analyzed	Number of labeled metaphases	
		absolute	%
0	500	0	0
20	500	0	0
30	410	397	96,8
40	600	562	93,7
46	700	110	15,7

Note. Two chromatids of metaphase chromosomes were labeled.

TABLE 2. Incorporation of Thymidine-H^3 into Circulating Leukocytes of Monkeys M. Mulatta (cultures fixed 72 h after seeding)

Age of culture at moment of labeling (in h)	Number of metaphases analyzed	Number of labeled metaphases		Percentage of metaphases with chromosomes	
		absolute	%	having one chromatid labeled	having two chromatids labeled
0	500	0	0	0	0
10	500	0	0	0	0
20	500	0	0	0	0
30	516	140	27,1	3,9	6,9
40	725	573	79,0	23,2	48,3
50	725	710	97,9	0,8	95,0
62	1058	966	91,3	0	91,3
66	500	276	55,2	0	55,2
67	710	240	33,8	0	33,8
68	515	94	18,2	0	18,2

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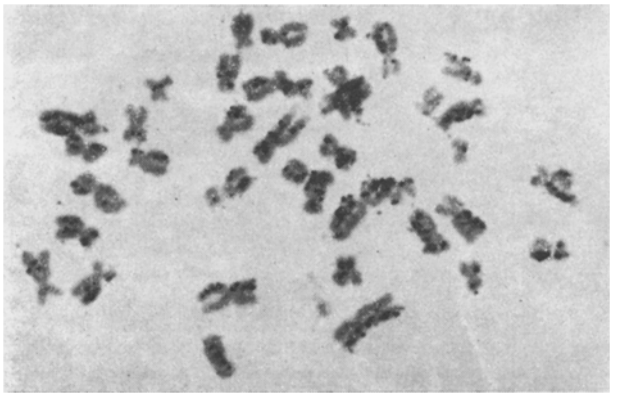

Fig. 1. Metaphase plate with chromosomes having one labeled chromatid.

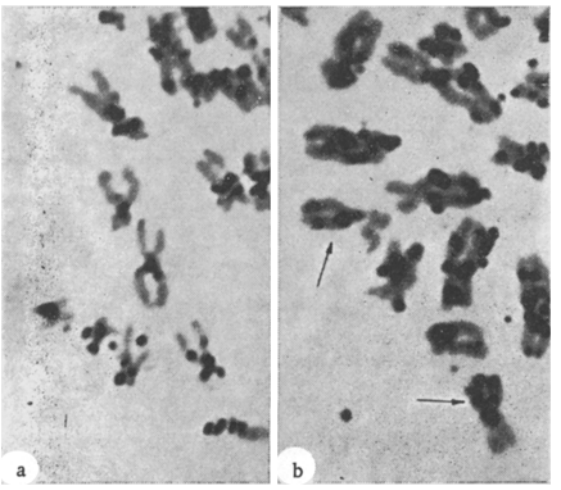

Fig. 2. Metaphases with both chromatids labeled. a) Label localized mainly in centromere region; b) asynchronous replication of DNA in homologous chromsomes (arrows point to label in both chromatids of chromosome in homologous segments).

Fig. 3. Sister exchange between chromatids (marked by arrow).

experiments of series I thymidine-H^3 was added either immediately after cultivation or 20, 30, 40, and 46 h later. Cultures were fixed 50 h after seeding with a mixture of glacial acetic acid and methyl alcohol (1:3). Colchicine was added 4 h before fixation. In the experiments of series II thymidine-H^3 was introduced immediately after seeding and 10, 20, 30, 40, 50, 62, 66, 67, and 68 h thereafter. The cultures were fixed 72 h after seeding, colchicine having been added to them 4 h previously. In some experiments thymidine-H^3 was added to a 40-h culture. Some flasks with cultures were fixed 50 h after seeding, others 72 h after seeding. The preparations were stained by the Feulgen method, covered with type M (NIKFI) photographic emulsion, and exposed for 18 days. After development of the autoradiograph, they were counterstained by Unna's method. The background of the autoradiograph was slight (0.7 grain per interphase cell). Metaphase plates with more than 5 grains per cell were regarded as labeled. Distribution of the label among chromatins was analyzed by the usual method [2].

EXPERIMENTAL RESULTS

As the results in Table 1 show, if thymidine-H^3 was added immediately after seeding and 20 h after the beginning of cultivation, none of the metaphase chromosomes contained label, i.e., at the moment of addition of thymidine-H^3 the cells were in stage G_1. Radioactive label, although to a slight degree, was nevertheless incorporated into DNA of the chromosomes when added to a 30-h culture. The number of labeled metaphases when thymidine-H^3 was added at this time was 96.8%. Label was actively incorporated into DNA of the chromosomes when the isotope was added to the culture 40 h after seeding. The number of labeled metaphases if thymidine-H^3 was added at this time was 93.7%, most of them being labeled intensively. Consequently, 6-10 h before the beginning of mitosis the cells were in stage S. If thymidine-H^3 was added to the culture 46 h after seeding and the material was fixed after 50 h, the number of labeled metaphases was 15.7%, but many of them were labeled only slightly. Consequently, if the cells were fixed 4 h after addition of thymidine-H^3, cells which had been in stage G at the moment of labeling (84.3%) were collected in metaphase.

In the experiments of series II, when cultures were fixed 72 h after seeding, incorporation of the label began 30 h after the beginning of cultivation. The number of labeled metaphases was 27.1% (Table 2). All

the dividing cells were labeled weakly. If thymidine-H^3 was added 40 h after the beginning of cultivation, the number of labeled metaphases increased strongly to 79%. Cells were labeled with different intensities. The intensely labeled metaphases included many cells whose chromosomes contained only one labeled chromatid (Fig. 1). The number of cells having one labeled chromatid was 23.2% (Table 2), i.e., about one-third of all the labeled mitoses by 72 h had entered stage X_2. Both chromatids were regarded as labeled if the grains of silver lay distinctly in homologous areas of the chromatids of the chromosome and their presence could not be attributed to the emission of radioactive particles from one chromatid or to the presence of a definite background. The latter possibility was ruled out if the background was slight, provided that only metaphases which contained not one, but several chromosomes having two labeled chromatids were taken into account (Fig. 2b).

When the label was added 50 h after the beginning of cultivation, the number of labeled metaphases fixed after 72 h was 97.9%. There were few metaphases whose chromosomes had one labeled chromatid (0.8%). Cells in stage G_2 (X_1) were not labeled when thymidine-H^3 was added at this time, and some of them were evidently capable of starting the second mitosis toward 72 h, because they had more time to pass through the second cycle than cells in stage S. That is possibly why the total number of labeled cells was less than 100% (97.9%). This must be allowed for when the duration of period G_2 max is established.

When label was added 62 h after seeding the number of labeled metaphases in the case of fixation after 72 h was 91.3%. Both chromatids contained label in all metaphases (Fig. 2b). The number of labeled metaphases 66, 67, and 68 h after the beginning of cultivation fell sharply to 55.2, 33.2, 18.2% respectively. At the end of the period of DNA synthesis label in most chromosomes was localized in the region of the centromere (Fig. 2a).

When label was added 68 h after the beginning of cultivation, mainly cells in the prophase and prometaphase stages of mitosis contained the label. The mean duration of period M + G_2 was 5 h, although for some cells it was rather more than 10 h. In cells with both chromatids labeled, granules were distributed unevenly between sister chromatids. It was apparent on marker chromsomes with constrictions in their short limbs (Fig. 2b). that the homologs are characterized by asynchronism of DNA replication. A heterogeneous degree of labeling of metaphase cells was observed after addition of thymidine-H^3 at the same time of cultivation. This shows that the duration of mitotic cycles in cultures of monkeys' leukocytes varies. Different types of cells apparently begin blast-transformation and DNA synthesis at different times. It can also be considered that the time required for transformation of lymphocytes into cells of blast type varies from one cell to another. The cells thus begin mitosis asynchronously.

The first mitoses in culture of monkeys' leukocytes began to appear, just as in a culture of human leukocytes, 40 h after the beginning of cultivation [1], and their number gradually increased thereafter. When cultures were fixed 72 h after the beginning of cultivation, mitoses were far more numerous than in cultures fixed 50 and, in particular, 40 h after seeding.

Observations on distribution of grains among chromatids showed that the second mitotic cycle can be passed through in a shorter period of time, so that by 72 h the cells have begun stage X_2. This is shown by the fact that when label was added 40 h after the beginning of cultivation and the cultures were fixed 72 h after seeding, label was found in one chromatid in about 30% of labeled metaphases. When thymidine-H^3 was added 50 h after the beginning of cultivation and fixation carried out after 72 h, grains in nearly all the intensely labeled cells were distributed above both sister chromatids. Probably during this short time interval cells synthesizing DNA had not succeeded in starting mitosis for a second time by 72 h. The experiments in which thymidine-H^3 was added to 40-h culture and the cells were fixed 50 and 72 h after seeding showed that the number of grains in metaphases fixed at 50 h was 2.13 times greater than in metaphases having one labeled chromatid and six at 72 h. Hence, lymphocytes converted into blast cells in the first cycle in vitro (in the lag phase) gave rise to daughter cells with a shorter mitotic cycle, with a duration of about 22 h (72-50). As was pointed out above, when thymidine-H^3 was added to the culture 30 h after seeding, the cells were labeled weakly. Among cells labeled 40 h after the beginning of cultivation were a very few metaphases containing a small number of grains. In these weakly labeled metaphases it is difficult to determine the character of distribution of the grains above the sister chromatids, so that the doubtful cases were not included in columns 5 and 6 of Table 2. The uneven distribution of grains was evidently due to asynchronism of DNA replication in the sister chromatids, and also to exchanges between labeled and unlabeled chromatids. There are indications that such exchanges can considerably complicate the picture [4]. In intensely labeled cells they are readily demonstrated (Fig. 3).

LITERATURE CITED

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