The Cell Cycle of an Established Line of *Drosophila melanogaster* Cells in vitro

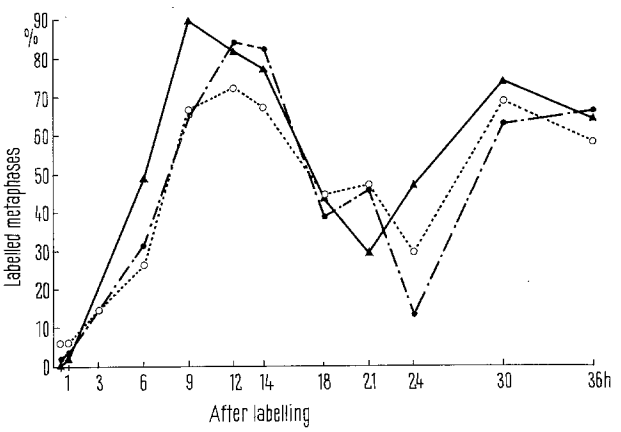
Several cell lines have been established by ECHALIER and OHANESSIAN[1] from primary cultures of embryonic cells of *Drosophila melanogaster*. This material is of interest for studies on several biological problems. A knowledge of the cell cycle and of karyotypic variation in these cells is a useful basis for any further investigation. Moreover, it is of interest to compare this kind of data with those previously obtained on embryonic cells of the same species in short-term cultures by DOLFINI and TIEPOLO[2]. In this note we report on the cell cycle and the duration of its different phases of one of these cell lines, determined by means of pulse labelling with tritiated thymidine.

We recall that DNA is synthesized during only a part of the interphase which is called the S period. Between the end of telophase and the beginning of S there is a period called G_1 and between the end of S and the beginning of prophase a period called G_2.

Material and method. Cell cultures: The analysis was performed on the line named Kc. At the time of the experiments, the line had been cultivated for 15 months with 27 passages. A karyotypic analysis of the cells had previously shown that they were diploid (ECHALIER and OHANESSIAN[1]). The cells were grown at 26 °C on coverslips in Leighton tubes in an appropriate medium (ECHALIER, OHANESSIAN and BRUN[3]) supplemented with 20% foetal calf serum.

Determination of the periods of the cell cycle: We followed the pulse labelling method, which consists in fixing samples of the cell population at various intervals after a short exposure to ^{3}H-thymidine and in plotting the percentage of labelled metaphases scored against the time after the pulse (HOWARD and PELC[4]). ^{3}H-thymidine (Amersham, specific activity 3 C/m*M*) at a final concentration of 0.5 μC/ml was added to 3 series of 12 tubes; each series was obtained by transferring the cells of one culture flask 2 days before the beginning of the experiment. The cells were washed twice in Locke's solution at 26 °C and incubated in fresh medium with cold thymidine at a concentration 100 times higher than that of ^{3}H-thymidine. At various intervals after labelling ($^1/_2$, 1, 3, 6, 9, 12, 14, 18, 21, 24, 30, 36 h) the cells were treated with hypotonic solution (1% sodium citrate) for 30 min, fixed with a 3:1 mixture of methanol and glacial acetic acid for 30 min, air-dried and stained with acetic orcein. In each slide, about 50 metaphases were scored. Autoradiographs were prepared by the stripping film method, using AR 10 Kodak film and exposed for 12 days. The percentage of labelled metaphases was determined, scoring a cell as labelled when the ratio between the number of grains on the cell and the number of background grains was higher than one. The amount of background was estimated by counting the number of grains over an adjacent area of approximately the same size.

Results and discussion. The results of the 3 parallel experimental series are shown in the Figure and in the Table. The average durations of G_1, S and G_2 phases were estimated at 1.8, 10.0 and 7.2 h respectively. The



Percent of labelled metaphases plotted against the time after the pulse. ○—○—○, experiment I; ●-·-●-·-●, experiment II; ▲—▲—▲, experiment III.

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[2] S. DOLFINI and L. TIEPOLO, II Intern. Colloq. Invertebrate Tissue Culture (Istituto Lombardo, Milan 1968), p. 182.
[3] G. ECHALIER, A. OHANESSIAN and G. BRUN, C. r. Acad. Sci., Paris *261*, 3211 (1965).
[4] A. HOWARD and S. R. PELC, Heredity *6* (suppl.), 261 (1953).

Number of metaphases scored at each time interval and percentage of labelled metaphases

After labelling (h)	Experiment I			Experiment II			Experiment III		
	No. of scored metaphases	No. of labelled metaphases	Labelled metaphases (%)	No. of scored metaphases	No. of labelled metaphases	Labelled metaphases (%)	No. of scored metaphases	No. of labelled metaphases	Labelled metaphases (%)
$^1/_2$	50	3	6.0	51	1	2.0	50	0	0.0
1	49	3	6.1	57	2	3.5	54	1	1.8
3	55	8	14.5	–	–	–	–	–	–
6	56	15	26.8	48	15	31.2	55	27	49.1
9	48	32	66.7	29	19	65.5	49	44	89.8
12	51	37	72.5	51	43	84.3	50	41	82.0
14	52	35	67.3	52	43	82.7	49	38	77.5
18	54	24	44.4	54	21	38.9	25	11	44.0
21	51	24	47.1	52	24	46.1	51	15	29.4
24	51	15	29.4	53	7	13.2	55	26	47.3
30	52	36	69.2	52	33	63.5	51	38	74.5
36	53	31	58.5	39	26	66.7	48	31	64.6

duration of the total generation time or G_T was 18.8 h on an average.

The findings concerning the length of G_2 are confirmed by experiments of continuous labelling. In a series of cultures, labelled for 3, 4, 5, 6, 7, 8, 9 h, set up for the study of the replication patterns of the heterochromatin, the first labelled metaphases were found in the 7–8 h samples.

In a previous experiment of pulse labelling on short-term cultures of embryonic cells of *Drosophila melanogaster*, a similar duration of the G_2 period of 7 h was determined (DOLFINI and TIEPOLO[2]). The length of S and G_2 appeared to be rather constant in each of the present experiments while those of G_1 showed a higher variability. This finding agrees with the general observations of DEFENDI and MANSON[5], of TERASIMA and TOLMACH[6] and of SISKEN and MORASCA[7] on mammalian cells. According to these authors, not only does the average duration of G_1 vary considerably from one cell type to another, but also this is the phase in which most of the variation between individual cells occurs within the same cell population and which is affected to a greater extent by physiological and/or environmental factors.

It is interesting to point out that, in spite of the very specific conditions of culture of these insect cells, particularly the relatively low temperature (26 °C), the duration of the total cell cycle and of its different phases is approximately within the same range of those obtained in mammalian cell cultures (CLEAVER[8,9]).

Riassunto. La durata media delle fasi del ciclo cellulare (G_1, S e G_2) e del tempo di generazione totale (G_T) in una linea stabilizzata di cellule di *Drosophila melanogaster* risulta essere rispettivamente di 1.8, 10.0, 7.2 e 18.8 ore.

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[9] We are grateful to Profs. C. BARIGOZZI, G. ECHALIER and M. FRACCARO for helpful discussion and critical review of the manuscript.

Some New Data on the Number of Chromosomes of Teleost Fish Obtained by Means of Tissue Culture in vitro

In a previous note in this journal[1] some of us referred to preliminary research made by means of tissue culture in vitro on the somatic chromosomes of some species of teleost fish. This research has recently been developed in our laboratory and the chromosome number of somatic cells of other species of fish has been accurately determined by the same method. The species studied belong to different families and for almost all of them no caryological data were available in the literature. A list of the species studied with their chromosome number is presented here (Table). A detailed morphological description of their caryotype is in preparation and will be sent for publication shortly.

Numbers of chromosomes of some teleost fishes

Taxa	$2n$
Centrarchidae	
Lepomis gibbosus (Linnaeus, 1758)	46
Characidae	
Hemigrammus caudovittatus (E. Ahl, 1923)	50
Cyprinidae	
Danio devario (Hamilton-Buchanam, 1822)	50
Danio malabaricus (Jerdon, 1849)	50
Brachydanio rerio (Hamilton-Buchanam, 1822)	50
Brachydanio albolineatus (Blyth, 1860)	50
Leuciscus souffia muticellus (Bonaparte, 1837)	50
Leuciscus aula (Bonaparte, 1837)	50
Leuciscus cephalus (Linnaeus, 1758)	50
Alburnus albidus alborella (De Filippi, 1844)	50

Riassunto. In questa nota vengono riportati nuovi dati sul numero dei cromosomi di alcune specie di pesci teleostei.

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Aneuploids in Pearl Millet

The classical work of BLAKESLEE and coworkers on *Datura*, that a change in the relative proportions of a group of genes due to variations in chromosome number had an effect on the phenotype, evoked interest of plant geneticists to build up stocks carrying an extra chromosome (Trisomics). In wheat, barley, maize, rye, tomato and peas, such stocks have been developed and found useful for establishing linkage groups[1]. Pearl millet, *Pennisetum typhoides* (Burm.) S. & H. ($2n = 14$), an important grain and forage crop in Asia and Africa and