

It is possible to find some mitoses in the chromaffin cells. In the entire adrenal medulla, there are 12–16 in 60–80 days old animals. This number is reduced to 6–7 in 350-day-old animals.

The mitoses appear frequently condensed in small zones, sometimes they are located near the cortex, sometimes in the center of adrenal medulla; however they do not show a particular localization.

Most of the dividing cells observed are in prometaphase, the remaining in anatelophase. Only 2–4 mitoses of connective cells can be found in the entire adrenal medulla. No mitoses of the ganglion cells, which can be found in small clusters between the adrenal medullary cells, have been observed.

Circadian rhythm of mitoses in albino rats treated with colchicine 3 h before sacrifice

h of day	No. of animals	No. of mitoses in Wistar rats	No. of mitoses in Italic rats
03.00	1	12	13
	2	14	16
	3	12	12
09.00	1	20	27
	2	19	37
	3	18	32
12.00	1	32	33
	2	35	36
	3	34	35
18.00	1	29	36
	2	21	25
	3	18	27

In the 60–80-day-old animals treated for 3 h with colchicine, the number of mitoses varies depending upon the hour of the day at which the animal is killed. In animals killed at 03.00 the number of mitoses ranges from 12–16 and increases throughout the day. A maximum is reached at noon. The mitoses (almost all metaphases) present the same distribution as that found in non-treated rats. The medullary cells in cariocinesis show the chromaffin reaction, although to a lesser degree than the quiescent ones.

These observations show that mitoses are present in adrenal medullary cells, although in a very small number.

Their number varies at different time of the day, showing a peak at noon. This finding can be interpreted as evidence of circadian rhythm of mitotic activity, as observed in other tissues.

The presence of the chromaffin reaction shows that the cells in mitoses are differentiated elements of the adrenal medulla^{11,12}.

Riassunto. Nella presente ricerca si dimostra che nella midollare surrenale di ratto adulto, esaminata completamente con sezioni in serie, esistono mitosi delle cellule cromaffini. Se ne stabilisce il numero ed i rapporti con il ritmo nictemerale.

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The Generation Time of Two Day Chick Neuroepithelial Cells

Neuroepithelial cells in the chick embryo synthesize DNA in the depth of the neural tube wall, following which they round up to the lumen to divide and then elongate back into the wall (MARTIN and LANGMAN¹). The duration of the above pattern has been investigated recently, however the results obtained have varied widely (FUJITA², ATLAS and BOND³ and KAUFFMAN⁴). The present study was undertaken therefore to investigate the duration of the cell cycle in neuroepithelial cells of the 2 day chick embryo.

Materials and methods. White leghorn chick eggs, incubated at 101 °F for 48 h, were removed from the incubator, and an opening made in the shell over the embryo. Three drops of thymidine H³, concentration 10 µc/ml, were dropped onto the embryo, the opening in the shell closed and the eggs returned to the incubator. At half hour intervals following treatment, embryos were removed, fixed, dehydrated and embedded in Paraplast, serially sectioned at 5 µ, prepared for radioautography by the coating method of KOPRIWA and LEBLOND⁵, and exposed for 5–7 days.

Results. The percentage of labeled mitotic figures, plotted against time between treatment with thymidine H³ and fixation, is represented in Figure 1. The following values were found: G2 = 1.5 h, M = 1.0 h and a genera-

tion time of 10.5 h which agreed with a 10 h cell cycle found in an earlier study using colchicine (MARTIN⁶). By extrapolating the descending curve, assuming the rate of descent equals the rate of ascent, the time between the midpoints of the curves, equal to 8.5 h, gave the duration of the 'S' stage. When the values of G2 + M + S were compiled however the value of 11 h was found to be greater than the generation time of 10.5 h. Furthermore, according to the above method the G1 stage of the cell cycle was completely non-existent.

To investigate the above problems grain counts were made on 50 labeled mitotic figures at each time interval used to produce the preceding curve. The average grain count/mitosis is represented in Figure 2. Assuming that cells incorporate thymidine H³ at a constant rate through-

- ¹ A. MARTIN and J. LANGMAN, *J. Embryol. exp. Morph.* **14**, 25 (1965).
- ² S. FUJITA, *Expl Cell Res.* **26**, 52 (1962).
- ³ M. ATLAS and V. P. BOND, *J. Cell Biol.* **26**, 19 (1965).
- ⁴ S. L. KAUFFMAN, *Expl Cell Res.* **42**, 67 (1966).
- ⁵ B. KOPRIWA and C. P. LEBLOND, *J. Histochem. Cytochem.* **10**, 269 (1962).
- ⁶ A. MARTIN, unpublished.

out the 'S' phase the number of silver grains/mitosis should have increased for the duration of the 'S' phase or until the thymidine H^3 was no longer available. Since the number of silver grains increased from 1.5–3.5 h after treatment the 2 h period was taken as the availability time of thymidine and as advocated by KOBURG⁷ the plateau of the curve, equal to 6.5 h, was taken as the duration of the 'S' phase.

A further method was employed to check the value of the 'S' stage. Knowing the generation time, 10.0–10.5 h, and the labeling index 65%, the following formula can be applied (QUASTLER and SHERMAN⁸):

$$\frac{NS}{NC} = \frac{TS}{TG} = 65 = \frac{TS}{10-10.5} = 6.5-6.8.$$

This 6.5–6.8 h value for the 'S' stage agrees reasonably well with the value arrived at by the grain count method.

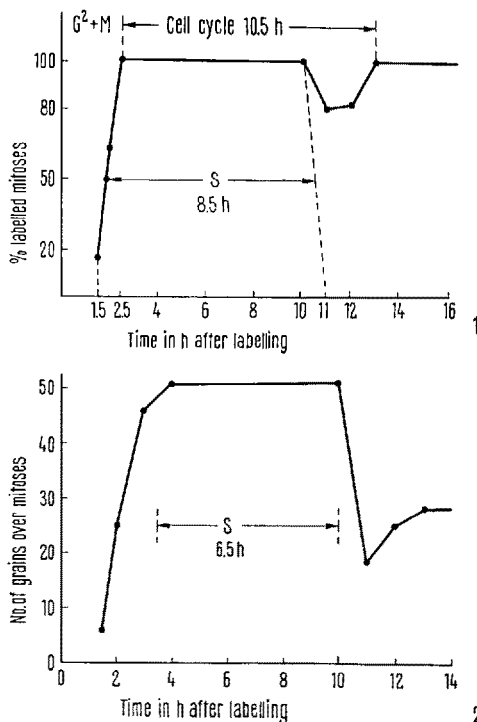


Fig. 1. Curve representing the percentage of labeled mitoses plotted against time between treatment with thymidine H^3 and sacrifice.

Fig. 2. Curve demonstrating the average number of silver grains per mitosis plotted against time between treatment with thymidine H^3 and sacrifice.

Discussion. As is apparent from the results, a discrepancy exists in the values for the 'S' stage with the 8.5 h value derived from the method of labeled mitoses being least compatible. This value was arrived at assuming that the thymidine H^3 was available for only a short period of time. As shown, however, the thymidine H^3 was available for at least 2 h during which time cells, initially outside the 'S' stage, could enter and incorporate the label thus lengthening the value of the synthesis stage. By utilizing the 2 h availability time we find an 'S' stage (8.5–2), equal to 6.5 h, a value which agrees with the time derived from the other 2 methods. Furthermore, if the values of $G_2 = 1.5 + M = 1.0 + S = 6.5$ are now added together and the value of 9.0 h subtracted from the generation time of 10.0–10.5 h we have a value of 1.0–1.5 h for the G_1 phase of the cell cycle.

In Figure 1 the descending curve does not fall below 80% labeled mitoses. A reasonable explanation may be as follows: the number of cells in a particular stage of the cell cycle is proportional to the time spent in that stage, therefore approximately 65% of all the neural tube cells are in the 'S' stage at any one time. Of the 35% unlabeled cells, approximately half will enter the 'S' stage and incorporate the label during the 2 h availability time of thymidine H^3 . This means that at least 80% of all the cells within the neural tube will be labeled and, since there is a non-synchronous movement of cells through the cycle, as evidenced by the slope of the ascending and descending curves, the number of labeled cells should never drop below this 80% mark.

Résumé. Des études autoradiographiques effectuées sur les stades du cycle cellulaire dans les cellules neuroépithéliales de poulet de 2 jours, ont donné les résultats suivants: $G_2 = 1,5$ h; $M = 1,0$ h; $S = 6,5-6,8$ h; $G_1 = 1,0-1,5$ h et le temps de génération = 10,0–10,8 h. On a également constaté que la thymidine H^3 administrée à l'embryon comme dans l'expérience ci-dessus mit au moins 2 h à être incorporée.

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⁷ E. KOBURG, in *Cell Proliferation* (Blackwell Scientific Publications, Oxford 1963).

⁸ H. QUASTLER and F. G. SHERMAN, *Expl Cell Res.* 17, 420 (1959).

Short-Term and Long-Term Radioprotective Effect of Magnesium Pemoline

Radioprotective effect of magnesium pemoline was recently reported¹. Further investigations showed that this drug is more potent than pemoline alone (made up 75% weight of the drug) in exhibiting protection against ionizing radiations. Results from the above studies have strongly indicated that magnesium pemoline offers significant degree of protection against lethal doses of X-irradiation for both short-term and long-term. In subsequent experiments we have studied explicitly this aspect and results are presented in this paper.

Methods. Two experiments were performed. In the first experiment, 540 CF-1 male mice (20–22 g), 50–60 days old were randomly divided into 3 groups. On the first day of the experiment half of the mice in each group were injected i.p. with 0.6 cm³ of magnesium pemoline prepared in

¹ H. LEVAN, *Experientia*, 23, 1058 (1967).