THE CELL GENERATION CYCLE OF THE ELEVEN-DAY MOUSE EMBRYO

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ABSTRACT

The incorporation of tritiated thymidine into the DNA of erythroblasts, primitive ependymal cells, and mesenchymal cells of 11-day mouse embryos was studied by radioautography at different times between 25 minutes and 18 hours after injection intraperitoneally. There was no labeling of mitotic figures until 1 hour after injection. Following this, mitotic figures were labeled for about 5.5 hours in primitive ependymal cells and mesenchymal cells, and for a longer period in erythroblasts. The percentage of the labeled primitive ependymal cells at various times after injection indicate a periodic migration into and out of the mitotic zone. The cell generation cycle of primitive ependymal cells and mesenchymal cells is similar to some kinds of adult cells. The cycle of the erythroblasts is more like that of the cells of aging mice.

The relation of deoxyribonucleic (DNA) synthesis to the cell generation cycle has become more definite since the demonstration by ultraviolet spectrophotometry that the synthesis occurs during the interphase (1, 20). The concept that the interphase of the cell generation cycle is divided into a presynthetic phase (G_1), a DNA synthetic phase (S), and a postsynthetic phase (G_2) was proposed after autoradiographic experiments with P^{32} and C^{14} (11, 12). It has been confirmed and widely studied in adult tissues *in vitro* and *in vivo* through the use of tritiated thymidine and high resolution autoradiography (7, 8). There is general *agreement* that the thymidine is incorporated into DNA (6).

This work was undertaken to study the generation cycle of cells in the mouse embryo. It makes use of the knowledge that thymidine is incorporated into the cells of the mouse embryo (2) and that the time pattern of labeled mitoses can be utilized to characterize the cell cycle.

MATERIALS AND METHODS

Embryos were obtained from Swiss albino mice. Their age was determined through observed matings or by finding of copulation plugs in females which had been paired with males the preceding night, counting as day 1 the day on which the copulation plug was found. At 11 days after the time of mating, the pregnant females were injected intraperitoneally with tritiated thymidine (one microcurie per gram body weight, sp. act. 1.8 millicuries per millimole). At different intervals (25 minutes, 45 minutes, 1 hour and 25 minutes, 2 hours, 3 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 12.5 hours, 16 hours, and 18 hours) after injection, the mothers were etherized and the embryos removed and fixed in acetic-alcohol (1 : 3). After imbedding in paraffin and sectioning at 5 micra, the deparaffinized sections were covered with AR 10 stripping film for 28 days. After development they were stained with Harris haematoxylin.

The sections were studied for the uptake of tritiated thymidine as indicated by the presence of grains over the nuclei. Nuclei with at least four grains were considered labeled. Background was approximately one grain per nucleus. Data were obtained from tissues which had sufficient numbers of mitoses. These were primitive erythroblasts dividing in the blood, primitive ependymal cells, and mesenchymal cells. Since early stages of prophase were difficult to identify, observations were limited

to late prophase, metaphase, anaphase, and early telophase. One hundred mitotic figures per data point were counted to obtain a suitable sample.

RESULTS

Many cells were labeled in all tissues at 25 and 45 minutes after injection of tritiated thymidine; however, no mitotic figures were labeled at these times. The pattern of labeling of primitive ependymal cells was noteworthy. In this tissue, the mitotic population of metaphases and anaphases is limited to the single layer surrounding the neurocoele (Fig. 1). Peripherally, the nuclei are in interphase and are close together. Cell boundaries are not clear. The tissue has been described as a pseudostratified columnar epithelium in the chick

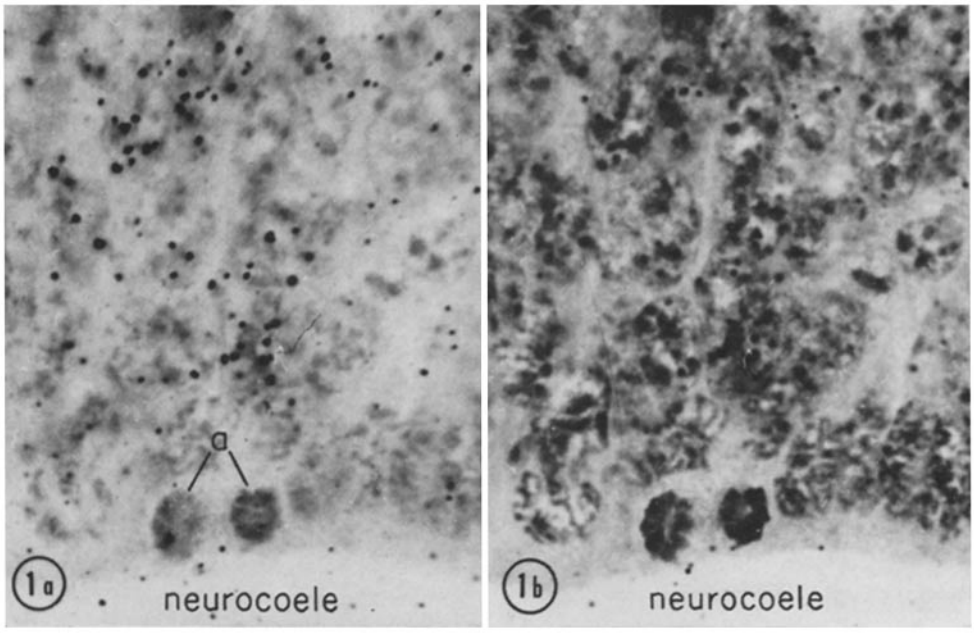

FIGURE 1 Neural tube 45 minutes after injection of tritiated thymidine. *A*, focuses on grains; *B*, focuses on mitotic figures and nuclei. Mitotic figures are not labeled; labeling is restricted to the periphery of the neural tube. *a*, anaphase. × 1200.

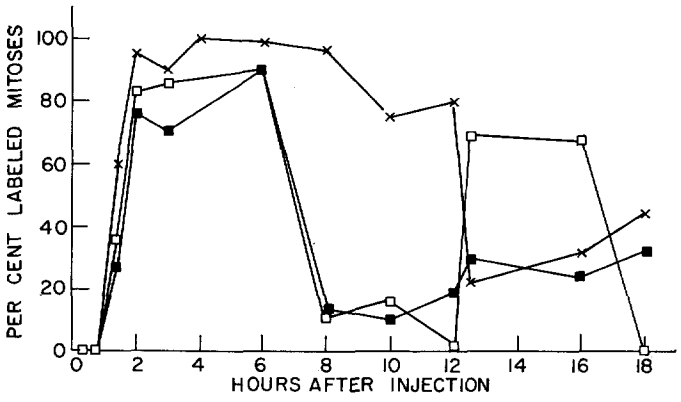

FIGURE 2 Per cent of mitotic figures which were labeled at different times after injection of tritiated thymidine. X, Primitive erythroblasts dividing in the blood. □, Primitive ependymal cells. ■, Mesenchymal cells.

and pig by Sauer (17). This has been more recently confirmed for the mouse by Berry *et al.* (3), who have found by silver and other methods that the scanty cytoplasm is connected with the central and peripheral borders of the tube by long processes. We found no evident differentiation in these cells and they may be considered part of a homogeneous population. The absence of labeling was the same in brain and spinal cord. In some areas, cells were labeled up to the mitotic layer. In others, the labeled region extended a few layers into the periphery of the neural tube. As a result, there were large unlabeled zones which extended from the mitotic layer outwards. Since the mitotic figures are localized, this complete lack of label is striking.

The percentage of labeling of primitive ependymal cells was difficult to determine because of difficulty of determining nuclear boundaries. Also the closeness of cells in this tissue, and the nature of their staining under radioautographic film rendered the cell outlines indistinct. However, a general survey indicated that, in most places, at least 50 per cent of the primitive ependymal cells were labeled at 25 minutes. At this time, 60 per cent of the erythroblast and 40 per cent of mesenchyme interphases were labeled. The high degree of labeling at this time indicates the availability of thymidine to cells of the embryo which were synthesizing DNA and shows, therefore, that the nonlabeling of mitotic nuclei is not due to a placental interference with thymidine penetration. Grain counts varied from zero to 32, with an average of 13 for erythroblasts at 25 minutes. Grain counts were similar in other tissues. Grain counts at different times after injection could not be compared since there was a difference in density of the label in different females.

Metaphases and anaphases were definitely labeled in all tissues, beginning at 1 hour and 25 minutes (Fig. 2). At this time, approximately 60 per cent of the erythroblast, 27 per cent of the mesenchymal, and 36 per cent of the ependymal cell mitoses were labeled. The per cent labeling of each tissue rose rapidly after this to a maximum at about 2 hours. The maximum value obtained for the erythroblast population was essentially 100 per cent, and this value was maintained until the 8th hour (Fig. 2). There was then a slow decline to about 80 per cent, followed by a very rapid decline at 12 hours. The maximum of the mesenchymal and ependymal cell labeled mitoses was

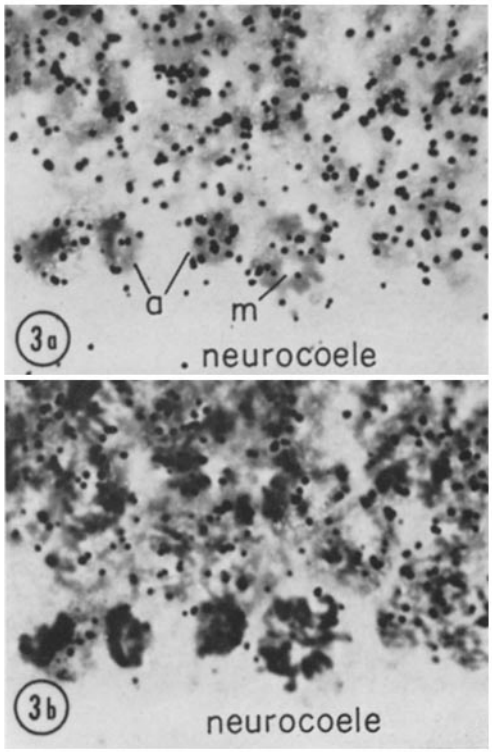

FIGURE 3 Neural tube 6 hours after injection of tritiated thymidine. *A*, focuses on grains; *B*, focuses on mitotic figures and nuclei. *a*, anaphase labeled; *m*, metaphase labeled. × 1200.

maintained until 6 hours (Fig. 3). After this time, the number of labeled mitotic figures dropped precipitously to reach a value of 13 per cent for mesenchymal and 10 per cent for ependymal cells at 8 hours. The ependymal cell value fell to almost zero at 12 hours. At 12.5 hours, there was a rise in the number of labeled mitoses to 69 per cent in the ependymal cells. This increase remained constant until 16 hours, when it started to decline, reaching zero at 18 hours. For mesenchymal cells, there was a corresponding small rise at 12.5 hours which remained constant thereafter. The time of beginning and end of labeling of mitotic figures was the same for spinal cord and brain cells. However, determinations of per cent figures is limited to spinal cord primitive ependymal cells.

DISCUSSION

The data reported here indicate that the thymidine is incorporated into the nuclei of the mouse embryo only at a particular stage of the cell cycle.

Until 45 minutes after injection, only interphases showed the tritium label. This is the period before the cells, which have incorporated tritiated thymidine into their DNA, have reached the mitotic phases. By extrapolation in Fig. 2 to zero labeling time, it is possible to estimate the time (G_2), from the first appearance of labeled mitoses to be about 1 hour. If the time of 50 per cent labeling is taken as a reference point for the beginning and end of the synthetic period (S) for the ependymal and mesenchymal cells, a value of about 5.5 hours is obtained. The minimum mitotic time (M), the time from zero to 100 per cent label, is about 1 hour. This corresponds to the average value recorded for mouse tissues (9). The total generation time, the time from the start of the first wave of labeled mitoses to the start of the second, may be estimated to be about 11 hours. From this, the presynthetic time (G_1) may be determined by subtracting G_2, S, and M times (7.5 hours) from the generation time. G_1 is, therefore, about 3.5 hours. The events in the erythroblasts are somewhat different from those in the cells of the other two tissues. The G_2 seems a little shorter, but the difference may not be significant if we consider the variations inherent in the method. In this case, it is not possible from the data to separate G_2 and S. The generation time is also about the same as that of cells of the other two tissues—about 11 hours.

The only related previous work on a tissue studied in this paper has been that of Fujita (10) who found a value of 5.6 hours for spinal cord of the 6-day chick embryo. Wegener *et al.* (21), in a study of embryonic tissues of the rat, reported that values of S ranged from 5.2 to 6.0 hours for various digestive and kidney epithelia. A similar estimate for mouse adult duodenum has also been made (14). Somewhat larger values (5.2 to 7.7 hours) have been published by Pilgrim and Maurer (16) for various epithelia, lymph node, and Kupffer cells of adult mouse and rat, and by Cameron and Greulich (5) for various digestive epithelia of the adult mouse. The latter authors have suggested the possibility of a constant S period. The data of Defendi and Manson (8) suggest that 6 hours is a minimum value for the S period of adult mammalian tissues, particularly for tumor cells. However, Cameron (4) has found a value of 5 to 6 hours for 2.5-day cockerels and 7 hours for mouse placenta. He has suggested that one cause of variation in S is a difference of temperature between embryo and adult tissues. The work of Fujita (10) indicates that another cause of variability is the stage of development, since he finds a total generation time of 5 hours in the 1-day chick embryo compared with 16 hours in the 6-day embryo. With the facts at hand, it is possible at present to assert only the low variability of S compared with other parts of the cell generation cycle found in many tissues of embryo and adult mammals and birds.

The generation time of 11 hours reported for the tissues studied in this report agrees with that for early cleavage (1) and adult duodenal tissue of mice (14) and intestine of embryo rat (21). Mouse cells *in vitro* have shown an average intermitotic time of 12 hours (9); there was a range of 6 to 18 hours, which may be due to the conditions of the culture not being optimum. However, in other tissues of rat embryo, the time ranged from 15 hours for salivary gland to 46 hours for hibernating gland. It is worth noting that the adult tissues with the low values of generation time are those which have a rapid turnover rate and are more likely to have generation times similar to cells of the embryo. The other fetal tissues studied were from 20-day pregnancies in which the embryonic period has been long passed. As mentioned previously, the generation time may increase as development proceeds. The curve for the erythroblasts is different from those for cells of the other two tissues studied. It resembles that for mature erythroblasts of the dog (15), and intestinal cells of aging mice (14). It is not possible, from the data, to separate G_1 and S. It may be that the primitive erythroblasts which are finishing their generation cycles are behaving like the cells of aging mice which are more heterogeneous.

The primitive ependymal cells seem to remain in step better than the cells of the other tissues during the second cycle. This may be correlated with the observation that cell division is accompanied by apparent migration. It has been suspected that the nuclei of the developing brain and spinal cord undergo their intermitotic and early mitotic stages in the periphery of the wall of these organs. To go through the later stages (*i.e.*, metaphase and anaphase) they migrate to the lumen (17). This has been confirmed by radioautographic studies on the chick (18, 10, 13) and the rodent (19, 3). The present work further bears this out. During the 1st hour after injection of tritiated thymidine, the labeling is limited to

interphase stages in the layers more or less distant from the neurocoele layer (Fig. 1). Following this, the labeling is found in the mitotic stages adjacent to the neurocoele (Fig. 3). There must consequently have been a migration of cells from the peripheral to the more central area. This movement for the most peripherally labeled nuclei would require a passage through a distance of 50 micra in 5 hours. This might be well within the potential of a cell of this size, but the mechanism whereby the cell could do this is obscure. It seems to be a mass movement of nuclei rather than the movement of isolated nuclei. According to Berry *et al.* (3), there is a migration of nuclei through the elongated ependymal cells which have one of their ends attached to the neurocoele boundary and the other to the peripheral membrane. After migration, the nuclei undergo mitosis and divide without the occurrence of cytokinesis. They then move back to the periphery. Although their description is for later stages of the brain of the rat fetus, the nuclear arrangement and pattern of migration fits closely that of the 11-day mouse embryo. It is not possible to determine whether both daughter nuclei migrate back through the original cells they were in, which Berry *et al.* believe to be the case. If they do, the question arises as to where the additional cells necessary to increase the length and height of the tube come from. It should be pointed out that the movement is not uniform throughout the wall of the tube, since at the beginning of the experiment some patches of labeled cells are already adjacent to the mitotic zone whereas others are farther away. Nevertheless, all the cells seem to reach metaphase and anaphase together, since labeled mitotic figures are found at 1½ hours in all sections of the tube, irrespective of the original variations. After 5 hours, the labeled mitoses are at a minimum. This indicates that the mass of cells which was exposed to tritiated thymidine has moved away from the mitotic zone. When the labeled cells pass through the second G_1, S, and G_2 stages, they reach the mitotic zone again about 11 hours after the first wave and continue to provide labeled mitotic figures in this region until they are again replaced by unlabeled cells. From this, it may be surmised that the primitive ependymal cells of the 11-day mouse embryo pass through two generation cycles and migrations during this day.

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