tiplication for all 3 strains studied is characterized by: a) an initial lag phase of 2-3 days, b) a phase of active cell multiplication, reaching maximum concentrations around 9-10 days, and c) a phase beginning around 12 days with no further increase in cell numbers. Under conditions described, maximum cell yields were obtained by collecting one-half the cells from populations in phase (b) each 3-4 days.

We wish to thank Dr. Edmund Mayer for help in formulation of problem, and E. Manoogian for assistance in preparation of manuscript.

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Received May 5, 1959. P.S.E.B.M., 1959, v101.

Radioautographic Study of Interkinetic Nuclear Migration in the Neural Tube.* (25014)

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Mitotic figures in the early neural tube occur only adjacent to central canal. This led to the view that cells near the lumen constitute a distinct type, which alone divide, and thus serve as "mother cells" for the entire wall. However, histological studies(1,2) indicated a migration of nuclei in the neural tube, *i.e.*, after division at luminal margin, a nucleus moves into the depths of wall during interkinetic period, and returns to the free surface as it passes into the prophase of the next division. Nevertheless, a recent discussion(3) continues to favor the long held germinal cell theory. The response of the early neural tube to colchicine(4,5) gave the first experimental confirmation of the migration concept. Additional evidence came from measurements of amount of deoxyribonucleic acid (DNA) in cells of neural tube. Sauer and Chittenden(6) found that mean amount of DNA in nuclei at some distance from the lumen considerably exceeded the 2n value. Since synthesis of DNA precedes each cell division during duplication of chromosomes, this indicated that deep nuclei undergo changes preliminary to division, as the concept of migration requires. A demonstration of the site of formation of DNA in the early neural tube with tritium-labeled thymidine, known to be incorporated only into new DNA,

* This investigation supported by research grants from NINDB and Dental Institute, NIHPHS.

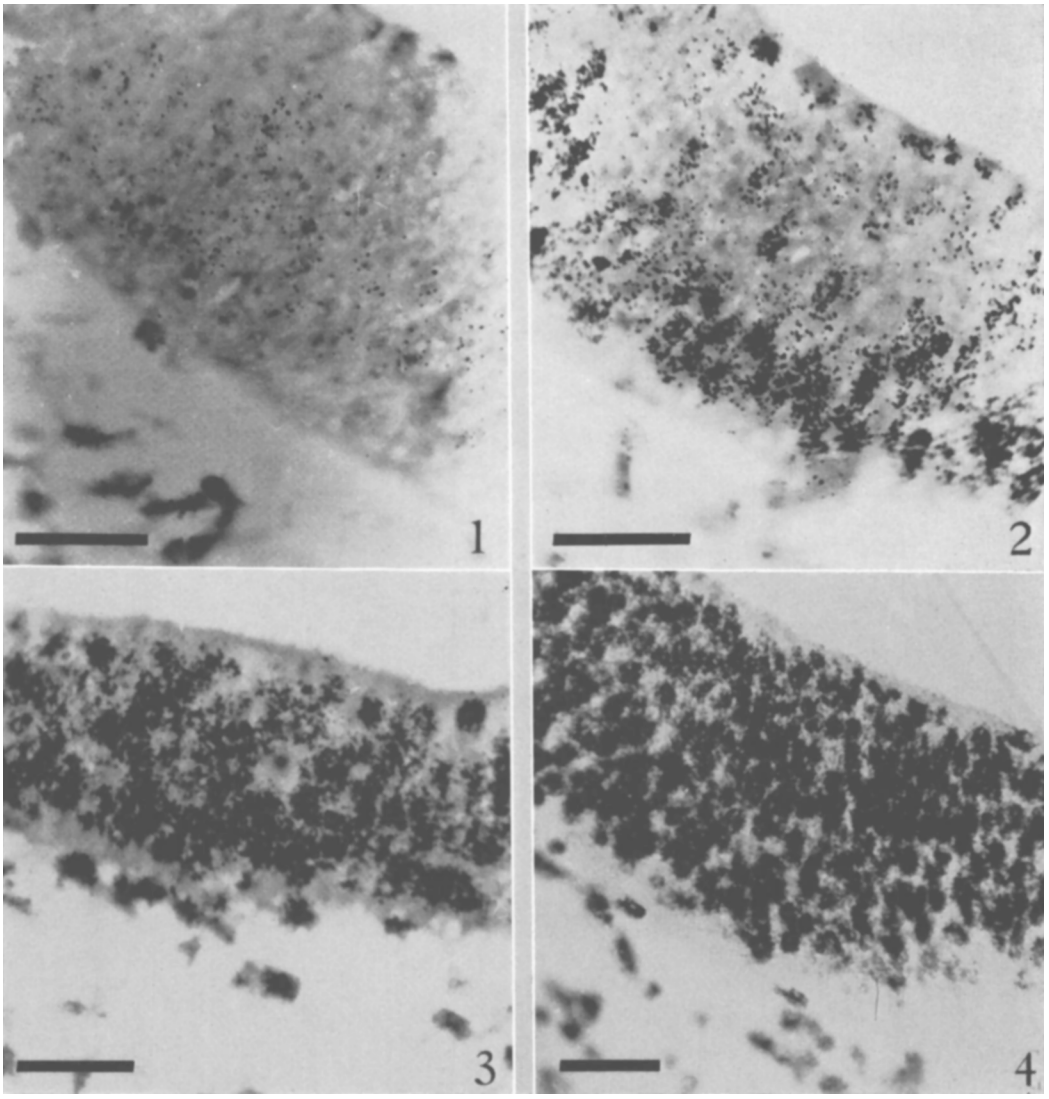

FIG. 1-4. Radioautographs of neural tube (brain) of chick embryos fixed at varying intervals after administration of thymidine-H^3. Lumen at top. Exposed 15-16 days. Hematoxylin. Line indicates 25 μ. FIG. 1. 1 hr, stage 16 embryo. FIG. 2. 4 hr, stage 18. FIG. 3. 8 hr, stage 16. FIG. 4. 12 hr, stage 17.

should provide a graphic test of the migration concept. The present communication enlarges upon a preliminary report(7) of such a study in the neural tube of the chick embryo.

Procedure. Eggs containing 2½ day old embryos were opened by the Harkmark-Graham(8) method. Thymidine-H^3 was dropped onto each embryo (dosage/embryo, 50 μc in .05 ml). Further incubation time ranged from 1 to 24 hours, giving embryos of stages 16 to 19(9). These were fixed in Serra's fluid, embedded in paraffin and sectioned at 6 μ. Sections were processed for radioautography by the dipping method of Messier and Leblond(10), using NTB3 emulsion (courtesy Eastman Kodak Co.), and developed after exposure times ranging from 15 to 112 days.

Results. The descriptions below apply to radioautographs of the neural tube. Time intervals refer to the interval between administration of thymidine-H^3 and fixation of the

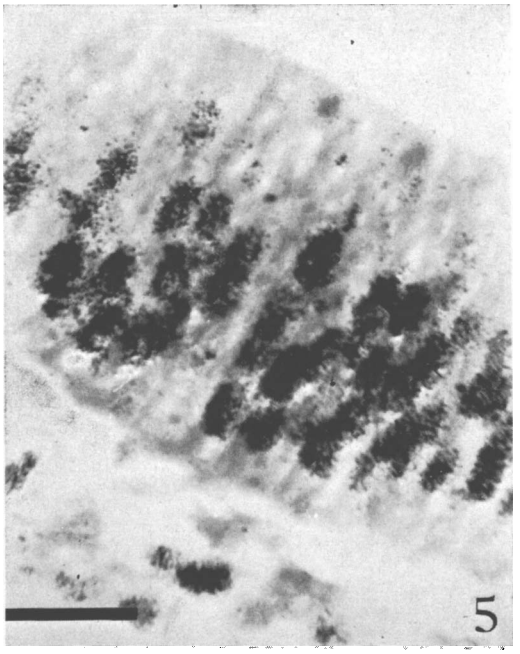

FIG. 5. Neural tube from caudal half of a stage 16 embryo. Lumen at top. 2 hr after treatment. Exposed 112 days. Aqueous basic fuchsin. Line, 25 μ.

embryo. Exposures for the radioautographs illustrated in Figs. 1-4 were all of the same duration (15-16 days); that in Fig. 5 was much longer (112 days).

1 and 2 hour intervals. These intervals are characterized by radioactivity distributed uniformly in the outer half to two-thirds of the wall, and a lack of radioactivity at the luminal margin. The labeling is light (Fig. 1) as compared to similarly exposed radioautographs of the longer time interval embryos. In order to obtain a denser radioautograph for comparison, a prolonged exposure (112 days) was made of sections from a 2 hour interval embryo (Fig. 5). Although radioactivity increases between the 1 and 2 hour intervals, the marked difference between Figs. 1 (1 hr) and 5 (2 hr) is a matter of exposure. Prolonged exposure may bring out light labeling in the inner third of the wall or involve an occasional metaphase at the lumen; however, the general distribution of radioactivity is the same, regardless of length of exposure.

4 hour interval (Fig. 2). Radioactivity extends throughout the wall, but is not distributed uniformly. A layer of greater density is located at the periphery. Although the pattern in the neural tube varies somewhat with level and age, a dark band in the outer third or fourth of the wall usually characterizes this period. The area between the dense peripheral zone and the lumen contains many lightly radioactive nuclei and a few heavily labeled nuclei. Mitotic figures at the lumen are now radioactive.

8 hour interval (Fig. 3). Strongly radioactive nuclei extend uniformly from periphery to lumen.

10, 12, 16 and 24 hour intervals (Fig. 4). These show no change beyond the previous interval, other than a progressive increase in density up to 16 hours, when practically all nuclei are labeled. The 24 hour interval embryo resembles the 16 hour.

Discussion. The results obtained in the early neural tube with thymidine-H^3 clearly verify the concept of an interkinetic migration of nuclei(1,2). Radioautographs of the 1 and 2 hour interval embryos demonstrate that only nuclei in the peripheral region of the tube, away from the lumen, synthesize DNA. Therefore, new nuclei produced by mitosis at the lumen must move away from the lumen and enter the peripheral zone before synthesizing any significant quantity of DNA. Nuclei remain in the peripheral zone, while synthesizing DNA, for at least 4 hours, since the most radioactive nuclei of the 4 hour interval embryos occur in this zone. The nuclei then return to the luminal border to divide. Some nuclei are already part way through their synthetic period at the time of application of the isotope, and, therefore, incorporate less than the maximum quantity of thymidine-H^3. In the 4 hour interval radioautographs these are the weakly radioactive nuclei of the juxtaluminal zone. Since this zone also contains a few strongly radioactive nuclei, probably 4 hours is almost long enough for a complete period of synthesis of DNA.

At 8 hours, heavily labeled nuclei are moving both to and from the lumen. Consequently, the layering effect does not appear at this time interval, or at later intervals. However, the density of the radioautographs does increase with time, indicating cumulative incorporation of thymidine-H^3 during subsequent periods of

DNA synthesis. The continued availability of thymidine-H^3 to the chick embryo from a single application is in contrast to its fate in mammals, where it is rapidly metabolized, and therefore labels nuclei over only a short period of time(11). A preliminary report(12) of work similar to the present study illustrates this species difference, while confirming nuclear migration in the neural tube.

It should be emphasized that the fundamental concept involved here is not that of nuclear migration in itself, but rather a recognition of the structure of the early neural tube as a columnar epithelium(13,14). Nuclear migration is a necessary accompaniment to the rounding up of the cell in mitosis and its subsequent elongation. The cell maintains its firm terminal bar attachment at the lumen as long as it is an element of a columnar epithelium, hence, the nucleus is drawn toward the lumen as the cell rounds up in preparation for division. Following mitosis, the nucleus is carried peripheralward as the cell elongates to renew its attachment to the outer limiting membrane.

Conclusions. Radioautographs of early neural tube of chick embryos treated with thymidine-H^3 for varying intervals of time give evidence substantiating the concept of interkinetic migration of nuclei. Synthesis of DNA occurs only in nuclei of peripheral part of wall, and does not take place in those of juxta-luminal zone. The period of DNA synthesis continues for at least 4 hours, but does not extend much beyond this time.

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Received May 12, 1959. P.S.E.B.M., 1959, v101.

Serum Adenosine Deaminase Activity in Cancer.*† (25015)

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Straub and his associates(1) reported that adenosine deaminase, the enzyme that catalyzes the deamination of adenosine to inosine, was present in plasma and was elevated in 92% of the 527 cancer patients studied. A similar frequency was reported by Letnansky and Seelich(2). Such a high frequency of elevated serum or plasma enzyme activity indicated possible diagnostic utility. Our previous studies on other serum enzyme activities in patients with cancer(3,4,5) showed that elevations in these activities were related more directly to growth or activity of tumor, rather than to its presence. It was accordingly decided to determine, first, activity of the serum adenosine deaminase in groups of normal persons and patients with

* This work supported by Am. Cancer Soc., Damon Runyon Memorial Fund for Cancer Research, and Cancer Inst. of N.I.H., P.H.S.

† The authors wish to acknowledge technical assistance of Anita C. Smith. The patients were from services of Memorial Center for Cancer and Allied Diseases, chiefly the Clinical Chemotherapy Service.