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DEOXYRIBONUCLEIC ACID SYNTHESIS IN FREE CELLS OF THE COELOMIC FLUID OF NEWTS, *TRITURUS PYRRHOGASTER*

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Introduction

A few cytological studies on free cells in the coelomic fluid of newts have hitherto been undertaken by Ohuye (1936), Mizutani and Nakahara (1961) and Seto (1970). In these papers morphological features of cells with Russell's bodies (so-called Russell's cells) were particularly noticed in control and experimental conditions. However the characteristic cell has never been clarified on the origin and the function in the abdominal cavity. In the previous study (Seto 1970), characteristic Russell's cells were supposed to occur by the transformation from lymphocytes or monocytes *in situ*. Although the Russell's cells scarcely show their mitotic figures in a control animal the proliferative ability has not been perfectly made negative proof. It is needed to ascertain whether lymphocytes or monocytes could be the ancester of Russell's cells or not.

Mitosis is believed to be initiated during a postmitotic gap (G_1) of the cell division cycle since induced cell proliferation first results in DNA synthesis (Harding and Srinivason 1961, Sherman *et al.* 1961, Gelfant 1962, Mazia 1963, and others). Thus the population of cells capable of multiplication are thought to proceed a phase of DNA synthesis(S), followed by a short premitotic gap (G_2) prioring to enter the mitotic phase. The phases of S, G_2, and mitosis generally seem to be closely linked ; therefore, they are referred to as a group by the term "doubling sequence" in the mitotic cycle.

On the basis of the definition, the present study was undertaken by an autoradiography method to examine occurrence of DNA synthesis in free cells of the coelomic fluid *in vivo* for detecting the proliferative ability in the cell population.

Materials and Methods

Adult male and female newts, *Triturus pyrrhogaster*, have been stocked in the laboratory before use. A routine cytological examination was the same as previously reported (Seto 1970). Free cells in the coelomic fluid were aspirated with a capillary pipette and put to use for the smear preparation, following by the procedures of methanol-fix and May-Grünwald Giemsa stain.

Proliferative cells which underwent a synthesis of DNA in the nuclei were detected by an autoradiograph. Tritiated thymidine was used as a precursor of DNA. Cells in the synthetic phase of the division cycle incorporated the radioactive precursor, which were marked by dark grains in the photographic emulsion. Each animal received a single intraperitoneal injection of tritiated thymidine (H-TdR, specific activity 5.0

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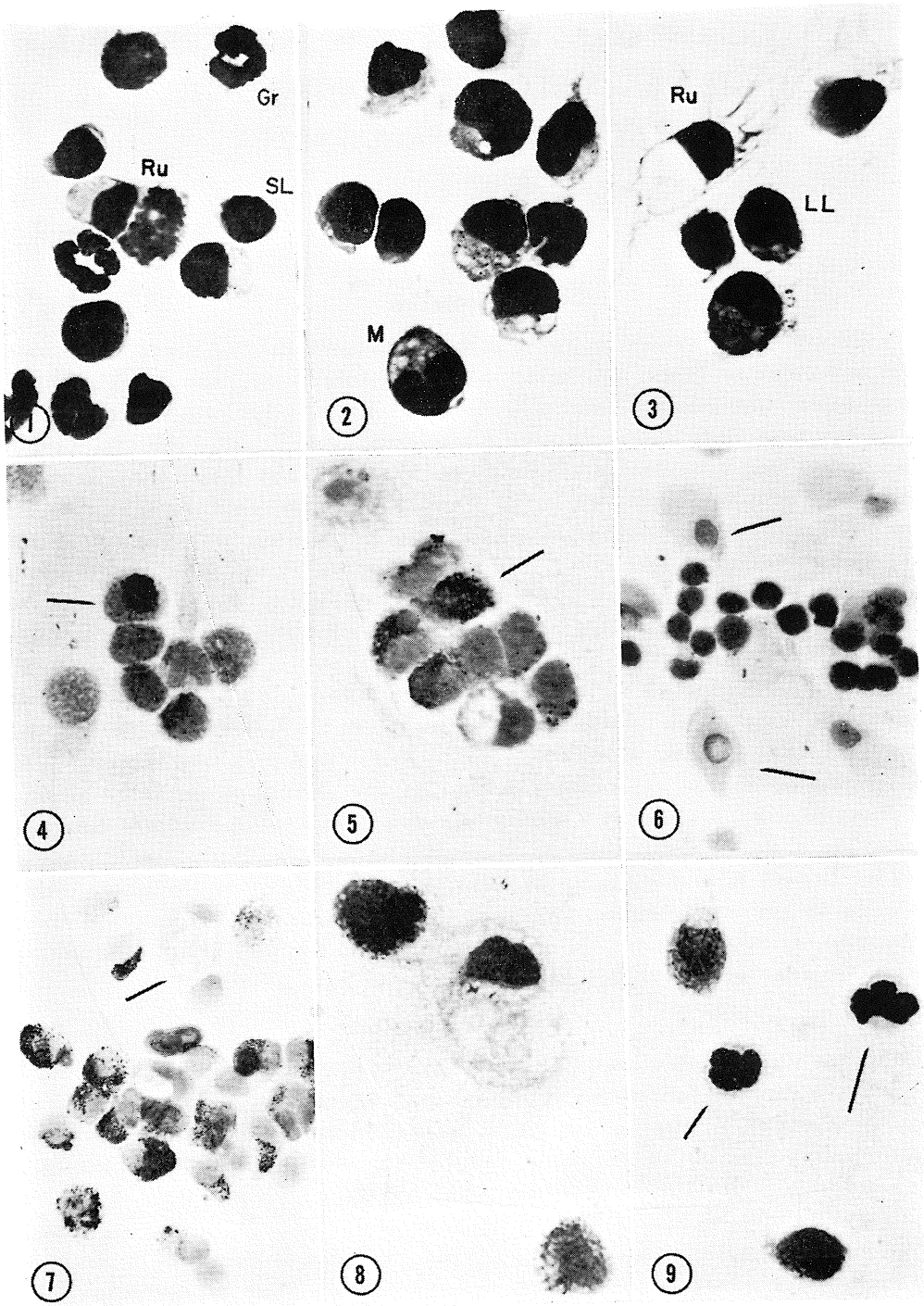
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c/mM, Daiichi Pure Chemicals, Tokyo) at dosage of 2-3 μc/g body weight. Labeled cells were taken out from the abdominal cavity and fixed at 3, 6, 12, and 24 hour after the treatment. Autoradiography was made with a stripping film (Sakura NR-M2), which was mounted on a slide in 0.02 per cent of potassium bromide solution. The slide was dried in a vertical position for about an hour and stored at 4°C in sealed boxes containing desiccant. After exposure of 1 to 12 weeks the film on the slide was developed in photographic developer (Sakura SDX-1) at 17°C for 15 minutes. Preparations were stained with Delafield's hematoxylin or phenol-gentian violet solution after autoradiographic exposure and development.

Results and Remarks

Coelomic free cells of newts generally consist of large and small lymphocytes, monocytes, granulocytes, histiocytes, and Russell's cells (Figs. 1-3). The morphological features of these cells have been described in detail in the previous papers (Mizutani and Nakahara 1961, Seto 1970).

Treated cells with ^{3}H-TdR were fixed at intervals of 3, 6, 12, and 24 hour after a single intraperitoneal injection. The point of observations was to determine whether the Russell's cell still retains the multiplying ability or be a fully differentiated cell that is uncapable of renewal. Another question was which cell population is a main group of the proliferation in the peritoneal fluid.

Labeled cells were first detected by autoradiographs of the preparation fixed at 3 hour after the treatment. At 12 and 24 hour after the treatment labeled cells showed heavior grains than the earlier preparations. The type of free cells which incorporated ^{3}H-TdR were only restricted to lymphocytes and monocytes (Figs. 4 & 5). Large lymphocytes and monocytes actively synthesize DNA in the normal condition, as judged by the rate of ^{3}H-TdR incorporation. With increasing times of labeling, the more and more amount of radioactivity appeared as dark grains on the emulsion attached to the lymphocytic and monocytic nuclei. Among free cells which were treated for 24 hours about 60 per cent of large lymphocytes and monocytes were labeled *in vivo*. Nuclei of small lymphocytes were lightly labeled, while granulocytes have consistently unlabeled by the radioisotope in any preparation (Fig. 9).

Russell's cells have never shown any radioactivity of ^{3}H-TdR even in the preparations of 24 hour treatment (Fig. 6-8), indicating that no Russell's cell passed on a phase of DNA synthesis. A primitive type of the Russell's cell which is characterized

Figures 1-3. Photomicrographs of free cells in the coelomic fluid of newts, consisting of small lymphocytes (SL), granulocytes (Gr), Russell's cells (Ru), monocytes (M) and large lymphocytes (LL). All cells from the same slide. A smear preparation, methanol-fix, and May-Grünwald Giemsa stain. ×400.

Figures 4-9. Autoradiographs of the coelomic free cells after ^{3}H-TdR incorporations, showing grains in the emulsions on particular cell nuclei. Stripping-film method, hematoxylin stain. 4 & 5, labeling 3 hr before fixing the cells, large lymphocytes was heavily labeled (*arrows*), and small lymphocytes lightly labeled. × 300. 6 & 7, labeling 12 hr before fixing the cells, large lymphocytes and monocytes were labeled, while Russell's cells (*arrows*) were consistently unlabeled. × 120. 8 & 9, labeling 24 hr before fixing the cells, monocytes and large lymphocytes incorporated ^{3}H-TdR, while Russell's cells and granulocytes (*arrows*) did not show incorporation. ×400.

by a small-sized cytoplasmic inclusion and undeveloped-protoplasmic processes was lightly labeled infrequently, but active incorporation of DNA precursor have not been detected in the cell nuclei. So far as observed, large lymphocytes and monocytes were recognized as main groups of proliferative cells. It is hardly believed that Russell's cells multiply predominantly by their own divisions in the abdominal cavity. The results suggest that Russell's cells could possibly be derived from other type of cells either lymphocytes or monocytes by the cellular transformation.

Although Russell's cells have not undergone the DNA synthesis the proliferative ability was not perfectly deniable. Since Gelfant (1962), investigating mouse epidermal cells, has challenged this concept by concluding that there is a population of cells fully prepared to divide but blocked in G_2, until they receive an appropriate stimulus to divide. If mitosis of a primitive type of Russell's cell is initiated after DNA synthesis in the G_2 period, the majority of mitotic figures and the interphasic nuclei would not be labeled under the present treatments. However the autoradiographic experiment did not extend to the cells which was induced mitosis. Further studies on quantitative analysis in DNA synthesis in free cells and on the timing of interphasic gaps (G_1, S, and G_2) of mitotic cycle in proliferative cells should be continued to solve this question.

Summary

Deoxyribonucleic acid synthesis was selectively observed in coelomic free cells of newts *in vivo*. Lymphocytes and monocytes were labeled with ^{3}H-TdR while granulocytes and Russell's cells have not shown any silver grains of autoradiography on their nuclei.

The result suggests that Russell's cell does not predominantly multiply *in vivo*, but are transformed from other type of cells which are actively synthesizing DNA *in vivo*, possibly from a large lymphocyte or a monocyte.

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