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Ribonucleic Acid and Lens-Regeneration[1]

The fact that ribonucleic acid (RNA) is especially abundant where cells are intensively multiplying[2], as in developing embryos[3] or in proliferating cancer tissues[4], has led to the idea that cytoplasmic RNA is closely connected with cellular protein synthesis. The same relation seems to hold also for regenerative proliferation: for example, DRABKIN *et al.*[5], and NOVIKOFF and POTTER[6] reported an increase in the cellular RNA in the regenerating liver of the rat, and WEITZMANN[7] observed intensified basophilia in the regenerating tissue of the Oligochaeta and ascribed it to RNA.

As is well known, the lens of Urodela can be regenerated from the margin of the iris after its operative excision. The pigmented cells of the free margin of the iris lose their pigment to form a transparent spherical aggregate. The morphological picture of the subsequent development of this vesicle into the lens, together with its rapid growth, seems to suggest that here an intensive synthesis of protein occurs, followed by its profound denaturation. It is very probable that cytoplasmic RNA plays a significant rôle also in this master example of regeneration[8].

From the adult newt (*Triturus pyrrhogaster*) the lens was removed by an operation performed by SATO. The operated animals were kept separately in glass vessels and decapitated on the 7th, 10th, 14th, 25th, and 30th day after the operation. The removed heads were fixed in 10 per cent neutralized formalin. The isolated eye-balls were imbedded and sectioned as usual. The original series of sections from one eye-ball was divided into three series, every third section being allotted to the same series. Every series was mounted on a separate slide, and designated as *A*, *B* and *C* respectively. The series *A* was incubated in 0·1 mg/cm³ aqueous solution of crystalline ribonuclease buffered to pH 7·4 for 1 hour at 40°C and stained with toluidine blue or thionin. The series *B* was treated with distilled water buffered to pH 7·4 at the same temperature and for the same duration as the series *A* and stained with toluidine blue or thionin. The series *C* was tested for its basophilia by applying the same dyes after the treatment with trichloroacetic acid (5%) or perchloric acid (10%). In some special series Feulgen's nucleal reaction was applied to investigate the behaviour of DNA. The ribonuclease was prepared according to MCDONALD[1] by M. SHIMOMURA of the Chemical Institute of Nagoya University.

Results: (1) *7-day regenerate.* The margin of the iris is thickened. Especially at the upper margin of the iris many cells have lost their cytoplasmic pigment. They show strong basophilia in their now transparent cytoplasm. In the ribonuclease-treated series this basophilia is totally absent. In contrast to these regenerating cells, the cells of adjoining tissues do not show any tendency of basophilia.

(2) *10-day regenerate.* Now the regenerate has formed an incomplete vesicle attached to the upper margin of the iris, the outer and inner layers of the iris being continuous to the outer and inner walls respectively of the vesicle. The cytoplasm of the regenerate is completely depigmented and stains intensively when treated with toluidine blue. On the other hand, in the series treated with ribonuclease, the cytoplasm is completely unstainable with this dye (Fig. 1 and 2).

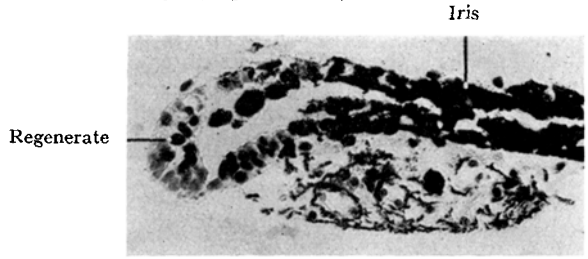

Fig. 1.–10-day regenerate. Treated with ribonuclease (0·1 mg per ml ribonuclease in distilled water, pH 7·4, 1 h, 40°C). The basophilia is completely disappeared in the cytoplasm but not in the nucleus.

(3) *14-day regenerate.* The regenerate is still attached to the margin of the iris. The internal wall of the lens-

Fig. 2. 10-day regenerate. Control (distilled water, pH 7·4, 1 h, 40°C). Adjoining section from the same regenerate as Figure 1. Note strong basophilia of the cytoplasm.

[1] Aided partially by the Governmental Research Fund for Science.

[2] T. CASPERSSON (1941), cited in Symp. Soc. Exp. Biol., No. 1, 127 (1947). – J. BRACHET (1941), cited in *Embryologie Chimique*, (Masson & Cie., 2nd ed., Paris 1947).

[3] J. BRACHET, Cold Spring Harbor Symp. Quant. Biol. *12*, 18 (1947). – A. B. NOVIKOFF and V. R. POTTER, J. Biol. Chem. *173*, 233 (1948).

[4] R. C. MELLORS and K. SUGIURA, Proc. Soc. Exp. Biol. Med. *67*, 242 (1948).

[5] D. L. DRABKIN, J. M. FETSKO, and B. L. LECRONE, J. Biol. Chem. *171*, 395 (1947).

[6] A. B. NOVIKOFF and V. R. POTTER, J. Biol. Chem. *173*, 223 (1948).

[7] W. WEITZMANN (1941), cited by J. BRACHET in *Embryologie Chimique* (1947).

[8] To examine this point, a histochemical study of nucleic acids in the regenerating lens of *Triturus pyrrhogaster* was undertaken under the direction of Professor TUNEO YAMADA. The author is especially indebted to Professor TADAO SATO for suggesting this work and his kind offer of the operated animals. Thanks are also expressed in appreciation of the assistance and aids given by Professor FUJIO EGAMI and Mr. MICHIO SHIMOMURA of the Laboratory of Organic Chemistry of the Faculty.

[1] M. R. MCDONALD, J. Gen. Physiol. *32*, 39 (1948).

vesicle is thickened forming the rudiment of lens-fibres, while the remaining part of the wall of the vesicle is converted into lower, one-layered epithelium. Both components of the vesicle show strong basophilia, which is completely lost after the use of ribonuclease (Fig. 3 and 4).

(4) *17-day regenerate.* The regenerate is separated off from the upper margin of the iris. The ribonuclease-sensitive basophilia of the cytoplasm of both components of the regenerating lens is as strong as before. In the now strongly elongated fibre cells, the nuclei are anisometric with its longest diameter along the long axis of the cell. Small vacuoles which are unstained with basic dye are visible in the cytoplasm of fibre cells which retains its basophilia.

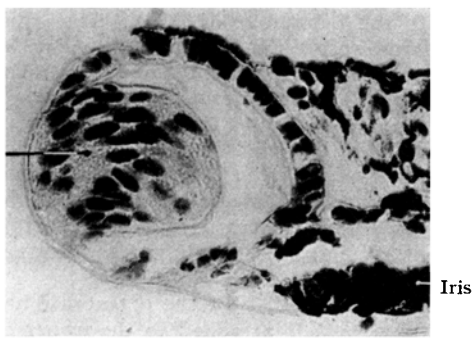

Fig. 3. 14-day regenerate treated with ribonuclease. The same treatment as for Figure 1.

(5) *25- and 30-day regenerates.* The morphogenesis of the regenerating lens is approaching its end. The ribonuclease-sensitive cytoplasmic basophilia of all components of the regenerate is still strong. In the central region of the lens, the nuclei of lens-fibres show degenerative changes, being irregularly fragmented or forming vacuoles. On the other hand the lens-epithelium always contains nuclei of normal appearance.

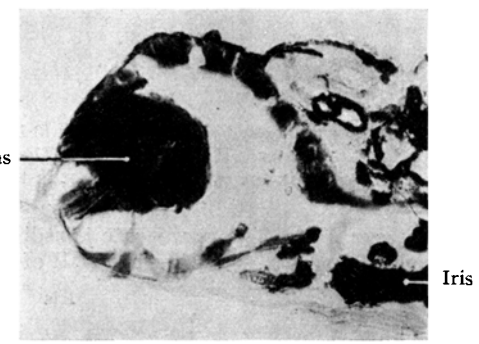

Fig. 4. 14-day regenerate. Control. The same treatment as for Figure 2.

(6) *Intact lens.* In the intact lens of the adult, the lens-epithelium shows no cytoplasmic basophilia, while the lens-fibre shows a weak basophilia in its cytoplasm. However, in the series treated with ribonuclease, the latter basophilia is unaffected.

(7) It may be added that no indication of the presence of nucleoli was obtained throughout the phases of regeneration investigated, despite special endeavour to demonstrate them as a negative image with Feulgen's test or as a positive image with toluidine blue combined with ribonuclease.

From the data presented it is highly probable that the cytoplasm of all components of the regenerate contains a large amount of RNA and no significant change in its quantity occurs throughout all regenerating periods examined. Even in the later stage of regeneration, where the structure of the regenerate is almost comparable with the normal lens, the cytoplasm of the regenerate is still rich in RNA. This is in remarkable contrast to the almost complete absence of this substance in the normal adult lens. This fact may indicate that rich RNA of the regenerate is closely related to the cytoplasmic protein-synthesis, which underlies the growth and differentiation To this general suggestion might be added some further considerations: Microscopical observations show that during the period from the 7th to the 10th day the cells multiply most intensively. Here the cytoplasmic RNA seems to take part in the regenerative cell-multiplication. During the period between the 14th to the 30th day, differentiation of fibre occurs parallel to cell-multiplication. Here it is not impossible that the cytoplasmic RNA participates in fibre-differentiation as well as in cell-multiplication.

As described above, the nuclei of central fibre region show characteristic degenerative changes in the last phase of regeneration. To examine the possible alteration of the cytochemical nature of the nuclei during their degeneration, Feulgen's nucleal test and the staining with methyl green-pyronin were carried out. However, no sign of any qualitative change in DNA was obtained.

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Biological Institute, Faculty of Science, University of Nagoya, February 2, 1952.

Zusammenfassung

Das Verhalten von Nukleinsäuren während der Regeneration der Linse des erwachsenen Molches (*Triturus pyrrhogaster*) wurde untersucht, wobei hauptsächlich Toluidinblaufärbung kombiniert mit Ribosenuklease verwendet wurde. Am Zytoplasma aller Zellen des Regenerats wurde während sämtlicher Regenerationsstadien eine starke Reaktion auf Ribosenukleinsäure festgestellt. Die normale Linse zeigte im Zytoplasma keine Reaktion auf Ribosenukleinsäure.

Metabolic Chromosomes Isolated from Blood Cell Nuclei of Various Animals

Recently, YASUZUMI and coworkers[1] have succeeded in isolating the metabolic chromosomes from the blood cell nuclei of man, carp, and tortoise, and have demonstrated that the metabolic chromosome clearly consists of a double-coiled spiral in which the major spiral is double-stranded. In the present experiment the direction of spirals has been discussed in the metabolic chromosomes isolated from the blood cell nuclei of rabbit *Oryctolagus cuniculus* var. *domesticus*, triton *Triturus pyrrhogaster*, and carp *Cyprinus auratus*. In addition to such chromosomes composed of the spiral as this, in the present experiment another kind of the chromosome in

[1] G. YASUZUMI, Chromosoma *4*, 222 (1951). – G. YASUZUMI, G. MIYAO, Y. YAMAMOTO, and J. YOKOYAMA, Chromosoma *4*, 359 (1951).