# ELECTRON MICROSCOPE OBSERVATIONS ON THE METABOLIC NUCLEUS

by

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## INTRODUCTION

The ordinary light microscope revealed nothing more concerning the structure of nucleus than the stainable elements with basic dyestuffs. All the finer details, especially unstained elements, were hopelessly concealed. Some of the conditions necessary for the examination of objects with an electron microscope are very discouraging to biologists. It occurred to the present authors that the difficulty of obtaining small, thin objects might be overcome by preparing nuclei of hemolyzed fish erythrocytes whose nucleus membrane is thin and transparent to electron beams.

## METHODS

In this experiment the red blood cells of *Sebastodes matsubarae* were used. Blood was withdrawn from the caudal vein with a syringe containing a small quantity of hypotonic salt solution. The specimen was spread at once over the celloidin-covered glass slide in a manner similar to that employed in making blood smears for light microscopic study. It is important that the films be exceedingly thin. The thin films of blood cell layer were air-dried at 20° C, and then osmic acid was applied in vapour form from a 2% water solution for a short period. The contrast of the object was increased with the chromium shadow technique, which at the same time makes the collodion membrane so strong that it gets rid of the shrinkage when stripped off the slide glass. A magnetic type of the SHIMAZU electron microscope was used in this experiment.

## RESULTS

Several degrees of hemolysis were apparently provoked by the hypotonic solution employed in the preparations of the material. Thus, there are different degrees of opacity of the cytoplasm to electrons in each of the figures, but the cytoplasm of each of the hemolyzed erythrocytes contained in the figures is transparent to electrons as compared with the homogeneous opacity of unhemolyzed erythrocytes.

Fig. 1 indicates that the membrane is damaged at its lower border with a deformation which represents an opening through which the contents of the erythrocyte flowed out into the medium during the hemolysis. In hemolysis, such fissures regularly appear in membranes, as seen also at the right border in Fig. 2. The identification and interpretation of the fine structure of the nucleus is difficult because the nucleus components situated at different levels appear in focus simultaneously, owing to the great depth of focus of the electron microscope objective, so that it is often impossible to

*References p. 17.*

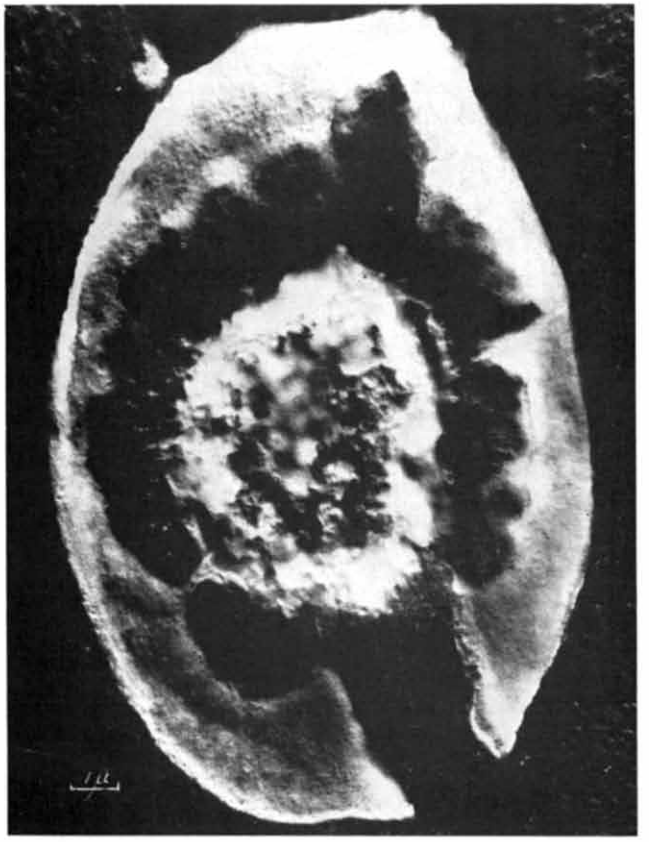

Fig. 1. The erythrocyte membrane of *Sebastodes matsubarae* is damaged at its lower border. The partially hemolyzed cytoplasm makes it possible to demonstrate the fine structure of the nucleus.

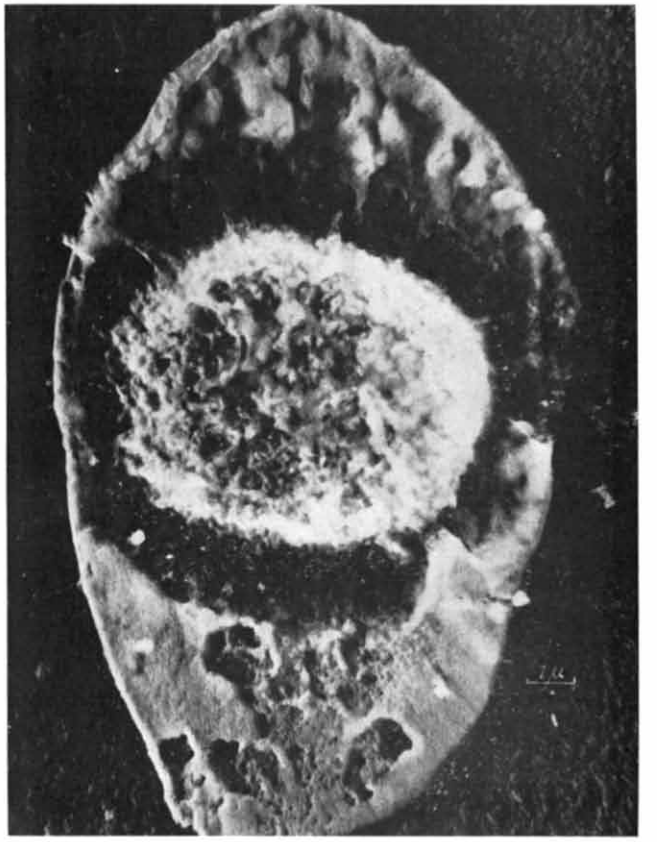

Fig. 2. The partially hemolyzed cytoplasm has the appearance of a coarse spongework. The nucleus is packed with a multitude of coiled fibrous elements.

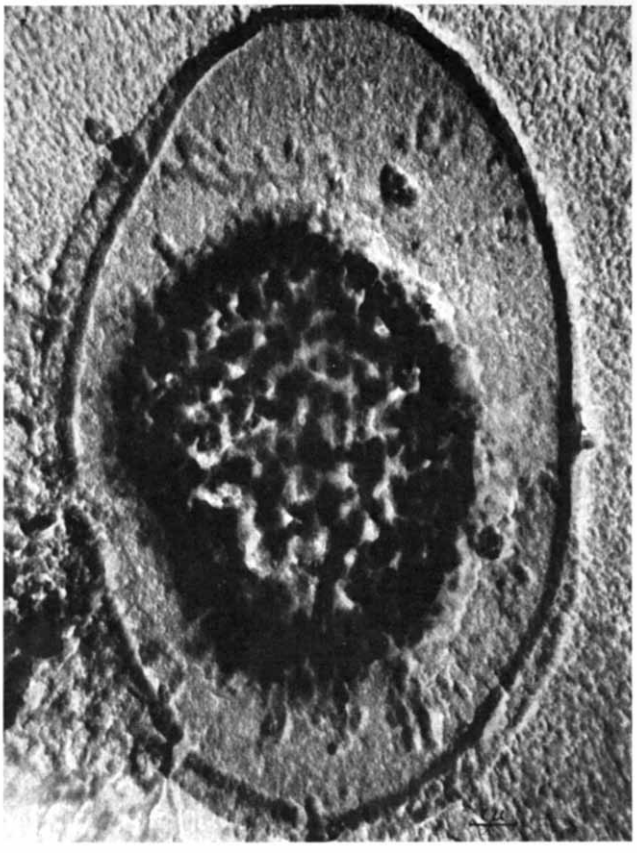

Fig. 3. The hemolyzed erythrocyte appears as double disk with thin walls and oval shape. The nucleus shows the complicated structure composed of the thread-like bodies.

ascertain their true position in the nucleus. Fig. 1 and 2 clearly indicate that the nucleus is packed with a multitude of the coiled fibrous elements, which often are suggestive of being composed of chromomeres, and gives an appearance of sponge.

The orginally more or less ellipsoid-like corpuscle changes to a flattened form as the result of hemolysis. The nuclei, in correlation with these changes in cell form, increase in size and become structurally incompact, and manifest a tendency toward a flattening in conformity with the shape of the cell body (Fig. 3). From consideration of electron scattering it is very likely that the dark outline in Fig. 3, 5, and 6 can be interpreted as arising from a cell wall or cell membrane. As can be seen from Fig. 3, as the hemolyzed cell body swells, the nucleus enlarges itself, showing the structure composed of thread-like bodies. In this stage it is difficult to detect the chromomere-like granules, while it is easy to demonstrate a pair of coils twisted around each other at the points marked by the arrows (Fig. 4).

As the nucleus membrane is damaged the karyolymph has been released retaining only the form-elements. The rod-shaped bodies, being of several sizes in length and width, are clearly visible as in Fig. 5. In Fig. 6, the nucleus assumes a more or less eccentric position, and the nucleus membrane is damaged at its right border with a

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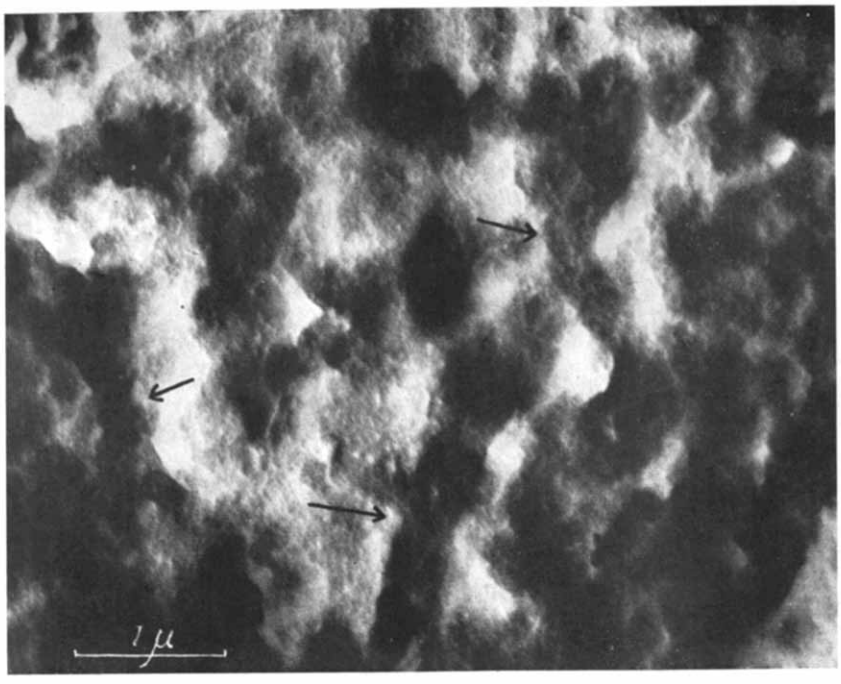


Fig. 4. The enlargement of part of Fig. 3 shows that the thread is composed of two coils twisted around each other, at the points marked by the arrows.

deformation which represents an opening through which the contents of the nucleus have slowly flown into the watery surroundings during the karyolysis. But the karyolysis like this is possible only when we have a blood corpuscle of low osmotic resistance. All the elements released out of the nucleus in Fig. 6 look like the coiled threads, being of several sizes of length and width. With the progress of karyolysis the thread-like bodies are broken into pieces; so that finally, there remain only the fine granules with the approximate dimensions of 80–160 mμ (Fig. 7). In the erythrocyte nuclei the nucleolus can neither be revealed by electron microscopy nor by UNNA-PAPPENHEIM's method.

## DISCUSSION

The structure of nuclei in the metabolic stage appears to be very complicated, hence it is generally called reticulum. In fact, even with suitable cytological methods (acetecarmin and iron-haematoxyline) exact observation concerning the structure of nuclei of animal tissues is not possible.

If the chromosomes are of the spiral or the thread structure, the reticulum would be interpreted as formed of the threads of the chromonemata through a slight change in their spiral construction without any discontinuity in the transformation. The results obtained by recent botanists are converging on the view that in the metabolic stage the chromonemata are rendered more or less loosely or even irregularly coiled, so that the chromosome territories become hardly distinguishable from one another, thus

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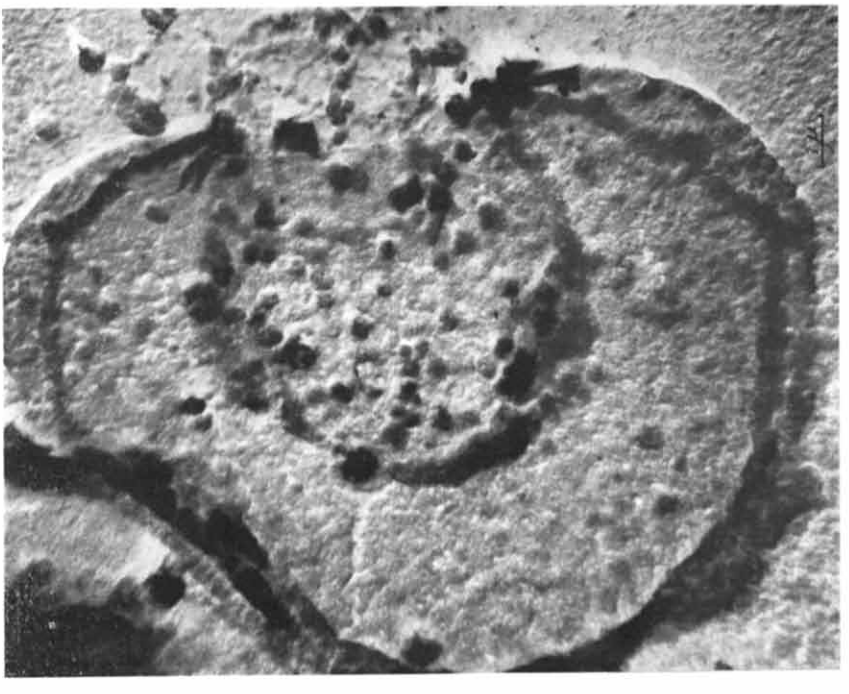

Fig. 6. The erythrocyte membrane is damaged at its right border with a deformation which represents an opening through which the contents of the nucleus are released. The released bodies out of the nucleus seem to be coiled threads.

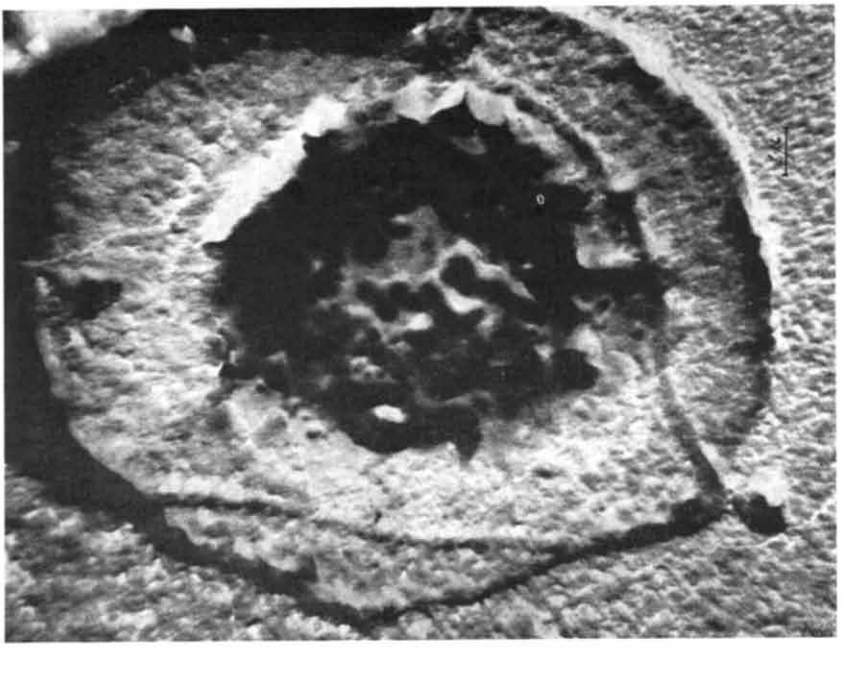

Fig. 5. The nucleus membrane is destroyed and the rod-shaped bodies, being of several sizes in length and width, are clearly visible.

Fig. 7. The figure shows the sharp border formed by the elasticity of the erythrocyte membrane. The nucleus shows the structure composed of the fine granules and their aggregation as the results of karyolysis.

forming a structure of the complex appearance, the reticulum, but still retaining their spiral character[1,2,3,4,5].

The intact nucleus seems to be rather ellipsoid, so that it is impossible to ascertain each chromosome in the nucleus. The hemolyzed erythrocytes are sufficiently thin to permit electron microscopy of the nuclear region, and structural detail has been revealed in the nucleus. With the progress of hemolysis the nucleus increases in size and manifests a tendency towards a flattening, resulting in isolating each chromosome which is composed of a pair of chromonemata.

Recently, YASUZUMI and co-workers[6,7,8] have succeeded in isolating the metabolic chromosomes from the blood cell nuclei of various animals by a Waring blendor and have demonstrated that the metabolic chromosome clearly consists of a double coiled spiral in which the major spiral is double-stranded. The present experiment supports YASUZUMI and collaborators's findings, illustrating the chromosomes in situ in the nucleus.

The rod-shaped bodies, which appear as the result of damage of the nucleus membrane, presumably correspond to the metabolic chromosomes whose microstructure

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has unfortunately been concealed by shrinkage in drying. The coiled bodies released out of the nucleus are perhaps the fragmented metabolic chromosomes. With the progress of karyolysis the chromosomes are finally broken into the fine granules which seems to be chromomeres or subunits.


## SUMMARY

The blood of *Sebastodes matsubarae* was spread over the celloidin-covered glass slide in a manner similar to that employed in making blood smears for light microscopic study. The contrast of the object was increased with the chromium shadow technique, which at the same time makes the collodion-membrane so strong that it gets rid of its shrinkage when stripped off the slide glass. The microstructure of the nucleus in partial hemolysis, total hemolysis, and karyolysis has been investigated. From these images it is concluded that the chromosomes exist in the metabolic nucleus as spiral threads composed of a pair of chromonemata. With the progress of karyolysis the chromosomes have finally been destroyed into the chromomere-like granules approximately 80–160 mμ in diameter.

## RÉSUMÉ

La structure du noyau des érythrocytes de *Sebastodes matsubarae* a été étudiée, après hémolyse et caryolyse, à l'aide du microscope électronique. Les contrastes de l'object ont pu être accentués par la technique des ombres au chrome. Ces photographies montrent les divers aspects de la texture macromoléculaire du noyau et de son contenu; les chromosomes avec une y semblent exister sous forme de filaments en spirale. Il ne subsiste dans les noyaux caryolysées que des grains, semblables à des chromomères, et de 80–160 mμ de diamètre.

## ZUSAMMENFASSUNG

In der vorliegenden Arbeit wurde die Struktur der Erythrozyten-Kerne von *Sebastodes matsubarae* nach partieller und totaler Hämolyse und Karyolyse elektronenoptisch untersucht. Zur Herstellung des Erythrozyten-Präparates strich man das Blut auf den Objektträger, auf den man vorher eine Kollodium-Membran aufgesetzt hatte. Die Kontraste des Objektes wurden durch Anwendung der Chrom-Schatten-Technik verstärkt. Im metabolischen Kern der Erythrozyten finden sich die Chromosomen, die im Zustande von Spiralen zum Vorschein kommen. Mit dem Fortschritte der Karyolyse haben die Chromosomen die Neigung zu zerfallen und noch weiter in chromomeren-artige Granula, deren Durchmesser 80–160 mμ betragen, zerstört zu werden.


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