

Differentiation between Metabolic Chromosomes and Micro-organisms with Coiled Structure

In our laboratory metabolic chromosomes isolated from blood cell nuclei of various animals have been revealed with the double coiled or triple coiled structure, and the flagellata-like bodies have been suggested to be sex chromosomes from the cytological standpoint¹. As many authors² have reported, we have also obtained many micro-organisms with coiled structure and one or a few flagellae (Fig. 1), and stalked bacteria from the

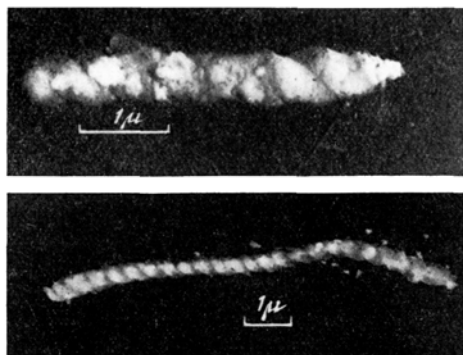


Fig. 1.—Electronmicrographs of micro-organisms with coiled structure.

distilled water which had been left in the room for a long time. In the present paper we have endeavoured to differentiate the metabolic chromosomes isolated from erythrocyte nuclei of triton *Triturus pyrrhogaster* from these micro-organisms. This was carried out with the SHIMAZU's magnetic electron microscope.

When the blood cell nuclei are disintegrated by a blender (3,000 R.P.M.) at 3°C, a lot of metabolic chromosomes appear in several shapes, lengths, and widths, but only in the same coiled structure. By means of shadow

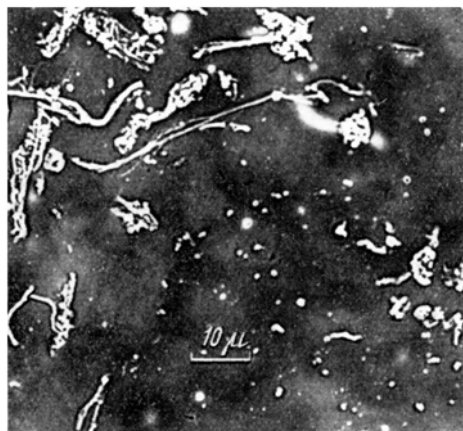


Fig. 2.—Light micrograph of metabolic chromosomes isolated from erythrocyte nuclei of *Triturus pyrrhogaster*. Chrom shadow casting.

¹ G. YASUZUMI, *Chromosoma* 4, 222 (1951). — G. YASUZUMI, G. MIYAO, Y. YAMAMOTO, and J. YOKOYAMA, *Chromosoma* 4, 359 (1951). — G. YASUZUMI, T. YAMANAKA, S. MORITA, Y. YAMAMOTO, and J. YOKOYAMA, *Exper.* 8, 218 (1952).

² JULIA M. COFFEY, *Ann. Rep. Div. Labor. Research, New York State Dep. Health* 1951, 25. — A. L. HOUWINK, *Exper.* 8, 385 (1952). — R. WIGAND und D. PETERS, *Z. wiss. Mikrosk.* 60, 405 (1952). — A. R. T. DENŨES (Private communication). — H. RUSKA (Private communication).

casting we can easily demonstrate the coiled structure of metabolic chromosome with the ordinary light microscope, as is seen in Figure 2.

For quantitative analysis of metabolic chromosomes we have measured and compared with the metaphase plates published by MUCKERMANN¹ and SATO². The metabolic chromosome shown in Figure 3 and the long type in metaphase plates of germ cells are identical in shape and size. As already shown in our previous papers³, the so-called chromatin-granules (heterochromatin?) are always detected, attaching to the vertex

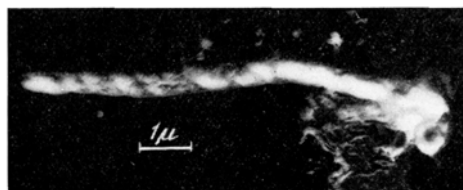


Fig. 3.—Electromicrograph of \uparrow -type metabolic chromosome attached to chromatin granule and nuclear membrane.

of loop-angle of metabolic chromosomes, if the nuclei are not markedly decomposed, as shown in Figures 2 and 3. But we cannot find evidence of any connection between micro-organisms and chromatin-granules.

The coiled structure of a metabolic chromosome is easily destroyed by mechanical agitation and uncoiled, so that it is sometimes difficult to obtain a complete metabolic chromosome. Each metabolic chromosome is composed of a pair of chromonemata and each chromonema of a pair of chromofibrils (Fig. 4). We have been able to follow the several degrees of disintegration of chromosomes, though it is not possible to show many micrographs in this paper. However, the micro-organisms offer resistance to mechanical agitation, as compared with metabolic chromosomes.

The micro-organisms obtained from the water have often one or a few flagellae, while the metabolic chromosomes have no flagella. A fibre found at the vertex of the metabolic chromosome is an artifact produced by mechanical agitation, that is, an elongated chromonema or chromofibril.

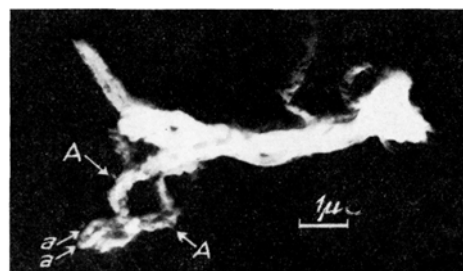


Fig. 4.—Electromicrograph of \vee -type metabolic chromosome partially decomposed. A Chromonema. a Chromofibril.

We have already succeeded in revealing the existence of metabolic chromosome in situ in the erythrocyte nucleus of *Sebastes matsuabarae*⁴. In the triton ery-

¹ H. MUCKERMANN, *La Cellule* 28, 231 (1913).

² I. SATO, *J. Sci. Hiroshima University* [B] 2, 33 (1932).

³ G. YASUZUMI, *Chromosoma* 4, 222 (1951). — G. YASUZUMI, G. MIYAO, Y. YAMAMOTO, and J. YOKOYAMA, *Chromosoma* 4, 359 (1951).

⁴ G. YASUZUMI, Z. SUGIOKA, and A. TANAKA, *Biochem. Biophys. Acta* 10, 11 (1953).

thocyte nucleus treated with ammonia, the coiled structure has been shown by means of the ordinary light microscope¹.

It will be discussed in the near future how the flagellata-like chromosomes are different from the stalked bacteria.

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Zusammenfassung

Elektronenoptisch wird die Differenz zwischen den metabolischen Chromosomen und den Mikroorganismen, die den ersteren in mancher Hinsicht ähnlich sind, nachgewiesen. Durch die mechanische Einwirkung werden die metabolischen Chromosomen leicht zerstört; so werden Chromosomen in Chromonemata und weiter zu Chromofibrillen zerlegt. Die Mikroorganismen leisten der mechanischen Einwirkung im Vergleich mit den metabolischen Chromosomen Widerstand. Die quantitative Analyse der metabolischen Chromosomen ist durch Vergleich mit der Äquatorialplatte in der Metaphase möglich.

¹ Y. KUWADA (Private communication).

Notes on the Chromosomes of the Porcupine and the Chinchilla

This short paper is a report on the chromosomes of two interesting forms of rodents, the New World porcupine and the chinchilla, which belong to the suborder Hystricomorpha of the order Rodentia. Testicular tissues, fixed with FLEMING's solution without acetic acid and stained with NEWTON's gentian violet, were used for study.

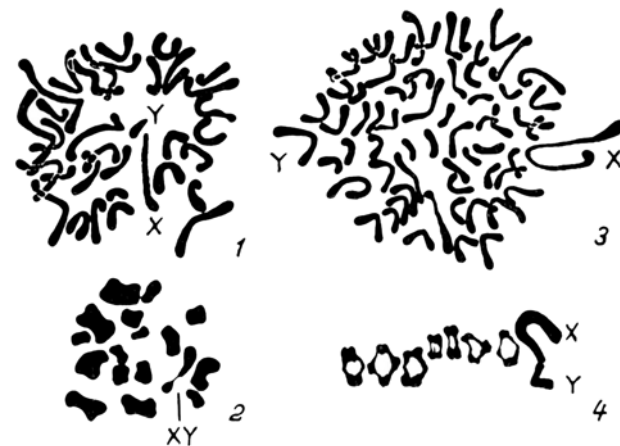
Chromosomes of the New World Porcupine. The porcupine occurring in North America is represented by only one species, *Erethizon dorsatum*, in the family Erethizontidae. This animal is a common forest inhabitant distributed throughout almost the whole range of the United States.

Based on five good metaphase plates of the spermatogonia, this animal was found to have the diploid number of 34 in the male (Fig. 1). The chromosomes have mostly subterminal and submedian centromeres assuming distinct J- and V-shape. Among them, however, two chromosomes are prominent because of their distinguishing features. One is a long rod-like element with slender, somewhat diffused outline. The other is a short rod-shaped one having a tapering end. Each one is without a partner corresponding in shape and size. There is thus sufficient reason to interpret the larger one as the X chromosome and the smaller as the Y.

Some first spermatocytes were found in process of division showing 17 bivalent chromosomes consisting of 16 autosomal bivalents and a heteromorphic XY-complex (Fig. 2).

According to MULDAL¹ the Old World porcupine, *Hystrix cristata* (which may not be closely related to *Erethizon*, according to WOOD²), has 48 chromosomes in

the diploid set. We have not had access to any detailed accounts of the chromosomes of *Hystrix* other than the diploid number. Therefore we are not in position to enter into any discussion of the difference of the chromosome number between these two forms, although both cytologists and taxonomists will be interested in this question.



Figs. 1–2.—Chromosomes of the porcupine (*Erethizon dorsatum*). 1, spermatogonial metaphase. 2, first spermatocyte metaphase. Figs. 3–4.—Chromosomes of the chinchilla (*Chinchilla laniger*). 3, spermatogonial metaphase. 4, first spermatocyte spindle, metaphase side view.

Chromosomes of the chinchilla. *Chinchilla laniger* is a large South American rodent belonging to the Chinchillidae. This animal has attracted attention because of the high value of its fur, and has been largely bred and domesticated.

The results of counting the chromosomes of clear metaphase plates in both the spermatogonia and two kinds of the spermatocytes revealed that the chromosome number of this animal was 64 in diploid and 32 in haploid. The diploid complement is provided with the chromosomes carrying subterminal and submedian centromeres, each one having two distinct arms (Fig. 3). Further, the diploid complex is very remarkable in showing two chromosomes which are prominent in shape and size. The one is a huge V-shaped chromosome, largest of all in size. The other is also a remarkable V-shaped element coming next in size. Both have submedian centromeres and remain unpaired since there is no homologous mate corresponding in shape and size. There is no doubt that these two particular chromosomes are to be regarded as the XY-complex (Fig. 3).

Primary spermatocytes show at metaphase 31 autosomal bivalents and a heteromorphic XY-complex which consists of a larger V-shaped X and a smaller V-shaped Y coming into contact by their shorter arms (Fig. 4).

Looking over the literature¹, the chromosomes of the animals belonging to the suborder Hystricomorpha have been reported in *Myocastor coypus* (Capromyidae), *Cavia cobaya* (Caviidae) and *Hystrix cristata* (Hystricidae). The nutria, *Myocastor coypus*, was found to possess 42 diploid chromosomes which are of atelomitic structure except three dot-like ones. In the guinea-pig, *Cavia cobaya*, 64 chromosomes were known in diploid, showing the formula of $2V's + 62r's$. The chromosome

¹ S. MULDAL, John Innes Horticultural Institution, 38th Annual Report 23 (1947).

² A. E. WOOD, *Evolution* 4, 87 (1950).

¹ R. MATTHEY, *Chromosomes des Vertébrés* (Edit. S. Rouge, Lausanne, 1948). — S. MAKINO, *An atlas of the chromosome numbers in animals* (Iowa State College Press, Ames, Iowa, 1951). — J. Fac. Sci. Hokkaido Univ. Ser. VI. Zool. 9, 345 (1947).