NEW EVIDENCE ON THE SPIRALIZED STRUCTURE OF RESTING CHROMOSOMES (ISOLATED HUMAN CHROMOSOMES)

by

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New contributions to the study of the resting nucleus both in normal and pathological conditions have been made by squash technique and by the technique of isolated chromosomes.

A critical review on this subject has appeared recently[1].

By means of squash technique it has been established that in botanical and zoological species the structure of the nuclei can be classified in four types, with the possibility of reciprocal transformation. According to these researches, the most common type of nucleus contains a number of filaments more or less intermingled and irregularly spiralized to give the appearance of a reticulum. Only certain portions of the chromosomes are not free, but form compact masses (chromocentres, heteropycnotic segments). We have no definite knowledge whether these filaments are single or multiple, and what special activities are to be ascribed to the heteropycnotic segments and to the chromocentres[2,3,4]. Further data on the morphology of the interphase isolated chromosomes are given by several authors[5–18].

The general procedure followed by these authors consisted essentially in a physical isolation of somatic nuclei (mainly in the resting stage), followed by differential centrifugation to obtain chromatin threads isolated from broken nuclei. These filaments were found to have aspects both of mitotic (constrictions, rigidity of the arms) and of interphase chromosomes (filamentous shape). From a structural point of view, it seems of outstanding interest to investigate further the relationship between the isolated resting chromosomes, which are irregularly spiralized, and the mitotic chromosomes, whose spirals are regularly and tightly packed. So far, electron microscope data have been available only from YASUZUMI and co-workers[13,14], who showed a spiralized structure in the resting chromosomes from different zoological species.

The aim of the present paper is to report data on the morphology of human chromosomes (in normal and pathological conditions), which has not hitherto been investigated.

The chromosomes were isolated from normal and leukaemic white cells of human blood. The technique has already been described[12]. Mounts for electron microscopy were made with unfixed material placed on the grid by means of a platinum wire. The excess was removed, and the Formvar films fixed in the dark for 5 to 10 minutes by vapour-fixation with osmium tetroxide (2 % aqueous solution). After drying, the grids were washed several times with glass-distilled water. Shadowing was carried out with gold at

References p. 220.

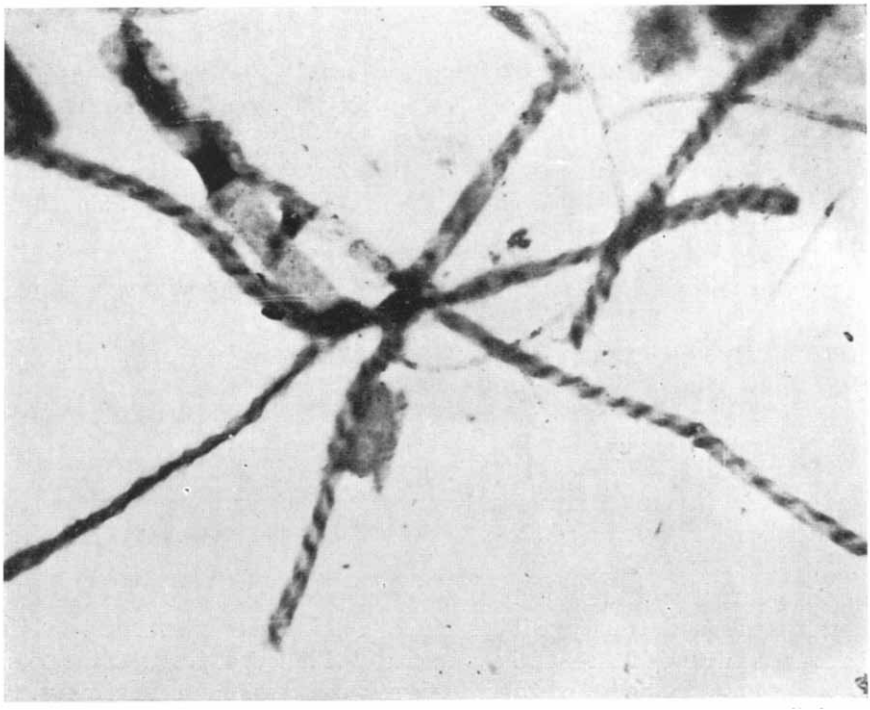

Fig. 1. A group of isolated human chromosomes from leukaemic cells showing a coiled structure Magnification 8,000. Vapour-fixation with 2 % osmium tetroxide in aqueous solution. × 60,000.

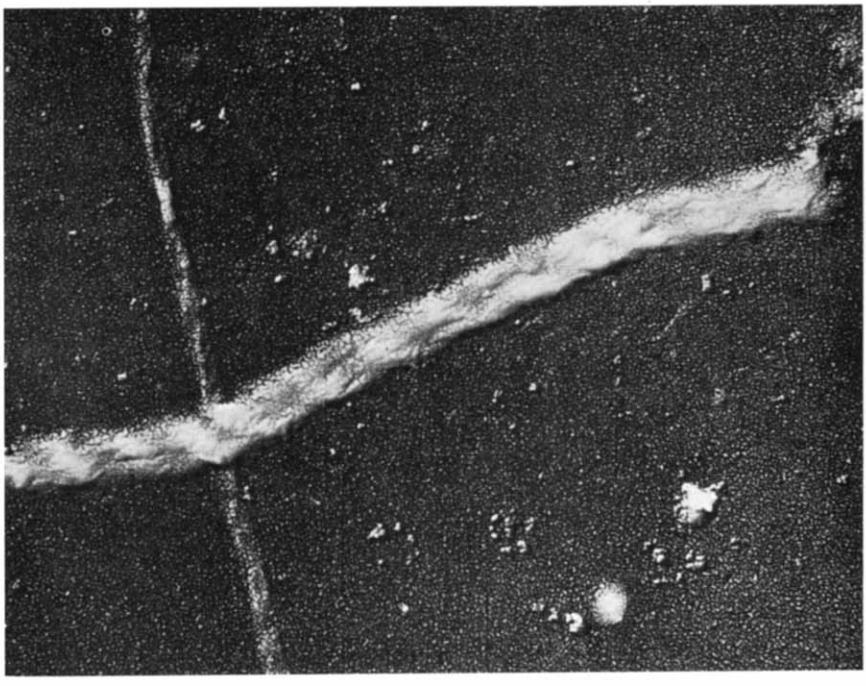

Fig. 2. Isolated human chromosomes from leukaemic cells. Note, at the left, the thin thread with traces of spirals. Magnification 19,000. Vapour-fixation with 2 % osmium tetroxide in aqueous solution. Shadowing with gold. × 60,000.

Fig. 3. Isolated human chromosome, from leukaemic cells, showing coiled structure. In the central portion a trace of a longitudinal split can be seen. Magnification 20,000. Vapour-fixation with osmium tetroxide in aqueous solution. Shadowing with gold. × 60,000.

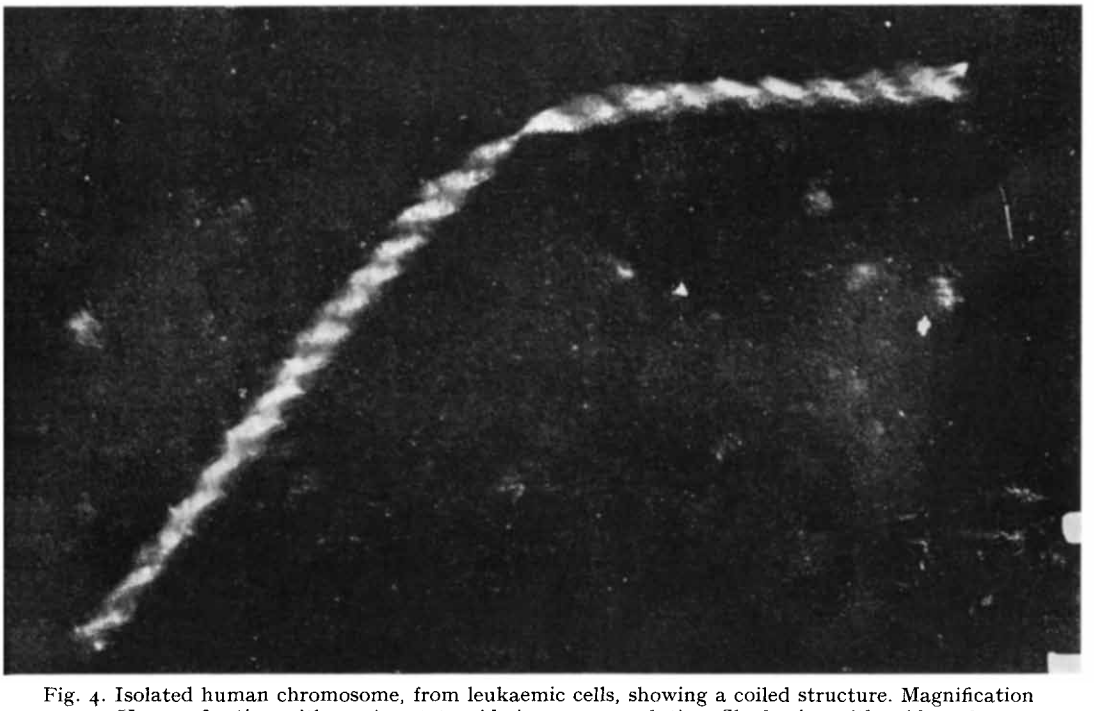

Fig. 4. Isolated human chromosome, from leukaemic cells, showing a coiled structure. Magnification 14,000. Vapour-fixation with osmium tetroxide in aqueous solution. Shadowing with gold. × 60,000. (At the right, note the length of 1 micron).

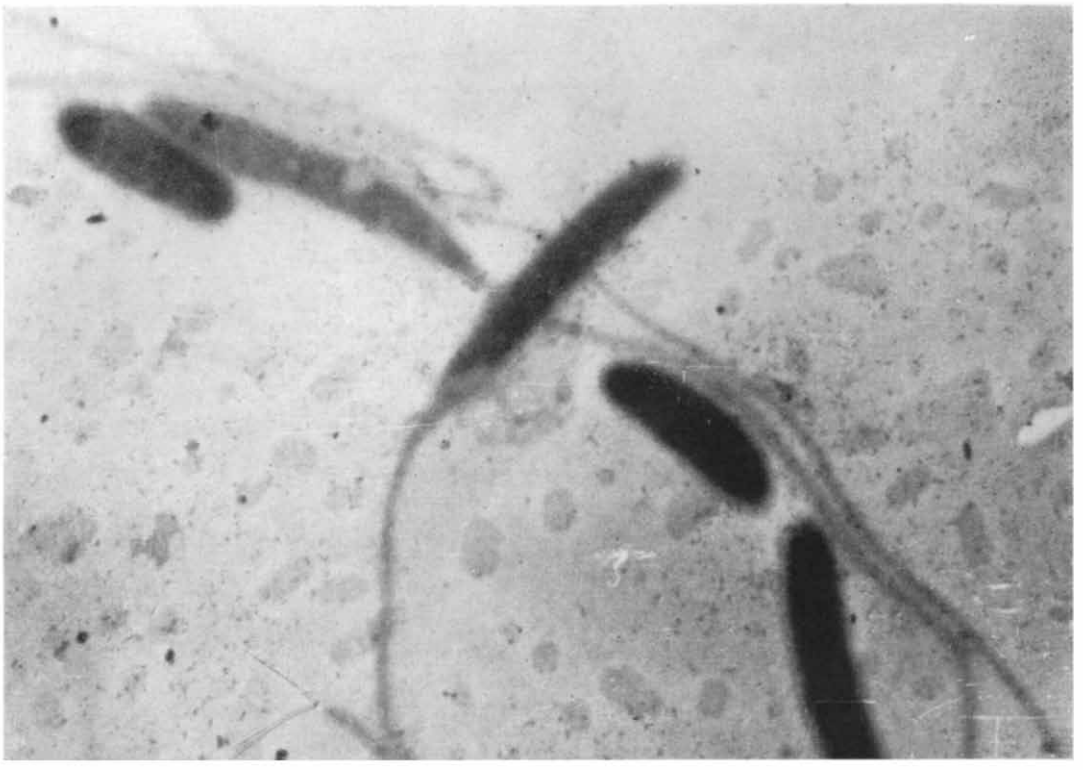

Fig. 5. Probable artefacts due to treatment. Magnification 20,000. Vapour-fixation with 2 % osmium tetroxide in aqueous solution. × 60,000.

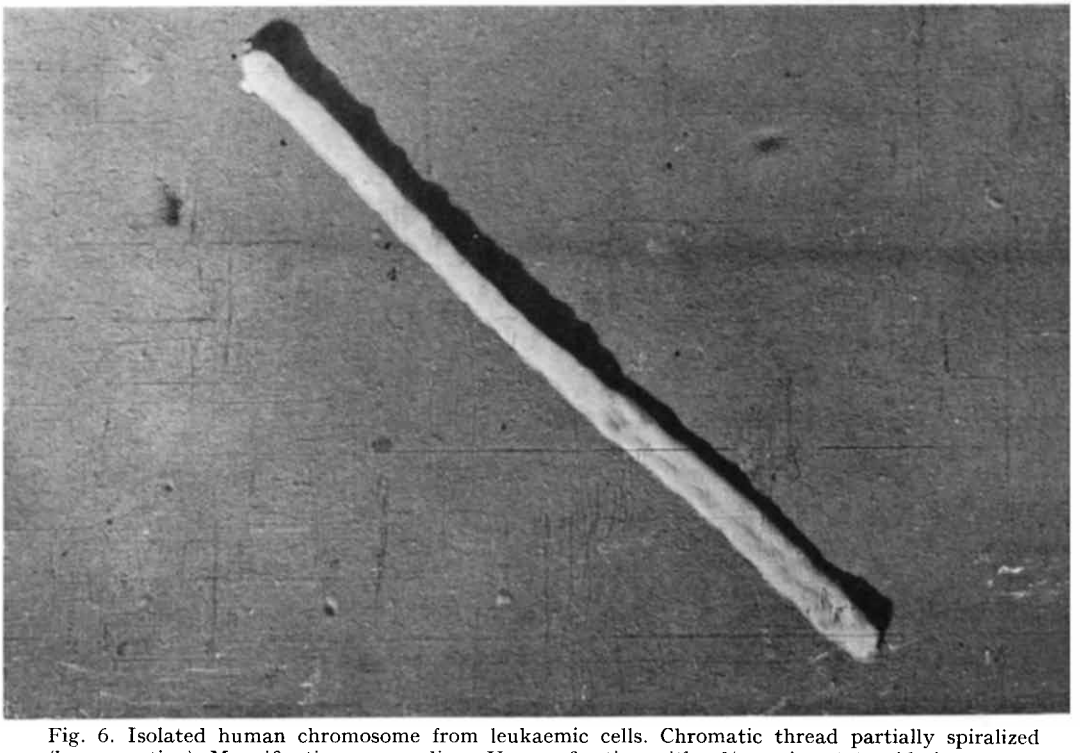

Fig. 6. Isolated human chromosome from leukaemic cells. Chromatic thread partially spiralized (lower portion). Magnification 15,000 diam. Vapour-fixation with 2 % osmium tetroxide in aqueous solution. Shadowing with gold. × 60,000.

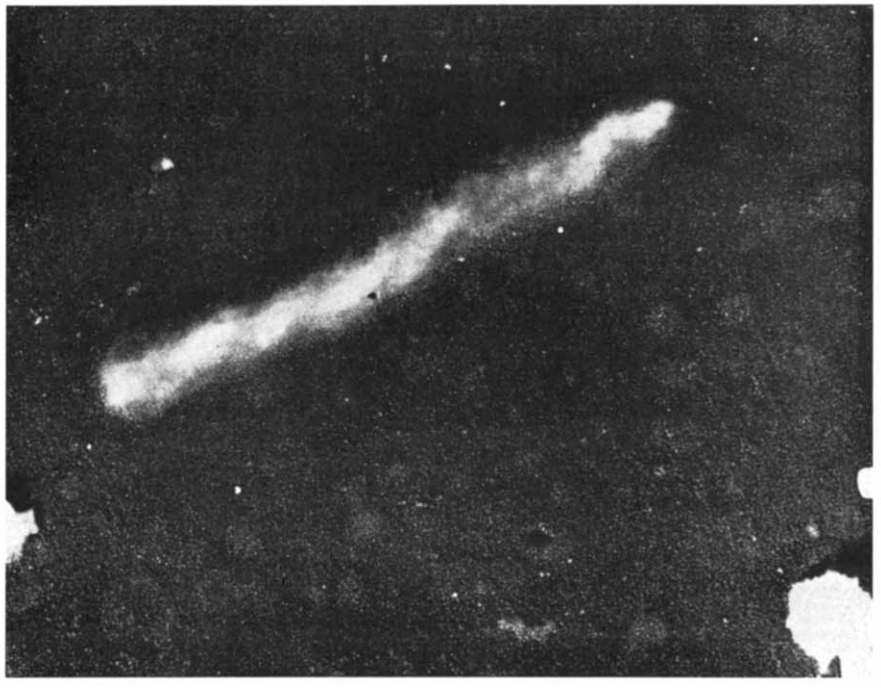

Fig. 7. Isolated human chromosome from leukaemic cells. Chromatic thread with despiralization restricted to a small portion. Magnification 20,000. Vapour-fixation with osmium tetroxide 2 % in aqueous solution. Shadowing with gold. × 60,000.

an angle of 25°. A Philips electron microscope was used, with the kind help of the personnel of the Inst. for Phys. Technique of the Polytechnical High School of Milan. At a magnification of 15,000–20,000 diam. chromosomes are seen as threads which are regularly and tightly spiralized (Figs. 1, 2, 3, 4). Some chromosomes show a longitudinal split (Fig. 3), and in the lightest prints the coils are clearly right-handed (Figs. 3, 4). The number of turns varies from 4 to 15, the length per turn being 0.4–0.5 μ. The ends of these filaments are generally blunt, though sometimes thin filaments of various lengths seem to branch off from the ends (Fig. 5). This feature was also described by YASUZUMI *et al*[17]. It seems likely that these thin filaments are artefacts arising from the physical treatment, such an interpretation being suggested by the excessive variability of their length (from 2 to nearly 40 times).

The spiralized bodies appear with a frequency of 2 % in a few thousand filaments examined. Nearly 10 % show clearly intermediate stages between spiralized and opaque homogeneous chromosomes (Figs. 6, 7), the latter representing nearly 88 % of the total filaments observed.

In some cases very thin threads appear with traces of spirals (Fig. 2). Variation in results is probably due to the difficulty in achieving suitable fixation.

The present data may be discussed in connection with several problems. First of all, the possibility must be considered of artefacts produced during the isolation procedure and during exposure in the electron microscope. Thus, for example, it is doubtful at the moment whether the spirals of the chromosomes seen in the electron micrographs correspond to the true spiralization of chromatin threads *in vivo*. If future investigations

References p. 220.

confirm that electron micrographs give a picture which does not differ consistently from the living state, and that the packing of the resting chromosomes is true, a revision will be necessary of the theory of the mitotic cycle, classically understood as a cycle of spiralization and unspiralization of the chromatic threads.

SUMMARY

The present note describes a morphological study of chromosomes extracted from human resting nuclei, both of normal and leukaemic leucocytes. The isolated chromosomes were examined in the electron microscope. At a magnification of 15,000–20,000 times they appeared to consist of a pair of regularly and tightly spiralized filaments. The observations are discussed in relation to the structure seen *in vivo*.

RÉSUMÉ

Dans ce mémoire l'auteur décrit une étude morphologique de chromosomes extraits de noyaux de leucocytes humains normaux et leucémiques à l'état de repos. Les chromosomes isolés ont été examinés à l'aide du microscope électronique. A un grossissement de 15,000 à 20,000 ils semblent être constitués d'une paire de filaments régulièrement enroulés en spirale serrée. L'auteur discute ces observations par rapport à la structure observée *in vivo*.

ZUSAMMENFASSUNG

Die vorliegende Notiz beschreibt die morphologische Untersuchung von Chromosomen, die aus Kernen von normalen und leukämischen menschlichen Leukocyten im Ruhestadium extrahiert wurden. Die isolierten Chromosomen wurden unter dem Elektronenmikroskop untersucht. Bei einer Vergrösserung von 15,000–20,000 schienen sie aus einem Paar regelmässig und dicht aufgewundener Fäden zu bestehen. Die Beobachtungen werden in Verbindung mit den *in vivo* beobachteten Strukturen besprochen.

Addendum

After the presentation of this paper for publication, Dr DENUES kindly informs me, that spiralized filaments can be observed with the electron microscope, which should be interpreted as contaminant bacteria. These objects are similar to those referred to by YASUZUMI and by myself as isolated chromosomes. I do not object to Dr DENUES' observation, and I assume, too, that bacteria as well as fungel filaments can show the same spiralized structure as the chromosomes do. On the other hand; the great majority of my preparations is composed of chromatic filaments taken from isolated nuclei. Evidence of this fact is presented in my preceding works on these filaments (see ref. 12 and 15), which show a higher degree of Feulgen positivity, which fails to appear in bacteria. Thus I assume that only a casual minority of the analysed filaments could be contaminants.

REFERENCES

1 C. BARIGOZZI, *Experientia*, 8 (1952) 133.
2 T. CASPERSSON, *Naturwissenschaften*, 29 (1941) 33.
3 C. D. DARLINGTON, *Recent advances in Citology*, London, 1937.
4 P. C. KOLLER, *Symp. Soc. Exp. Biol.*, 1 (1947) 270.
5 A. E. MIRSKY AND A. W. POLLISTER, *Proc. Natl. Acad. Sci.*, 28 (1942) 344.
6 A. CLAUDE AND J. S. POTTER, *J. Exptl. Med.*, 77 (1943) 345.
7 A. E. MIRSKY AND H. RIS, *J. Gen. Physiol.*, 31 (1947) 1.
8 W. HOVANITZ, *Genetics*, 32 (1947) 500.
9 W. HOVANITZ, A. R. T. DENUES AND R. M. STURROCK, *Wasman Collector*, 7 (1949) 233.
10 H. H. PFEIFFER, *Experientia*, 6 (1950) 334.
11 H. RIS AND A. E. MIRSKY, *Exptl. Cell. Res.*, 2 (1951) 263.
12 E. E. POLLI, *Experientia*, 7 (1951) 138.
13 G. YASUZUMI, *Chromosoma*, 4 (1951) 221.
14 G. YASUZUMI, G. MIYAO, Y. YAMAMOTO AND J. YOKOYAMA, *Chromosoma*, 4 (1951) 359.
15 E. E. POLLI, *Chromosoma*, 4 (1952) 621.
16 A. R. T. DENUES, *Exptl. Cell. Res.*, 3 (1952) 388.
17 G. YASUZUMI, T. YAMAMKA, S. MORITA, Y. YAMAMOTO AND J. YOKOYAMA, *Experientia*, 8 (1952) 218.
18 Q. VAN WINKLE, M. W. RENOLL, J. S. GARVINE AND A. F. PREBAN, *Science*, 115 (1952) 711.

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