Chromosome Structure; Half-Chromatids in Chloroform Treated Metaphases[1]

Several hypotheses on the chromosome structure have been forwarded, yet they differ as to the number of filaments within a chromatid. Investigations using light microscopy (LM)[2] and electronmicroscopy (EM)[3–5] indicated a compound structure for the chromosome. 'Half-chromatids' in amphibian lampbrush chromosomes were recently described at the EM[6]. But, other authors[7,8] reject this hypothesis in their ultrastructure studies. According to DUPRAW[7], the duplex or quadriplex aspect is an artifact ascribable to acid or enzymatic digestion, inducing a secondary aggregation of chromatin fibers of 230 Å which enables their perception at the LM. Such aggregations were already demonstrated by BARNICOT in 1967[9].

This paper is a tentative interpretation of the fibril complexity at the chromatid level. It is based on a cytological investigation of chloroform-treated preparations by light and electron microscope. A binemic model for the chromosome structure is proposed.

The material we used consisted of chromosomes of the tetraploid ($4n = 44$) and diploid ($2n = 22$) *Odontophrynus americanus*, in which the DNA content corresponds with the chromosome number[10–12], and *Eleutherodactylus binotatus*, a diploid species ($2n = 22$) with large chromosomes containing a fourfold DNA content in relation to the other species of the same genus and the same diploid number[13]. For the LM, the following technique was used: the animals were inoculated with a 1% colchicine solution (0.02 ml/g) 2 h prior to the cytological preparation. Small fragments of the intestine were placed in cold distilled water for 15 min, fixed in 50% glacial acetic acid for 15 to 30 min, and immersed in chloroform for 15 min at room temperature. Each fragment was then transferred to a slide covered with a drop of 50% acetic acid and squashed. The coverslip was removed in dry ice. After hydrolysis in HCl N at 60 °C for 5 min, the slides were stained by the Feulgen or Giemsa method. Preparations for the EM were done on a 1% collodium covered slide. After phase microscope examination, the preparations were directly transferred to metal grids[14]. The grids containing the material were stained in 2% uranyl acetate dehydrated in the alcohol series and examined in an Elmiskop I at 80 K_v.

Chloroform-treated metaphase chromosomes showed uncommon aspects: 1. The chromatids uncoil, elongate and are sometimes distinctly separated into 'half-chromatids', suggesting the quadriplex aspect proposed by some authors[2]. Both elongation and unfolding of only one of the chromatids were often found. The course of the unfolded fibrils could be followed for a considerable distance up to the limit of LM resolution. Satellites at both 'half-chromatids' were also observed (Figures 1, A–F and 2). Chromatin loops whose width is at the limit of LM resolution were found along the half-chromatid's axial fibres. (Figure 2).

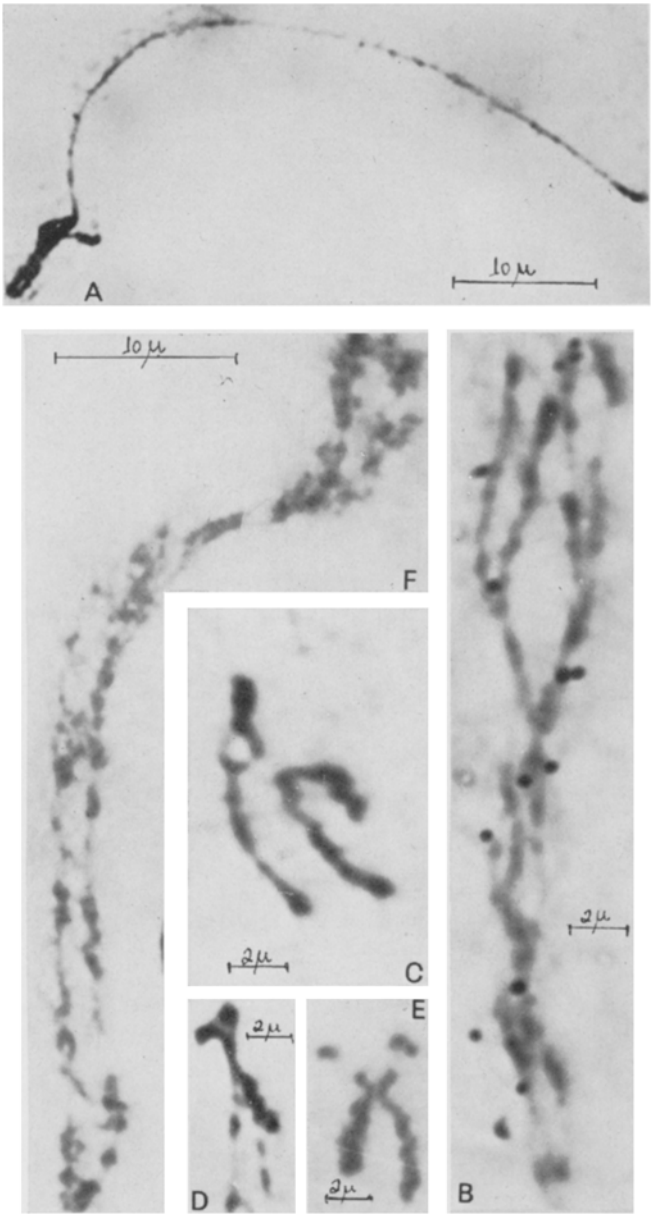

Fig. 1. Metaphase chromosomes from squash preparations of intestine treated with chloroform and stained with Giemsa: A) distorted chromosome in which one arm was pulled off; B) unfolding of chromatids in half-chromatids; C) localized separation of the half chromatids; D) separation in only one chromatid (*O. americanus*, $4n$); E) 2 satellites at the right chromatid (*O. americanus*, $2n$); F) chromatid separated in half chromatids (*E. binotatus*).

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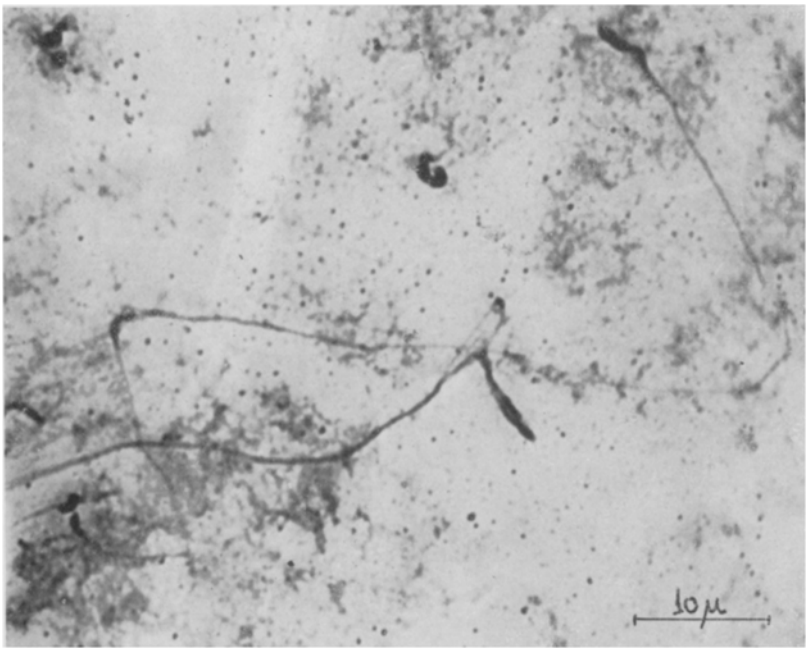
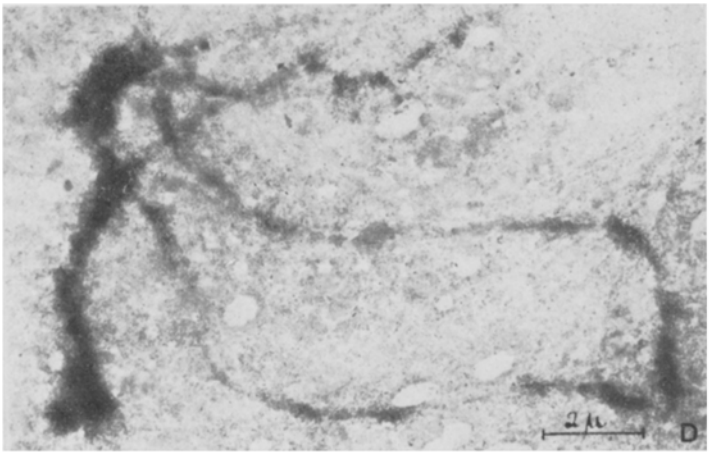
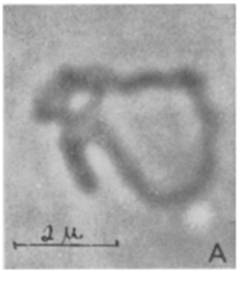
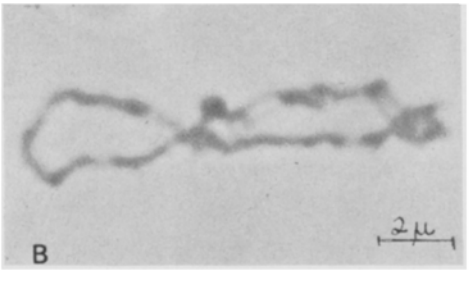


Fig. 2. Metaphase chromosome of *O. americanus* intestine 4*n*, treated with chloroform and stained with Giemsa, showing separation of half-chromatids and loops along the axial fibres.







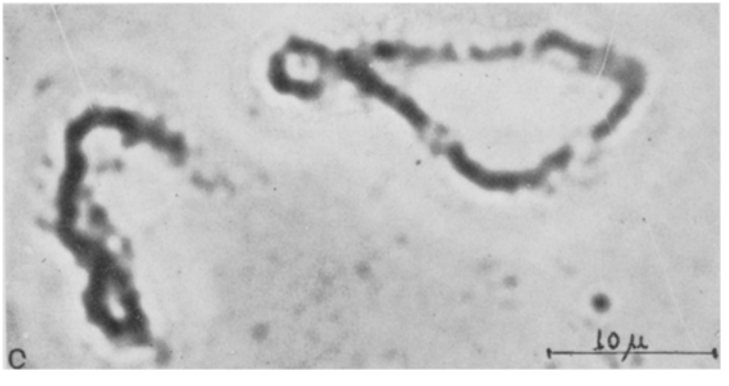


Fig. 3. Metaphase and anaphase circular chromosomes from squash preparations of intestine, treated with chloroform. A) The circle at right consists of 2 half-chromatids separated at the centromere but still connected at the telomeres (*O. americanus*, 4*n*, Feulgen); B) each circle of the eight-like configuration is formed by half chromatids connected by the telomeres but separated at the centromere (*O. americanus*, 4*n*, Feulgen); C) 2 anaphase chromosomes in an eight-like configuration. (*E. binotatus*, Giemsa); D) electron micrography of a metaphase chromosome of *O. americanus*, 4*n*, treated with chloroform. At right, the circle consists of half chromatids separated at the centromere but joined at the telomeres. Note the similarity of the aspects with those of Figure A).

2. Each metaphase chromatid showed two separate filaments (half-chromatids) usually connected at the centromere and telomere regions. Depending on the degree of the separation at the centromere level, the metaphase chromosome may exhibit ring-shaped or eight-like chromatids. At the EM, a gap between both annular 'half-chromatids' could be observed (Figure 3, A, B, C, D). Although the half chromatids showed longitudinal striae, the number of the component filaments could not be determined. There remains the question whether the striae end as free subfilaments at the telomere, or constitute a part of a single, longitudinally bent filament at the telomere region.

The morphology of the chromosomes after chloroform treatment suggests some similarity with the model proposed by STUBBLEFIELD and WRAY[4] for the mammal chromosome. According to this model, each anaphase chromatid consists of 2 half-chromatids, each of which has 2 deoxyribonucleoprotein ribbons linked to a single core and presenting epichromatin loops, laterally attached.

The chloroform effect has been followed up in several experiments. Figure 1 and 2 show aspects usually seen, Figure 3 shows a most uncommon aspect. In preparations treated over long periods of time, as well as in preparations treated with chloroform prior to fixation, the chromosomes were found to lose their integrity making any analysis difficult.

Our observations on the morphology of chromosomes after chloroform treatment suggest a multistranded or at least a binemic model. How could an uninemic model fit the observations of well defined half-chromatids with evidently split telomeres? We suggest that the interphase chromosome consists at least of two DNA strands. During DNA synthesis they uncoil and the DNA helices replicate. The distribution of sister helices to the same or to different chromatids of the metaphase chromosome can only be elucidated by an investigation of TH_3 incorporation patterns.

The hypothesis of an unineme model was favoured by the finding of TH_3 heterolabelling in the second generation of DNA replication of mitotic chromosomes[15], while an isolabelling pattern has recently been described by PEACOCK[16]. Accepting the binemic model, TH_3 isolabelling or heterolabelling would depend on the distribution of the sister helices of each of the M_1 DNA molecule to the very same or different chromatids. More recently it was shown that mammal sex chromosomes become isolabeled at M_2[4].

Our autoradiographic findings, discussing the binemic hypothesis, will be reported elsewhere.

The existence of 'half-chromatids' should not be interpreted as an indication that drastic variations in DNA content among species with similar karyotypes are a consequence of differential polynemy. As a matter of fact, all species examined by us presented 'half-chromatids'. Yet, this also does not necessarily exclude polynemy, since elucidation with respect to the number of filaments within the 'half-chromatids' still needs better resolution.

The considerable difference in DNA content in the 2 anuran species of *Odontophrynus* used in this investigation is demonstrably the result of polyploidy. The high DNA content in *Eleutherodactylus binotatus* is assumed to be a consequence of remote polyploidization and/or interstitial duplications[13].

Zusammenfassung. Metaphasechromosome lassen nach Chloroform-Behandlung eine Aufteilung in Halbchromatide erkennen. Zytologische Befunde der Untersuchungen (Licht- und Elektronen-Mikroskop) weisen auf eine binemische Struktur des eukaryotischen Chromosoms hin.

MARIA LUIZA BEÇAK

Serviço de Genética, Instituto Butantan, Caixa Postal 65, São Paulo (Brazil), 8 April 1974.

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Chromosomal Identity of Black Rats (*Rattus rattus*) from the Galápagos Islands, Ecuador

Considerable interest has been given in recent years to the documentation of chromosomal variability in the cosmopolitan human commensal rodent, the black rat *Rattus rattus* (Linneaus). Two karyotypic morphs have been described: a $2n = 42$ morph from eastern[1] and southeastern[2] Asia and the Indian subcontinent[3]; and a $2n = 38$ morph from south India[4], western Asia[5], Europe[6], Africa[7], North America[8], South America[9], and Australasia[10]. The exact cytological relationships between these variants are now known from Giemsa-banding patterns, and consist of two Robertsonian fusions and 1 to 2 pericentric inversions[11]. In addition to the general polytypic nature of variability in diploid number summarized above, intrapopulation polymorphic systems have also

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