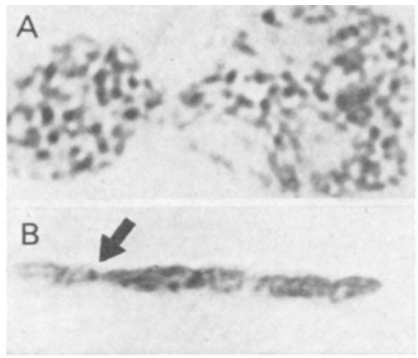

Fig. 1. (A) Mesenchymal cell nuclei dividing amitotically. A clear chromatinic strand connecting the 2 originated nuclei is present. (B) Continuous chain of several connected nuclei in a myoblast. The arrow signals a chromatinic strand connecting a separating nucleus to the rest of the structure. Adult mosquito specimen (*Culex bonariensis* Brèthes). Feulgen staining. × 1000.

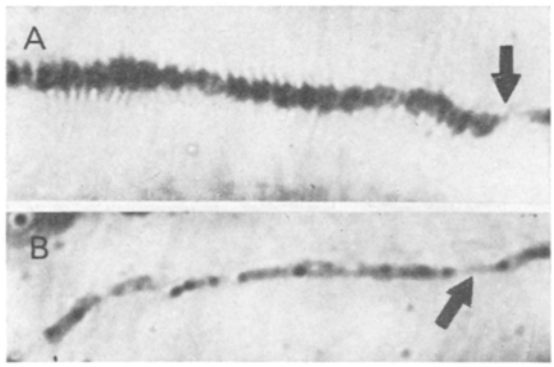

Fig. 2. (A and B) Clear distinct figures of multiple amitotic nuclear division in cord-like nuclear structures (Polykaryonema) of young muscle fibers. In (A) multiple periodical constrictions can be seen. The arrows indicate commissural chromatinic filaments. Adult fly specimen (*Dermatobia cyaniventris* Macquart). Feulgen staining. About × 3000.

flight muscle were carefully excised, placed in albumenized slides, squashed and fixed for 15 min in Carnoy's fluid. The smears obtained by these 2 methods were stained with Feulgen, Giemsa and Toluidine blue stainings.

The study of the cytological preparations disclosed distinct nuclear pictures corresponding to different stages of amitotic division. Giant nuclei were observed in many cases 10 times longer than normal and showing periodic constrictions. Frequently the 2 or more originated nuclei – in many cases different in size – were connected by a strand or by a tenuous chromatinic filament (Figure 1A).

In striated muscle fibers the increase in size preceding the amitotic nuclear division occurs along the long axis of the nucleus, giving rise to very typical nuclear structures which we have found in myoblasts and young muscle fibers of the insects studied (Figures 1B and 2). These consist of truly chromatinic cords, varying in diameter, which by successive constrictions and fragmentations originate the rows of independent nuclei found in the central axis of the adult striated muscle fibers.

We suggest the term *Polykaryonema* to designate this cord-like or filamentous nuclear structure which by successive divisions will give origin to several nuclei. We propose the term *clasmatotenesis* or *clasmatotenic division* for this new type of complex amitotic nuclear division.

Résumé. On décrit quelques aspects morphologiques de l'appareil nucléaire des fibres musculaires striées des insectes. Le nom Polykaryonéma est proposé pour désigner les noyaux filamenteux géants qui par fragmentations successives produisent des chaînes de noyaux indépendants, placées dans le centre des fibres musculaires adultes. Ce phénomène offre un bon exemple de division nucléaire amitotique.

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Feulgen-Cytophotometric Determination of DNA Content in the Germ Cell Nuclei of the Female Chicken Embryo During Premeiosis

In a previous work[1,2], one of us investigated DNA synthesis during premeiosis in the ovarian germ cells of the chicken embryo both in vitro and in vivo, using the autoradiografic technique, following the incorporation of ^{3}H-thymidine. The successive developmental germ cell stages found during this period in the cortex of the ovary of the chicken embryo are represented in Figure 1. Only during the preleptotene stage of the germ cells, chiefly occurring in the central part of the ovarian cortex of 15- to 17-day-old embryos, does nuclear incorporation of ^{3}H-thymidine take place[3]. Germ cells with a reticulated nucleus, characterized by a more regular and much finer chromatin distribution than the oogonia at interphase, do not incorporate the DNA-precursor. Large numbers of these cells are found in the central part of the ovarian cortex of 14- and 15-day-old embryos. Since there is morphological evidence of a transition between cells with a reticulated nucleus and cells in the early preleptotene stage[4], we consider the former to be the precursors of the latter. The meiotic divisions of the female germ cells in the chicken are concluded months or years after the observed ^{3}H-thymidine incorporation wave. Hence, this DNA synthesis may represent:

(1)A premeiotic reduplication of the germ cell nuclear DNA; (2) a metabolic DNA synthesis[5] ('specific gene amplication')[6] or DNA turnover; (3) a combination of these two hypotheses. The present study was undertaken to check these explanations by comparing the DNA content of reticulated nuclei and leptotenes.

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Fig. 1. Drawings of the successive developmental stages of the germ cells found in the ovarian cortex of a 15-day-old female chicken embryo, exactly as seen under the microscope. From left to right: oogonium, oocyte with reticulated nucleus, oocyte in early preleptotene stage, oocyte in late preleptotene stage and oocyte in leptotene stage of meiosis (fixation of the ovary with acetic-alcohol and coloration with Feulgen-fast green).

Material and methods. Fertile eggs of White Leghorn chickens were incubated at 38.5 °C for 15 and 17 days. 4 ovaries of each age were fixed in A.F.A. (alcohol 96%: 70 parts, formaline: 20 parts and chilled acetic acid: 5 parts), dehydrated in graded alcohols, embedded in paraffin and cut at 10 μ thickness.

In order to enable a uniform processing of the material from the 2 age groups, sections from 15- and 17-day-old embryos were placed on the same slide. After hydrolysis in 1 *N* HCl during 12 minutes at a constant temperature of 60 ± 1 °C, the sections were stained with Schiff's reagent during 45 min and rinsed in 3 successive baths of a freshly prepared So_2 solution. For the Feulgen-DNA determinations we used the Lison cytophotometer in visible light[7]. The nuclei were projected on a screen and their light-absorption was measured through a diaphragm of about $^1/_4$ of their projected surface area. Nuclei which on manipulating the fine adjustment appeared cut were not measured. 50 nuclei of each cell group were investigated, 5 measurements are made over each nucleus and their arithmetic mean was computed as the nuclear extinction. The Feulgen-DNA content was expressed in arbitrary units (AU) as the product of the nuclear extinction and the projected nuclear surface area. Since the Feulgen-DNA contents of the nuclei may be assumed to follow a logarithmic distribution within a given ploidy class, the mean values were calculated after logarithmic transformation of the distribution-curve. The absolute nuclear surface area of nuclei in both groups is irrelevant to the present study. Hence it was not computed separately but also expressed in arbitrary units (AU).

Results. The results of the Feulgen-DNA measurements are summarized in Figure 2 and in the Table. The mean value of Feulgen-DNA found for the reticulated nuclei is 1364 AU and for the leptotene nuclei it is 3645 AU. The average surface area of the reticulated nuclei is only slightly larger than that of the leptotenes (Figure 3). This confirms the impression gained by microscopical observation that the 2 groups can hardly be differentiated on the basis of their nuclear size.

Discussion. If the mean nuclear Feulgen-DNA content of reticulated nuclei is taken as 100%, the leptotene group has a mean of 267%. In other words, if we consider the DNA-content of the reticulated nuclei to be at the diploid level, then the leptotenes would be largely hypertetraploid, suggesting aneuploidism or DNA of combined origin. If, alternatively, we assume that the leptotenes are tetraploid, the reticulated nuclei would be on a hypodiploid level. This second hypothesis seems the most likely when the conditions of the measurements are taken into account.

(1) The particular asymmetrical shape of the histogram of reticulated nuclei, with its numerous very low Feulgen-DNA values (Figure 2), is in sharp contrast with the symmetry of the histogram of leptotene nuclei. This asymmetry can most readily be explained by an almost

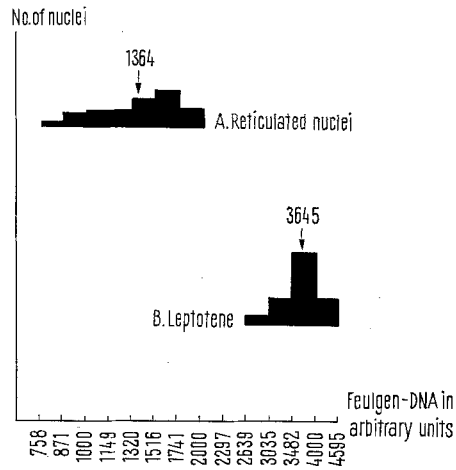

Fig. 2. Feulgen-DNA content in arbitrary units of reticulated and leptotene nuclei.

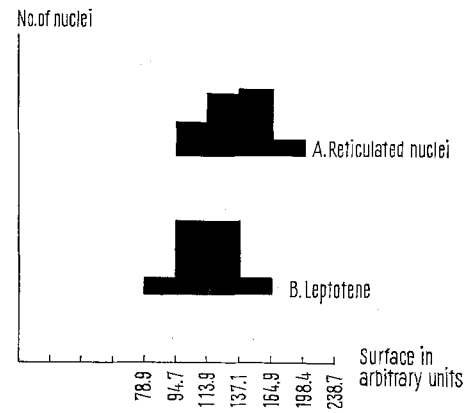

Fig. 3. Surface area in arbitrary units of reticulated and leptotene nuclei.

Feulgen-DNA content of reticulated and leptotene nuclei. $\bar{x}$, logarithmic mean; s, standard deviation; s log $\bar{x}$, standard error.

	$\bar{x}$	log $\bar{x}$	s	s log $\bar{x}$
Reticulated nuclei	1364	3.13469557	0.10344	0.014798
Leptotene nuclei	3645	3.57034185	0.045825	0.0065038

[7] L. Lison, *Histochemie et cytochimie animale* (Gauthier-Villars, Paris 1960).

unavoidable inaccuracy in the selection for measurement of the reticulated nuclei. Indeed because these cells are very pale, it is difficult to ascertain by manipulating the fine adjustment of the microscope whether or not a more or less important part of the nucleus has been cut. This difficulty therefore may have led to the measurement of incomplete reticulated nuclei. As suggested by the symmetry of their histogram, the selection of the leptotenes, which are much darker, was more precise.

(2) The extinction values in the reticulated group oscillate around 10–12% and in the leptotene group around 30%. Since the minute extinctions of the reticulated nuclei often fall just beyond the range of the linear section of the cytophotometer's absorption curve, these measurements are less accurate and, moreover, bring about an underestimation of the Feulgen-DNA content[8].

(3) In reticulated nuclei, many of the fine chromatin granules are studded against the inner surface of the nuclear membrane and may have escaped measurement since we had to keep the projection screen diaphragm clear of the nuclear membrane. Thus this particular distribution of DNA brings about another underestimation of extinction in the reticulated nuclei. Summarizing, error (1) causes the insertion of measurements over incomplete nuclei into the calculation of the mean Feulgen-DNA content, thus lowering the mean of the reticulated nuclei. It also accounts for the asymmetrical shape of their histogram. Errors (2) and (3) provoke an overall underestimation of the Feulgen-DNA content in the reticulated nuclei.

These limitations of the technique in this material may suffice to explain that the mean Feulgen-DNA content of the reticulated nuclei is less than half that of the leptotenes.

Conclusion. The results of the present quantitative study show that the germ cells at the leptotene stage contain at least twice as much nuclear Feulgen-DNA as the germ cells with reticulated nuclei. Considering the sources of error in this material, a simple 2/1 (tetraploid/diploid) rate is suggested, but could not be ascertained. Our data, however, indicate that during the DNA synthesis at the preleptotene stage, the Feulgen-DNA content in the germ cells of the female chicken is at least doubled. The germ cells appear to go through a period of premeiotic doubling of their DNA content, analogous to the S-phase of a regular cell cycle. Our data neither support nor rule out the possibility of a concomitant DNA synthesis of other origin at this stage of premeiosis[9].

Résumé. Chez l'embryon de poulet femelle nous avons comparé à l'aide de la technique cytophotométrique de Lison la teneur en ADN des cellules germinales à noyau réticulé à celle des cellules au stade leptotène. Comme pendant la phase S d'un cycle cellulaire régulier, la quantité de DNA au cours du stade préleptotène, semble au moins doubler. Néanmoins nos résultats ne permettent pas d'affirmer ou d'exclure la possibilité d'une synthèse de DNA supplémentaire d'autre origine.

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[9] Acknowledgments. The authors are gratefull to Professor L. VAKAET (University of Antwerp) and to Professor H. ROELS (University of Ghent) for their valuable suggestions.

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Monoamine Oxidase- and Catechol-*O*-Methyltransferase Activity in Umbilical Vessels of the Human Fetus[1]

Catecholamines may be inactivated by monoamine oxidase (MAO) or by catechol-*O*-methyltransferase (COMT). Since noradrenaline penetrates the human placenta[2] it was considered of interest to assess the activity of noradrenaline inactivating enzymes in the vessels connecting the placenta with the fetus. Special interest was focused on the ductus venosus, which has an adrenergically innervated sphincter mechanism[3], the physiological significance of which is not yet properly understood.

The material consisted of 25 fetuses, classified according to their crown-heel length as noted in the Table. Immediately after legal abortion, the tissue was prepared from: (1) the umbilical arteries from the umbilicus down to the level of the urinary bladder, (2) the free intraabdominal part of the umbilical vein, and (3) the entire ductus venosus. 2 transverse sections (3–4 mm in length) of the umbilical cord (4 and 5) were prepared at 1 and 5 cm distance from the skin level thus consisting of both venous and arterial vessels including the Wharton jelly. Liver tissue (6) was simultaneously removed for analysis. (Numbers in brackets refer to the Figure.) Material from each site within each group of fetuses had to be pooled for analysis. Tissue of the ductus venosus was only available in sufficient amounts in fetus group II.

All the legal abortions were performed by laparatomy. For anaesthesia the patients received the following drugs: thiopental sodium, fluothane, and nitrous oxide together with suxamethonium chloride to secure muscular relaxation.

The tissue was removed within 15 min after the operation, and then stored at -70 °C for up to 12 days. Control analysis showed no decrease in COMT or in MAO activity within this period. The supernatant ($8000 \times g$, 15 min) of tissue homogenate in 40 volumes (w/v) of ice-cold isotonic KCl solution served as enzyme solution.

COMT was measured by a modification of a method described by AXELROD et al.[4]. Instead of radioactive

[1] This study was supported by part by a grant of the Medical Faculty, University of Lund, the Magnus Bergwall's Foundation and Expressen's Prenatal Foundation.

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