THE THREE-DIMENSIONAL RECONSTRUCTION OF THE XY CHROMOSOMAL PAIR IN HUMAN SPERMATOCYTES

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ABSTRACT

The spatial reconstruction of the XY pair of chromosomes from human spermatocytes has been made by the study of serial sections 1000 A in thickness. The sex pair during zygotene-pachytene forms a condensed mass of chromatin that has two filamentous, electron-opaque cores: the long and the short core. During early pachytene both cores have a common ending region, about 0.4–0.8 μ long. This common end is a synaptonemal complex, and each of the cores forms a lateral element of that complex. The cores become more convoluted during middle pachytene forming "ringlike bodies." Nucleoli from spermatocytes have three distinct regions: (*a*) granular; (*b*) dense fibrillar; and (*c*) clear intermediate. Occasional association of the XY pair and the heteropycnotic "basal knobs" results in apparent association of nucleoli and the sex pair in a minority of cells. The evidence presented is interpreted as a strong support of the hypothesis of homologous regions in the human XY pair.

INTRODUCTION

The nature of the relationship between the X and Y chromosomes in human spermatocytes has not been clearly explained by the extensive cytological studies of the last 40 years (see reviews by Mittwoch [15], Eberle [2], and Luciani [13]). That information has great importance for the interpretation of sex linkage data as well as for the understanding of disjunction of the sex chromosomes during meiosis in the human species. The sex chromosomes of man and other mammals form a condensed mass during zygotene-pachytene in the spermatocyte nuclei. That dense body or "sex vesicle" has been extremely difficult to analyze by light microscopy (25, 22, 35). The first analysis of the ultrastructure of the heterochromatic sex pair ("sex vesicle") of mouse spermatocytes showed that some problems could be solved by electron microscopical methods (29). During recent years we have provided data on the histochemistry (32), the ultrastructural development (30), and the three-dimensional ultrastructure (31) of the sex chromosomes of mouse spermatocytes. Thus, it has been possible to show a reasonably clear picture of the behavior of the XY pair in the mouse (31).

The human XY pair has been previously studied by us (33). From these results and those on the mouse, it was concluded that the existence of a synaptonemal complex in the human XY pair could be conclusively proved only by the analysis of serial sections.

The aim of this paper is to present the reconstruction of the whole XY pair ("sex vesicle") during pachytene in human spermatocytes and to discuss some of its implications for human cytogenetics.

MATERIALS AND METHODS

Testicular biopsies were obtained from 10 men selected from a group of patients attending infertility clinics. Histological examination of the biopsies showed a normal seminiferous epithelium. Clinical and laboratory tests discarded any injury in the spermatogenic cells in these cases. Although the electron microscopical features of the sex vesicle were identical in all of the men examined, the present study pertains mainly to two of the men, aged 40 and 29. Six complete reconstructions of the XY pair were made from these two men. Pieces of tissue (about 2 mm in diameter) were immediately fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 6.9, for 2 hr, washed with buffer, and postfixed in 1% buffered osmium tetroxide for 1 hr. The tissue was embedded in Maraglas and sectioned with a Porter-Blum ultramicrotome (Ivan Sorvall, Norwalk, Conn.). Serial sections, nominally 1000 A in thickness, were collected on single-hole grids with Sjöstrand's technique (28). Other sections, 500 A thick, were used for the study of special elements of the sex pair. Sections were stained with uranyl acetate in methanol and then with lead citrate. Micrographs were taken with a Siemens Elmiskop I electron microscope at fixed magnifications that were checked, after each series of micrographs, with a carbon replica grating. The micrographs of serial sections were traced with china ink on celluloid sheets 0.5 mm thick. The sheets were spaced with glass slides in such a way that the spaces between the sheets corresponded to the section thickness at the magnification of the micrographs.

The celluloid models formed by the overlapping sheets were photographed with a Reprovit II copier (E. Leitz Inc., Wetzlan, Germany) on Panatomic-X film. The assemblage of the overlapping sheets was made with the use of at least four reference points or lines on the micrographs (generally a cell or a nuclear membrane, edges of large vacuolae and nucleoli). From the six complete reconstructions of XY pairs, four were made with thin (1000 A) sections and the remaining two were made with thicker sections (0.20–0.25 μ in thickness). Although the latter (thick) sections permitted obtaining an over-all picture of the array of the filaments inside the "sex vesicle," it was only with the thin sections that the accurate reconstruction of the path of the filaments was made.

OBSERVATIONS

Number and Characteristics of the Filamentous Structures (Cores) of the Human Sex Pair

Two filamentous cores were observed in all the reconstructions of the sex pair. These cores are different in length, and the length is characteristic for each core. Thus the two cores will be referred to as the *long* filament and the *short* filament (or core) (Figs. 1–24). Lengths of the cores can be measured approximately by passing a thin copper wire through the celluloid sheets along the path of the filaments and then measuring the wire length. The length ratio of the short to the long core is about 1:2.9.

Both cores are formed by electron-opaque, tightly packed fibrils about the same diameter as the thinnest chromatin fibrils. The widths of the cores vary from 900 to 400 A. These variations may be related to the doubleness of the cores, because when the cores are clearly double each component measures about 400 A. While the short core appears to be single along most of its path, the long core is visibly double at many places (Figs. 6, 7, and model in Fig. 23). Consecutive sections show that the core seems to be formed by two elements or fibers plectonemically coiled around each other. These elements become slightly separated at some points (Fig. 23).

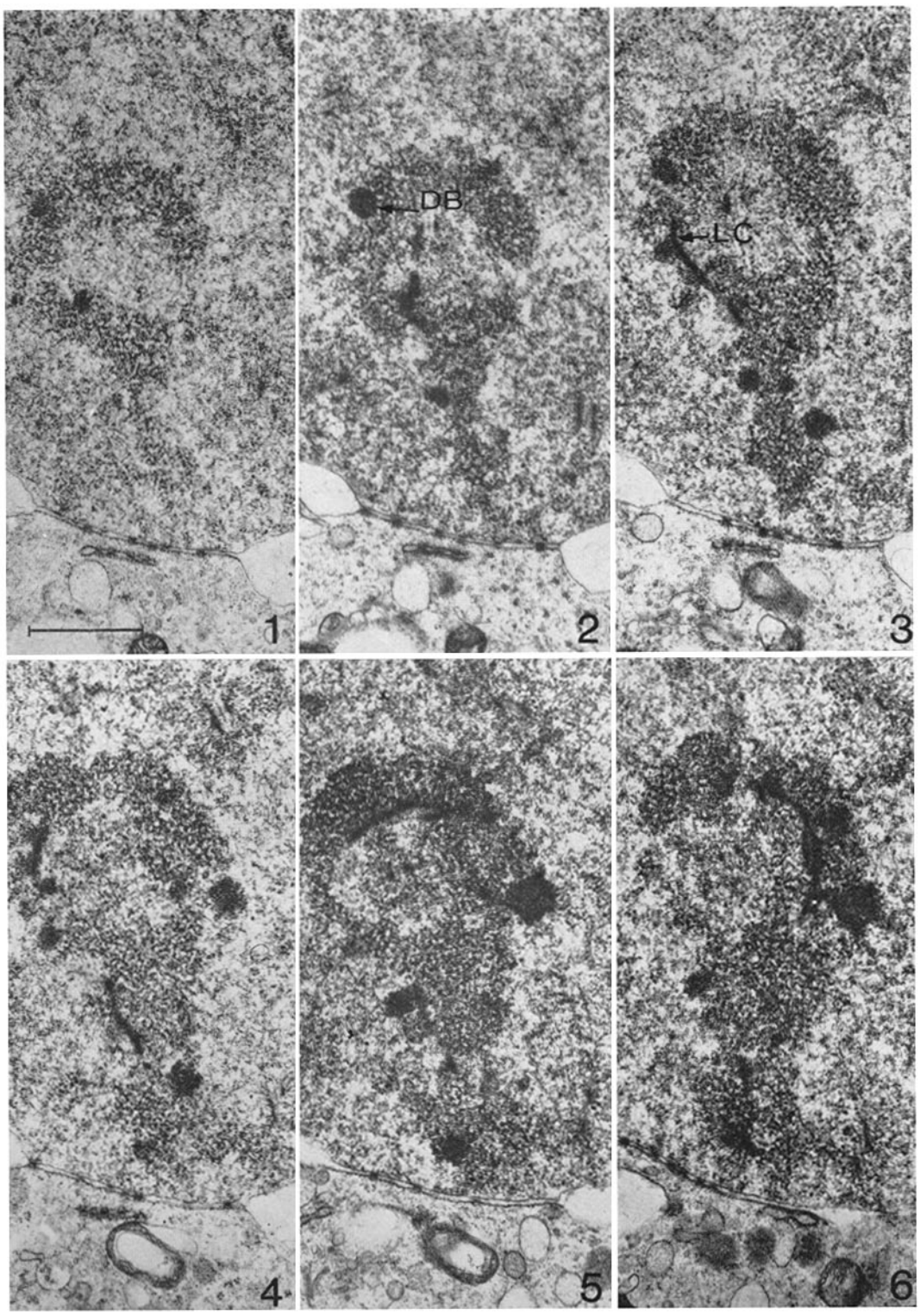
Both the long and the short cores are laterally connected, at some points, to dense, round, or oval masses. These dense bodies have a somewhat different structure, being more granular in appearance (Figs. 3–6, 10–13). These bodies are frequently near one end (the free end) of the short filament and along the large loop made by the long core (Figs. 2–7).

The Ending Regions of the Cores

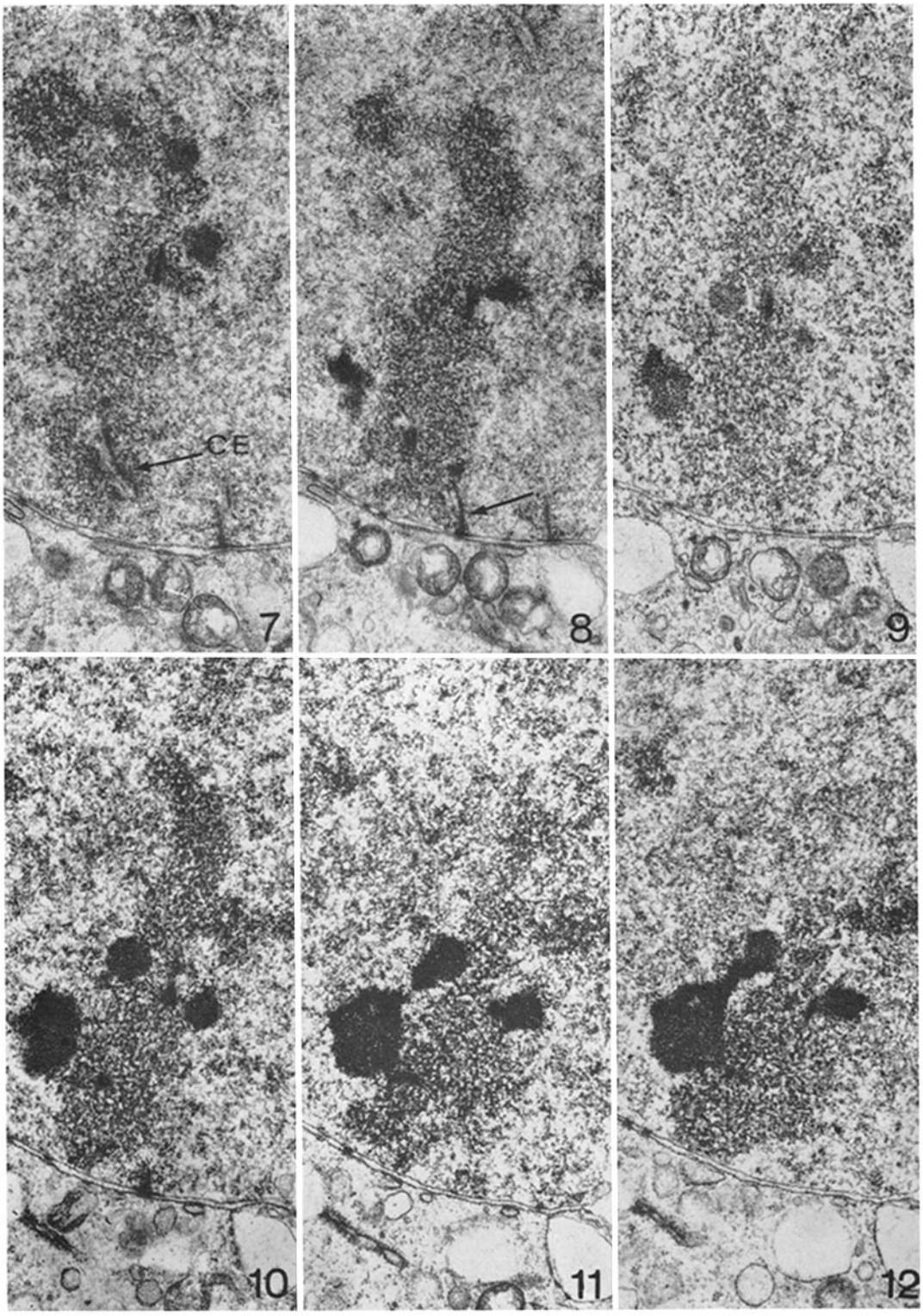
Both cores start and end against the nuclear membrane. In all cases three ending regions were present in the sex vesicle. Both cores have one *common end region*, where they form a synaptonemal complex, and they have also one *free end* (Fig. 23).

The *common end* is formed by the pieces of both cores that come close to each other and then continue parallel to each other up to their touching the nuclear membrane (Figs. 7–8, 23). That region then consists of two lateral elements formed by each core, and a third, central element which is thinner (about 100 A wide) and sometimes barely visible. Thus, the common end region is identical to a synaptonemal complex.

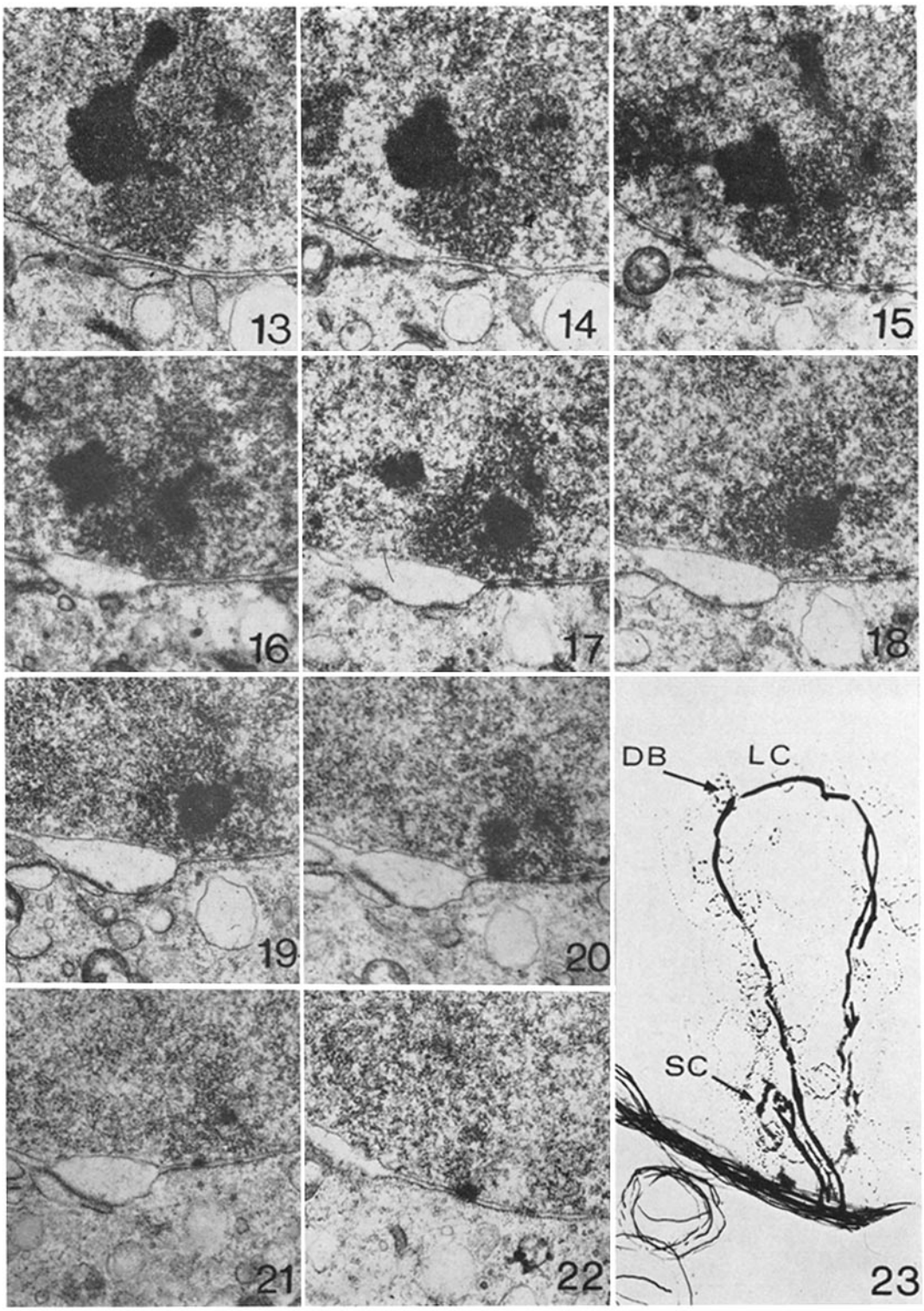
The common end region is short (from 0.8 to 0.4 μ long) and flexuous. Thus, the possibility of detecting this synaptonemal complex in single sections is rather small. Besides, this region shortens progressively from zygotene to late pachytene, and its morphology becomes distorted, thus lessening



FIGURES 1–23 Electron micrographs of serial consecutive sections through the sex vesicle in human spermatocyte in early pachytene. One section is missing between Figs. 21 and 22. The long core (*LC*) forms a large loop visible in Figs. 3–7. The common end (*CE*) is formed by a synaptonemal complex visible in Figs. 7, 8 (arrows). The short core (*SC*) is almost perpendicular to the sections except at the common end. Dense bodies (*DB*) are observed along the cores. Fig. 23 is a photograph of the complete model. Mark: 1 μ. × 16,800.



FIGURES 7–12 See legend under Figs. 1–6.



FIGURES 13–23 See legend under Figs. 1–6.

the possibility of recognition of the common end as a synaptonemal complex. The free ends of both filaments appear as dense rods which expand slightly when touching the nuclear membrane. During early pachytene the free end of the short core is most atypical, as frequently a dense, round body is found near the core ending on the nuclear membrane (Figs. 18–20).

The Formation of Ringlike Bodies by the Cores

In a previous paper "ringlike" bodies were observed inside the human sex pair (Fig. 25). Although at that time we observed connections among the ringlike bodies and the cores, the origin of these bodies remained without explanation. Serial sections show that the ringlike bodies are always interposed in the path of the cores, and that they are formed by the self-coiling of some regions of the cores. At these places the core is double. Both components of the core, curved onto themselves, form the ringlike bodies. These bodies are not present at zygotene or early pachytene (Figs. 1–23) but they are seen at later stages during pachytene (Fig. 25). At that time the cores become more convoluted and their path becomes very irregular and extremely difficult to analyze.

The Dense Bodies of the Human Sex Pair

As already mentioned, dense bodies from 0.2 to 0.6 μ wide are associated with the cores along their path, especially at early stages (Figs. 3–6, 10–13). Their number varies from more than 15 for the long core to a few large ones for the short core. No such bodies are associated with the common end region (synaptonemal complex) (Figs. 7, 8). Near the free end of the short core the path of the core is lost as it touches a prominent terminal dense body (Figs. 18–20). Thus the possibility exists that the core is mixed with or contributes to the formation of that body.

The Nucleoli of Human Spermatocytes and their Relationship to the Sex Pair

Several nucleoli are present in human spermatocytes during pachytene. The bigger one is a round body about 1.5 μ wide that has a short stalk connecting the nucleolus to a condensed chromatin region (Fig. 24).

The spheroidal nucleolus has two distinctly different ultrastructural regions (Fig. 26): an outer, thick shell formed by dense granules 150–200 A wide, and a fibrillar, dense core, about 0.4 μ wide. The connecting stalk has a low density, round region which is partially surrounded by a cuplike region of dense chromatin. That mass of chromatin contacts the nuclear membrane at the opposite side (Fig. 26). A synaptonemal complex (and in some instances two) lies inside the condensed chromatin and ends against the nuclear membrane. Thus, the structure of this chromatin region is that of the "basal knobs" described by Woollam and Ford (36) (Fig. 27).

Most generally, the main nucleolus is far from, and opposite, the "sex vesicle" (Fig. 24). However, a small nucleolus seems to be associated with the sex pair in a small number of cells (Fig. 28). That "association" seems not to be based in a true functional relationship, because those small nucleoli always have a stalk connecting to a different piece of chromatin, generally well differentiated from that of the "sex vesicle." This fact is also related to the occasional association of an autosomal synaptonemal complex with the sex pair. Serial sections have shown that that synaptonemal complex pertains to a "basal knob" which bears the small nucleolus that lies beside the sex pair.

DISCUSSION

The Relationship of the X and Y Chromosomes as seen with the Light Microscope

The question about the existence of chiasmata between the human X and Y chromosomes has not been resolved by light microscopy. Koller (12) assumed the existence of partial synapsis and chiasma formation between the human sex chromosomes. However, most of the cytologists who have used the more refined techniques of squashing and air drying have denied that the existence of chiasma and synapsis could be proved (1, 2, 11, 13, 27).

Most of the observations coincide in the description of the XY pair after diplotene (2, 11, 13). The sex pair is then joined in an end-to-end relationship which has received two different interpretations. For some authors (2, 13, 25), that joining is due to the remainder of a hypothetical material constituting the sex vesicle, or to an undefined nonchiasmatic attachment. For a minority of observers (5, 6), that joining is due to a terminalized

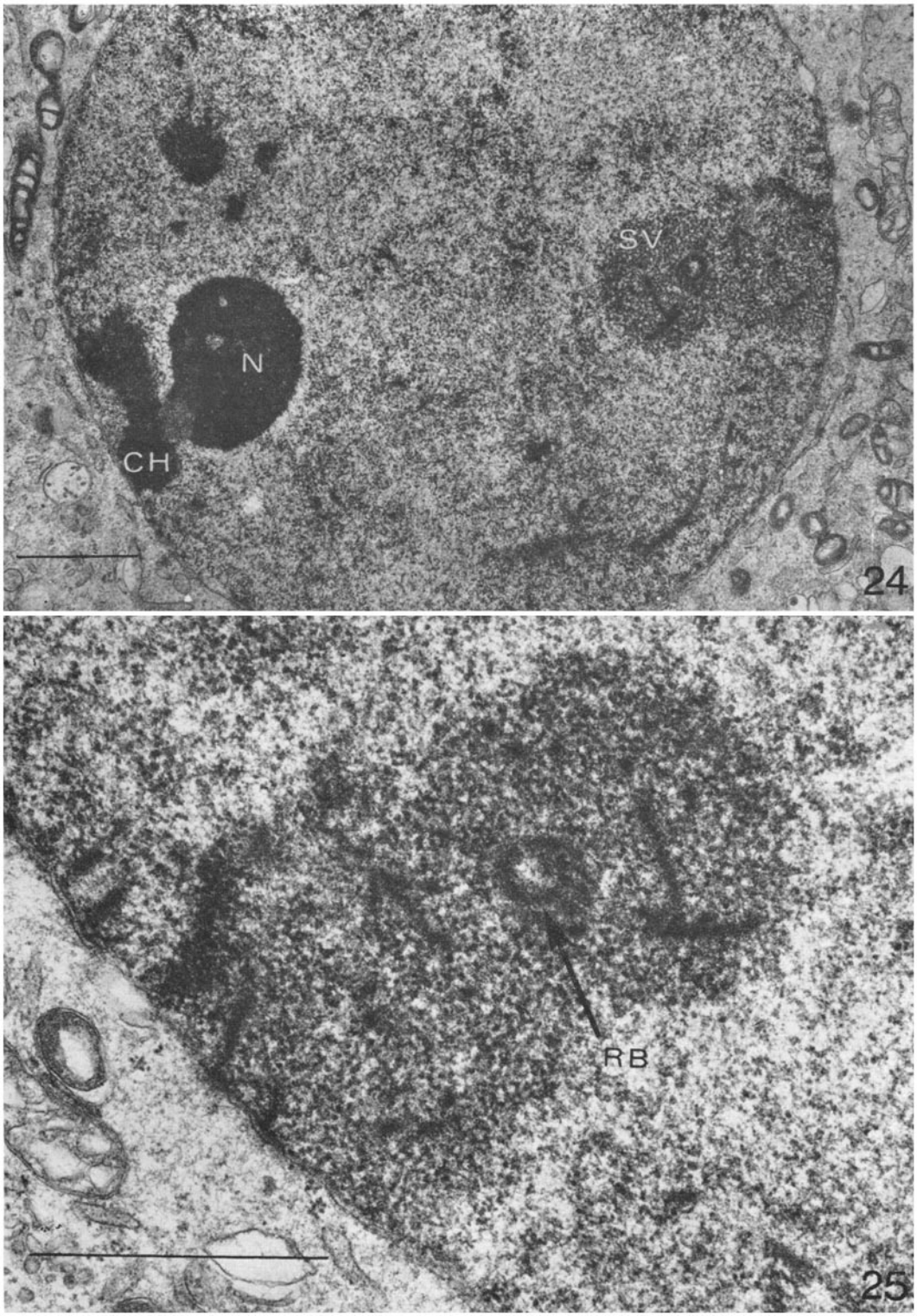


FIGURE 24 Low magnification micrograph of a human spermatocyte nucleus showing the sex vesicle (SV) opposite the main nucleolus (*N*). The nucleolus is attached to a dense chromatin mass (*CH*). Mark: 1 μ. × 18,000.

FIGURE 25 Sex vesicle during middle to late pachytene. Several sections of cores are observed. A ringlike body (*RB*) is formed on the path of the long core. Mark: 1 μ. × 45,000.

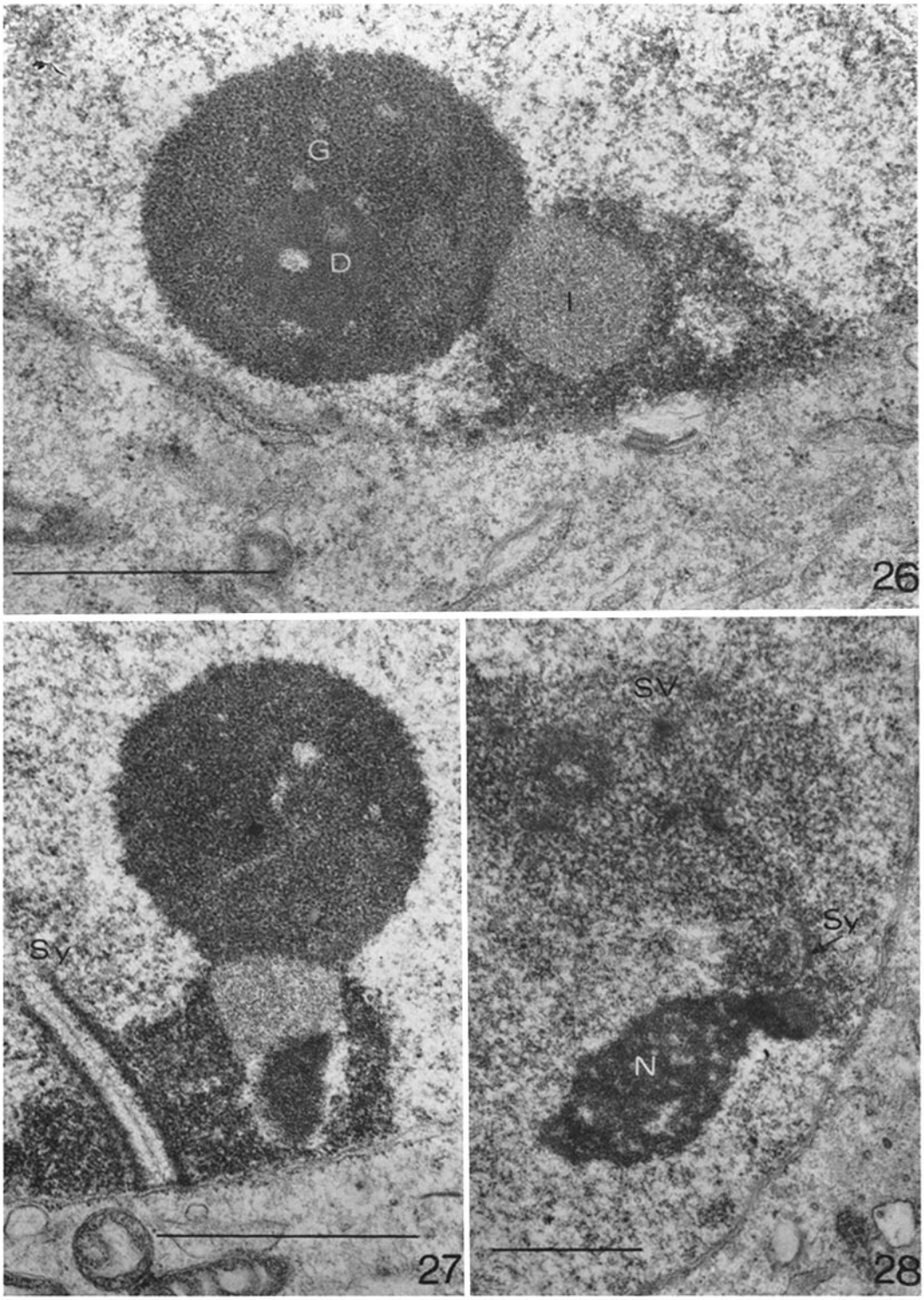


FIGURE 26 The main nucleolus, showing its structural regions: granular (*G*), dense fibrillar (*D*), and the intermediate low-density region (*I*) surrounded by dense chromatin. Mark: 1 μ. × 39,000.

FIGURE 27 Electron micrograph of the main nucleolus, showing its association with a dense mass of chromatin that is crossed by a synaptonemal complex (*Sy*). Mark: 1 μ. × 39,000.

FIGURE 28 Electron micrograph of the "sex vesicle" (*SV*) lying near a secondary nucleolus (*N*). The nucleolus is attached to a dense intermediate piece and to an autosomal synaptonemal complex (*Sy*). Mark: 1 μ. × 22,500.

chiasma made between minute homologous regions of the XY pair.

The existence, in a variable frequency, of the X and Y chromosomes as univalents in the first meiotic metaphase supports the idea that the relationship in the XY pair is more able to lead to precocious disjunction than in the case of the autosomes. The frequencies of sex univalents vary from 6 to 28% (11, 13).

Most authors regard the tip of the short arm of the X chromosome as the part of this chromosome that is involved in the association with the Y (11, 13, 27). There is no agreement, however, on which part of the Y chromosome is involved in this association. The distal end of the Y chromosome is strongly condensed as a round mass, and two constrictions often have been described in the Y chromosome (11, 13). The X chromosome presents its distal arm visibly coiled with the end rather condensed, while the associated end is thin and straight (11, 13). Thus, a definite pattern of condensation and constrictions is observed in the XY pair at diplotene and diakinesis with the light microscope. It seems reasonable to compare the coiling of the distal part of the X chromosome with the progressive coiling and formation of ringlike bodies in the *long core* of the sex pair as described in Results.

The condensation of the distal part of the Y chromosome may be related to the presence of a large, dense body near the free end of the short core and the formation of a ringlike body at late pachytene. A better correlation between the light microscope pattern and the ultrastructural observations is not possible, mainly because of the lack of identification of centromeres at the ultrastructural level.

Significance of the Presence of a Synaptonemal Complex in the "Sex Vesicle"

The rationale of the ultrastructural analysis of the "sex vesicle" in mammals has been presented by Solari (29, 31). The presence of a synaptonemal complex in the "sex vesicle" would support, according to Moses' hypothesis (19), the existence of synapsis between the sex chromosomes. The observation that the synaptonemal complex is a distinctive nuclear structure during meiotic prophase was made by Moses (18) and by Fawcett (3). Moses (19) proposed that the synaptonemal complex had an intimate relationship with the synapsis of homologues. This hypothesis has received abundant support (19), although special cases have been shown in which synaptonemal complexes or composite bodies do not express a simultaneous synapsis of homologues (24, 34, 17, 20). According to the available data, the presence of a synaptonemal complex at zygotene and early pachytene is more significant for the demonstration of synapsis than its presence at later stages. Based on this criterion, ultrastructural proofs of partial synapsis in the XY pair of the mouse have been provided (31).

The idea that each core is formed by each sex chromosome is suggested by the similarities between the cores and the sex chromosomes after diplotene (see above). This idea is strengthened by the length ratio of the cores, as this ratio is nearly the same as the length ratio of the mitotic X and Y chromosomes, 2.6–2.9 (2).

The two cores of the human "sex vesicle" are paralleled by the two cores extensively studied in the "sex vesicle" of the mouse (30, 31). In the latter species, evidence has been presented that each core is an axial structure of each sex chromosome (31).

Electron microscopic observations on the sex chromosomes of the mouse (30) and the rat (in preparation) coincide with the present results on the human. A common end region bearing a small synaptonemal complex is present in these three species. Although a general conclusion cannot yet be established, we suggest that the end-to-end joining is actually a chiasma in the majority of mammals.

The observations of Ford and Woollam on the golden hamster seem to show that partial synapsis is also found in this species (7); the same thing seems to occur in other mammals (9, 21, 23).

The Relationship between the Nucleolus and the "Sex Vesicle"

Previous studies (4, 33) have shown that the nucleoli are independent from the human "sex vesicle" and that the latter does not contain histochemically detectable RNA (33). However, the association of secondary nucleoli with the "sex vesicle," in a small frequency, has been recorded (2, 13). Although there is no structural relationship between those two structures, this association may not be a completely random one. It has been ob-

served that heterochromatic regions of chromosomes have a tendency to associate with each other (14). As nucleoli are attached to heterochromatic "basal knobs," the occasional association of nucleoli with the "sex vesicle" may only reflect the tendency of heterochromatin to association, in the especially long meiotic prophase.

It has been hypothesized frequently that a special material, rich in RNA, fills the "sex vesicle" and surrounds the sex chromosomes (10, 26). Such a hypothesis may have arisen because of an inability to distinguish, with the light microscope, the nucleolus from the chromatin part of the "sex vesicle" of the mouse (in the mouse the sex chromosomes bear the nucleolus organizer) and because of the occasional association of nucleoli with the sex pair in other mammals. Electron microscopic observations (29, 30) and histochemical evidence (32) make that hypothesis untenable. Furthermore, the need of a special material in order to explain the end-to-end joining of the sex chromosomes can be abandoned, as synapsis is supported by electron microscopical data. It may be remarked that the name "sex vesicle" assigned to the condensed XY pair during zygotene-pachytene is misleading and could be replaced by *heterochromatic sex pair* (30).

Some Implications for Human Genetics

If synapsis occurs in a limited region of the XY pair, as shown in this paper, the possibility exists that crossing-over occurs in that region. This crossing-over should be reflected in the partial sex linkage of genes located in such a region of the X chromosome. As data on partial sex linkage in the human are poor and confusing (8, 16), a search for new observations may be valuable. Some hypotheses have been suggested (5), on the assumption that crossing-over in the human XY pair could exist, but experimental evidence for those hypotheses is lacking at present.

Another problem related to the present results concerns the origin of some cases of sex aneuploidy in man. The idea, supported by Hulten et al. (11), of a precocious disjunction as the source of sex univalents during normal meiosis seems to agree with our results. The alteration by deletion or translocation of the small pairing region in the sex chromosomes would result in the inability to segregate regularly at the first meiotic anaphase, thus producing a high frequency of sex-aneuploid gametes. Such a case has not been clinically detected in the human, as aneuploid gametes seem to be produced accidentally.

We express our thanks to Prof. R. E. Mancini and to Dr. J. C. Lavieri for making this work possible.

This work was supported by the Consejo Nacional de Investigaciones Cientificas y Tecnicas and a grant from the Population Council. Both authors are established investigators in the C.N.I.C.T.

Received for publication 25 July 1969, and in revised form 17 November 1969.

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