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# Arrangement of Centromeres in Mouse Cells

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*Abstract.* Applying a staining procedure which reveals constitutive heterochromatin to cytological preparations of the mouse (*Mus musculus*), one detects heterochromatin pieces at the centromeric areas of all chromosomes except the Y. The Y chromosome is somewhat heteropycnotic in general but possesses no intensely stained centromeric heterochromatin. The arrangement of the centromeric heterochromatin in interphase cells is apparently specific for a given cell type. In meiotic prophase, centromeric heterochromatin may form clusters among bivalents. From the location of the centromeric heterochromatin of the X chromosome in the sex bivalent, it is concluded that the association between the X and Y (common end) in meiosis is limited to the distal portions of the sex elements.

## Introduction

Yasmineh and Yunis (1970) have reported that most of the satellite DNA of the mouse, *Mus musculus*, is located in the heterochromatin fraction of disrupted interphase nuclei. Using the *in situ* DNA/RNA hybridization technique, Pardue and Gall (1970) found that this satellite DNA is specifically localized in the centromeric areas of mouse chromosomes. They also were the first to report, as far as we are aware, centromeric heterochromatin in the chromosomes of the mouse. Arrighi and Hsu (1971) found that the various treatments used for *in situ* DNA/RNA hybridization were responsible for revealing constitutive heterochromatin.

All mouse chromosomes are telocentric, and all except one (the Y chromosome) possess centromeric heterochromatin. Furthermore, heterochromatin does not occur outside of the centromere regions. Centromeric heterochromatin is therefore useful as a marker to trace the orientation or arrangement of the centromeres in the interphase nuclei. This paper reports observations on centromeric arrangement in cells of certain somatic tissues and male germ cells of *Mus musculus*.

## Materials and Methods

Laboratory mice of the Swiss strain were used for this study. For light microscopy studies, animals received an injection of Velban (Eli Lilly, Indianapolis, Indiana) at 2.5 μg/g body weight, approximately 2 hours before sacrifice. Bone marrow, cerbellum, and testicular tissues were used for squash preparations. The staining procedure was the same as that described by Arrighi and Hsu (1971).

For electron microscopy studies, the seminiferous tubules were dissected in 3% glutaraldehyde in pH 7.4 phosphate buffer (Millonig, 1961) and diced into 1 mm segments. After fixation for 1 hr at room temperature, the tubules were rinsed twice in phosphate buffer, and postfixed in 1% osmium tetroxide for 1 hour. The tissue was dehydrated in a graded series of ethanol, embedded in Epon 812, and ultrathin sections were cut with a diamond knife using an LKB Ultratome III. Sections were picked up on collodion-coated slotted grids and stained in alcoholic uranyl acetate, followed by lead citrate.

Whole mount preparations were made by a modification of the procedure used by Gall (1966). Seminiferous tubules were pipetted repeatedly until a single-cell suspension was obtained. The suspension of cells was pelleted and resuspended in Pipes buffer at pH 6.8. The cell suspension was cast upon an aqueous surface in a teflon dish and picked up on formvar-coated 200 mesh copper grids. The material was fixed for five minutes in aqueous uranyl acetate, dehydrated in a graded alcohol and amyl acetate series, and dried by the Anderson critical point method (Anderson, 1951). Grids were examined with an Hitachi HU-11C electron microscope operating at 75 kV.

## Results

### *Mitotic Metaphase*

Centromeric heterochromatin was detected in all metaphase figures from bone marrow preparations. All 40 chromosomes in female cells exhibited centromeric heterochromatin (Fig. 1a). In the male cells, however, only 39 showed centromeric heterochromatin (Fig. 1b). The single chromosome devoid of centromeric heterochromatin was identified as the Y, not only because of its size (Stich and Hsu, 1960), but also because of the lack of such a heterochromatin piece in the sex bivalents of meiotic prophase. In all metaphases of the male cells observed, the entire Y chromosome appeared to be more heteropycnotic than the euchromatic arms of other chromosomes. In the female cells, no heteropycnotic element, representing the inactivated X, was noted.

The amount of centromeric heterochromatin appeared to be different from chromosome to chromosome. Although we made no attempt to construct a mouse karyotype with relation to heterochromatin distribution, observations on the two shortest chromosomes which can be identified by morphology (pair 19) indicate that the amount appeared to be consistent for each chromosome. This is in agreement with the conclusion reached in human chromosome studies (Arrighi and Hsu, 1971).

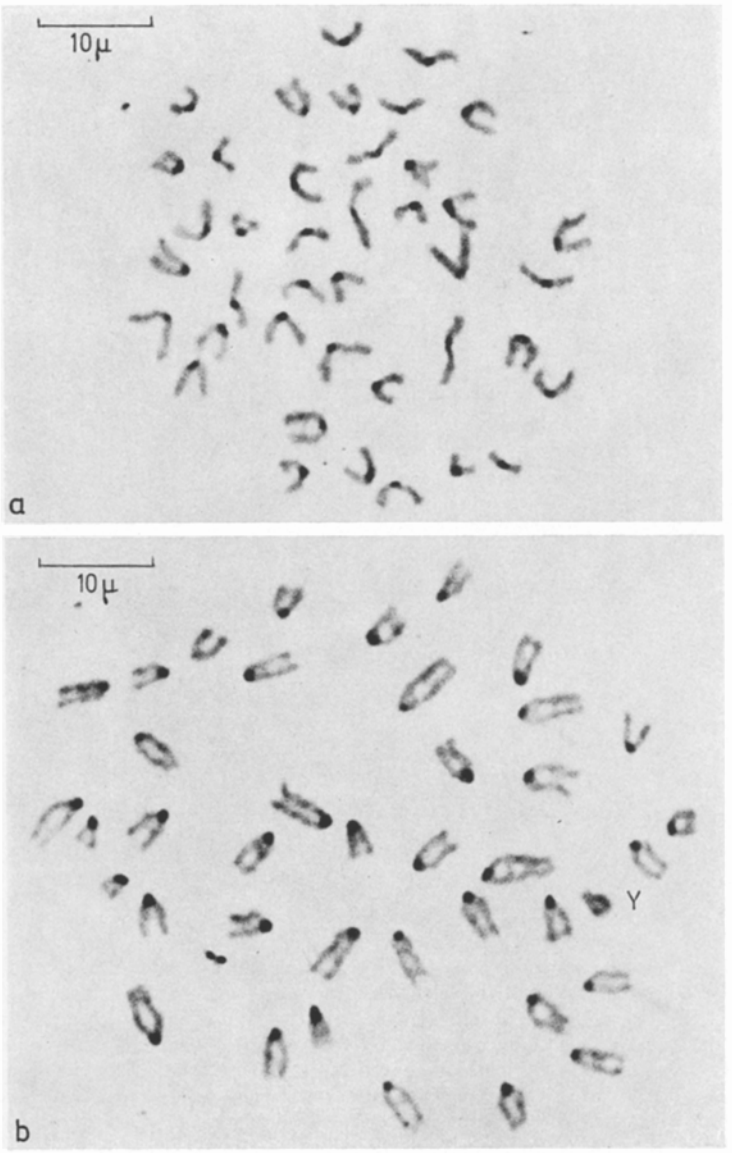


Fig. 1. a Metaphase from bone marrow of female mouse. Each chromosome exhibits a densely-stained block of heterochromatin near the centromere. b Metaphase figure from bone marrow of male mouse. Only 39 chromosomes have centromeric heterochromatin. The Y chromosome has no centromeric heterochromatin, but is more pycnotic than the other chromosomes

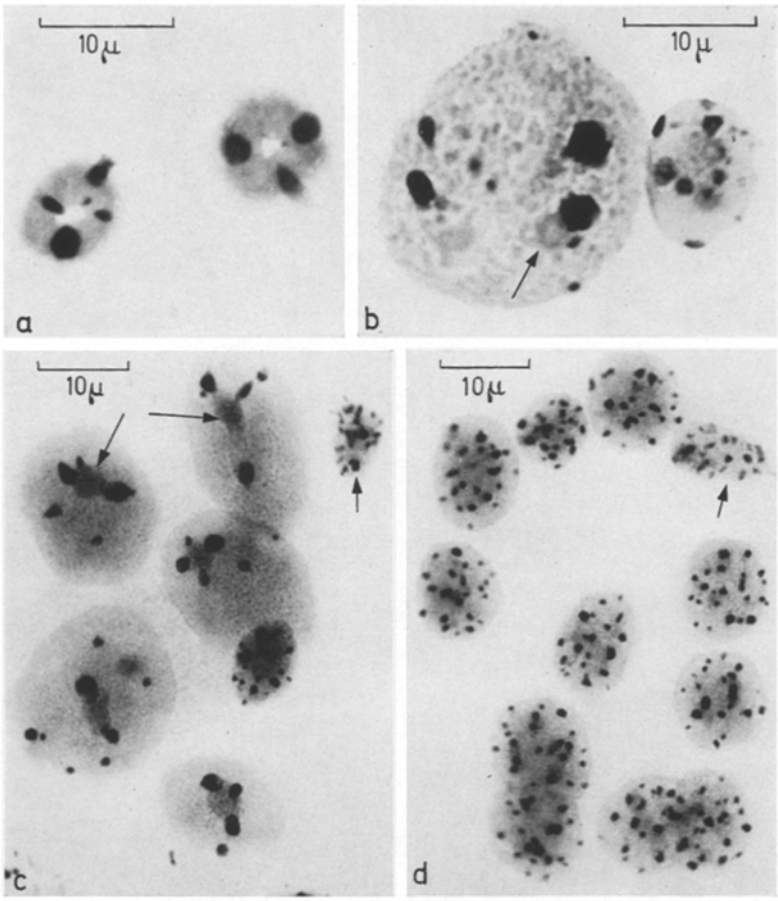


Fig. 2. a Granulocytes from mouse bone marrow. b Neuron and glial elements from mouse cerebellum. The neuron is distinguished by its large size and small number of large heterochromatin blocks as well as its prominent nucleolus (long arrow), whereas the glial nucleus has more numerous smaller heterochromatin blocks. c Mouse testis squash. Sertoli cells have few large heterochromatin blocks associated with a large nucleolus (long arrows). Tubule sheath cells (short arrow) have many smaller heterochromatin pieces. Note the tiny heterochromatin pieces, probably from single chromosomes. d Spermatogonia from mouse testis. Note the large number of dense heterochromatin blocks, apparently distributed at random. An interstitial cell is easily distinguished (arrow)

### *Somatic Cell Nuclei*

In the bone marrow the granulocytes are easily identified because of their characteristic doughnut-shaped nuclei (Fig. 2a). They were therefore chosen as one cell type for the study on heterochromatin. Granulocytes contained a low number of large heterochromatin blocks, more or

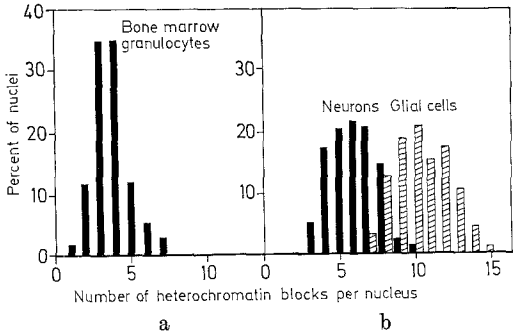


Fig. 3a and b. Distribution of heterochromatin blocks in bone marrow granulocytes, cerebellar neurons and glial cells from mouse

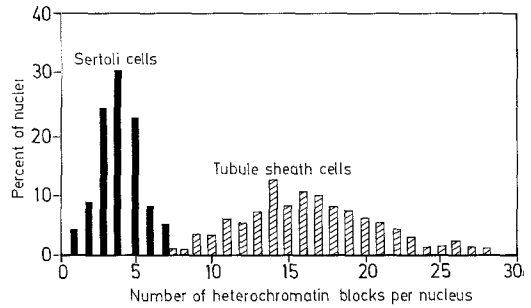


Fig. 4. Distribution of heterochromatin blocks in Sertoli cells and tubule sheath cells from mouse testis

less evenly spaced along the outer or inner membrane. As shown in Fig. 3a, the number of heterochromatin blocks did not exceed 7, and the majority of granulocytes contained only 3 or 4. In all other cells of the bone marrow, heterochromatin blocks were smaller in size but more numerous.

In the cerebellum, the large neurons and the smaller glial elements are distinguishable by the nuclear size. There are usually one or more conspicuous nucleoli in the neurons, and some heterochromatin blocks are in close association with the nucleoli (Fig. 2b). The distribution of heterochromatin blocks provides a further clear distinction between these two cell types. Like granulocytes, neurons generally contain only a small number of large blocks, whereas glial elements display a greater number of smaller heterochromatin blocks. The histogram in Fig. 3b shows that, although the number of heterochromatin blocks per nucleus of the two cell types overlap, the two cell populations were entirely different in respect to the arrangement of centromeres in interphase.

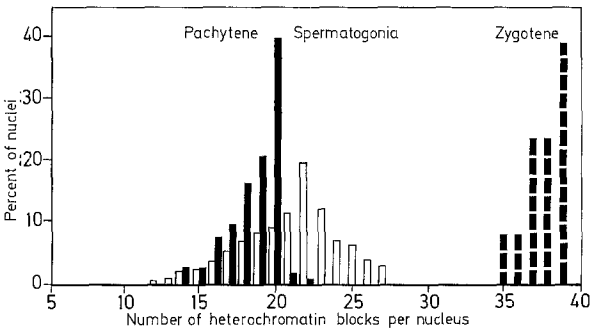


Fig. 5. Distribution of heterochromatin blocks in spermatogonia and in meiotic prophase nuclei from mouse testis

Two types of somatic cells are recognizable in testicular preparations, the Sertoli cells and the tubule sheath or interstitial cells (Fig. 2c). The nuclei of the Sertoli cells are large, round, and relatively homogeneous with one or more prominent nucleoli. As in the case of neurons, at least one, and generally two, large heterochromatin blocks are in close association with the nucleolus. The heterochromatin blocks are usually large, but tiny ones, each probably representing that of one chromosome, are not uncommon. Nevertheless, as shown in Fig. 4, the total number of heterochromatin blocks (both large and small) in the Sertoli cells is so low that its upper limit (7) barely overlaps the lower limit for the tubule sheath cells. The narrow distribution of the average number of heterochromatin blocks for the Sertoli cells, with an average of 4 per nucleus, strongly contrasts with the broad distribution for the sheath cells, with an average of 17.

### *Spermatogonia*

The nuclei of spermatogonia, regardless of the type or size, were recorded together. In general, the heterochromatin pieces of the spermatogonial cells were small but numerous (Fig. 2d). However, the number never reached the expected 39 (Fig. 5), indicating fusion of some of the centromeric heterochromatin pieces. The highest number recorded was 27, and the lowest, 12.

Fig. 6a–f. Meiotic prophase nuclei from mouse testis. Sex vesicle (*X*) is prominent in each nucleus. Note only one heterochromatin piece is present in each sex vesicle, presumably that of the X chromosome. Short arrow, heterochromatin at the terminus of each bivalent. Double-shaft arrow, nonhomologous association between heterochromatin pieces of different bivalents. Long arrow, connecting strands between nonhomologous heterochromatin. a Zygonema, with about 36 het-

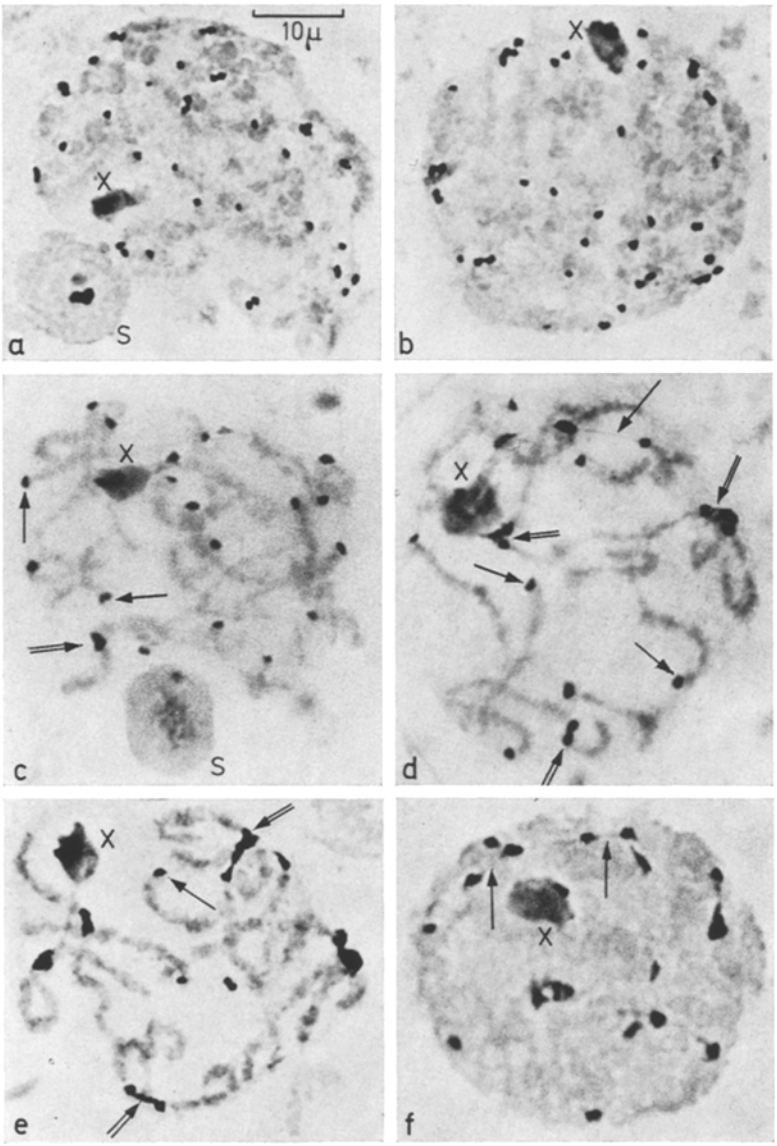


Fig. 6a—f

erochromatin blocks, either single or arranged in pairs. Note spermatid nucleus with a single large heterochromatin block (*S*). b Zygonema. The pairing of heterochromatin blocks is more obvious, and probably represents synapsis of homologs. c Early pachynema. Note spermatid nucleus (*S*) with dispersed heterochromatin. d Pachynema. e Late pachynema. f Nucleus with morphology resembling that of interphase. Sex vesicle and centromeric heterochromatin arrangement identify this as a pachytene nucleus

*Spermatocytes*

In mammalian meiosis, it is generally difficult to identify the prepachytene stages. Using the heterochromatin staining procedure, the extended chromosomes may become completely obliterated, but the heterochromatin pieces would become comparatively more pronounced. We believe that the zygotene stage can be identified by the "pairing" behavior of heterochromatin pieces. Figures 6a and 6b are presumably zygonemas. Note the single, as well as the paired, heterochromatin blocks, suggestive of a synapsis process between homologous centromeres. The sex vesicle (X) is visible in all zygotene and pachytene stages. In each sex vesicle only one heterochromatin block that of the X chromosome, is evident. Counting each single heterochromatin block as one, and each paired block as two, the highest number of heterochromatin pieces found in zygonema was 39, and the lowest, 35 (Fig. 5).

In pachynema, all heterochromatin pieces are paired and often fused to present the appearance of single blocks, larger than in zygonema (Fig. 6c–f). When a bivalent is not in contact with another bivalent, the heterochromatin is always situated at the terminal position of each bivalent (short arrows, Fig. 6c–e). However, in many cases there appeared to be nonhomologous association between the paired heterochromatin bodies (double arrows, Fig. 6c–e). Sometimes several heterochromatin pairs formed a coalesced mass, or showed thin connections between them (long arrows, Fig. 6d, f).

In late pachytene stages, the paired heterochromatin pieces may be so closely associated in each bivalent that unequivocal counts of individual heterochromatin blocks would be highly inaccurate. Coalesced pairs caused further problems in heterochromatin counts. Nevertheless, in a large proportion of cases, 20 heterochromatin pieces (19 autosomal pairs plus the single X) were counted. The number seldom exceeded 20 and was frequently below this value (Fig. 5).

In a number of pachytene cells the euchromatin of the bivalents was overexpanded by the treatments and the nuclei showed a smooth, interphaselike appearance (Fig. 6f). The presence of the sex-vesicle and the arrangement of the centromeric heterochromatin, however, became useful criteria for identification.

The centromeric heterochromatin of meiotic prophase in mouse cells can be observed also in conventional electronmicrographs. Fig. 7a shows two synaptonemal complexes, each of which is situated directly against the nuclear envelope and is surrounded by condensed chromatin, presumably the centromeric heterochromatin. Fig. 7b demonstrates that, although a sharp demarcation exists between the condensed heterochromatin and the diffused euchromatin, the synaptonemal com-

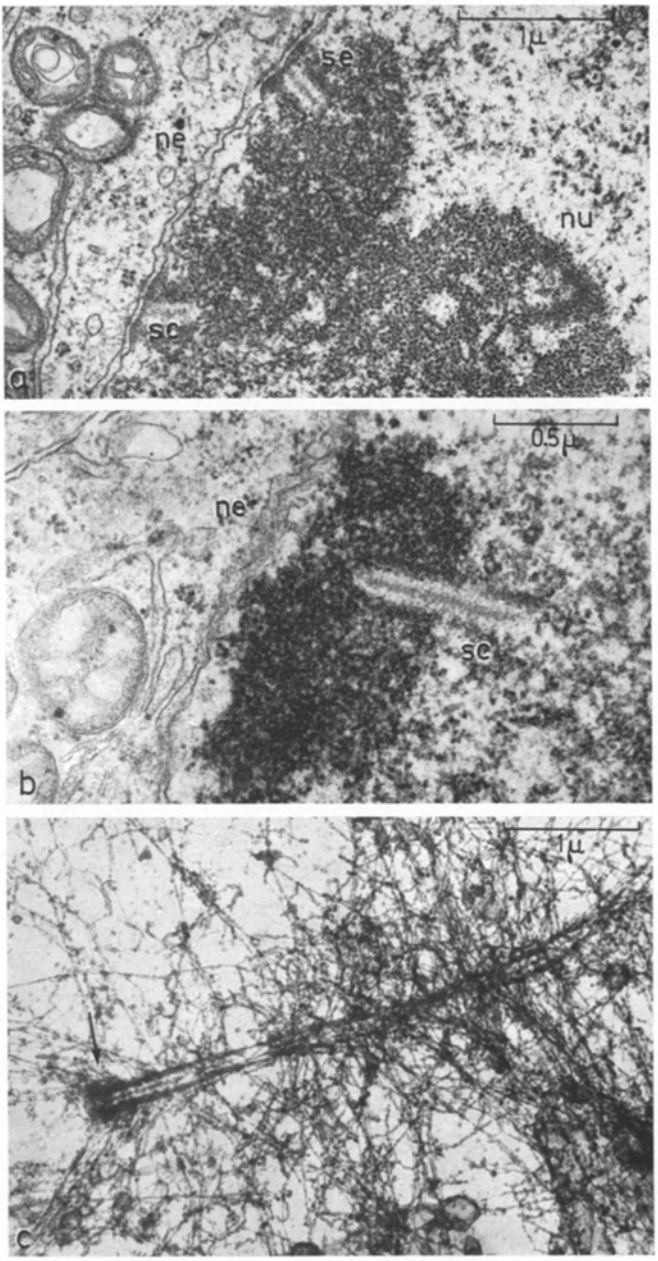


Fig. 7a–c. Electron micrographs of pachytene chromosomes showing centromeric heterochromatin. a Two sets of synaptonemal complexes (*sc*) ending at the nuclear envelope (*ne*). Note the electron dense fibers which probably represent centromeric heterochromatin. A nucleolus (*nu*) is also evident in this section. b Synaptonemal complex is continuous through diffuse euchromatin and adjacent dense heterochromatin. c Condensed heterochromatin (arrow) is evident at the terminus of this isolated water-spread synaptonemal complex

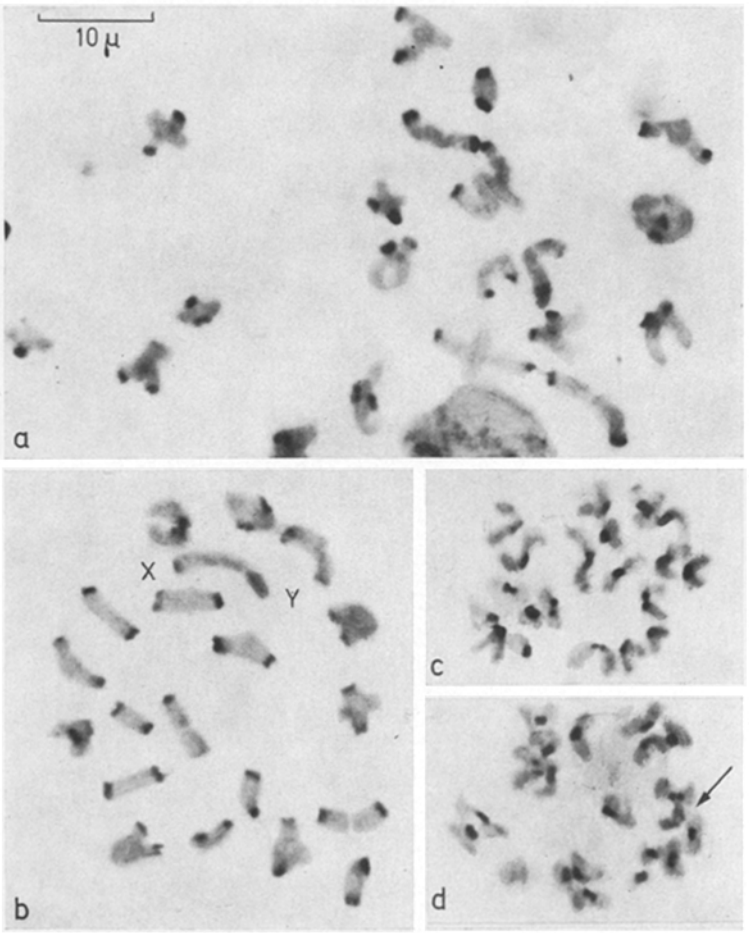


Fig. 8a–d. Meiotic figures from mouse testis. a Diakinesis, showing 20 bivalents. The heterochromatin blocks show the relative position of centromeres in each bivalent. b Metaphase I, showing 20 bivalents. The sex bivalent is asymmetric, with only one terminus displaying centromeric heterochromatin (*X*); the shorter component is considered to be the Y chromosome. c Metaphase II. All 20 chromosomes have centromeric heterochromatin, identifying this as an X-bearing cell. d Metaphase II. Only 19 chromosomes display centromeric heterochromatin, with one heteropyknotic chromosome without heterochromatin at the centromere (arrow), presumably the Y

plex is continuous through both regions. This phenomenon was also observed in whole mount preparations (Fig. 7c).

In later stages of meiotic prophase and first metaphase, the centromeric heterochromatin is extremely conspicuous (Fig. 8a, b). In Fig. 8b,

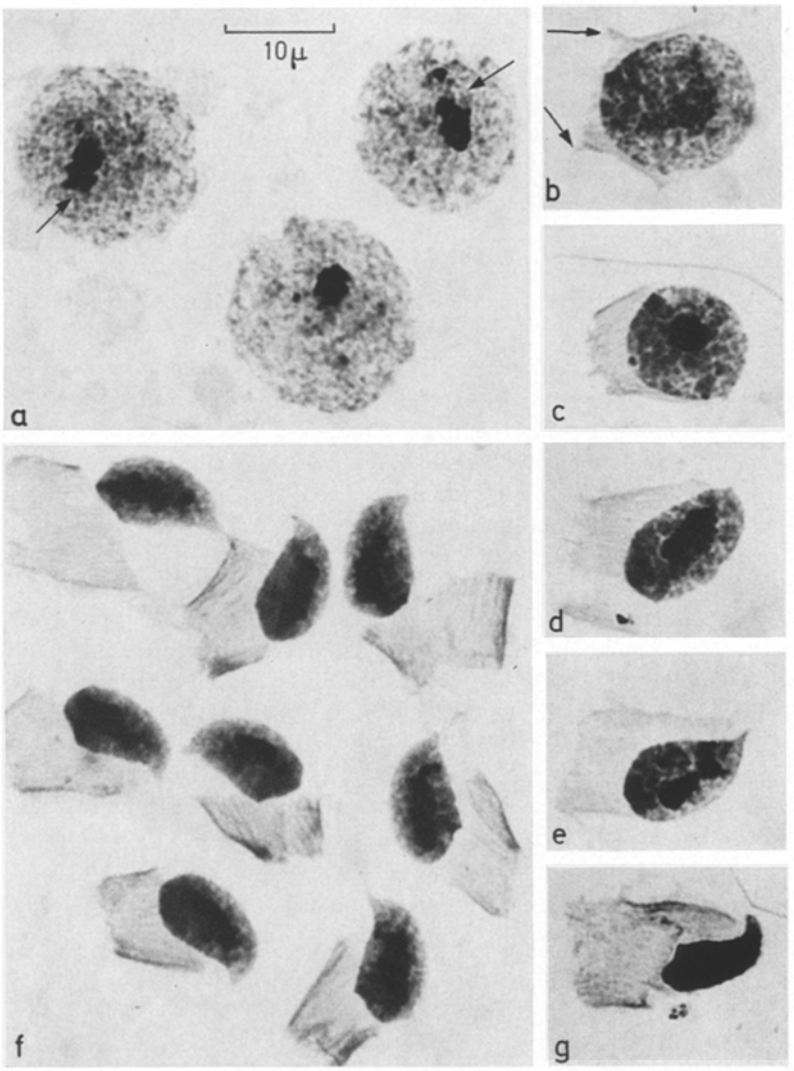


Fig. 9a–g. Morphologic changes in spermatid nuclei of mouse testis during spermiogenesis. a Note the single large block of heterochromatin in each nucleus, closely associated with a prominent nucleolus (arrows). b Nucleus is smaller, more condensed, and centromeric heterochromatin is dispersed; a short manchette (arrows indicate the extent of the manchette) surrounds one end of the nucleus. c Heterochromatin is aggregated again in the center of the nucleus. d and e Nucleus elongates; note central mass of heterochromatin. f Nuclei are more condensed, making differentiation of central mass of heterochromatin more difficult. Manchette is associated with each late spermatid nucleus. g The nucleus is extremely condensed and densely-stained, and the central mass of centromeric heterochromatin is no longer distinguishable

one bivalent is clearly unequal in length, with centromeric heterochromatin on the longer component only. The shorter component is in general more densely stained, although no centromeric heterochromatin is present. This bivalent is considered to be the sex bivalent, with the longer component as the X, and the shorter element as the Y. Two distinct types of metaphases in the second meiotic division were noted, one with heterochromatin on all 20 chromosomes (Fig. 8c) and the other with heterochromatin on only 19 chromosomes (Fig. 8d). Presumably, they were the X-bearing and the Y-bearing cells, respectively.

### *Spermiogenesis*

The behavior of centromeric heterochromatin during mouse spermiogenesis can be described in the following sequence. Probably the first stage is represented by the most commonly observed type of early spermatid nucleus, showing a single large, dense, centrally-located block of heterochromatin, closely associated with a prominent nucleolus (Fig. 9a). The heterochromatin then disperses into many small blocks, each perhaps representing one chromosome, aggregated at the center of the smaller, more condensed nucleus (Fig. 9b). At this time, a sheath of short fibers, the manchette, becomes visible surrounding one end of the nucleus. The heterochromatin then coalesces again into a large dense mass, and the manchette elongates (Fig. 9c). The nucleolus is no longer visible at this stage. The nucleus undergoes a change in morphology as it condenses further (Fig. 9d–f), finally resulting in a very densely-stained mature spermatozoan nucleus (Fig. 9g). Throughout these last stages, the heterochromatin remains condensed in a large central mass, and the manchette is visible in all these stages.

## Discussion

If an interphase nucleus is formed with the chromosomes arranged according to the telophase configuration, all centromeres should aggregate in one area or in a few neighboring areas. In several cell types analyzed in this study, e.g., Sertoli cells, neurons, and spermatids, this seems to be the case; in many others, however, the centromeres appear to disperse over the entire nucleus. Our data suggest that the pattern of centromere arrangement differs in different cell types, and that there is a tendency for more differentiated cell types to possess fewer heterochromatin blocks (indicative of centromere aggregation) than cells with a potential to proliferate.

One of the possible reasons for the aggregation of centromeres, at least in the mouse, is that secondary constrictions (nucleolus organizers) are located near the centromeres of several chromosomal pairs

(Levan *et al.*, 1962). They may be either directly inside the heterochromatin zone or very close to it. Schildkraut and Maio (1968) found a 3 to 4-fold enrichment in satellite DNA in isolated nucleoli as compared with DNA from whole nuclei. Realizing that the nucleolus organizer is close to the centromeric heterochromatin, the data of Schildkraut and Maio appear highly reasonable, viz., the satellite DNA was an unavoidable contaminant. Thus the aggregation of centromeric heterochromatin may be the result of sharing nucleoli by the nucleolus organizers e.g., the Sertoli cells). If this is the case, cells with dispersed heterochromatin must have a different arrangement of the nucleolus organizers as well as the centromeres.

The observations on spermatogonia, spermatocytes and spermatids in terms of heterochromatin arrangement strongly indicate that the arrangement of centromeres follows a definite pattern according to the stages of cellular development. The coalescence of, and the connecting strands between, heterochromatin blocks of nonhomologous bivalents in prophase nuclei probably can be explained by the similarity in the base composition of the satellite DNA. The situation is analogous to the chromocenter formation in the polytene chromosomes of *Drosophila*. It is not known, however, whether or not pairing of bivalents begins with the centromeric regions. Since all centromeric regions possess the same type of DNA, nonhomologous synapsis is conceivable if this should be the case.

Of interest is the possible correlation between the centromeric heterochromatin found with light microscopy and that found with electron microscopy. Although verification still awaits data from *in situ* hybridization using electron microscopic techniques, it is highly probable that the condensed chromatin material in the electronmicrographs (Fig. 7) corresponds to the centromeric heterochromatin observed with light microscopy.

In our study, the X and the Y chromosomes of the sex bivalent are associated end-to-end (Fig. 8 b), but the centromeric heterochromatin of the X chromosome is at the opposite end of the association. This strongly indicates that if a partial synapsis is formed in the sex bivalent (the "common end" of Solari, 1970), it is at the distal portions of both the X and the Y chromosomes, and not at the centromeric ends.

In this connection we may add that many research projects during the past few years have utilized the Y chromosome of the mouse as a marker to identify male cells. The heterochromatin characteristics of the Y should be very useful for unequivocal identification in somatic cell hybrids or in tissue chimeras.

Dispersal of condensed heterochromatin blocks in spermatids at the time of manchette formation may be functionally related to the appar-

ent loss of the nucleolus. The subsequent reaggregation of the heterochromatin blocks without a demonstrable nucleolus probably suggests that no more ribosomal RNA synthesis is demanded for the remaining stages of spermiogenesis.

One of the most vexing problems connected with the findings of Pardue and Gall is how all the centromeres of the mouse acquired the same DNA molecules. Presumably, repetitious DNA was formed by gene duplication. Thus, repeated nucleotide sequences should be clustered on one chromosome, at least at the beginning. Translocations and inversions may carry a portion of the cluster to other areas and the duplication process may be repeated. In the case of satellite DNA, however, the translocation process must be very precise since every centromere has the same molecules. This is especially puzzling when we consider the fact that not all rodents possess this satellite DNA. In fact, two species from the Orient, *Mus caroli* and *M. cervicolor*, considered closely related to *M. musculus*, failed to exhibit the satellite DNA (F. E. Arrighi, personal communication). Prior to the acquisition of the satellite DNA, the ancestral form must have had a certain type of base sequence or sequences for the DNA of its centromeric heterochromatin (our unpublished data show that no mammalian species is devoid of centromeric heterochromatin), most likely repetitious. Such DNA molecules must be replaced by the satellite DNA when *M. musculus* was established. It would be extremely interesting to conduct studies on the molecular homology among many subgenera and species of the genus *Mus*, using biophysical as well as cytological approaches.

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