Observations on the So-Called Sex Chromatin in Man

by

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During the last few years attention has been drawn to certain heterochromatic bodies of mitotic interphase nuclei in several tissues of man. These bodies are especially evident during deep resting stage, when the chromosomes normally have faded away leaving only small heterochromatic granules. These heterochromatic bodies differ in size and shape between male and female tissue, and they are, therefor, often referred to as the sex chromatin. Since its quantity is larger in female than in male tissue (Moore, Graham and Barr, 1953), it seems probable that the X chromosome should be mainly responsible for this heterochromatin.

Since that sex difference has significance for diagnostic purposes, as sex determination in hermaphrodites, pre-natal sex determination etc., it has attracted a wide interest (cf. e.g. Sachs, Serr and Danon, 1956, Shettler, 1956). The present investigation is an attempt to identify any characteristic structure of the X and the Y chromosome, involved in the sex chromatin.

The technic employed is the one recently published (Reitalu, 1957), viz. fixation in Kahle's modified fixative followed by various combinations of differential staining of chromatin and nucleoli. In the present study the combination azur A + acid fuchsin yielded the best results.

The material consisted of three male and three female embryos, 10-25 cm in lenght, which were obtained by legal abortions at the Clinic of Obstretics and Gynecology of the Lund Hospital. The observations here reported are from liver tissue. Other tissues, brain, cerebellum, kidney, gonads were also examined, with results corroborating those from the liver.

1. Female Interphase Nucleus

The sex chromatin of female nuclei is usually described as planoconvex in outline and characteristically seen just within and in contact with the nuclear membrane (Moore and Barr, 1954, Shettler 1956). In the nuclei of the female liver cells of this study the sex chromatin takes the shape of two similar, oblong bodies in close contact with each other (Fig. 1 *a-f*, Pl. I). Usually they are parallel, but they may be lying

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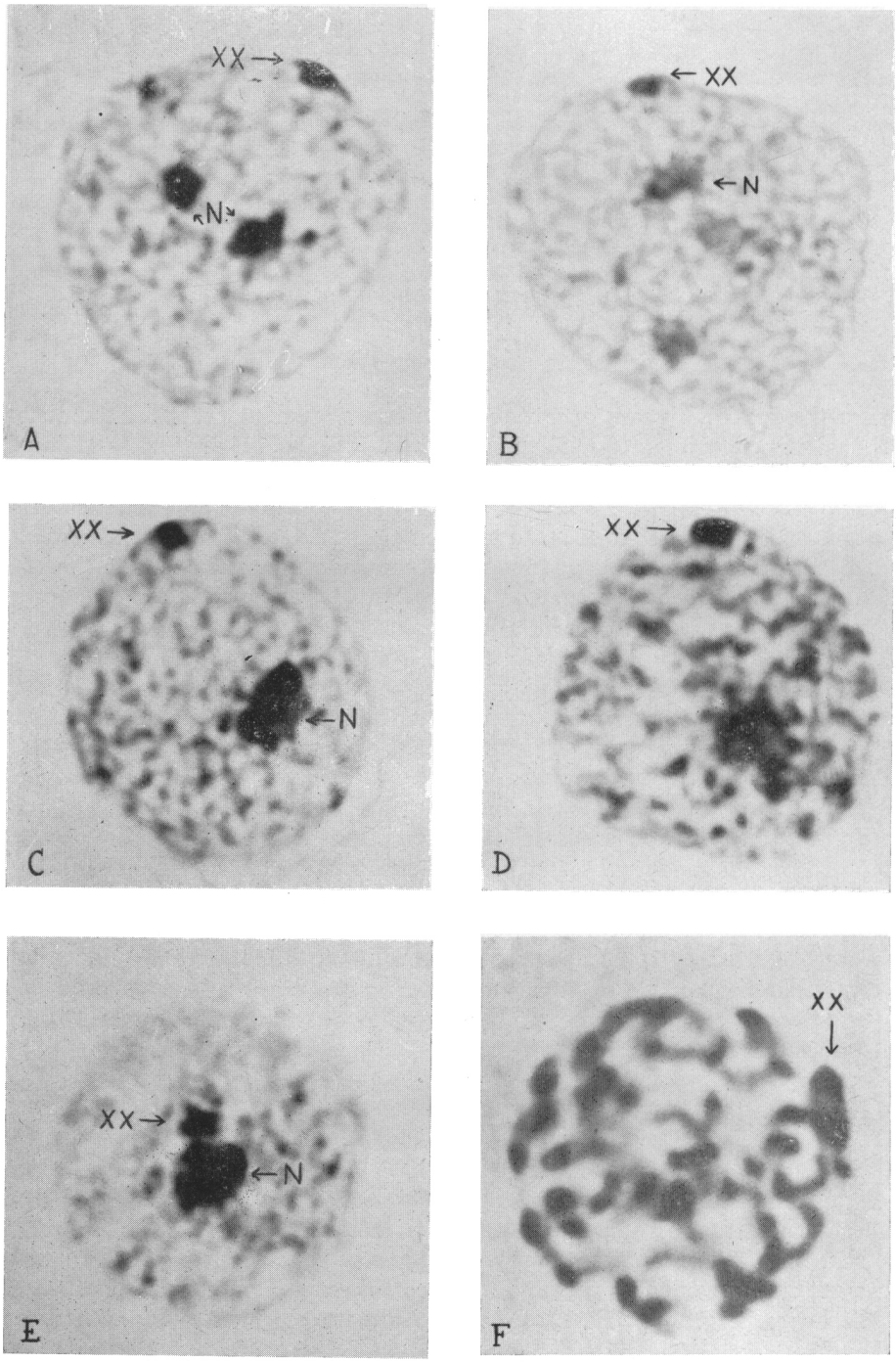

Plate I. A, B, C and E: some positions of the sex heterochromatin (XX) in female diploid resting nucleus. D, F: the same at prophase stage. N = nucleolus. × 2125

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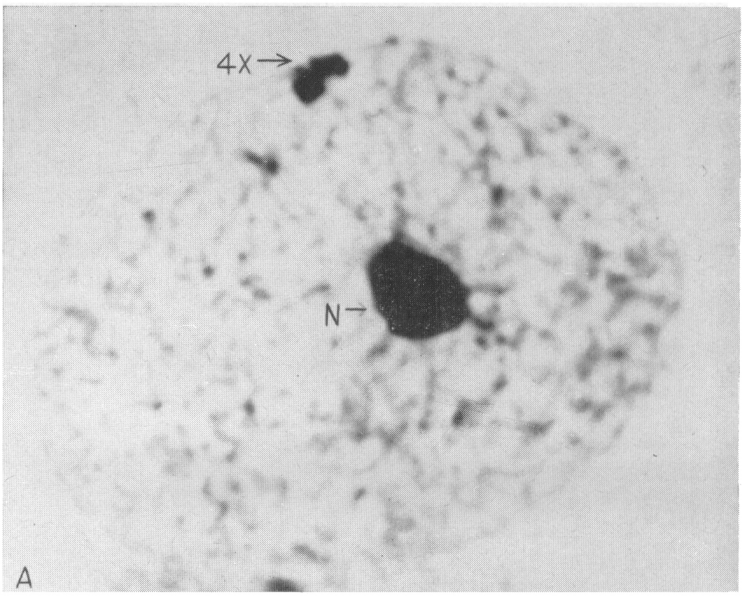
across each other (Fig. 1 *d*). The appearance of the sex chromatin of female cells is determined by several factors, as the relative position of the two elements involved, their original position in the nucleus, their position after the squashing etc. It will take the triangular or rectangular shape usually described, only when it is close to the periphery of the nucleus (Fig. 1 *a-c*, Pl. I*A-D*). Actually, a clearer understanding of the real structure of the sex chromatin is obtained, when it is in the center of the nucleus (Fig. 1 *d-f*, Pl. I *E*), its form then not being influenced by the nuclear membrane, allowing the symmetry of its two parts to become apparent.

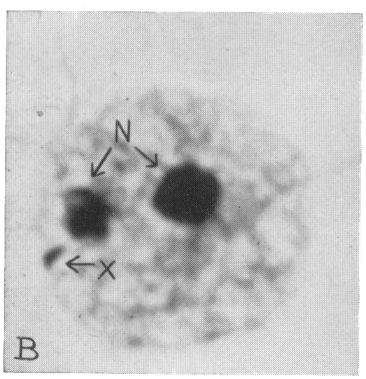
It is usually seen that each of the two parts of the sex chromatin continues as a fine thread from the sex chromatin to one of the nearest nucleoli. The two threads may be followed from the sex chromatin all the way to the nucleolus, and certain marks may be seen on the threads. Thus at the point of attachment of the threads to the nucleolus one or two small heterochromatic knobs are present. In addition, on each thread one small body lies constantly about halfway between the sex chromatin and the nucleolus (Fig. 1 *a-d*, Pl. I *A*). The length of the threads is extremely variable; sometimes they are completely concealed, the sex chromatin being directly in contact with the nucleolus which may be rather small. In Fig. 1 *f*, two small heterochromatic bodies may be seen on the other side of the minute nucleolus, these bodies evidently corresponding to the bodies usually at the point of attachment at the nucleolus. As a rule, the longer the distance between the sex chromatin and the nucleolus, the more tension is shown by the attachment threads.

While the sex chromatin is most apparent at deep resting stage, it may be identified at telophase (Fig. 1 *l*) and prophase (Pl. I *D*, *F*).

As is well known, liver tissue contains both diploid and polyploid cells, the latter having larger cellular and nuclear volume. This gave an opportunity of observing the sex chromatin, when present in duplicate. In nuclei with approximately 1,5-2 times the diameter of normal nuclei, and thus presumably tetraploid, the quantity of the sex chromatin is doubled (Pl. III *B*, *C*). It may be seen to consist of two twin groups of oblong bodies (Fig. 1 *g*, *i*, Pl. II *A*, III *C*). In other cases the twin groups may be separated from each other (Fig. 1 *h*). In conformity with the conditions of normal diploid nuclei the sex chromatin of tetraploid nuclei is connected by thin threads with the nucleolus. Usually the four threads end up in the same nucleolus (Fig. 1 *g*, *i*), but sometimes each of the two groups is attached to its separate nucleolus.

From the observations now reported it may be concluded that the so-called sex chromatin consists of a large heterochromatic segment of the two X chromosomes and is continued by a euchromatic segment, appearing as a thin thread. This thread has two heterochromatic lumps, both of much smaller diameter than the large heterochromatic segment. The second of these lumps, counted from the sex chromatin, is in contact with the nucleolus and may constitute a nucleolus-organizer of the X chromosome. Since no continuation of the thread has been observed beyond the nucleolus-organizer, this may constitute one of the ends of the X chromosome.





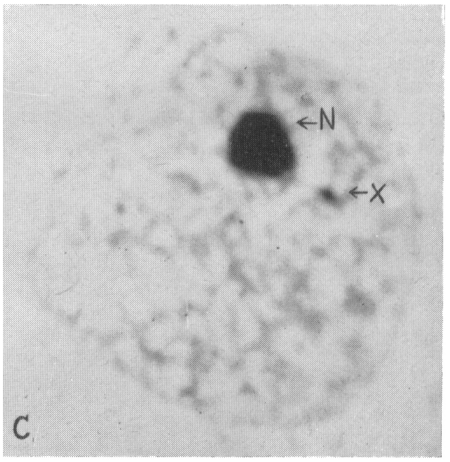


Plate II. A: the sex heterochromatin (4X) in female tetraploid resting nucleus. B,C: sex heterochromatin (X) in male diploid resting nucleus. N = nucleolus. × 2125

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2. Male Interphase Nucleus

The heterochromatic part of the X chromosome of male nuclei is recognizable as a single oblong body, similar to one of the elements of the sex chromatin of female nuclei (Fig. 1 *m-t*, Pl. II *B*, *C*, III *A*). As in female nuclei the heterochromatic part of the X chromosome is continued by a thread-like structure.

While the identification of the X chromosome thus met with little difficulty, the Y chromosome is more elusive. The detailed analysis of the X chromosome, however, gave some leads as to the probable appearance of the Y heterochromatin. The euchromatic section of the X chromosome in male nuclei exhibits an extra heterochromatic body, not observed in female nuclei. This extra body is usually attached directly to the nucleolus (Fig. 1 *n*, *r*, *s*, Pl. II *B*) or connected to the nucleolus by a thin thread (Fig. 1 *q*). Exceptionally, no connection to the nucleolus was seen (Fig. 1 *o*). In the latter case three heterochromatic bodies of unequal size are observed, attached to each other by a thread. Similar structures have been seen around one nucleolus (Fig. 1 *p*). The largest and smallest of these bodies belong to the X chromosome, while the medium-sized body at the end of the row, may be part, or the whole, of theY.

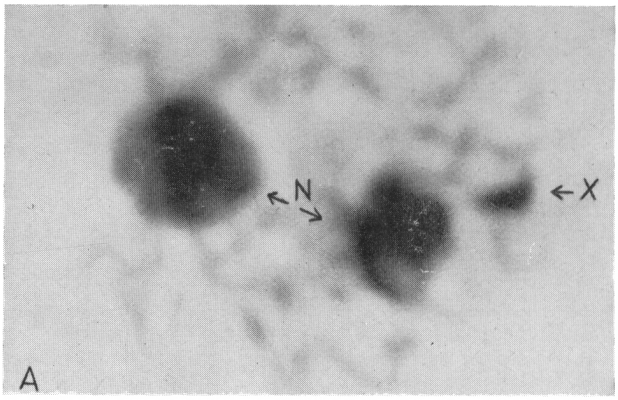
In tetraploid male nuclei, which contain two X and two Y chromosomes, somatic pairing between the heterochromatic bodies of the two X chromosomes is not the rule. Paired XX structures, similar to those typical of diploid female tissue, are seen only exceptionally. No somatic pairing between the two Y chromosomes has ever been observed.

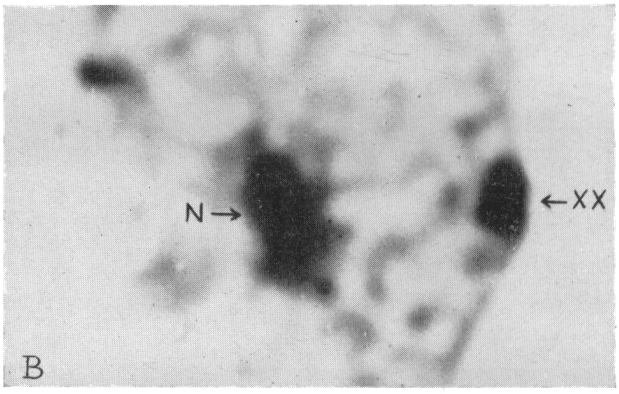
3. Conclusions

The present study has shown that the so-called sex chromatin of female nuclei in man consists of large heterochromatic segments of the X chromosomes lying side by side. It has also appeared that the X chromosome has a euchromatic segment, which, at interphase, may be of considerable length, ending up with a heterochromatic knob probably active at the nucleolus formation. In male nuclei, one X chromosome of essentially similar structure is seen. Here a heterochromatic knob, somewhat larger than the nucleolus-associated knob of the X, was often joined terminally to the euchromatic segment of the X. This knob probably constitutes the Y chromosome.

It thus seems that the X chromosome of man may show a considerable euchromatic segment during somatic interphase. It has often been observed that the X and the Y chromosome form a nucleolus-like heterochromatic body during meiotic prophase of various mammals, among them man. No morphologically visible euchromatic segment is observed during these stages (Sachs, 1954, 1955, Tijo and Levan, 1956). Evidently one chromosome may appear completely heterochromatic at one stage in a certain tissue, while at another stage in other tissues the same chromosome may exhibit a longitudinal differentiation between heterochromatic and euchromatic segments.

The basis for the morphologic difference of the sex chromatin of male and female





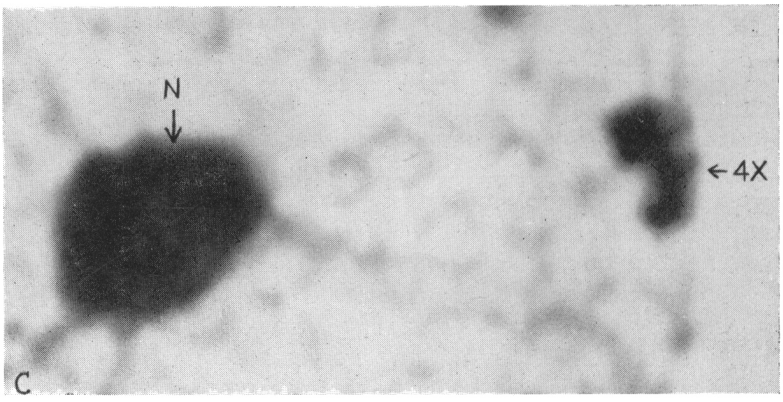


Plate III. A: the sex heterochromatin (X) in male diploid resting nucleus. B: the sex heterochromatin (XX) in female diploid resting nucleus. C: the sex heterochromatin (4X) in female tetraploid resting nucleus. N = nucleolus. × 5400

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cells is the pairing of the large heterochromatic segment of the X chromosomes, which form a lump large enough to be observed in female cells, while the lump formed of the single X chromosome in male cells is less striking. Various mechanisms for pairing of the two X chromosomes may be thought of. There may exist a specific attraction between homologous segments, comparable with the zygotene pairing, or with the somatic pairing of the Diptera. Or there may be a general tendency of the two large heterochromatic bodies to fuse with one another, as the autosomes disappear at telophase. Or, finally, the two heterochromatic segments may be brought together at the poles by the anaphase movement and then remain in juxtaposition because of stickiness. The last-mentioned situation would be favored in telophase nuclei with one predominating pair of heterochromatic bodies, as seems to be the case in man. The two heterochromatic segments will form a chromocenter in a similar way as the proximal chromosome parts form chromocenters in Drosophila or Dipcadi, only in man the chromocenter is separated by a euchromatic segment from the nucleolus organizers of the chromosomes involved.

The typical structure of the sex chromatin is found only in a fraction of all female cells. Glenister (1956) reports sex chromatin in 30-50% of the interphase nuclei of female embryonic tissues, while Shettler (1956) found nuclei with sex chromatin in cells of the amniotic fluid in a frequency varying between 28-65%. It is natural that the sex chromatin should not be detected in every cell, it often being concealed by other heterochromatic bodies. It has also been observed that the two X chromosomes may be lost through lagging at anaphase, Fig. 1 *j-k* being such instances. It is of course questionable whether cells deprived of their X chromosomes would survive.

In male nuclei the sex chromatin is more difficult to recognize. Since it is only half the size of the female sex heterochromatin, it may easily be confused with other heterochromatic bodies present, especially as small heterochromatic granules may fuse into larger units. The Y heterochromatin is still more difficult to identify, it being considerably smaller than the X heterochromatin. As discussed above, it may be found as a small, somewhat rod-shaped body connected with the end of the euchromatic part of the X chromosome.

The description and the discussion given above represent an interpretation of the nuclear structure in these liver cells. It should be mentioned, before leaving the subject, that other interpretations might also be conceivable. Thus, the comparatively rare occurence of male cells showing the typical X chromosome pattern might suggest that this pattern in the male cells may not represent the real X chromosome, but it might instead be a casual arrangement of autosomal chromocentres. It is conceivable that the X chromosome is heteropycnotic in the female sex only and does not show any heteropycnosis on the male side. There exist cases where the sex chromosomes show a different degree of heteropycnosis between the two sexes; thus, the X chromosomes of grasshoppers are heteropycnotic during meiosis in the males but not in the females (Coleman, 1943). Such an interpretation could explain why the « X chromosomes " of the tetraploid liver cells of human males do not show that mutual pairing which is so characteristic of the two X chromosomes of female cells.

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Summary

The difference in nuclear structure between male and female tissues in man has been examined in liver tissue from three embryos of each sex. The so-called sex chromatin consists of a large heterochromatic segment of the X chromosome, thus existing in duplicate in female diploid cells. The two segments have a tendency of juxtaposition resulting in a larger heterochromatic body in female than in male cells. Beside the large heterochromatic segment the X chromosome has, in the tissues studied, a euchromatic segment attached through a small terminal heterochromatic knob to a nucleolus. In male cells the euchromatic segment of the X chromosome is often joined terminally to a small heterochromatic segment believed to belong to the Y chromosome.

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SOMMARIO

La differenza di struttura nucleare fra i tessuti maschili e femminili nell'uomo è stata esaminata nel tessuto del fegato su tre embrioni di ciascun sesso. Ciò che si chiama la cromatina-sessuale consiste in un grande segmento eterocromatico di cromosoma X, esistente però in doppio nelle cellule diploidi femminili. I due segmenti hanno una tendenza di giustapposizione formante un corpo eterocromatico più grande nelle cellule femminili che in quelle maschili. A parte del grande segmento eterocromatico il cromosoma X possiede, nei tessuti studiati, un segmento eucromatico attaccato ad un nucleolo da una piccola protuberanza eterocromatica terminale. Nelle cellule maschili il segmento eucromatico del cromosoma X è sovente attaccato, alla sua estremità, ad un piccolo segmento eterocromatico che si crede appartenga al cromosoma Y.

SOMMAIRE

La différence de structure nucléaire entre des tissus masculins et féminins chez l'homme a été examinée dans le tissu du foie sur trois embryons de chaque sexe. Ce qu'on appelle la chromatine sexuée consiste dans un grand segment hétérochromatique du chromosome X, existant ainsi en double dans des cellules diploïdes féminines. Les deux segments ont une tendance de justaposition formant un corps hétérochromatique plus grand dans des cellules féminines que dans les masculines. A côté du grand segment hétérochromatique le chromosome X possède dans les tissus étudiés un segment euchromatique attaché à un nucléolus par une petite protubérance hétérochromatique terminale. Dans les cellules masculines le segment euchromatique du chromosone X est souvent attaché à son extrémité à un petit segment hétérochromatique qu'on croit appartenir au chromosome Y.

ZUSAMMENFASSUNG

Der Unterschied des Kernbaus zwischen männlichen und weiblichen Geweben im menschlichen Körper ist im Lebergewebe von drei Embryen jedes Geschlechtes untersucht worden. Die sogenannte Geschlechtschromatin besteht aus einem grossen heterochromatischen Segmente des X-Chromosoms, das also in weiblichen diploiden Zellen vorkommt. Die zwei Segmente haben eine Neigung zur Nebeneinanderstellung und bilden in weiblichen Zellen einen grösseren heterochromatischen Körper als in männlichen. Neben dem grossen heterochromatischen Segmente hat das X-Chromosom in den untersuchten Geweben ein euchromatisches Segment, das durch einen kleinen heterochromatischen Endhöcker mit einem Kernkörperchen verbunden ist. In männlichen Zellen ist das euchromatische Segment des X-Chromosoms oft am Ende mit einem kleinen heterochromatischen Segmente verbunden, von dem man glaubt, dass es zum Y-Chromosom gehört.

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