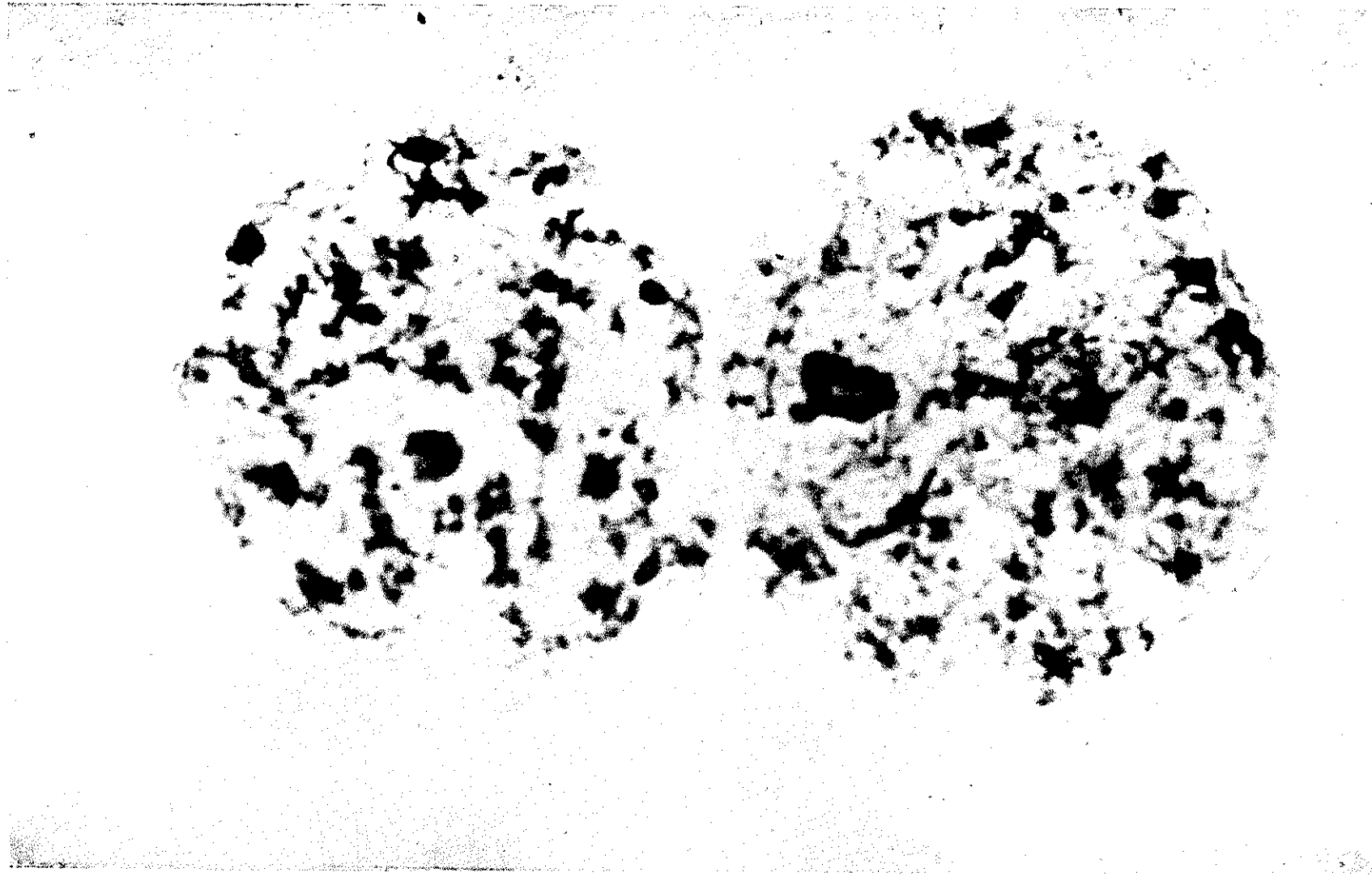

Frontispiece: Spermatogonial interkinesis of the rat. Two sister interkinesis nuclei, the X chromosome in the nucleus to the right, the Y in the nucleus to the left.— × 4200.

NOTE ON THE SEX CHROMOSOMES OF THE RAT DURING MALE MEIOSIS

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In connection with a recent investigation into the chromosomes of the Yoshida sarcoma of the rat we needed a detailed analysis of the normal mitotic chromosomes of this animal. These were studied in spermatogonial mitoses and in various somatic tissues, specifically in regenerating liver (TJIO and LEVAN, 1956). We found no difficulty in identifying the Y chromosome at mitosis, this being the smallest rod chromosome present. It was especially striking in the spermatogonial mitoses because of its positive heteropycnosis. The heteropycnosis of the Y was less pronounced, or even missing, in liver tissue.

The X chromosome escaped immediate recognition, since it is isopycnotic with the autosomes in the tissues studied. It was apparent, however, from the idiogram analyses made that the X must be one of the longer rod chromosomes, probably the fifth longest one. We wished, however, to obtain somewhat closer information about the X chromosome and to this end undertook the present study of the male meiosis of the rat.

The sex bivalent turned out to be easily observable from pachytene to interkinesis, and we thus obtained a good idea of the size relation between the X and Y, which largely corroborated our conclusions from the mitotic chromosomes. In addition, our study touched upon the interesting problem of the mode of pairing of the sex bivalent, and on the problem of tissue-specific heteropycnosis. So, although we originally intended this study as a chapter of the paper on the Yoshida chromosomes quoted above, we found it more appropriate to extract it as an independent paper.

The material of rat testes was obtained from an inbred strain of laboratory rats, used at various institutes in Lund. The slides were acetic orcein squashes, prepared according to TJIO and LEVAN (1954).

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I. SPERMATOGONIAL MITOSIS

The detailed description of the mitotic chromosome complement of the rat was summarized in our paper (1956) as a diagram based on measurements of the chromosomes of ten spermatogonial cells (l. c., Fig. 3). It is seen from this diagram that the rat chromosomes

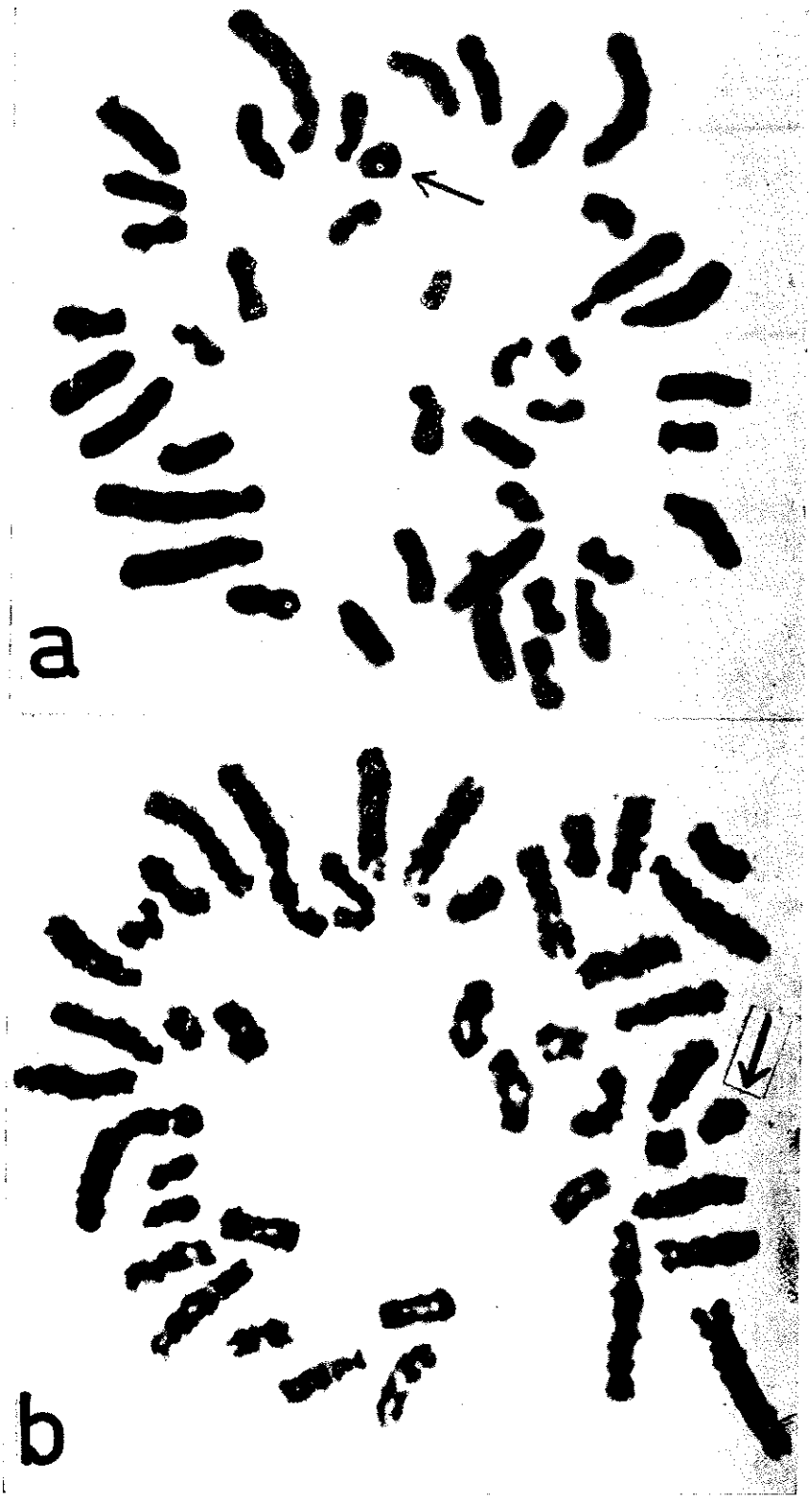

Fig. 1.—Two spermatogonial metaphases of the rat. The Y chromosome (at the arrows) shows positive heteropycnosis. — × 1900.

are of three morphologic types: V-, J-, and rod-type. From the location of the centromere these three types may be called median, subterminal and terminal, respectively, or M, S, and T chromosomes. While the M group comprises only small chromosomes, the S group also includes large chromosomes. The T group consists exclusively of large chromosomes with the one exception of the small Y chromosome.

A typical spermatogonial metaphase is represented in Fig. 1 *a*, in which the three morphologic classes may be distinguished. The Y chromosome, which is especially noticeable in the present connection, is marked with an arrow. It shows positive heteropycnosis, being of a somewhat more advanced contraction stage than the other chromosomes. This difference in contraction is emphasized still more in Fig. 1 *b*, which is a deviating type of spermatogonial metaphase with unusually low contraction. The Y stands out with a much denser staining. Certain segments of the other chromosomes show heterochromaty, as especially the centromeric regions. It will be shown later that these regions are often heterochromatic during various meiotic stages.

The Y chromosome is heteropycnotic all through the mitotic cycle in spermatogonial tissue. Its appearance during the resting stage is seen from Fig. 2 *a*. Here it forms a long and slender, heavily stained body. The other heteropycnotic bodies seen in this nucleus are mainly centromeric regions. The X chromosome is not heterochromatic in the spermatogonial tissue.

II. PROPHASE I.

During the first meiotic prophase the X and Y chromosomes are developed as a peculiar nucleolus-like vesicle. The sex bivalent thus shows a strikingly different behaviour from the autosomal bivalents.

This remarkable development of the XY bivalent has been long known (it was described in the rat, for instance, by MINOUCHI, 1928). However it seems to have escaped the attention of several later workers. Thus, KOLLER and DARLINGTON (1934) describe a normal pachytene structure of the XY pair of the rat with an extensive zone of pairing (l. c. Text fig. 15 *a*, *b*). According to SLIZYNSKI (1949 and 1955), the XY bivalent of the mouse at pachytene consists of two parts, viz. the heterochromatic vesicle (by him called «the puffy region»), which goes over into a fairly long euchromatic part, representing the homologous segments of the X and Y. According to him the euchromatic segment includes the centromere in such a position as to allow of chiasma formation on both sides of it.

Recently SACHS has directed the attention to the heteropycnotic structure of the XY bivalent during meiotic prophase of man (1954), the mouse (1955), and several other mammals. According to his opinion the heteropycnosis of the XY pair during the period of chromo-

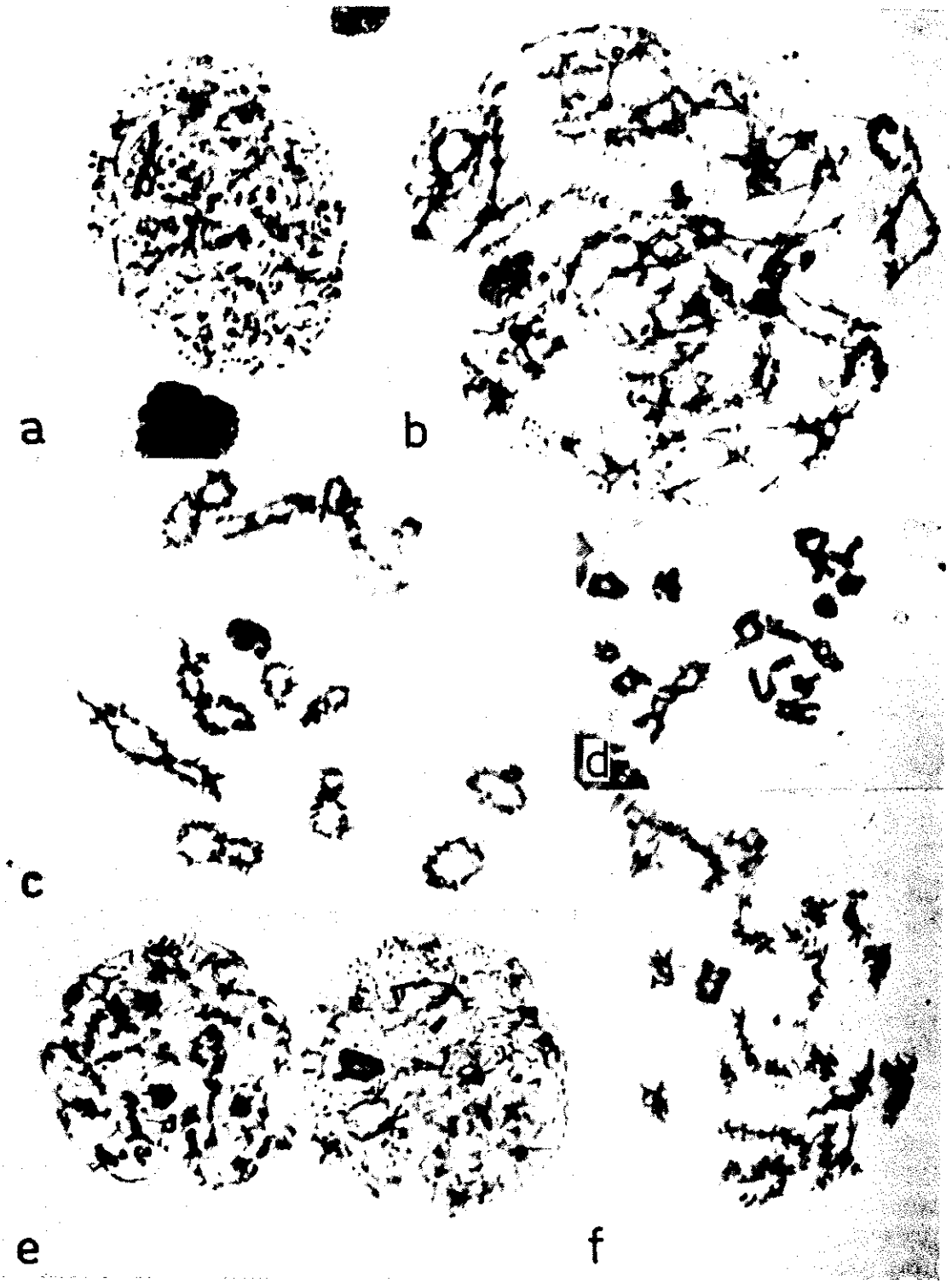

Fig. 2.—*a*: resting nucleus of the spermatogonial tissue; the Y chromosome is heteropycnotic; *b*: diplotene; *c*, *d*: early and later diakinesis; *e*: two sister interkinesis nuclei, the X chromosome in the nucleus to the right, the Y in the nucleus to the left; *f*: one interkinesis nucleus squashed to show the heterochromatic Y chromosome and the heterochromatic centromere regions of the autosomes. — × 1700.

Fig. 3.—*a*: pachytene; *b*: diplotene; *c*, *d*: diakinesis: *e*: early metaphase I, squashed, in side view; *f*: one pair of sister interkinesis nuclei; *g*, *h*: metaphase II; *i*: anaphase II; *j*: telophase II; *k*: sixteen instances of metaphase I in polar view showing the orientation of the sex bivalent. — *a-j*: × 1150; *k*: × 700.

some pairing should prevent normal chiasma formation between the X and Y. Nor does he find any visible chiasmata within the XY pair during the further development of it in the animals studied by him. This is essentially different from the observations of KOLLER and DARLINGTON (l. c.), who describe chiasmata on both sides of the centromere in the XY bivalent of the rat.

Our observations agree closely with those of SACHS. At pachytene (Fig. 3 *a*) the characteristic sex vesicle is always present. Often a darkly stained spot is found on the vesicle, probably corresponding to the centromeric regions, which generally stand out by a darker tone at this stage. We were unable to find any euchromatic section of the XY attached to the vesicle, but the possibility of a minimal such section can hardly be excluded. Chiasma formation within such a hypothetical section would not appear as visible chiasmata at later stages, but the X and Y would hang together end-to-end.

As prophase proceeds, the sex vesicle decreases in volume (Fig. 2 *b*, 3 *b*, 5 *a*, *b*), and the outlines of the two chromosomes inside it begin to appear (Fig. 2 *c*). An ordinary nucleolus, very faintly stained in our technique, is often visible in contact with the sex vesicle (Fig. 5 *b*). Usually the two sex chromosomes are situated in a ring around the margin of the vesicle; this may be an effect of the squashing, however. They seem to touch each other at their centromeric ends. The smaller chromosome (the Y) is considerably darker and more compact at this stage. Sometimes the X is divided by a constriction into one longer and one shorter part (Fig. 5 *b*).

At diakinesis a rather dramatic change takes place with the sex bivalent: the vesicle disappears and the two chromosomes straighten out (Fig. 2 *d*, 3 *c*, *d*). Now the X and Y may be studied in detail. Several instances of this stage are represented in Fig. 5 *c*. Y is still a little more deeply stained and more contracted than the X, which makes the estimation of their length relations somewhat doubtful. At a certain moment Y grows isopycnotic with the autosomes, while X remains negatively heteropycnotic (Fig. 3 *e*). The division into chromatids is evident in both the sex chromosomes. Constrictions, somewhat erratic in appearance, are often noticed. In the X the constrictions are observed mainly in two regions, dividing the chromosome in thirds. The Y often has one constriction on its middle.

III. METAPHASE I.

The sex bivalent is of a very striking appearance at the first metaphase. The rat meiosis constitutes a highly favorable material for a study of the sex bivalent. In Fig. 4 several metaphase plates are photographed in polar view, somewhat earlier stages are collected in the upper part of the picture and later stages in the lower part of it. In all of them the sex bivalent stands out clearly. The Y is al-

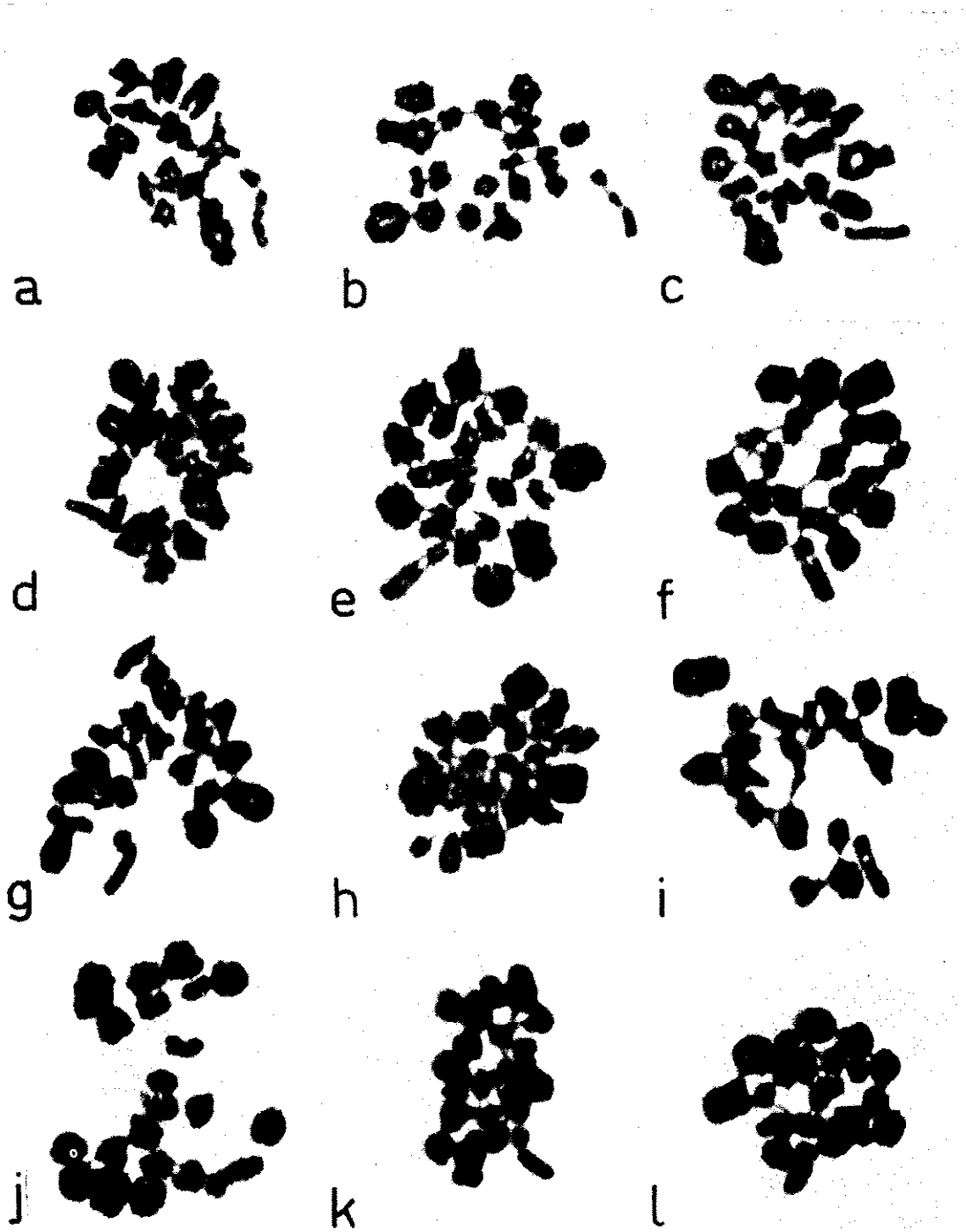

Fig. 4. Twelve instances of metaphase I plates in polar view. The sex bivalent is seen in the lower part of each plate. — × 1700.

ways situated close to the plate, or inside it, while the X is stretched out radially. In Fig. 3 *k* some more metaphases in polar view are sketched in order to show the orientation of the sex bivalent. Several sex bivalents are drawn separately in Fig. 5 *d*.

It may be seen from these pictures that the X is a T chromosome of somewhat more than double the length of the Y. As mentioned already, we concluded that X is the fifth longest T chromosome of the mitotic set, so there are two pairs of longer T autosomes present. However, we do not claim to have identified conclusively the somatic chromosome corresponding to the X. Unfortunately, the secondary constrictions seen on the X during meiosis do not help, since several of the possible T chromosomes have constrictions during mitosis.

As seen from our figures, the X and Y were always joined end-to-end. No chiasmata were ever seen. The appearance of the XY bivalent, as well as its orientation on the spindle, indicates that the X and Y are joined at their centromeric ends. It is true that single observations may suggest that the Y centromere should be at the free end (as in Fig. 5 *c*, bivalent nr. 3, or *d*, bivalent nr. 7), but we found this appearance to be exceptional. The free Y end shows no traction towards the pole. Often the interspace between the X and Y is filled by a faintly stained matter (Fig. 5 *d*), which may constitute a residue of the nucleolus, or diminutive shorter arms. In the latter case it is, of course, impossible to know whether any chiasmata have been present. No interstitial chiasmata were observed.

IV. INTERKINESIS

At anaphase I, X and Y fall apart and proceed to different poles. Since they are perfectly isopycnotic with the autosomes at this stage they are extremely hard to detect.

It is very fortunate that both the X and the Y are equally heteropycnotic during interkinesis. This is accordingly a very convenient stage to study them. Even their size relations are better understood at this stage than at metaphase I, since they seem to be at identical contraction state at interkinesis. The appearance of this stage is seen in Fig. 2 *e*, *f*, and Fig. 3 *f*. Whenever pairs of sister interkinesis nuclei kept together through the squashing, it was the rule that they contained different sex chromosomes, X was found in the one, Y in the other (Fig. 2 *e*, the frontispiece picture).

We had here an opportunity to directly observe the result of the separation within the sex bivalent at the first meiotic division. As is well known, different results are to be expected if chiasmata are formed on both sides of the centromeres, viz. the so-called reductional and equational division of the sex bivalent, respectively. In the former case the two chromatids of each interkinetic sex chromosome should be equal in length, in the latter case each sex chromosome should consist of one long and one short chromatid. This latter case

Fig. 5.— *a:* the sex vesicle at pachytene; *b:* at diplotene and diakinesis; *c:* the sex bivalent at diakinesis; *d:* at metaphase I; *e:* metaphase I in side view; *f:* ten instances of the Y chromosome (upper row) and the X chromosome (lower row) at interkinesis. — × 2750.

would result from chiasma formation on the long arms of the X and Y. In the investigation of KOLLER and DARLINGTON (l. c.) this happened in no less than 10 % of all cases, as judged from the structure of the first metaphase bivalents.

We scrutinized some hundred interkinesis nuclei, and only in one doubtful case something was seen that could be interpreted as a chromosome with unequal chromatids. Ten randomly drawn X and Y chromosomes at interkinesis are given in Fig. 5 *f*. Our results strongly suggest that if chiasmata are formed at all, they are located in the diminutive shorter arms.

The second meiotic division was observed only cursorily. The different morphologic chromosome types can be identified at the second metaphase and anaphase (Fig. 3 *g-i*). The sex chromosomes, however, are isopycnotic at these stages and therefore escape observation.

V. CONCLUDING REMARKS

The present observations stress the fact that the mammalian sex chromosomes may behave differentially during meiosis. While the autosomes go through the conventional development resulting in metaphase I bivalents of the usual type with interstitial and terminal chiasmata, the sex bivalent has a deviating type of meiosis. During the pairing stages the sex chromosomes are enclosed in a nucleolus-like vesicle, which persists until diakinesis.

This extraordinary prophase development of the sex chromosomes ends up with a deviating type of metaphase bivalent. The end-to-end association of the X and Y at this stage strongly suggests other means of association than chiasmata. It appears morphologically as if the small heads of the two chromosomes were sticking together. Evidently the centromeres are capable of coorientation, since the sex bivalent is kept on the equator and is separating regularly at anaphase I.

The study of the interkinesis leads to the conclusion that no chiasmata could have been present on the longer arms of the sex chromosomes. To exclude the possibility of chiasma formation even on the shorter arms is more difficult, even if this undoubtedly would be the most reasonable conclusion from the pictures observed. However, it can hardly be denied that a very small euchromatic, homologous segment may be present on the short arms and may undergo pairing during prophase. This would lead to chiasma formation in the short arm, which would appear at metaphase I as an end-to-end junction. The assumption of such a chiasma formation is purely hypothetical and without morphologic support. However, if the genetic data should require the occurrence of crossing-over between X and Y in the rat, this possibility cannot be neglected. Therefore, in our

opinion, the conclusion of SACHS, that no such crossing-over can occur in the sex bivalent, is not absolutely unobjectionable.

It was found that the heteropycnotic development of the sex chromosomes is largely depending on the tissue studied. Thus, both the X and the Y are heteropycnotic during meiosis (i. e. from pachytene to, and including, interkinesis), only the Y is heteropycnotic in spermatogonial tissue, while neither of them is heteropycnotic in liver tissue. It is very suggestive that the differential appearance of entire chromosomes, or chromosome segments, known as heteropycnosis, is a function of the differentiation pattern of the organism.

SUMMARY

The behaviour of the sex chromosomes has been followed through meiosis of the male rat. The development of the XY bivalent as a sex vesicle during meiotic prophase was studied. At metaphase I the X and Y are joined at their centromeric ends without any visible chiasma formation. No interstitial chiasmata were ever seen in the sex bivalent, nor the equational mode of separation expected from such chiasmata.

The consequence of this behaviour for crossing-over within the sex bivalent was discussed. The heteropycnotic development of the sex chromosomes is dependent on the tissue studied: both X and Y were heteropycnotic during meiosis, Y was heteropycnotic in the spermatogonial divisions, neither of them was heteropycnotic in liver tissue.

RESUMEN

(NOTA SOBRE LOS CROMOSOMAS SEXUALES EN LA MEIOSIS DE LA RATA MACHO)

Se estudia el comportamiento de los cromosomas sexuales durante la meiosis de la rata macho. También se estudia el desarrollo del bivalente XY, como vesícula sexual, durante la profase meiótica. Los cromosomas X e Y, en metafase I, se unen por sus extremos centroméricos, sin formación visible de quiasma. No se vieron quiasmas intersticiales en el bivalente sexual, ni tampoco se observó el modo de separación ecuacional que podría esperarse si existieran tales quiasmas.

Se discuten las consecuencias que supone esta conducta para el sobrecruzamiento dentro del bivalente sexual. El desarrollo heteropicnótico de los cromosomas sexuales depende del tejido estudiado. Tanto el X como el Y son heteropicnóticos durante la meiosis; el Y lo es también en las divisiones espermatogónicas y ninguno de ellos lo es en tejido hepático.

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