

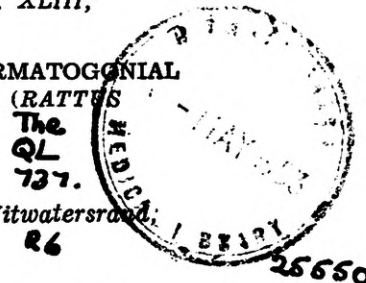
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THE CHARACTERIZATION OF THE SPERMATOGONIAL
CHROMOSOMES OF THE ALBINO RAT (*RATTUS*
NORVEGICUS ALBINUS)

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INTRODUCTION.

The spermatogonia of the albino rat have been an object of frequent studies in the past. Much of the earlier work was devoted to the determination of the chromosomal number of this species. Pincus (1927) has summarised the counts made prior to that time. It is evident from his table that considerable discrepancies were recorded, owing no doubt to the poor fixation obtained under the older technique. In 1918, Allen came close to the accepted figure when he reported 37 chromosomes in the spermatogonia. He was able to identify eighteen pairs of homologous autosomes, and a sex-determining X-chromosome. Rauh (1925) quoted by Minouchi, counted seven equatorial plates of the male rat and found 40 chromosomes in six instances, and 41 in the seventh.

Painter (1926) first reported the accepted figure of 42 chromosomes in spermatogonia of albino rats. This count was confirmed by his study of somatic mitoses in amniotic cells of male and female embryo rats. He found, too, that sex in the rat is governed by an XX-XY mechanism, the male being the heterogametic sex. The diploid number of chromosomes was verified by investigators in the United States (Pincus, 1927; Swezy, 1927), in Japan (Minouchi, 1928) and in England (Bryden, 1932; Koller and Darlington, 1934).

While much attention was devoted to the chromosomal configurations at meiosis in the rat, little attempt was made to describe or characterize the individual members of the mitotic chromosomal complement. Pincus, Painter and Bryden arranged the mitotic metaphase chromosomes in serial alignment. They paired forty of the chromosomes on the basis of general similarity in size and appearance. The remaining two elements constituted a heteromorphic pair, which these workers regarded as the X-Y pair. However, as each member of this heteromorphic pair fell within a group of chromosomes approximately equal in size, there could be no certainty that the true X and Y chromosomes had been identified as such.

The only chromosomal measurements which have been provided are those of Koller and Darlington. According to these workers, the chromosomes of the largest pair are about 3.6μ in length, while the rest of the complement ranges in length down to 0.6μ .

It is manifest from a brief survey of the literature that the meiotic chromosomes of the rat, and particularly the heteromorphic pair have been thoroughly studied, whereas the mitotic chromosomes have been largely neglected. The present study was designed to remedy this deficiency by providing as complete a characterization of the mitotic chromosomes as is possible in a species with so large a complement.

MATERIAL AND METHODS.

The testes of four young albino rats (*Rattus norvegicus albinus*) were removed under ether anaesthesia. One testis from each animal was fixed in Bouin's solution for 24 hours, dehydrated, cleared and embedded in paraffin wax. Sections were cut at 7μ and stained with Newton's crystal violet and the Feulgen reagent. While the sectioned material was not favourable in most instances for detailed measurements of metaphase chromosomes, it nevertheless provided useful information about the association of metaphase plates with other cell types in the same tubule. Squash preparations were made from the other testes, after fixation in 25 per cent. acetic alcohol. These were re-stained with Ehrlich's acid haematoxylin or Heidenhain's iron haematoxylin, according to the method recommended by Brenner (1946). The squash preparations were used for making detailed observations including measurements.

Measurements made directly on the preparations with an ocular micrometer can introduce a significant error. This was obviated by measuring the lengths on camera lucida drawings, which were made at a magnification of 2,550 x (immersion objective 97x and eyepiece 15x). The final length was corrected to the first decimal place; any errors of measurement or of drawing became insignificant when divided by the magnification.

The difficulties of overlap or foreshortening were eliminated by focussing with the fine adjustment. Where this was not possible the plates were rejected.

OBSERVATIONS.

1. *Chromosomal Number.*—In twelve spermatogonial metaphase and prometaphase plates in squash preparations, the chromosomal number was consistently found to be forty-two. The validity of this number received verification from the 21 diads observed in primary spermatocyte nuclei. The observation served as additional confirmation of the number reached by previous workers on-sectioned material.

2. *Chromosomal Shape*.—Centric constrictions are most easily observed in late prophase or prometaphase nuclei when they appear as short achromatic gaps in the chromosomes. For reasons to be mentioned later, it was necessary to establish the shape at metaphase as well as at prometaphase.

In prometaphase plates (Figs. 1, 12) twenty-eight chromosomes possessed sub-terminal constrictions, and the remainder median constrictions. These results are not entirely in accordance with the observations of Koller and Darlington as depicted in their illustration of a mitotic metaphase plate. Their plate portrayed 27 elements with sub-terminal constrictions and 15 with median constrictions. The disparity may be due to the confusion of a sub-median for a sub-terminal constriction, or vice versa, in one of the smaller elements.

Two types of sub-terminal constriction were observed. In one, the achromatic gap representing the centric constriction was situated some distance from the end of the chromosome. The distance ranged from about $1/10$ of their length in the larger chromosomes to about $1/5$ of their length in the medium-sized elements. The second type of sub-terminal constriction was so close to the end of the chromosome that only a small chromatic granule was visible distal to the constriction. It is probably the same or a similar structure which Minouchi named the "polar granule". Several chromosomes had sub-terminal constrictions intermediate in position between these two types.

The possession of median constrictions was confined to the smaller elements. Hence it was not possible to measure the two limbs on either side of each constriction with accuracy, nor to determine whether any of the so-called median constrictions were actually sub-median in position.

At metaphase, it was impossible to observe constrictions in every chromosome (Fig. 13). The most favourable plates contained fourteen elements with median constrictions. Such a chromosome appeared as two small granules lying side by side and separated by a thin region corresponding to the centric constriction. Of the rest of the complement, a variable number contained obviously sub-terminal constrictions, while the remainder were uniformly rodlike. In no instance was a centromeric granule observed in the indented portion of the chromosome.

It is apparent from these findings that different conclusions would have been drawn if only prometaphase or only metaphase plates had been examined. A study of metaphase plates alone would have led to the conclusion that a certain number of chromosomes possessed no centric constrictions and were terminally-attaching or telomitic; whereas, from a study of both stages, it is possible to say that the rod-like appearance of

some elements resulted from the difficulty of detecting terminal granules in chromosomes with immediately sub-terminal constrictions.

3. *Chromosomal Length.*—The chromosomal length is the most variable of the features mentioned so far. In spermatogonial chromosomes of the albino rat, Pincus and Minouchi both found variation in length. However, they did not attempt to give exact mathematical expression to the size variation for the whole complement.

The size range was assessed for the chromosomes of the albino rat. In this description, "size-range" refers to the varying lengths of one chromosome recognisable in different metaphase plates. For this purpose, the lengths of the chromosomes in eight spermatogonial plates from the same tubule were determined. The results are represented diagrammatically in Figs. 2-9. The chromosomes are arranged in order of length, but no effort has been made to pair them off in this diagram, nor to distinguish the heteromorphic pair. The lengths at any one position in the linear series cannot be assumed to represent the same chromosome in different plates, unless that chromosome has been accurately characterized on criteria other than size. This assumption cannot be made because a chromosome may become slightly more contracted in one plate than in another, thus altering its position in the series. The composite graph (Fig. 10) indicates the size range in any part of the series. In addition, the broken lines can be regarded as a measure of the size range of a particular chromosome only when this element occupies a constant position in the series. The five largest and four smallest chromosomes fall into this group (Fig. 11).

Simpson (1941) has pointed out that it cannot be assumed that the observed range of variation represents the true or population range. The latter can only be determined by an examination of every spermatogonial plate in every existing albino rat—a highly impracticable procedure. The spermatogonial plates examined in the present investigation represent only a sample of the possible total. Thus it is improbable that this sample contains the true largest and smallest lengths for any one chromosome. Nevertheless, it would not be expected that the true size range is much greater than the observed range, owing to the relative constancy in length of the spermatogonial chromosomes.

DISCUSSION.

At metaphase, it has been shown, not all the chromosomes appear to have centric constrictions (Fig. 12). Hence, if it is desired to pair off the metaphase chromosomes accurately, it is essential to establish a correspondence between the shapes observable at prometaphase and the lengths measured at metaphase. Even if this could be effected completely, the two

characters of size and position of centric constriction do not provide sufficient information to pair off all the chromosomes. Accordingly, a third feature was necessary, and this was found in the general appearance of the chromosomes. By general appearance of the chromosomes is meant such features as thickness, evenness of contour, degree of tapering between the end of the chromosome and the centric constriction.

By the combined use of these three features, the chromosomes of the rat were characterized as completely as possible.

The four largest chromosomes possessed sub-terminal constrictions and fell into two pairs on the basis of size. Fig. 11 represents the maximum and minimum observed lengths of these two pairs, as well as of the fifth element, the two smallest pairs and the presumptive Y-chromosome. Where there are differences in length between two members of a pair, there can be no certainty that, in different plates, the same element will invariably be the larger. Hence the size range for one member of the longest pair may be taken as the mean range for both members. This is depicted by the horizontal broken line in Fig. 11. The same applies to the second pair.

The fifth largest chromosome could not be mated. It has a sub-terminal constriction and its range ($2.8-3.1\mu$) is smaller than the ranges for the other large chromosomes. It is presumed that this fifth element is the larger member of the heteromorphic pair found by earlier workers, and it may be called the presumptive X-chromosome. The size range reported corresponds well with Koller and Darlington's figure of 2.9μ as the length of the larger of two chromosomes sometimes found lying off the metaphase plate.

The four smallest chromosomes consist of a larger pair with median constrictions and a smaller pair with immediately sub-terminal constrictions. In the latter pair, the terminal granule is not detectable at metaphase, the two chromosomes appearing oval. The size ranges of the smaller chromosomes have a small absolute value. In addition, relative to their chromosomal lengths, the ranges are smaller than those of the longer chromosomes.

Between these two well-defined groups of chromosomes are ten larger pairs with sub-terminal constrictions, six smaller pairs with median constrictions, and an unmatched chromosome. The intermediate sixteen pairs of chromosomes could not be characterized individually, but only as groups. The members of the groups exhibit a gradation of size and shape, so that it is impossible to distinguish the same pair within two plates with complete certainty.

The smaller unmatched element is the presumptive Y-chromosome. It is the smallest chromosome with a sub-terminal constriction apart from the pair already described. It

is recognisable at metaphase when its length in various plates was found to be $1.2-1.3\mu$. The length of Koller and Darlington's presumptive Y-chromosome was 1.1μ . In six plates it occupied position 34 in the linear series, while in three others it was 28, 32 and 36. This illustrates the earlier statement that one chromosome may lie in different positions in the linear series in several plates.

Table 1 summarises the final characterization. The size ranges quoted for any group represent the range between the extreme lengths of the smallest and largest members of the group.

TABLE 1.

Chromosome or Group	Length	Position of centric constriction
Pair 1	$3.6-4.4\mu$	Sub-terminal
Pair 2	$2.9-3.9\mu$	Sub-terminal
Presumptive X	$2.8-3.1\mu$	Sub-terminal
Pairs 3—12	$1.4-3.1\mu$	Sub-terminal
Pairs 13—16	$1.2-2.9\mu$	Median
Presumptive Y	$1.2-1.3\mu$	Sub-terminal
Pairs 17—19	$0.8-1.3\mu$	Median
Pair 20	$0.8-0.9\mu$	Sub-terminal

The type-graph (Fig. 11) is an attempt to represent these results graphically. If possible variations in the linear order are borne in mind, this graph may be used as the type-graph for the species, in comparisons with the chromosomal complements of closely related species.

SUMMARY.

1. Studies have been made on the mitotic chromosomes of the albino rat to characterize them as accurately as possible.
2. The diploid chromosome number has been found to be forty-two in dividing spermatogonia.
3. The complement has been characterized on the basis of chromosome length, position of centric constrictions, and general appearance. In this way, it has been established that the complement consists of thirteen pairs with sub-terminal constrictions, seven pairs with median constrictions, and a heteromorphic pair, the presumptive X- and Y-chromosomes.
4. It has been found that mitotic chromosomal length has a range of values for any chromosome pair in the rat, and is not a constant single value. Size ranges have been established for the complement.

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ILLUSTRATIONS.

Fig. 1.—Linear alignment of the chromosomes of a mitotic prometaphase plate. Lengths in . In this figure the positions of the centric constrictions are indicated.

Figs. 2-9.—Linear alignments of the chromosomes of eight mitotic metaphase plates.

Note that the chromosomes are not paired off, nor is the heteromorphic pair indicated.

Fig. 10.—Composite Graph of size range and position of centric constrictions in the chromosomal complement of the Albino Rat.

Fig. 11.—Size ranges of four largest and four smallest chromosomes, and of Presumptive X- and Y-Chromosomes.

Fig. 12b.—Drawing from a photomicrograph of mitotic prometaphase plate. Acetic orcein squash preparation re-stained with Ehrlich's Haematoxylin x4000.

Fig. 13b.—Drawing from a photomicrograph of mitotic metaphase plate. Acetic orcein squash preparation re-stained with Heidenhain's Haematoxylin x4000.

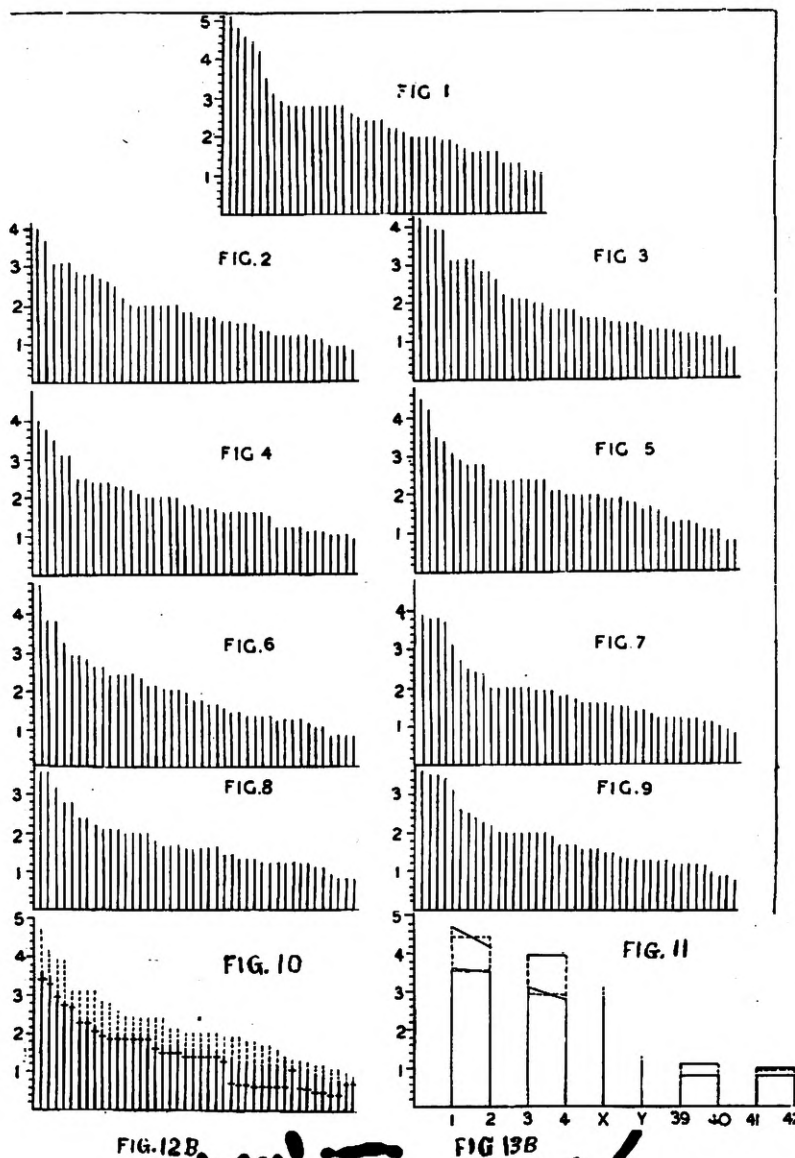


FIG. 12B



FIG. 13B

