APPENDIX

PAPERS AND ABSTRACTS

SILA BANDYOPADHYAY (née DE), M. Sc.
GENETICS LABORATORY
DEPARTMENT OF ZOOLOGY
UNIVERSITY OF BURDWAN
BURDWAN, WEST BENGAL
INDIA 713 104
LIST OF PAPERS


LIST OF ABSTRACTS


REPLICATIVE SEQUENCE OF HUMAN RING X-CHROMOSOME

S. K. GHOSAL, S. DE, T. K. BANDYOPADHYAY, S. CHATTOPADHYAY
and N. C. CHAKRABARTY

Zoology Department, Burdwan University, Burdwan 713 101, West Bengal.

Summary

Ring X-chromosome from a human female exhibiting Turner's syndrome stigmata was labelled with tritiated thymidine at one hour interval throughout the S period. Radioautographic analysis of both the amount and the rate of DNA synthesis suggested that despite being 'late replicating', the ring X-initiates its replication shortly after at least five G group chromosomes start their DNA duplication at early S period. Synthesising at least 20% of its DNA, ring X-chromosome's replication either stops or synthetic rate decreases considerably (for about an hour) by the mid S. Ring X-chromosome synthesises approximately 25% and 55% of its DNA in the third and final quarters respectively of the S period. The DNA synthesis of the 'late replicating' X-chromosome appears to be characterised by (i) its initiation at early S, (ii) a short interruption (or very little synthesis) at mid S, and (iii) a burst of synthetic activity in the late S period.

Introduction

In mammalian females one of the two X-chromosomes is genetically inactive (Lyon, 1961), forms sex chromatin in interphase nuclei (Barr and Bertram, 1949) and is late in terminating its DNA synthesis (Mukherjee et al., 1968). Structurally abnormal X (Mukherjee et al., 1966), with a few exceptions (Miller, 1972), is usually late replicating. It was earlier presumed that the particular late replicating X is also late in initiating its DNA synthesis (Hsu, 1964; Priest et al., 1967). Several workers (Kikuchi and Sandberg, 1964), however, suggest that both the Xs start DNA replication almost simultaneously, but the X destined to be late replicating after synthesising about 20% of its DNA undergoes an interruption of this synthetic activity (Wright et al., 1970), followed by a sudden burst in the replicating activity at the later part of the S period (Mukherjee et al., 1968). Present experiment was designed to study the replicating behaviour of this marker X (ring-X) chromosome at different intervals within the S period.

Materials and Methods

Leucocyte cultures were set up from an XX/X ring-X/XO human mosaic by standard technique (Mukherjee et al. 1961) using TC-199 (Difco), phyto (Difco) and new born calf serum (Microbiological). (A) For studying the initial replication pattern, H3-thymidine (sp. act. 0.36 Ci/mM, NEN) at a final concentration of 2μ Ci/ml medium was added at the time of setting up the culture. At 55 hrs (Mukherjee et al. 1966) cells were washed thrice in BSS and incubated in isotope free medium containing "cold" thymidine. At 68 hrs colcemide (CIBA) (5×10^-4M) was added and at 72 hrs cells harvested. (B) For determining terminal (i.e., "late") replication cultures were set up in 'cold' medium. H3-TdR and colchicine was added at 69 hrs and...
cells harvested at 72 hrs. (C) In order to determine the isotope incorporation at short duration within the S period, a 5-min pulse labelling with H³-TdR was done at 48 hrs. Cells were washed and incubated in 'cold' medium without colchicine. Cells were harvested covering a period from 1.5 to 16.8 hrs post-labelling (Wright et al, 1970). Kodak NTB-3 emulsion was used for radioautography (Ghosal et al 1972).

Results and Discussion

A total of 327 well spread metaphases from culture (C), harvested at various intervals in S period, was analysed. The number of grains on the ring-X

<table>
<thead>
<tr>
<th>Cells harvested at following hours after isotope (H³-TdR) administration</th>
<th>Number of grains on the ring-X chromosome</th>
<th>Number of grains on the most heavily labelled C-group chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.8</td>
<td>2-3</td>
<td>0-5</td>
</tr>
<tr>
<td>2.8</td>
<td>18-22</td>
<td>4-8</td>
</tr>
<tr>
<td>3.6</td>
<td>31-39</td>
<td>7-13</td>
</tr>
<tr>
<td>4.8</td>
<td>37-42</td>
<td>14-22</td>
</tr>
<tr>
<td>7.8</td>
<td>26-32</td>
<td>14-22</td>
</tr>
<tr>
<td>9.8</td>
<td>18-20</td>
<td>7-11</td>
</tr>
<tr>
<td>10.6</td>
<td>2-7</td>
<td>5-7</td>
</tr>
<tr>
<td>11.0</td>
<td>0-3</td>
<td>4-8</td>
</tr>
<tr>
<td>11.9</td>
<td>3-6</td>
<td>2-4</td>
</tr>
<tr>
<td>12.8</td>
<td>3-5</td>
<td>2-4</td>
</tr>
<tr>
<td>13.0</td>
<td>2-4</td>
<td>2-4</td>
</tr>
</tbody>
</table>

and those on the most heavily labelled chromosome belonging to group C were recorded. It may be mentioned that the labelled metaphases appearing shortly after the isotope administration come from those cells which had been at the later part of the S period at the time of H³-thymidine addition (Wright et al, 1970). Data indicate that although the ring-X is not the first chromosome in C group to start replication, it does so within a short period after 1-5 C-group chromosomes enter their S phase. About $2 \pm 0.4$ hrs. of initiation, of its DNA synthesis (i.e., replicating about 20% of its DNA), ring X-chromosomes replication nearly stops and synthetic rate remains
considerably decreased for about 1 ±0.3 hrs. by the mid-S period. Ring X-chromosome synthesises approximately 25% and 55% of its DNA in the third and final quarters respectively of the S period. A few workers, however, have suggested that the late replicating X-chromosome incorporates no thymidine at the beginning of S period; but these workers used DNA synthesis blocks (inhibiting thymidylic acid synthesis), e.g., FUdR (Hsu, 1964; Priest et al, 1967) or aminopterin (Peterson, 1964) to synchronise the cells prior to the addition of those chemicals which are used to label DNA. DNA synthesis blocks may interfere with the normal physiology of these cells. Experiments without using any chemicals have earlier demonstrated almost simultaneously initiation of DNA synthesis in both the X-chromosomes of bovine females (Mukherjee et al, 1968, 1961; Wright et al, 1970). The evidence presented here shows that human ring-X, which is late replicating, is not correspondingly late in initiating its DNA synthesis. This finding is in perfect agreement with similar pulse labelling experiments on human (Kikuchi and Sandberg 1964) and bovine females. The DNA synthesis of the "late-replicating" X-chromosome appears to be characterised by (i) its initiation at early-S, (ii) a remarkable drop of synthetic activity at mid-S, and (iii) a burst of synthetic activity in the late-S period.

Acknowledgement

CSIR grant-in-aid 38 (164)-GAU. II is gratefully acknowledged.

References

Hsu, T C, J Cell biol 23 (1964) 53.
Kikuchi, Y and Sandberg, A A, J natl cancer inst 32 (1964) 1109.
Peterson, A J, J Cell biol 23 (1964) 651.
Reprint from the proceedings of the
Symposium on Use of Radiations and Radioisotopes in
Studies of Animal Production
Izatnagar, Dec. 16-18, 1975
ESTIMATION OF PRE-MEIOtic DNA SYNTHESIS PERIOD
IN THE DOG SPERMATOCYTES
S.K. Ghosal, T. Bandyopadhyay, S. Chattopadhyay, S. De and L.J. Beauregard
Zoology Dept., Burdwan University, Burdwan, and Genetics Lab., Rhode Island Hospital, Providence

Introduction
Present knowledge regarding duration of pre-meiotic S (DNA synthesis) period and chronology of meiosis in mammals is obscure. Although attempts have been made to calculate the pre-meiotic duration of the S period of mouse oocytes, spermatocyte's S duration has only been estimated in the mouse and golden hamster. Present investigation was designed to calculate the duration of pre-meiotic S period in the Canine spermatocytes.

Material and Method
About 10 µCi of H-thymidine (sp. act. 2 Ci/µM, NEN, Boston) was injected under tunica at four arbitrary sites in each testis of 6 dogs. Biopsies were taken at intervals covering a period from 0.40 to 49.19 days post-injection. Slides were prepared by conventional air-drying method, stained with acetic orcein and radioautographed with the Kodak NTB3 liquid emulsion. The progression of labelled spermatocytes through various meiotic stages was recorded (Table I).

Method for calculating pre-meiotic S duration in the spermatocytes is a simple modification of the technique.
employed for estimating the S period duration in mitotic
spermatocytes. The interval of time between isotop administration and the appearance of labelled diakinesis for the
first time represents minimum time required by labelled
spermatocytes to complete all meiotic stages preceding
diakinesis. Theoretically the proportion of labelled
diakinesis will (i) rise (ascending limb) thereafter
finally reaching 100%, (ii) remain at this plateau for
several hours, and (iii) fall (descending limb) later
to approximately 0%. The maximum duration of all pre-
diakinetic meiotic stages would be the time post-labelling
when 100% diakinesis will be radioactive. The interval
between two 50% points on the ascending and descending
limbs of the curve is the mean duration of S period.

Results and Discussions

Preliminary work on the kinetics of spermatogenesis
in dog by standard technique demonstrated that (Table I)

Table I

<table>
<thead>
<tr>
<th>Most advanced</th>
<th>Days post-injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>stage(s)</td>
<td>Detected for the first time in later biopsies</td>
</tr>
<tr>
<td>Leptotene</td>
<td>0.40</td>
</tr>
<tr>
<td>Zygote</td>
<td>4.04</td>
</tr>
<tr>
<td>Pachytene</td>
<td>4.04</td>
</tr>
<tr>
<td>Diplotene, Diakinesis and Metaphase-I</td>
<td>20.96</td>
</tr>
<tr>
<td>Spermatids</td>
<td>22.20</td>
</tr>
<tr>
<td>Mature spermatozoa</td>
<td>42.15</td>
</tr>
</tbody>
</table>

---
leptotene, syngamy and pachytene stages appeared labelled for the first time at 0.40, 4.04 and 4.04 d respectively whereas diplotene, diakinesis and metaphase-I do so almost simultaneously at 20.95 d following the administration of \(^3\)H-thymidine. As the chronology of spermatogenesis is very uniform in a given species, the percentage of labelled diakinesis obtained from all the biopsies obtained from 20.96 to 22.20 was recorded (Table II). The diakinesis-

**Table II**

| Serial Nos. | Days post-injection (p.i.) | Percentage of labelled diakinesis of biopsied cell | Total number of labelled cell
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.96</td>
<td>8.9</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>21.02</td>
<td>22.6</td>
<td>378</td>
</tr>
<tr>
<td>3</td>
<td>21.45</td>
<td>77.2</td>
<td>363</td>
</tr>
<tr>
<td>4</td>
<td>21.54</td>
<td>84.7</td>
<td>273</td>
</tr>
<tr>
<td>5</td>
<td>21.54</td>
<td>86.2</td>
<td>295</td>
</tr>
<tr>
<td>6</td>
<td>21.70</td>
<td>81.9</td>
<td>225</td>
</tr>
<tr>
<td>7</td>
<td>21.86</td>
<td>61.3</td>
<td>312</td>
</tr>
<tr>
<td>8</td>
<td>22.02</td>
<td>46.4</td>
<td>445</td>
</tr>
<tr>
<td>9</td>
<td>22.20</td>
<td>9.6</td>
<td>535</td>
</tr>
</tbody>
</table>

being an easily identifiable stage having short duration, as compared to leptotene, syngamy and pachytene, was chosen. The percentage of labelled diakinesis was plotted against days post-injection (Figure 1). This curve is similar to the curves as in case of the spermatocytes of the golden hamster and mouse. In dog 20.96 days is the
maximum duration of leptotene, zygotene, pachytene and diplotene (plus any post DNA synthesis gap, if any). The percentage of labelled diakinesis sharply increased to its maximum frequency at 21.54 d and dropped to 9.6 at 22.20 d. The ascending limb represents the rate at which spermatocytes labelled at late S period leave the pre-

![Graph showing labelled diakinesis percentage over days post-injection.](image)

**Figure 1**

diakinetic stages and enter diakinesis. The shape of transition from peak to trough can be attributed to the rate at which spermatocytes enter the pre-meiotic S phase. The S duration of dog, as in other mammals, apparently varies in individual spermatocytes as the ascending limb is not a mirror-image of the descending one; however, this asymmetry of the curve may result from variability of the duration of the prediakinetic stages, too. This variability explains: (i) lack of a well defined peak, (ii) the
absence of 100% labelled diakinesis in any biopsy and (iii) the fact that this percentage never dropped to zero. It is interesting to note that the S duration of mitotic cells is rather short as compared to that of meiotic cells of mammals. This duration is 10.5—14 hrs in mouse oocytes. The mean duration of pre-meiotic S period in Canine spermatocytes is 20.4 hrs as compared to 29 and 40 hrs in the spermatocytes of the mouse and golden hamster respectively.

Summary

The kinetics of the progression of dog spermatocytes labelled with ³H-thymidine in vivo was studied. The data suggest that the mean duration of pre-meiotic S period of Canine spermatocytes is 20.4 hrs.

Acknowledgements

We thank Professor P.C. Fraser, Professor Barid B. Mukherjee and Dr. P. H. LaMarche for encouragement. Both RIH-243 and CSIR 3E(164)/74-GAN.II grants to SKG are gratefully acknowledged here.

References

Reprint from the
Proceedings of the symposium on
Genetics Applied to Human Needs
Bhabha Atomic Research Centre
Bombay
January 10-11, 1977
CYTOGENETIC STUDY OF A HUMAN DICENTRIC Y CHROMOSOME

and T. Bandyopadhyay

Zoology Department, University of Burdwan,
Burdwan, West Bengal 713 101

INTRODUCTION

Various techniques, e.g., autoradiography\(^{(1)}\), giemsa
banding\(^{(2)}\), fluorescence microscopy, etc.\(^{(3)}\) have facili-
tated the identification of those normal or structurally
abnormal chromosomes as are not readily detected by simple
karyotyping. A 6-year old baby, due to the apparent pres-
ence of an enlarged clitoris, was initially diagnosed as a
female \((2n = 46)\) having, besides a normal \(X\), an \(X\) with pa-
rtial long arm deletion. Autoradiography, due to late DNA
replication of this "C-group" chromosome with typical sum-
ble shaped morphology, suggested it as a morphologically
abnormal \(X\) (viz., iso-\(X\)\(^{(4)}\), ring-\(X\)\(^{(5)}\)) which almost invari-
ablly is late-replicating. A further investigation was
done because of this individual's total absence of sex chro-
matin in the buccal smear.

MATERIAL AND METHODS

Metaphase slides, prepared from leucocyte cultures by
standard technique\(^{(1)}\), were stained with either (a) acetic
orcein for morphological analysis or (b) "stained" with
0.5% quinacrine dihydrochloride for fluorescence work.
OBSERVATIONS AND CONCLUSIONS

Of 347 acetic orcein stained complements, this metacentric chromosome had a heteropycnotic appearance at the distal (to the centromere) halves of both arms. These light microscopic darker areas indeed exhibited high fluorescence, as is a property of human Y chromosome (6), in each of 246 well selected metaphases. This suggested a clear-cut translocation involving 2 Y chromosomes forming an apparently metacentric one. In 9 (3.6% of all) fluorescence microscopically examined and in 17 (4.8%) of the acetic orcein stained metaphases, there had been two such chromosomes in each complement (FIGURE I). The possibility of

FIGURE I

Origin and behaviour of Y-Y translocation at metaphase

[Diagram showing normal disjunction and breakage or nondisjunction for monocentric and dicentric Y chromosomes]
Y-Y translocation with centric fusion was ruled out as the centromere is much extended in this chromosome. The dicentric nature of this chromosome was further conjectured from the occasional (4.210.6% of all metaphases scanned) presence of two such “metacentric chromosomes” in each of these metaphases resulting from nondisjunction, which a dicentric chromosome is more prone to.

SUMMARY

A metacentric chromosome, originally suspected as a deleted X chromosome due to late DNA replication, required further cytogenetic investigation as no sex chromatin was later found in the buccal smear of the patient having an apparently enlarged clitoris. High-fluorescence as well as heteropycnosis of both the terminal segments of this metacentric chromosome, in addition to its having a very distended centromere, suggested it as a dicentric chromosome.

ACKNOWLEDGEMENTS

Our sincere thanks are due to Professor D.K. Choudhuri, Head of Zoology Department of this University, Professors P.C. Fraser and B.B. Mukherjee (McGill University) and to CSIR and UGC for grants (38(164)/74-GAU-II and T.23-S48/75/SR-II respectively) to SKG. TM is a CSIR JRF (No. 7/25(55)/76-BMR-I) at this Department.
REFERENCES


DISCUSSION

M.F. Rajasekharachetty: Dicentric chromosome you have reported could be due to an inversion (paracentric) in the two chromosomes and chiasma forming in the inversion. Meiotic chromosomes and their behaviour must be examined before one could come to a conclusion.

T. Midya: He would study the meiosis and thanked for the suggestion.

Comments (S.K. Ghosal): Attempts will be made to see the meiotic (diplotene and diakinesis) chromosome pairing. We do not know the testicular situation as exploratory laparotomy has not been conducted thus far. However, an increase of mitotic nondisjunction (in 4.2 ± 0.6% of leucocyte metaphases) may indicate a dicentric nature which presumably interfered with orientation of this Y-Y chromosome in mitotic spindle. This translocation apparently took place in any post-syngamy division after the non-disjunction from a XY syngamy.
Reprint from the
Proceedings of the symposium on
Genetics Applied to Human Needs
Bhabha Atomic Research Centre
Bombay
January 10-11, 1977
SEX CHROMOSOME PAIRING IN AN XXY HUMAN MALE

S.K. Ghosal, T. Bandyopadhyay, L. Ray, T. Midya, S. De and S. Joardar

Zoology Department, Burdwan University, Burdwan, and Genetics Lab., Rhode Island Hospital, Providence

INTRODUCTION

According to the classical concept, irrespective of plicidy, trisomy or tetrasomy of a meiotic cell, two chromosomes (and never more than two) are always paired in a particular segment whatever may be the number of homologues (1). In mammalian spermatocytes X and Y chromosomes form heteropycnotic sex vesicle at pachytene and exhibit an end-to-end configuration at diplotene, diakinesis and metaphase-I (2). The present work was designed to study the sex chromosome pairing in an 18-year psychopathic male whose XXY condition was earlier ascertained by H3-thymidine autoradiography and fluorescence microscopy of leucocyte metaphases and testicular biopsies (3).

MATERIAL AND METHODS

Testis biopsies of the XXY male were incubated in 0.7% sodium citrate (37°C, 10 min) and slides were prepared by standard technique (4). Acetic orcein stained slides were photographed and quinacrine dihydrochloride "stained" ones examined in a Zeiss fluorescence microscope ("BG-12").
OBSERVATIONS AND CONCLUSIONS

Sex vesicle size $^{(6)}$ was measured. It occupied 4.93 ± 0.72% of total area of each of 571 pachytenes scored from 3 karyotypically normal XY males. The pachytenes of this XXY male were of two categories according to the number and size of heteropycnotic areas (TABLE I).

<table>
<thead>
<tr>
<th>Individuals</th>
<th>Category I</th>
<th>Category II</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Total n. of pachytenes analysed)</td>
<td>Large Sex Vesicle</td>
<td>Normal SV</td>
</tr>
<tr>
<td>XY (571)</td>
<td>$^{5.74±0.71}$</td>
<td>4.93±0.44</td>
</tr>
<tr>
<td>XXY (209)$^f$</td>
<td>146</td>
<td>$^{224±347}$</td>
</tr>
<tr>
<td>(840)$^a$</td>
<td>621</td>
<td>219</td>
</tr>
</tbody>
</table>

* Size of heteropycnotic area expressed as percentage of total area of pachytene. Superscript f and a refer to the quinacrine dihydrochloride and acetic orcein stained slides respectively.

Of all pachytenes of the XXY individual, each of 74% figures had a single precociously condensed large sex vesicle (Sl) occupying $^{5.74±0.71}$% of total pachytene area and were grouped in category I. Each of the pachytenes in category II had two (deeply stained in acetic orcein and brightly fluorescent in quinacrine dihydrochloride slides) areas differing in size. The larger area, which did mimic the normal (Sn) sized sex vesicle, may represent the XY bivalent whereas the smaller area (Ss) was presumably due the Y univalent (FIGURE I). These findings may suggest that both the Y chromosomes, despite being homologous $^{(6)}$,
do not exhibit meiotic pairing (FIGURE II). In case of the golden hamster and Chinese hamster, diplotene, diakinesis and metaphase-I figures occasionally demonstrate that both the chromatids of the X and Y chromosomes show very typical pairing, as in case of the homologous autosomes, along certain length (7) (FIGURE I). An occasional lack of YY pairing in the XY human male be regarded as some property of the synaptic behaviour of the human Y chromosome.

**FIGURE I**

Diagrammatic representation of meiotic pairing in:

1) triploid/trisomy and tetraploid/tetrasomy, 11) hamster, and iii) XY and XYY spermatocytes.
SUMMARY

Human pachytene spermatocytes from an XXY male were grouped into two categories. Category I had larger-than-normal sex vesicles presumably representing XXY trivalent, while category II had two heteropycnotic sex: one of normal size and other of smaller size; probably representing XY bivalent and Y univalent respectively. An occasional lack of Y-Y pairing in 20% of all XXY pachytene scored, may be a unique monopoly of human Y chromosome.

ACKNOWLEDGMENTS

We are thankful to Professors D.K. Choudhuri, M.B. Mukherjee, P.C. Fraser and P.R. Jha and gratefully do acknowledge CSIR 33(154)/74-44U.II and UGC F.23-348/75/SR-II grants to SKG.

REFERENCES

3. S.X. Chosal and B.S. Mukherjee; Nucleus (in press).
7. T. Utakoji; Chromosoma 18, 449 (1968).
Initiation of sex chromosomal DNA replication in a XX/X ring-X/XO human mosaic.

S. De, S. Chattopadhyay, T.K. Bandyopadhyay and S.K. Ghosal

Department of Zoology, Burdwan University

The particular X chromosome, which is genetically inactive in mammalian females, forms sex chromatin in somatic interphase nucleus and undergoes late DNA replication. Structurally abnormal X is almost always late replicating. Present experiment having a marker ring-X chromosome was designed to investigate if this late replicating-X late in initiating its replication. Leucocytes labelled at early S with $^3$H-thymidine suggests that this chromosome despite being late replicating, starts its replicating along with other chromosomes of comparable length in group C and is not the last one in the complement to start its replication.
Cytogenetic analysis of drumsticks in mammals including man.

N.C. Chakraborthy, S.K. Ghosal, S. De, T.K. Bandyopadhyay and S. Joardar

Department of Zoology, Burdwan University.

According to Lyon's hypothesis one of the 2X chromosomes in normal mammalian female is genetically inactive. H\(^3\)-thymidine radioautography indicates that the inactive X is late in DNA synthesis, and forms both sex chromatin (Barr body) in somatic interphase nuclei, and a drumstick in the neutrophils. The present investigation was designed to study the drumsticks in human beings having multiple X chromosomes, e.g., Klinefelters, Turners, rare XX males etc., suggest that whereas in patients having more than 2Xs number of Barr body equals n-1 (n = no. of X chromosome), the drumstick differs both in size and number. The frequency of Loris and mongoose was also studied. The diagnostic value of drumstick and management of patients will be discussed.
Sexual dimorphism in the leucocyte of *Herpestes auropunctatus* (Indian Mongoose)

S. Chattopadhyay, T. Bandyopadhyay, S. De and S.K. Ghosal

Department of Zoology, Burdwan University.

Sexual dimorphism in leucocytes of *Herpestes* was studied. About 5.7% neutrophils, 10.5% eosinophils and 6.4% basophils contain drumsticks presumably formed by single X chromosome which is late DNA replicating.

The diameter of drumstick ranges between 5 and 1.2 μ modal diameter being 1 μ.
CYCLE OF THE SEMINIFEROUS EPITHELIUM IN THE GOAT

S. Joardar, S. De, T. K. Bandyopadhyay and S. K. Ghosal

Zoology Department, Burdwan University, Burdwan, W.B. 713 101.

Histological studies on PAS stained goat testis sections initially suggested i) 12 stages of spermiogenesis and ii) 8 distinct cellular associations (stages) of the "cycle of the seminiferous epithelium". Germ cell differentiation studied thereafter indicated that a) some cells divide at the end of stage VIII producing intermediate spermatogonia while the rest remain dormant until the next cycle, b) stage II terminated with the production of type B spermatogonia from intermediate ones, and c) primary spermatocytes, detected first in stage V, divide late in stage VII of following cycle forming secondary spermatocytes, which rapidly divide into young spermatids marking the end of stage VIII. About 4.5 cycles are required for the completion of the goat spermatogenesis.
Quantitative analysis of seminiferous epithelium of immature dogs.

T. Bandyopadhyay, D. Ray, S. De, S. Joardar and S.K. Ghosal

Department of Zoology, University of Burdwan.

Seminiferous epithelium of the testis of four dogs, each one and a half month old, was studied histologically. Seminiferous epithelium contains spermatogonia of A, intermediate and B types, besides sertoli cells and undifferentiated large ('resting') spermatocytes. However, any meiotic stage of the spermatocytes, spermatids and spermatocytes are totally lacking in the sections of these testes. Quantitative results expressed as a mean number of cells per tubular cross section reveal germ cell ratios as - type A : intermediate = 1 : 1.5 ; type A : type B = 1.0 : 2.0 ; type A : spermatocyte = 1.0 : 3.0 ; intermediate : type B = 1.0 : 1.7 and type B : spermatocyte = 1.0 : 1.3.
The cycle of the seminiferous epithelium in the mole, *Suncus murinus*.


Department of Zoology, Burdwan University.

Examination of aqueous Bouin's and Zenker-formol fixed mole's testis sections (4 μ) stained with PAS-haematoxylin reveals 8 steps of spermatid development. There are seven distinct cellular associations. Type A spermatogonia ending in step VII produce intermediate ones which transform to type B (stage IV), latter finally producing primary (stage V) and secondary (stage VII) spermatocytes. Spermiogenesis occupies about one and a half cycles. The criteria for dividing the cycle of the seminiferous epithelium into stages are (i) nuclear morphology and (ii) changes in the acrosome system of spermatids.
Proc. of the Symposium : Genetics applied to human needs,

Cytogenetic study of a human dicentric Y chromosome.

T. Midya, S. Joardar, S. De, D. Ray, S.K. Ghosal and
T. Bandyopadhyay.

Department of Zoology, Burdwan University.

Leucocyte culture of a 6 years old human male showed 46 chromosomes in each metaphase with 4 G group (apparently no Y) and 16 G (6-X-12) group chromosomes. One atypical G group meta-centric chromosome (comparable in length to chromosome number 10) appeared as a (partially long arm) deleted X due to late DNA replication in cells labelled at later part of S period with H3-thymidine. QM fluorescence technique employed thereafter, due to the male phenotype, exhibited typical Y like fluorescence of each arm of this chromosome suggesting it as a case of Y-Y translocation. Its dicentric nature is proposed due to (a) the extended nature of the primary constriction and (b) an occasional (4.2 ± 0.6 of all metaphases) presence of two of this atypical chromosome in the same cell - a fact readily described by the phenomenon of nondisjunction resulting from the simultaneous occurrence of two centromeres in the same chromosome involving Y-Y translocation.
Sex chromosome pairing in an XXY human male.


Department of Zoology, Burdwan University.

Air-dried meiotic chromosome slides were made from testis biopsy of an 18 years old psychopathic male whose 47 XXY condition was ascertained by H\textsuperscript{3}-thymidine autoradiography and QM fluorescence microscopy of leucocyte metaphases. A comparative study was done on the size of pachytene sex vesicle of this individual and of a normal male. Any size smaller and larger than normal vesicle (Sn) was scored as Ss and S1 respectively. The XXY pachytene were of 2 categories: 74% pachytene (category 1) had a single large (S1) precociously condensed sex vesicle presumably representing an XYY trivalent and remaining 26% (Category 2) had both an Ss and an Sn types heteropycnotic areas presumably representing a Y univalent and an XY bivalent respectively. An occasional lack of meiotic pairing, as in category spermatocytes. Between both Y chromosomes despite their genetic homology may be a unique phenomenon.

Quantification of testicular cell types of Vespertilio sp. - a microchiropteran.


Department of Zoology, Burdwan University.

Quantification of male germ cells of immature and mature bats was done from histological slides stained with PAS-haematoxylin. One month old bat's seminiferous epithelium contains three spermatogonial types (A, Intermediate and B) and exclusively 'resting' spermatocytes, the respective percentage of these cells being 15.7, 14.6, 18.9 and 50.8. In mature bats the frequency of the cell types or stages of meiosis and spermiogenesis is A 8.41%, Intermediate 2.12%, B 2.78%, zygotene-pachytene spermatocytes jointly 28.91%, early spermatids 40.01% and late spermatids 16.79%. The relative value of these cells types in addition to the meiotic and spermiogenetic stages is invariably constant in this species and could be species-specific as in several other mammals.
Transportation of $^{3}$-Lysine from host tissue to the invasive larvae of Trichinella spiralis.


Zoology Department, Burdwan University & Biochemistry Department, Burdwan University Medical College, Burdwan.

$^{3}$-Lysine was injected into the diaphragm of each of 5 albino rats of 30 days post infection with T. spiralis. The kinetics of migration of labelled Isotope at 6, 22, 30.75, 53.50 & 97.50 hours post-labelled was recorded autoradiographically. The grains were localised at 6 hrs. and later specimens at host diaphragm, capsule and the body of the parasites. This indicates a) that lysine is transported from the host muscle to the parasitic tissue and b) that the capsule and the cuticle of the nematode, permit this transportation for the active protein synthesis in the trichinellid.
H\textsuperscript{3}-uridine autoradiography of normal and atretic human oocytes

A. SENGUPTA, S. BANDYOPADHYAY, T. BANDYOPADHYAY, P.K. MALICK,
S. BASU, R. RAY and S.K. GHOSAL, Burdwan

Ovaries from eight-week old baby and thirty-four-year old human female were incubated at 37\degree C for 30 minutes in TC-199 medium supplemented with H\textsuperscript{3}-uridine. The diplotene (diplotene) oocytes despite prolonged meiotic interruption are active in RNA synthesis. It is interesting to note that the atretic oocytes too incorporate tritiated uridine. Labelling is distinct both chromosomally and at nucleolus.
Stages of the cycle of the seminiferous epithelium in the ram


On the basis of the change in the PAS-positive acrosome system and nuclear morphology of developing spermatids of ram Ovis aries fourteen stages of the cycle of the seminiferous epithelium have been identified. Of eighteen steps of spermatids, the initial fourteen stage characterise the sequence of fourteen associations (I-XIV). Type B spermatogonia, the forerunners of spermatocytes, are confined to only four (IV-VII) stages. About 4.75 cycles are required for the completion of spermatogenesis.