

C Y T O L O G I C A L
A N D
C Y T O G E N E T I C A L S T U D I E S
O N T H E T E S T I S
O F T H E G E R B I L ,
T A T E R A B R A N T S I I D R A C O .

D E C L A R A T I O N

I hereby certify that this thesis
entitled "Cytological and Cytogenetical Studies
on the Testis of the Gerbil, Tatera brantsii draco"
is my own work, and that the work forming the
basis of the thesis has not been incorporated in
a thesis submitted for another degree by myself.

Signed A.V. Tolias

Dated 7th November 1952

"Search while thou wilt, and let thy Reason go,
To ransome Truth, even to the Abyss below;
Rally the scattered Causes; and that line,
Which Nature twists, be able to untwine."

(From "Religio Medici",

Sir Thomas Browne)

CYTOLOGICAL AND CYTOGENETICAL STUDIES ON THE TESTIS
OF THE GERBIL, TATERA BRANTSII DRACO.

by

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A C K N O W L E D G M E N T S

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P R E F A C E

THE SETTING OF THE THESIS.

Biology, having outgrown its purely descriptive phase, has, for nearly a century, been in an era of causal analysis. It has been a period of extreme compartmentalisation of the general field into many disciplines, each endowed with a defined range of problems, with peculiar materials of study and with special approaches and techniques.

From the nature of things, it is inevitable that each biologist should have been a specialist. The field of living things ramified so vastly that the species of scientist known as the biologist or naturalist became largely extinct: instead, there were geneticists, systematists, physiologists, embryologists, biochemists, cytologists and others. Specialisation did not stop even at that point for the systematists split into mammalogists, ornithologists, helminthologists, etc.; the geneticists into experimental geneticists, cytogeneticists, phenogeneticists, and so on.

Good and bad consequences flowed from this tendency. The advantage of specialisation was a great increase in the store of factual information; the disadvantage lay in the isolation between representatives of the various disciplines and in the absence of cross-pollination in the development and evaluation of concepts.

The lack of contact undoubtedly impeded the onward march of biology. At the same time, however, this brake probably had a beneficial effect for it prevented premature generalising on insufficient data and made possible the emergence of limited syntheses between defined parts of the total field.

To-day, one sees a third new trend arising in biology. For little more than a decade now, far-seeing biologists have felt that the time is ripe for the development of a grander, more synthetic outlook on the study of organisms, for the convergence of the mass of accumulated facts and the numerous concepts towards a set of general principles applicable to the entire realm of living matter. As Dobzhansky (1942) has put it, "Biology, it seems, is no longer in its childhood; as a science, it is approaching maturity."

UNITARIAN BIOLOGY:

The unifying trend appears in the early thirties and is apparent in the works of Fisher (1930), Haldane (1932), Rensch (1933) and Schindewolf (1936). Dobzhansky (1937) signalises, in his book, "Genetics and the Origin of Species", what Dunn calls, in the Preface, the Back-to-Nature movement. Dobzhansky's work heralded this movement in two senses: for not only did it betoken what Dunn intended, namely the return of geneticists from "an apparently narrow alley, hedged in by culture bottles of *Drosophila* and other insects" to that "ultimate laboratory of biology, free nature itself"; but also, in this return to the organic world, the unity of Nature

engendered anew the holistic approach and the specialist became once again the biologist.

With Waddington's "An Introduction to Modern Genetics" (1939), we see the new trend exemplified - for here was an experimental embryologist writing a text-book on Genetics! It was, however, a deliberate choice by the author for, as he tells us in his Preface, "the need has arisen primarily because the different kinds of biological cobblers have in the past stuck too closely to their lasts." He adds:

"I want to urge that the connection between genetics and the other branches of biology, such as cytology, embryology, the study of evolution and of the biochemical nature of cell-constituents, is much closer than is often admitted, and that the boundaries between these subjects deserve less attention than is usually paid to them."

(Preface, p.8)

Thus, the new striving received conscious formulation with Waddington.

A year later, two more books of importance to our present theme appeared. In his comprehensive analysis of "The Material Basis of Evolution", we find Goldschmidt (1940) drawing on data from genetics, embryology, palaeontology, biogeography, taxonomy and cytology. We see his broad synthetic view laying the foundation-stone for studies on Evolution as a discipline in its own right - not a specialised discipline but an avenue leading to the co-ordination of biology, for, from its very nature, Evolution cuts across every other field of biological thought.

The other significant work was a Symposium on "The New Systematics" - a phrase coined by the editor, Julian Huxley, to describe the new synthetic outlook and technique as applied to systematics. Taxonomists had reached a stage where it was necessary for them to pause and take stock, to re-assess the status and delineate the trends of systematics. Huxley's Foreword tells us that "the new systematics is not yet in being: before it is born, the mass of new facts and ideas which the last two or three decades have hurled at us must be digested, correlated, and synthesized." In this work, scientists labouring in many different fields bring the facts and the concepts of their disciplines to bear on a central theme, namely, the species and other taxonomic categories. One need only glance at the titles of some of the contributions to realise the revolution that has come about in biological thought - "Mutations and Geographical Variation" (Timofeeff-Ressovsky), "Taxonomic Species and Genetic Systems" (Darlington), "Ecology and the Future of Systematics" (Thorpe), "Embryology and Taxonomy" (de Beer).

In 1942, Mayr further developed the new ideas on taxonomy in his work on "Systematics and the Origin of Species". Clearly realising the need for a synthetic approach, Mayr states:

"We shall not be able to obtain a real insight into the different speciation processes and their distribution and relative importance in the various groups of plants and animals until the ecological factors (dispersal, various specializations) and the ethological factors (courtship patterns, mate preferences and aversions, and the like) have been correlated with the taxonomic facts (degree and speed of subspeciation, and so forth) and with the cytological and genetic findings (sterility, and so forth). The time has come for the naturalist to organize these data and to

present us with a better-balanced picture of species formation than can be given by the specialist in only one of the experimental or laboratory sciences."

(p.274)

If Goldschmidt's work in 1940 had given a blueprint of the new co-ordinating field of Evolution, Huxley's "Evolution: the Modern Synthesis" may be regarded as having established Evolution as a discipline in its own right. To quote Huxley:

"Genetics, developmental physiology, ecology, systematics, palaeontology, cytology, mathematical analysis, have all provided new facts or new tools of research: the need to-day is for concerted attack and synthesis. If this book contributes to such a synthetic point of view, I shall be well content."

(p.8)

Through all the cited works runs the modern unifying trend. Individual compilations differ in detail and in points of emphasis: some workers focus their unitarian biology on the gene; others concentrate the natural world about the species; still others see in evolution the great rallying point which is to re-integrate the biological sciences. It matters but little on what focal point the biologist makes nature converge. What is important is that the old isolation and compartmentalisation is breaking down. Clearly, biology still needs specialists, but it also needs the biological outlook to integrate the specialities, to cross-fertilise each field with concepts and facts from others.

To the modern biologist, the unitarian trend indicates the need for considering facts from many points of view. No matter how limited the study, its results can be interpreted in

devious ways, so as to illuminate more than one segment of the biological field. Since the present researches were undertaken against this background, we find here that a restricted set of cytogenetical facts are treated from a morphological and cytochemical, a genetical and an evolutionary viewpoint. These interpretations of biological fact correspond with Huxley's mechanistic-physiological, adaptive-functional and historical aspects (1942, p.40). The mechanistic-physiological aspect describes the components of the system e.g. the chromosomes, and their behaviour. The adaptive-functional role concerns the biological or genetical effect of these components on the organism as a whole. The historical aspect enquires into the development by which the present organisation of the chromosomes has come about.

None of these three broad aspects can be understood fully without reference to the others. We certainly cannot attempt to apply cytogenetical findings to evolution until we have a clear idea of the reactivity of chromosomes within a single species and even a single organism. The study of the chromosomes and of their behaviour, with which most of the present thesis is concerned, is an essential pre-requisite to the evolutionary studies.

Throughout the present work, therefore, I have tried to view my observations in this threefold light. In the new approach to biology, one single fact needs to be interpreted in many contexts for its full implications to be appreciated. In this

way, any piece of biological research, no matter how circumscribed, is influenced by, even depends upon the currently dominant biological preconceptions. Whether consciously or unwittingly, the research worker colours his interpretations with the atmosphere of his time. It is the atmosphere of unitarian biology which provides the setting for this thesis.

PART 1.

THE INTRODUCTORY SECTION.

C H A P T E R I.

I N T R O D U C T I O N.

THE AIMS OF THE THESIS:

The aim of this thesis is to present an integrated account of the morphology and behaviour of the chromosomes and the cells in the seminiferous tubules of the gerbil's testis. The relative constancies of the chromosomes at specific stages, revealed by the morphological study, will be considered first as the end-result of an evolutionary line of descent. By a comparative study of the karyotypic constancies of the gerbil and of other mammals, especially the Rodentia, generalisations will be sought on the patterns of chromosomal change and the developmental trends which have apparently led to the present-day scheme of chromosomal constants in extant mammals. In this 'cyto-phylogenetic' and 'cyto-taxonomic' part of the study, special attention is paid to the sex-chromosomes, the peculiar internal differentiation of which makes these chromosomes valuable indicators of directions in mammalian chromosomal evolution.

The modifications of mitotic chromosomal morphology make necessary an examination of the types, the determinants and the mechanisms of variation of the chromosomes. Both the first and the second aims are commonly included in cytogenetical studies with, perhaps, rather more emphasis on the constancies than on the variations of the karyotype.

Some of the cytoplasmic changes during mitosis, meiosis and spermiogenesis require close analysis and the observations are correlated with the sequence of nuclear events. By this means, two objectives are sought: firstly, to place the karyotypic constancies and modifications into a total cellular context and, secondly, to pave the way for a further part of the study, namely the patterns of differentiation of the cells in the tubule.

An explanation will be sought for some of the nuclear phenomena, the existence of which is uncovered in the earlier parts of the study. These phenomena include the relation between the chromosomes and the nuclear membrane, the reactivity of the sex-chromosomes and of heterochromatin. The features of plasmosomal physiology and of heterochromatic behaviour are explained in terms of the nucleic acid cycle and biochemico-morphological problems of the chromosomes are discussed. The biochemical differences between cells at different stages in the spermatogenic cycle are shown to offer a fresh yardstick, supplementing the morphological indicators, of cell differentiation in the adult testis and to provide evidence for the influence of general environmental factors on cell chemistry and morphology.

The nature of mitotic figures with excessively contracted chromosomes, previously reported in some mammals, is investigated in the light of observations on the gerbil. Two aspects of these special mitoses are considered: firstly, by what intra-cellular mechanism may the end-result of excessive chromosomal contraction be produced? Secondly, why do some testicular cells, as if out

of the blue, manifest this differential behaviour? To answer the second query, we must needs enquire into the position of the cells concerned in the moving spatial and chronological sequence of spermatogenesis.

The need to explain the appearance and varied behaviour of cytoplasmic components at specific stages in maturation, brings us to the same conclusion: the components must be placed in their correct positions in the procession of spermatogenesis, if we are to understand their significance.

Thus, inexorably, the emphasis of the study becomes altered from nuclear and cytoplasmic events to the pattern of cellular events within a larger milieu, that of the seminiferous tubule. This leads us to characterise and classify the cell-generations and cell-stages, whose compresence in various tubule-combinations marks the successive stages of the spermatogenetic wave-cycle. Many new problems arise in this part of the study, revolving around the causal mechanisms at play in normal spermatogenesis. If we can understand the factors influencing spermatogenesis, perhaps we shall have a key to the vexed and oft-discussed question of the interrelations between genotype and environment. Hence, in this section, we enquire into the nature of the spermatogenetic stimuli. We ask whether the events of spermatogenesis are to be accounted for by a self-sufficient development of the germ-cells, requiring only a trigger-stimulus to set off a chain of events; or whether the chain of reactions, once established, requires further external intervention to be carried through

to its consummation - the formation of the mature spermatozoon.

Lastly, then - and, perhaps, underlying many parts of the thesis as a whole - is an attempt to answer the major question, namely, what relation exists between the environment and the morphology and function of the chromosomes? Is there a direct influence at the chromosomal level? Or do the chromosomes, by manifesting a special differentiative stage of the cell, indirectly reflect the environmental impact on cell-differentiation? In other words, does the environment influence the chromosomes by affecting the bodily conditions controlling spermatogenesis, which process in turn imprints its cyclical fluctuations on the seminiferous cells?

Previous investigators have paid little attention to the moving background of the chromosomes in cytogenetical studies on mammals; while few studies on mammalian spermatogenesis have descended to the minutiae of the chromosomal level. Consequently, the two fields have tended to develop independently of each other. Once it is realised, however, that no study of chromosomal behaviour in the testis is complete without a concomitant study of spermatogenesis, unresolved problems of chromosomal morphology and behaviour (e.g. the meaning of the mitotic figures with excessively contracted chromosomes) rapidly approach a solution; while, per contra, the chromosomes provide a subtle gauge of the progress of spermatogenesis.

A combined study of the chromosomes and their context

is presented here in the hope that it may illuminate the dynamics of both the chromosomes and spermatogenesis.

In essence, this study uses the techniques of the cytologist to elucidate a sequence of events. At the outset, then, it would not be redundant to discuss briefly the cytologist's approach and methodology.

METHODOLOGICAL CONSIDERATIONS.

The fixed and stained preparation, which provides the starting-point for cytological research, presents a series of appearances, each isolated at a point in time. It provides a two-dimensional panorama (or, if thick sections, or serial sections, are used, a three-dimensional topography) of cellular morphology, from which the cytologist reconstructs a four-dimensional story. It is as though one attempts to create a continuity between a number of photographs by projecting them on to a screen in rapid succession, following the principle of motion pictures. However, the analogy is far simpler than are the prevailing conditions, because, while the moving picture may be assumed to produce little distortion which is not innate in the film, man perforce introduces a considerable subjective element in his dynamic interpretation of static appearances. The very labelling of a particular cell-morphology involves an act of judgment and, it is conceivable that almost as many hypotheses may be suggested to link a series of isolates as there are of cell-appearances.

The cytologist therefore takes all possible steps to

minimise the subjectiveness of his conclusions. One of these steps is the careful definition of the descriptive terms used. This involves the written or explicit formulation of a set of accepted criteria which are tacitly assumed by the investigator in his diagnosis of cell-types. Definitions are of their essence arbitrary. They are agreed upon among research workers to serve their own convenience. When a series of cell-appearances is involved, e.g. in the various stages of spermatogenesis, the definition of each would be of little value were it not fitted into the established sequence of events. For a cell-type may be defined solely on the basis of its morphology, or the definition may be formulated in terms of the cell's position in the temporal sequence, provided that the sequence has been convincingly established by experimental methods or in living preparations. Definitions of the second type are used in this study because it is accepted that the events of spermatogenesis follow a now well-established course.

To anticipate the charge of petitio principii, it may be stated that all studies on the cellular events of the reproductive cycle do involve certain assumptions. For instance, it is always accepted that pairing of the chromosomes occurs in the first meiotic prophase; more specifically, that it begins in the zygotene and ends at the beginning of the pachytene stages. If this assumption is made, then it is valid to define a cell-appearance in terms of whether or not its chromosomes have completed pairing. In fact, most modern cytogenetical studies

on the events of spermatogenesis assume a certain, highly-confirmed sequence of happenings and analyse the specific variations on this theme exhibited by the object of study. In the present investigation on the gerbil, I have used a similar approach while, at the same time, confirming as much of the accepted sequence of events as can be observed directly, or indirectly deduced with some validity.

These two factors, the use of explicit definitions and the assumption of an established series of happenings, minimise the degree of subjective judgment. Nevertheless, the basic limitation in this analysis inheres in the necessity for an extrapolation of static appearances into the fourth dimension, time. This limitation will apply wherever the facilities or technical requirements are lacking for a study of the changes in living cells. On the other hand, when living cells are studied, spatial considerations provide the limiting factor. For when one is examining the parts of a living tissue, only a few cells can be followed at any one time throughout their temporal extension. While this may not be a serious drawback, it certainly militates against the accurate observation of processes involving spatial and temporal extensions simultaneously e.g. the passage of a spermatogenetic wave along a seminiferous tubular system.

There exists here a profound limiting factor in current histological and cytological technique. In essence, this limitation is an inability to appreciate living cells throughout

their extension in space and time, except by the interposition of not inconsiderable subjective judgments. The cytologist therefore is constrained to make these judgments as logical as possible and, to formulate each step in his chain of reasoning with clarity and precision.

THE PRESENTATION OF THE THESIS.

The thesis is divided into six parts, dealing with the following topics:-

- Part 1 - Introductory Section.
- Part 2 - The Chromosomes.
- Part 3 - Descriptive Account of Spermatogenesis
- Part 4 - Chromosomal Behaviour during Spermatogenesis.
- Part 5 - Cytoplasmic Behaviour during Spermatogenesis.
- Part 6 - The Spermatogenetic Wave.

Part 1. Following the present introduction, an account is given of the origin and nature of the animals used and of the techniques employed, with their rationale.

Part 2. A preliminary study on the chromosomes of the albino rat is reported in Chapter III: this had two aims - the confirmation and extension of previous studies on this animal and the testing of a technique for characterising the chromosomes of an animal, such as the rat, with many small chromosomes. In the discussion, the general problems of chromosomal characterisation are reviewed, as exemplified by my own experience on the albino

The main investigation begins with the characterisation of the chromosomal complement of the gerbil (Ch. IV). Thereafter, the significance of the chromosomal number is discussed in the light of other rodent and mammalian chromosomal numbers (Ch. V).

Part 3. In Chapter VI, the chromosomes are placed in their moving context by an account of mitosis, meiosis and some aspects of spermiogenesis. The main emphasis is laid upon chromosomal and nuclear events, but the cytoplasmic constituents which were clearly demonstrated in the preparations used, viz. archoplasm, centrioles, spindle and chromatoid bodies, are traced through the stages of division and maturation and their behaviour is correlated with the chromosomal events.

Part 4. Following these studies, specific cytogenetical problems are taken up in greater detail: the relationship between the chromosomes and the nuclear membrane (Ch. VII A), the pre-meiotic mitoses (Ch. VII B), the behaviour of the plasmosomes (Ch. VIII) and the light they shed on chromosomal structure and nucleic acid metabolism. From this flows a consideration of those parts of the chromosomes most closely related to the nucleic acid cycle viz. the sex-chromosomes in particular (Ch. IX) and heterochromatin in general (Ch. X).

Part 5. In Chapter XI, the differentiation of the cytoplasm during spermatogenesis is discussed and the emergence of the archoplasm, the chromatoid bodies and a special type of centriole-spindle apparatus is shown to manifest a differentiative change

in the cytoplasm at the 1st meiotic stage.

Part 6. The cytoplasmic specialisation brings attention, in the last part of the thesis, to cell generations in the seminiferous tubule (Ch. XII). First, the sequence of events on the basement membrane of the tubule is established; thereafter, this sequence is correlated with that of the other three generations of cells in the seminiferous tubule. A modified conception of the spermatogenetic wave emerges, permitting a better understanding of cellular and chromosomal events.

The conclusion of the thesis diverts emphasis away from the chromosomes as isolates, towards the milieu within which the nuclear changes occur.

CHAPTER II.

TECHNICAL CONSIDERATIONS.

ANIMALS USED IN THE PRESENT STUDY.

As there are, in South Africa, four different species of gerbils belonging to the genus, Tatera, it is necessary, first, to consider their taxonomic position.

On the basis of their morphological characteristics and habitat the African gerbils of the genus, Tatera, fall into two species-groups, the Afra group and the Robusta group (Davis 1949). Each group, in turn, contains a number of species and sub-species:

<u>ROBUSTA</u>	<u>AFRA</u>
1. <u>T. robusta</u> (13 sub-species)	1. <u>T. afra</u> (2 sub-species)
2. <u>T. nigricauda</u> (5 sub-species)	2. <u>T. brantsii</u> (12 sub-species)
3. <u>T. guineae</u> (2 sub-species)	3. <u>T. valida</u> (11 sub-species)
4. <u>T. schinzi</u> (21 sub-species)	4. <u>T. giffardi</u> (7 sub-species)
	5. <u>T. leucogaster</u> (5 sub-species)
	6. <u>T. ruddi</u> (monotypic)

Both groups are represented in South Africa, Robusta by one species (T. schinzi) and the Afra group by three species (T. afra, T. brantsii and T. ruddi).

Morphologically, T. afra resembles T. brantsii more closely than it does T. schinzi, while T. ruddi appears closely related to T. brantsii. In fact, Davis suggests that T. ruddi

is "probably a relict offshoot of the stock that gave rise to the 'brantsii' group, and may indeed prove to be a sub-species of T. brantsii."

Geographically, T. afra is isolated from the other species, being confined to the coastal plains of the western Cape Province. T. ruddi is known only from its type locality in Zululand. The other two species, T. brantsii and T. schinzi, overlap in their distribution: T. brantsii, according to Davis, ranges over the highveld of the eastern and north-western Cape Province, Orange Free State, the southern Transvaal, Natal and Zululand, the Kalahari from Kuruman in the south to Ovamboland (South West Africa) and southern Angola, northern Bechuanaland and Barotseland (Northern Rhodesia). This range includes the Witwatersrand area in which the animals used in the present study were trapped. T. schinzi occurs in the Kalahari and its fringes in the north-west Cape and western Orange Free State, the bushveld and the lowveld of the northern Transvaal and, west to east across the sub-continent from southern Angola, through Northern Rhodesia, to Nyasaland and Portuguese East Africa.

The animals used in this investigation were trapped by the field staff of the Plague Research Laboratory, in the vicinity of Johannesburg. This area lies within the geographical range of the draco sub-species of T. brantsii and the animals were identified by Mr. D.H.S. Davis, the Government Ecologist and Chief Rodent Officer, as members of this sub-species.

One other sub-species, T. brantsii brantsii. occurs in South Africa, but it was not possible to procure any material from this animal for purposes of comparison.

The animals were trapped at varying intervals between the months of July and October. Although all trapped males showed active spermatogenesis, a higher percentage of tubules were actively functional in those animals killed in the later months.

Tatera brantsii draco was selected for several reasons. Firstly through the courtesy of the Chief Rodent Officer, Mr. Davis, there was a ready supply of animals. Secondly, no other species of the sub-family, Gerbillinae, of the Rodents had previously been studied from a cytogenetical point of view. Thirdly, a comparison of the chromosomal complements of various species of Tatera - towards which the present study provides the first step - might make it possible to unravel certain problems concerning the evolutionary divergence and speciation of Tatera.

TECHNICAL PROCEDURES.

Eight male animals and two females were procured. The testes and ovaries were removed under ether anaesthesia and, from these, sections, squash and smear preparations were made.

Half of each testis and one ovary from each female animal were fixed in Bouin's picro-formol-acetic fixing fluid for 24 hours. This fixative was chosen because of the excellent chromatin-preserving properties of the contained picric acid,

while the acetic acid ensures rapid penetration. The tissues were dehydrated, cleared and embedded in paraffin wax. Sections were cut at a thickness of 10 μ , 12 μ and 16 μ and were mounted, in most instances serially, on albuminised glass slides. After being deparaffinised with xylol, the sections were taken through the downgrade alcohols to water. The stains used were Heidenhain's iron haematoxylin, Ehrlich's acid haematoxylin, Newton's crystal violet and Feulgen's nucleal reagent (leuco-basic fuchsin).

Both forms of haematoxylin produce intense permanent staining of the chromosomes. Heidenhain's haematoxylin, after ferric alum mordanting, is a most valuable stain for preserving not only nuclear detail but also the accessory cytoplasmic structures of the male germ cells e.g. the chromatoid body, the division spindle, the centrioles, the neck granules, manchette and tail of the spermium. Carefully controlled de-staining may render the cytoplasm completely colourless, while the chromosomes stand out vividly. In sectioned preparations, it is often useful not to de-stain the cytoplasm entirely, since the presence of faint grey-stained granules in the cytoplasm and their absence from the nucleus facilitates the study of the nuclear membrane and, especially, of the non-chromatic karyotheca.

The crystal violet method was proposed first by Newton (1927). A chromic modification for deeper staining, suggested by La Cour (1937), was used in the present study and

does not prevent the tendency of crystal violet to fade after a few years.

The leuco-basic fuchsin technique is based on Schiff's aldehyde reaction, which was applied as a microchemical test for chromosomal desoxyribonucleic acid by Feulgen and Rossenbeck (1924). The technique involves:-

(i) mild acid hydrolysis, which splits the purine bases and carbohydrates of desoxyribonucleic acid and liberates the aldehyde groups of the aldo-pentose sugars;

(ii) a reaction between the free aldehyde groups and fuchsin-sulphurous acid reagent (leuco-basic-fuchsin), producing a reddish-purple pigment.

(iii) Following this focal microchemical reaction, these aldehyde groups are specifically and selectively adsorbed on to the remaining unhydrolysed parts of the nucleoprotein molecules which form part of the protein framework of the chromosome (Stowell 1945).

Although certain other classes of substance e.g. the polysaccharides, lignin and suberin, give a positive Schiff's reaction, it is accepted today that, under the carefully-controlled conditions of the Feulgen technique, a specific diagnosis of desoxyribonucleic acid in the cell should always be possible. These sections therefore provide valuable information about the nucleic acid cycle. When counter-stained with Light Green, it was possible to use these Feulgen prepara-

tions to study plasmosomal behaviour in relation to the nuclear changes of mitosis and meiosis.

Of the sectioned material, the haematoxylin and the Feulgen preparations were most useful for analysing the behaviour of the sex-chromosomes. As this study was interrupted by a considerable lapse of time, the crystal violet preparations, while originally showing the chromosomes with brilliant clarity, later proved quite useless either for photomicrographs or for study of the chromosomes, as the stain had faded almost completely. These faded sections, were, however, of value in the study of spermiogenesis, since the acrosomal cap and other accessory structures of the sperm-head showed up clearly. The sectioned material generally provided useful information about the association of cell-types in the tubules and formed the entire basis for the study of the spermatogenetic wave.

Most of the second halves of the testes and the remaining ovaries were fixed in 25% acetic acid in absolute alcohol. After 24 hours in the fixing fluid, the material was squashed in acetic orcein. This technique is based upon Belling's iron-aceto-carmin method (1926) for the study of pollen mother cells. It makes use of a combined stain-fixative. La Cour in 1941 proposed to substitute orcein for carmine owing to its greater selectivity and clarity. While originally the squashed material was fixed, stained and studied in aceto-carmin, it was found that in many species, the cytoplasm takes up too

(1929) found that staining of the cytoplasm is reduced by prior fixation in 1-and-3 fresh acetic alcohol for 12-24 hours. The gerbil material was therefore treated by acetic alcohol fixation prior to being stained with acetic orcein.

The squash preparations, fixed by albumin on the cover-slips, were floated off the slides in acetic alcohol and re-stained with Ehrlich's acid haematoxylin, Heidenhain's iron haematoxylin or Feulgen's leuco-basic fuchsin and light green. Brenner (1947) restained acetic-orcein squash preparations with haematoxylin and thereby obtained good results on Elephantulus. The sharp definition and clarity of fine detail made these preparations especially valuable for photomicrography and, for making counts and measurements of the chromosomes.

Some squash preparations were re-stained with Feulgen's leuco-basic fuchsin. Although this procedure yielded erratic results, in a proportion of successful slides, inner detail of pachytene threads was rendered clear.

Finally, some testicular material, as well as pieces of liver from the same animals, were smeared and fixed in 95% alcohol. These smears were then stained with Pappenheim's and Unna's methyl-green pyronin technique. This method clearly differentiates the plasmosome or true nucleolus from karyosomes or false nucleoli, the former taking up the pyronin and appearing red, the latter taking up the methyl green and appearing green

useful for a study of plasmosomal and karyosomal behaviour.

Observations were made using a 90x, 97x or 100x oil-immersion objective and 10x, 12.5x, 15x or 20x eyepiece. Drawings were made with the aid of a Zeiss camera lucida (magnification factor 1.8x).

Measurements made directly on the preparations with an ocular micrometer can introduce a significant error. This was obviated by measuring the lengths of the chromosomes on camera lucida drawings, which were made at magnifications of 3250x (immersion objective 90x, eyepiece 20x) or of 3500x (immersion objective 97x, eyepiece 20x). The final length was corrected to the first decimal place; it is assumed that errors of measurement or of drawing became insignificant when divided by the magnification. Difficulties of overlap or foreshortening were minimised by focussing with the fine adjustment. Where this was not possible, the plates were rejected.

TECHNICAL PROCEDURES OF PREPARATORY INVESTIGATION.

At the outset of the present investigation, I studied the chromosomes of the albino rat (Rattus norvegicus albinus) in order to provide as complete a characterisation of the mitotic chromosomes as is possible in a species with so large a complement (Tobias 1947). The albino rat was chosen because it had been the object of previous cytogenetical studies; the intention was to devise an analytical approach which could then be applied to other mammals.

Accordingly, the testes of four young adult albino rats 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 7 μ and stained with Newton's crystal violet or the Feulgen nucleal reagent. While the sectioned material was not favourable in most instances for detailed measurement 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 (1947). The squash preparations were used for making detailed observations including measurements.

The lengths of the chromosomes were measured on camera lucida drawings at a magnification of 2550x (oil 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 chromosomal complement of the albino rat was then characterised on the basis of the chromosomal length, the position of constrictions and the general appearance.

PART 2.

THE CHROMOSOMES.

CHAPTER III.

THE CHARACTERISATION OF THE
CHROMOSOMES OF A MAMMAL
(RATTUS NORVEGICUS ALBINUS) :ø
WITH SPECIAL REFERENCE TO
THE VALIDITY OF THE CHARACTERISATION.

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 (Rattus norvegicus albinus). Pincus (1927) has summarised the counts made up to that time:

<u>AUTHOR:</u>	<u>DATE:</u>	<u>NAME:</u>	<u>DIPLOID NUMBER:</u>	<u>HAPLOID NUMBER:</u>
Von Lenhossek	1898	Ratte	-	12 1st spc.
Von Ebner	1899	Wanderratte	?16 spg.	?8 1st and 2nd spc.
Regaud	1901) 1910)	Rat	20-30 spg.	12 1st spc.
Moore & Arnold	1905	Rat	32 spg.	16 1st spc.
Melissinos	1907	Mus rattus albus	-	8 1st and 2nd spc.
Sobotta & Burchard	1910	Weise Ratte	32 egg.	8 2nd spc.
Duesberg	1908	Mus decumanus albus	?24 spg.	?12 1st spc.
Van Hoof	1911	M.rattus albus & M. decumanus albus.	24+ spg.	?16 1st and 2nd spc.
Pratt and Long	1917	M. norvegicus albinus	40 pachy. threads of ooc.	-
Allen	1918	M. norvegicus albus	37 spg.	18+X 1st spc.
Painter	1926a	Albino rat (Wistar stock);	42 spg. and amnion.	21 1st spc.

TABLE 1.
 (After Pincus 1927)

CHROMOSOMAL ENUMERATIONS IN THE WHITE RAT PRIOR TO 1927.

It is evident from this table that considerable discrepancies were recorded, owing no doubt to the poor fixation obtained with the earlier techniques. In 1918, Allen came close to the accepted figure when he reported 37 chromosomes in the spermatogonia. He was able to identify 18 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 characterise 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 earliest measurements found in the literature are those of Evans and Swezy (1928, 1929). They quote a series of measurements of rat chromosomes, as measured on magnified camera lucida drawings of somatic mitoses in male and female rat embryos and in adult female rats. In the rat, as in the human, they found a constant significant difference between the lengths of chromosomes in the two sexes. Unfortunately, this comparison, like their comparisons on human material, was based upon "late prophasic nuclei, just before the dissolution of the nuclear membrane". As this is a dynamic, not a stable, phase, during which the chromosomes are still actively contracting, it may be questioned whether the establishment of this sexual difference is valid. The objection is offset, to a certain extent, by a statistical analysis: many cells of each sex were considered and, presumably, included within each group cells at different stages of late prophase. The only absolute lengths of rat chromosomes quoted are the total lengths ($\times 8,400$) of the first pair of chromosomes from 100 nuclei each, from male and female rat embryos. These figures are as follows:

<u>Chromosome Number:</u>	<u>Male</u>	<u>Female</u>
1	376.09 cms.	328.17 cms.
2	375.38 cms.	307.43 cms.

The figures give an average length of 4.5μ in the male and $3.7-3.9\mu$ in the female. These figures do not represent the minimum or metaphasic length of the chromosomes in the rat as measurements were taken in late prophase.

Later chromosomal measurements were provided by Koller and Darlington (1934). According to these workers, the chromosomes of the largest pair at metaphase 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. This preliminary study of the rat was designed partly to remedy this deficiency by providing as complete a characterisation as is possible in a species with so large a complement and, partly, to test the techniques and analytical approach on a well-known species before applying them to the gerbil, a species previously untouched by cytogeneticists.

OBSERVATIONS:

I. Chromosomal Number:

In twelve spermatogonial metaphase and pro-metaphase plates in squash preparations, the chromosomal number is consistently forty-two. The validity of this number receives verification from the 21 tetrads observed in primary spermatocyte nuclei.^Ø The observation serves as additional confirmation of the number reached by previous workers on sectioned material.

II. Chromosomal Shape:

Centric constrictions are most easily observed in late prophasic or pro-metaphasic nuclei when they appear as short achromatic gaps in the chromosomes. For reasons to be mentioned below, it has been necessary to establish the shape at metaphase as well as at pro-metaphase.

It should be added here that, when the squash technique is utilised, the actual recognition of the metaphase involves an assumption. This is due to the dislodgment of the chromosomes from the equatorial position, in terms of which metaphase is defined. However, in this study, the period during which the chromosomes stain most intensely has been accepted as co-extensive with metaphase. Hence this character is used to distinguish metaphasic chromosomes, despite the disruption of the typical metaphasic alignment by the technique in use. In practice, no difficulty is experienced in distinguishing metaphase plates from prophase or pro-metaphase plates on the basis of their

Figures a-e. Diplotene bivalents of the albino rat. Note the chiasmata and characteristic diplotene rings. 21 bivalents are present in each of the nuclei.

Acetic orcein squash preparation re-stained with Heidenhain's haematoxylin.

x 1500, 1600 and 2100





Figures f-h. Groups of bivalents in the first meiotic metaphase. 21 bivalents are present in each, while the sex-bivalent may be clearly seen in the upper three cells.

Acetic orcein squash preparation re-stained with Heidenhain's haematoxylin.

x 2100 and 2300

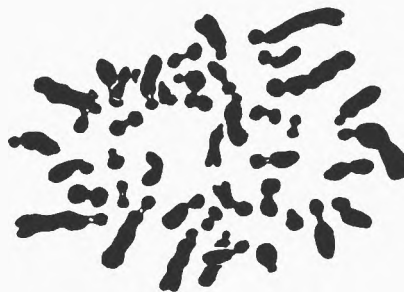
staining capacity. Although there is no direct evidence that a pro-metaphase, as defined above, occurs in every mitotic process, the term pro-metaphase has been applied to squashed nuclei^{ed} in which the chromosomes have not quite attained the degree of contraction nor of staining which serve to delineate metaphase.

In pro-metaphase plates (Figs. 1, 2) 28 chromosomes possess sub-terminal constrictions and the remainder median (or sub-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 portrays 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 are observed. In one, the achromatic gap representing the centric constriction is situated some distance from the end of the chromosome. The distance ranges 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 is so close to the end of the chromosome that only a small chromatic granule is visible distal to the constriction. It is probably the same or a similar structure which Minouchi named the "polar granule". Several chromosomes have sub-terminal constrictions intermediate in position between these two types.



A



Figures 1 and 2. Photomicrograph and camera lucida drawing of mitotic pro-metaphase plate (Plate A) of the albino rat, showing the diploid number of 42 chromosomes.

Acetic orcein squash preparation re-stained with Ehrlich's haematoxylin.

x 2550

The possession of median constrictions is confined to the smaller elements. Hence it has not been 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 are actually sub-median in position.

At metaphase, it is impossible to observe constrictions in every chromosome (Figs. 3-9). The most favourable plates contain 14 elements with median constrictions. Such a chromosome appears as two small granules lying side by side and separated by a narrow region corresponding to the centric constriction. Of the rest of the complement, a variable number contain obviously sub-terminal constrictions, while the remainder are uniformly rod-like. In no instance has a centromeric granule been observed in the indented portion of the chromosome.

It is apparent from these findings that different conclusions would be drawn if only pro-metaphase or only metaphase plates were examined. A study of metaphase plates alone would lead to the conclusion that a certain number of chromosomes possess no centric constrictions and are terminally-attaching or telomitic; whereas, from a study of both stages, it is clear that the rod-like appearance of some elements results from the difficulty of detecting terminal granules in chromosomes with immediately sub-terminal constrictions.



Figure 3. Camera lucida drawing of mitotic metaphase plate of the albino rat (Plate B) showing the diploid number of 42 chromosomes.

Acetic orcein squash preparation restained with Heidenhain's haematoxylin.

X2550.

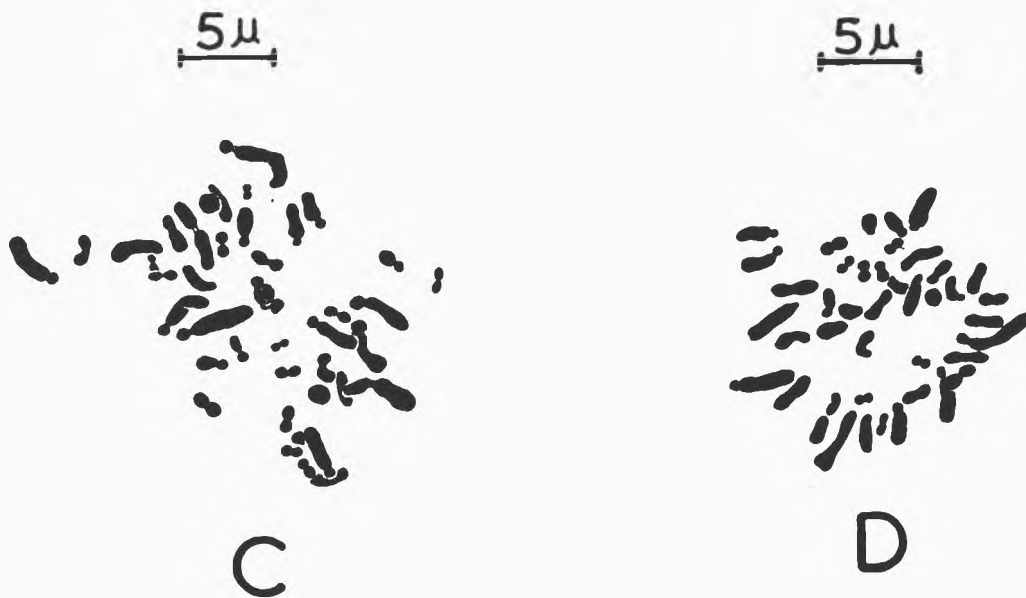


Figure 4. Camera lucida drawing of two mitotic metaphase plates of the albino rat (Plates C and D), each showing the diploid number of 42 chromosomes.

Acetic orcein squash preparation re-stained with Heidenhain's haematoxylin.

X2550.

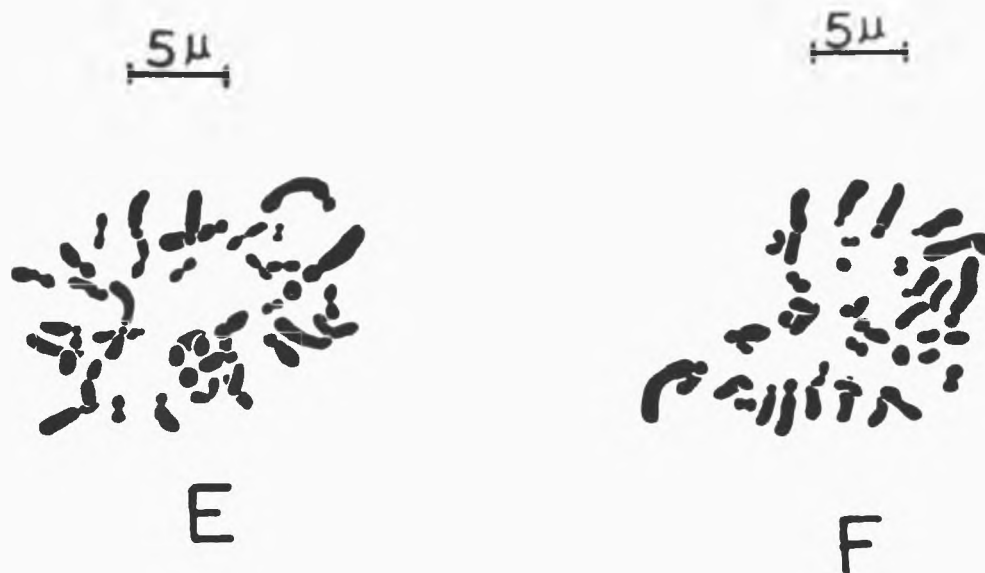


Figure 5. Camera lucida drawings of two mitotic metaphase plates of the albino rat (Plates E and F), each showing the diploid complement of 42 chromosomes.

Acetic orcein squash preparations re-stained with Heidenhain's (E) and Ehrlich's (F) haematoxylin.

X2550.



G



Figures 6 and 7. Photomicrograph and camera lucida drawing of mitotic metaphase plate (Plate G) of the albino rat, showing the diploid number of 42 chromosomes.

Acetic orcein squash preparation re-stained with Heidenhain's haematoxylin.

x ±3800



Figure 8. Camera lucida drawing of mitotic metaphase plate of the albino rat (Plate H), showing the diploid complement of 42 chromosomes.

Acetic orcein squash preparation re-stained with Heidenhain's iron haematoxylin.

X2550.

5 μ



I

Figure 9. Camera lucida drawing of mitotic metaphase plate of the albino rat (Plate I) showing the diploid complement of 42 chromosomes.

Acetic orcein squash preparation re-stained with Ehrlich's haematoxylin.

X2550.

III. Chromosomal Length:

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

In the present study, the size range has been 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 have been determined. The results are represented diagrammatically in Figs. 10, 11 and 12. The chromosomes in each plate are arranged in descending order of length on a pair of ordinates. No effort has been made to pair them off in the graphs, 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 characterised on criteria other than size. The reason why this assumption cannot be made is that a chromosome may become slightly more contracted in one plate than in another, thus altering its position in the series. The result of the graphical representation is a general correspondence in form of the graphs for all the examined plates.

Finally, a composite type-graph (Fig. 13) has been drawn by plotting the maximum and minimum lengths of the chromo-

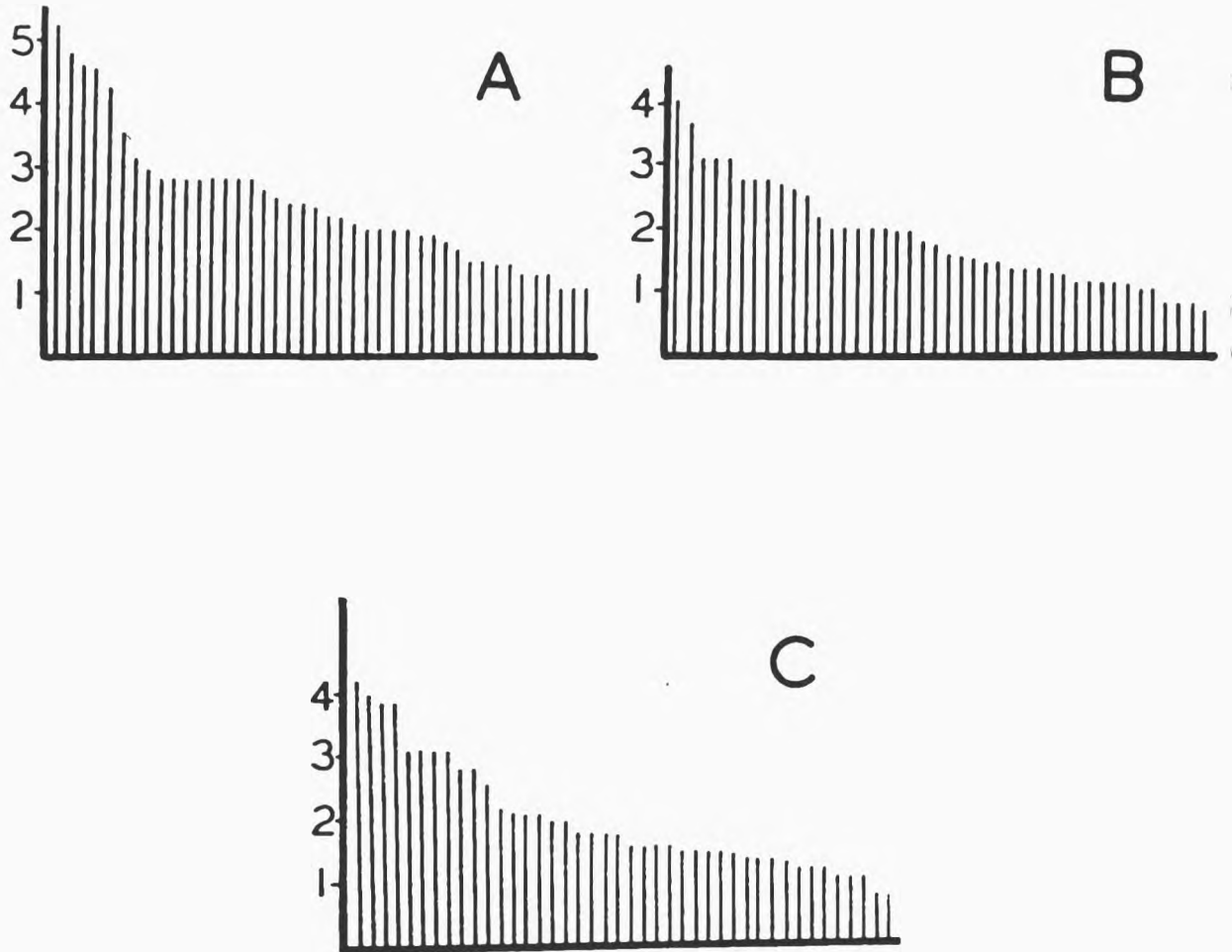


Figure 10. Graphs of the chromosomal complements of Plates A, B and C, of the albino rat.

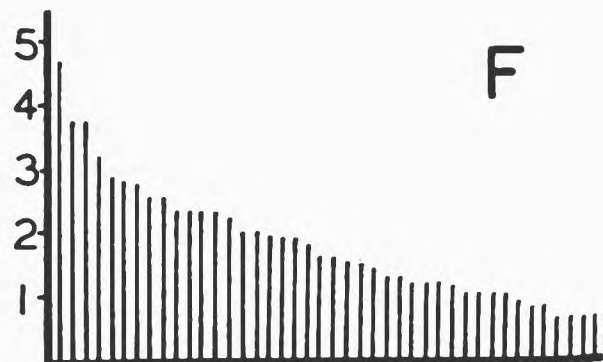
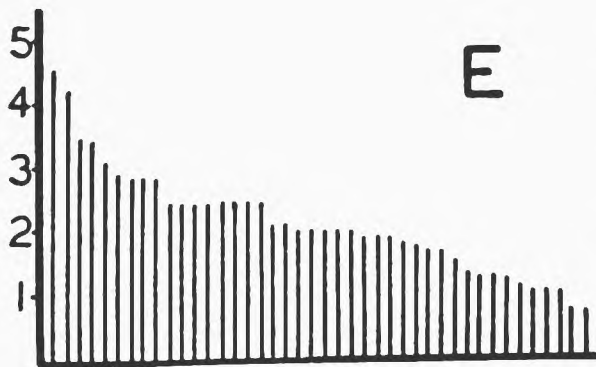


Figure 11. Graphs of the chromosomal complements of Plates D, E and F of the albino rat.

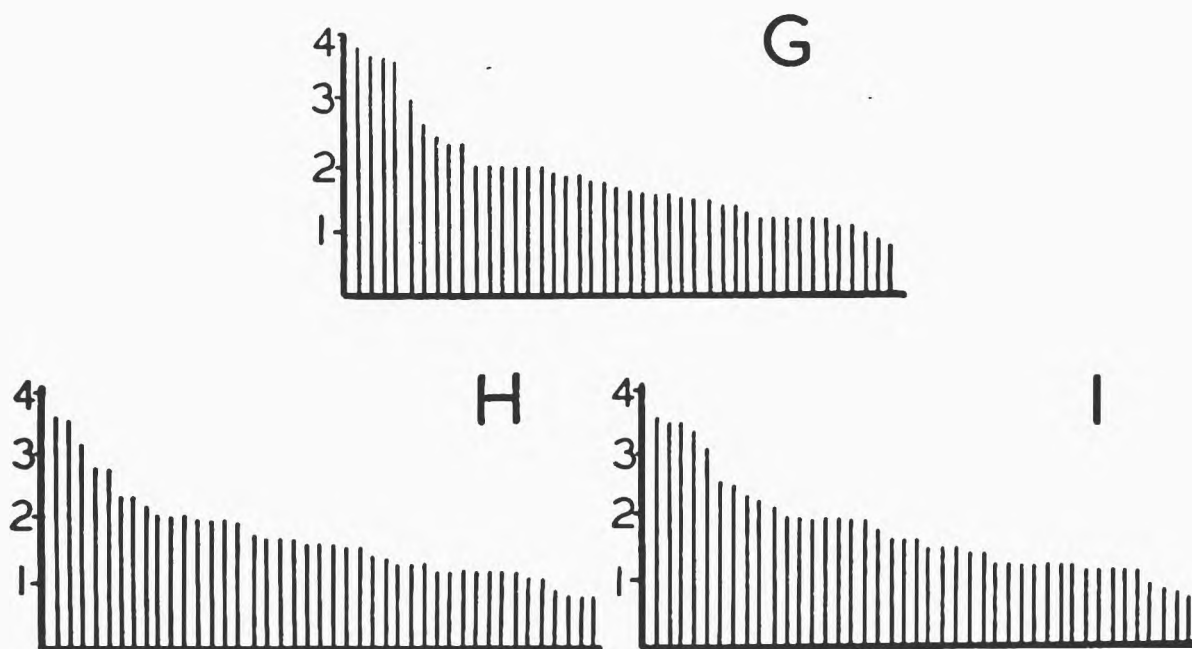


Figure 12. Graphs of the chromosomal complements of Plates G, H and I of the albino rat.

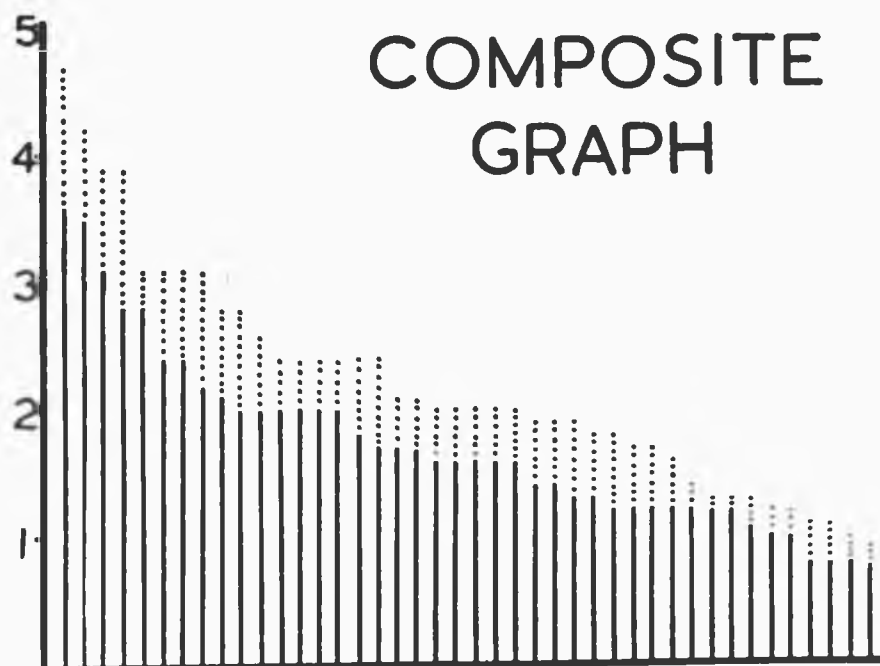


Figure 13. Type-graph of the chromosomal complement of the albino rat.

somes occupying corresponding positions in the linear series. Care must be exercised in the interpretation of this graph, in view of the possible variations in the linear order. In the final analysis, the graph can provide the following information:

(1) The broken lines represent the observed size range for only those chromosomes which occupy constant positions in the linear series i.e. the five largest and the four smallest. The mean range for the two members of a pair is accepted as the range for that pair, since there can be no certainty that the same homologue is invariably the larger where the two homologues differ slightly in size.

(2) The broken lines convey the order of magnitude of the size range for any part of the linear series. Thus, the longer chromosomes have greater size ranges, both absolutely and in relation to their lengths, than the smaller chromosomes. The presumptive X-chromosome is a notable exception to this generalisation.

It must be emphasised that the observed range of variation does not necessarily represent the true or population range. 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: THE CHARACTERISATION OF THE CHROMOSOMAL COMPLEMENT.

The chromosomal characterisation of many mammals is beset by extraordinary difficulties attendant upon the large number and the small size of the chromosomes. Of 169 mammalian species and sub-species whose chromosomal enumeration I have found in the available literature, 120 (including the albino rat) each possess 40 chromosomes or more and, of these, 47 possess fifty chromosomes or more. The tallies range up to a possible 84 and 86 for two members of the rodent family, Geomyidae (Cross 1931). The position is aggravated by the short lengths of the chromosomes when contrasted with plant chromosomes, or even with those of other Vertebrate orders, such as the Dipnoi, Urodela and Anura (Wickbom 1945). In most mammals where measurements have been made, the longest chromosome at mitosis has been found to measure 5 or 6 micra. The lengths of the numerous chromosomes present in mammals therefore lie within a comparatively small size range. The position is typified by my results in the albino rat. The 42 chromosomes of this species range in length from 0.8-0.9 μ , the length of the smallest pair, to 3.6-4.4 μ , the size of the largest pair. Consequently, the intermediate chromosomes show a very fine gradation in length, the elements often falling into groups of 6 or 8 which are equal in size. It is these considerations which render the squash technique particularly suitable for mammalian chromosome studies, since this technique tends to separate the chromosomes and open the plates.

At metaphase, it has been shown that not all the chromosomes appear to have centric constrictions. Hence, if it is desired to pair off the metaphase chromosomes accurately, it is essential to establish a correspondence between the shapes observable at pro-metaphase 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 have been characterised as completely as possible.

The four largest chromosomes possess sub-terminal constrictions and fall into two pairs on the basis of size, a larger with range 3.6-4.4u and a smaller with range 2.9-3.9u. 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 a pair may be taken as the mean range for both members.

The fifth largest chromosome cannot be matched. It

has a sub-terminal constriction and its range (2.8-3.1u) 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 2.9u 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 (0.8-1.1u) with median constrictions and a smaller pair (0.8-0.9u) 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 characterised 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 μ . The length of Koller and Darlington's presumptive Y-chromosome was 1.1 μ . In six plates it occupies position 34 in the linear series, while in three others it is 28th, 32nd and 36th. This illustrates the earlier statement that one chromosome may lie in different positions in the linear series in several plates. It is a consideration which appears to have been overlooked by Evans and Swezy (1929) in their tabulation of human chromosomal lengths. What has been said earlier about different chromosomes attaining their minimum lengths at varying times and in a variable order would indicate a priori a variation in the linear order. Apart from my observations in the rat, this is borne out by a survey of Evans and Swezy's measurements, from which it emerges that the larger member of the heteromorphic pair occupies widely varying positions in the linear alignment of different nuclei. As this holds for the presumptive X-chromosome in man and for the presumptive Y-chromosome in the rat - two chromosomes easily identified by their heteromorphism - it is not unreasonable to conclude that such variations may exist for the less-easily recognisable autosomes as well.

Table 2 summarises the final characterisation. The size ranges quoted for any group represent the range between the extreme lengths of the smallest and largest members of the group.

TABLE 2.

CHARACTERISATION OF THE CHROMOSOMES OF THE ALBINO RAT.

CHROMOSOME OR GROUP	LENGTH IN μ	POSITION OF CENTRIC CONSTRICTION.
Pair 1	3.6 - 4.4	Sub-terminal
Pair 2	2.9 - 3.9	Sub-terminal
Presumptive X	2.8 - 3.1	Sub-terminal
Pairs 3-12	1.4 - 3.1	Sub-terminal
Pairs 13-16	1.2 - 2.9	Median
Presumptive Y	1.2 - 1.3	Sub-terminal
Pairs 17-19	0.8 - 1.3	Median
Pair 20	0.8 - 0.9	Sub-terminal

If the position in the rat is typical of most mammals, then it would appear that the system of group characterisation will have to be used in characterising the chromosomes of many species. When this is supplemented by graphical representations of the complement (e.g. as used by Wickbom 1945, and White 1945), the morphological karyotype may be regarded as having been established as accurately as possible using the present techniques. The type-graph (Fig. 13) 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.

THE VALIDITY OF THE CHARACTERISATION.

Characterisation is essentially a procedure aimed at establishing relatively constant properties, typically associated with relatively stable states of a system.

In characterising the chromosomes of the rat, I have recorded a set of properties of cell-nuclei under certain conditions. Of a vast potential range of cells, of stages, of shapes and of sizes, I have singled out only certain cells, specific stages, particular shapes and sizes, to fulfil the purpose of characterising the chromosomal complement of the gerbil.

If we now wish to test the validity of the characterisation, we enquire whether the relative constancies (e.g. the chromosomal number and length) have been determined for defined states of stability. We require a knowledge of the more stable phases of the components (i.e. the chromosomes) of our system. Since the components are in a continual state of flux (e.g. mitotic cycles), to define their states of stability, we require to explore the limits of their instability; to find their constancy, we must weigh up their variability. Only then will we be in a position to assess what parts of the total range of variation may validly be selected for their stability or constancy.

For the purposes of this analysis, we shall choose arbitrarily the indicators which have been used in the study of the rat: these indicators are the number, the volume and the length, the shape and the patterns of internal differentiation of the chromosomes. These chromosomal properties will be our variables and we shall be concerned to observe what happens to each under varying sets of conditions.

Next, we may enquire the nature of the varying sets of

conditions, the determinants of variability. Although we shall find that each indicator yields to its own specific set of modifying factors, we may, in general, group the determinants of variability under certain broad heads:

- (a) the species and the sub-grouping within the species;
- (b) the sex;
- (c) the biological age of both organism and tissue;
- (d) the cell-type;
- (e) the functional state of the tissue;
- (f) the phase of the mitotic cycle;
- (g) residual factors.

The modifying rôle of each of the first six factors will emerge from the ensuing account, but a word should be added about the subtler and more enigmatical forces grouped here as "residual factors". To the residual factors must be ascribed a residue of variability, unaccountable by the more manifest factors. The residual factors are not obviously explained by the "functional state of the tissue", but reflect rather the state of reactivity of individual cells within the general structural and physiological fabric of a tissue. Thus, even if we were to study the range in a number of cells, all of the same type, at the same stage of division, of roughly the same age and during the same functional stage of the tissue or organ, in a series of animals of the same sex and of the same species, we might still expect to obtain a range of variation, albeit a minimal one. This range might be attributed only to the lesser-

known "residual factors".

We shall now consider the way in which each of the indicators of variability is influenced by the determinants enumerated above.

VARIATIONS IN CHROMOSOMAL NUMBER.

By far the most important determinant of variation in chromosomal numbers is the systematic group to which an organism belongs. The specificity of chromosomal numbers in various species has been known since the work of Flemming on the salamander (1882) and is discussed fully in Chapter V of the present thesis.

In considering variations in chromosomal number, we may disregard the sex of the animal, except in those few species in which the existence of an extra X or Y, or of an XO-XX sex-chromosomal mechanism has been claimed. An XY-XX mechanism has subsequently been demonstrated (Matthey 1938, Renaud 1938) in all of the alleged XO-XX species (e.g. the rodents, Apodemus speciosus ainu (Oguma 1934, 1937), Evotomys (Clethrionomys) bedfordiae (Oguma 1935) and Microtus montebelli (Oguma 1937)). Exceptional mammals are the insectivore, Sorex araneus, and two marsupials, Potorous tridactyla and Macropus ualabatus, in each of which a third sex-chromosome, an extra X or an extra Y, has been reported (see footnote, page 326). These exceptions apart, the chromosomal number does not vary with the sex of the animal.

Chromosomal number does not vary with age, but

important variations are encountered in comparisons between the cells of different tissues. For instance differences are known to exist between the chromosomal complements of germinal and somatic cells. Boveri (1907, 1909) was responsible for the classical demonstration of this in Ascaris megalocephala. In the univalent variety, dividing germ-cells have one pair of large chromosomes, while a much greater number, variously reported from 52 to 72, is present in dividing somatic cells (Geinitz 1915, Walton 1924). Similarly, in the variety A. megalocephala bivalens, there are 4 and 62-144 chromosomes in the germinal and somatic cells respectively. More recent studies, such as that by White (1936), have revealed that the large chromosomes are polycentric, i.e. that they have a number of spindle attachments. Such an arrangement would suggest that the polycentric chromosomes undergo fragmentation and so produce multiple somatic chromosomes.

Recently, Timonen and Therman (1950) and Therman and Timonen (1951) have reported wide variations in the chromosomal numbers of somatic cells of man, both embryonic and adult. Outside the germ-line, the chromosome number 48 is apparently not the commonest number; instead, the highest peak of frequency in embryonic tissues lies between 20 and 30 and, in adult uterine cells, between 20 and 25 with a much lower peak between 45 and 50. If their observations are confirmed, it seems that man is exceptional among the mammals so far studied in his preponderance of aneuploid numbers in somatic tissues.

In mammals, the commonest departure from the normal diploid chromosomal number is the state of polyploidy. Isolated tetraploid nuclei have been reported in the liver of the rat (Beams and King 1942, Bieseke 1944), in the human testis (Painter 1923, Evans and Swezy 1929) and in the testis of the gerbil (Tobias, unpublished observations). Several instances of octoploid nuclei have been recorded (e.g. Bieseke 1944). Hughes (1949) has demonstrated that, in spleen cultures of newly-born mice, filmed with phase-contrast microscope and time-lapse camera, polyploid cells can arise by the mitosis of binucleate cells. However, such nuclei are rare and exceptional in the cells of all healthy tissues so far examined: no healthy mammalian tissue is known in which the cells are regularly polyploid. Tumour cells exhibit a high proportion of polyploid nuclei, but they are unique in this respect among examined mammalian tissues. For purposes of our classification of determinants, the polyploidy of tumour cells may be regarded as a response to a particular functional state of a tissue - the state of neoplasia. That the incidence of polyploidy in a tissue may be altered by other functional states is suggested by unpublished observations of Gillman (1950) on the extraorbital lachrymal gland of the albino rat. The cell-nuclei of this gland undergo changes strongly suggestive of polyploidy, when the animals are put on to high dosages of oestrogen.

In considering variations in chromosomal number, we may disregard the phase in mitosis, because it has been abundantly

confirmed that the number remains constant from early prophase, when the chromosomes first become visible as discrete structures, to late metaphase. At anaphase, when the two daughter sets of chromosomes are moving apart, we have a cell containing the $4n$ or tetraploid number of chromosomes. This condition persists only until the formation of two daughter-cells is completed by cleavage of the cytoplasm. Then, once more, the diploid state prevails in each cell.

To summarise:- the most important variations in chromosomal number are met with in comparisons between different species. Exceptionally, variations occur within a species and these variations are to be related to the cell-type, the functional state of the tissue or residual factors determining variability.

VARIATIONS IN CHROMOSOMAL VOLUME AND LENGTH.

The most striking examples of chromosomal properties varying under an assortment of circumstances are afforded by the volume and length of the chromosomes. Since volume must be related to both the length and the diameter of the chromosomes, the volume could be gauged simply by the measurement of length and of diameter, were the nature of this relationship known. Although an inverse proportional relationship between chromosomal length and thickness has never, to my knowledge, been metrically established, it is none the less clear to the observer that the chromosomes shorten and thicken progressively and concurrently during the prophase of mitosis. Of these two dimensions, in

mammalian chromosomes, it is far easier to ascertain the length. The chromosomal length serves a useful purpose as an indicator of the changes which are proceeding in the chromosome and, in practice, there is a tendency to base conclusions on assessment of the length, rather than on volume determinations.

The different lengths of chromosomes are characteristic for each species and are therefore manifestations of the species genotype. Within the species, too, genotypic differences may reveal themselves in chromosomal variations. Hollingshead (1930) first demonstrated the genotypic control of the degree of contraction of the chromosomes. Darlington has discussed further examples in plants. In man (Koller 1937), the cat (Koller 1941b) and the dog (Ahmed 1941), groups of spermatogonia have been found with unusually contracted chromosomes and Koller has interpreted this peculiarity in the cells of the group as a derivation from one cell in which the genotypic control was altered by mutation. In other words, a change of genotype, which may be nothing more than a change in a single mutant locus in one chromosome, may alter the reaction of the entire set of chromosomes. However, a similar end-result may follow from a common genotypic response to special environmental conditions, created, for example, by the sex of the animal, the nature of its tissue and its physiological state.

Sexual Variation in the Lengths of Chromosomes.

Evans and Swezy (1928) have reported a sex-correlated

difference in chromosomal length in man and the albino rat. In these species, the chromosomes of the male are somewhat longer than those of the female. Whether the control of the difference is vested directly in the sex-differentiated genotype, or indirectly, in the varying physiological states associated with the two sexes, has not been ascertained and it may prove impossible to resolve these two factors apart in such a causal analysis.

Length Variation with Cell-type:

The chromosomes are often shorter and thicker in dividing spermatogonia than in dividing somatic cells: this is apparent from the camera lucida drawings of the opossum (Painter 1922), the macaque monkey (Painter 1924b) and the rabbit (Painter 1926) and, from the illustrations of Evans and Swezy (1929) on man. Differences of chromosomal length would be expected, too, between the complements of various somatic tissues, conditioned by cell- and nuclear-size and by other less well-established factors. Such dissimilarities have been found in a recent study of the albino rat by Liesele (1946): metaphasic chromosomal volume in aceto-carminic smears varied from organ to organ (actually from cell-type to cell-type) and this variability among the organs was least in embryos and greatest in the old animals.

Chromosomal Length Variations with Age and Physiological State.

A further type of length variation has been reported by Minouchi (1928) in the albino rat and confirmed by myself,

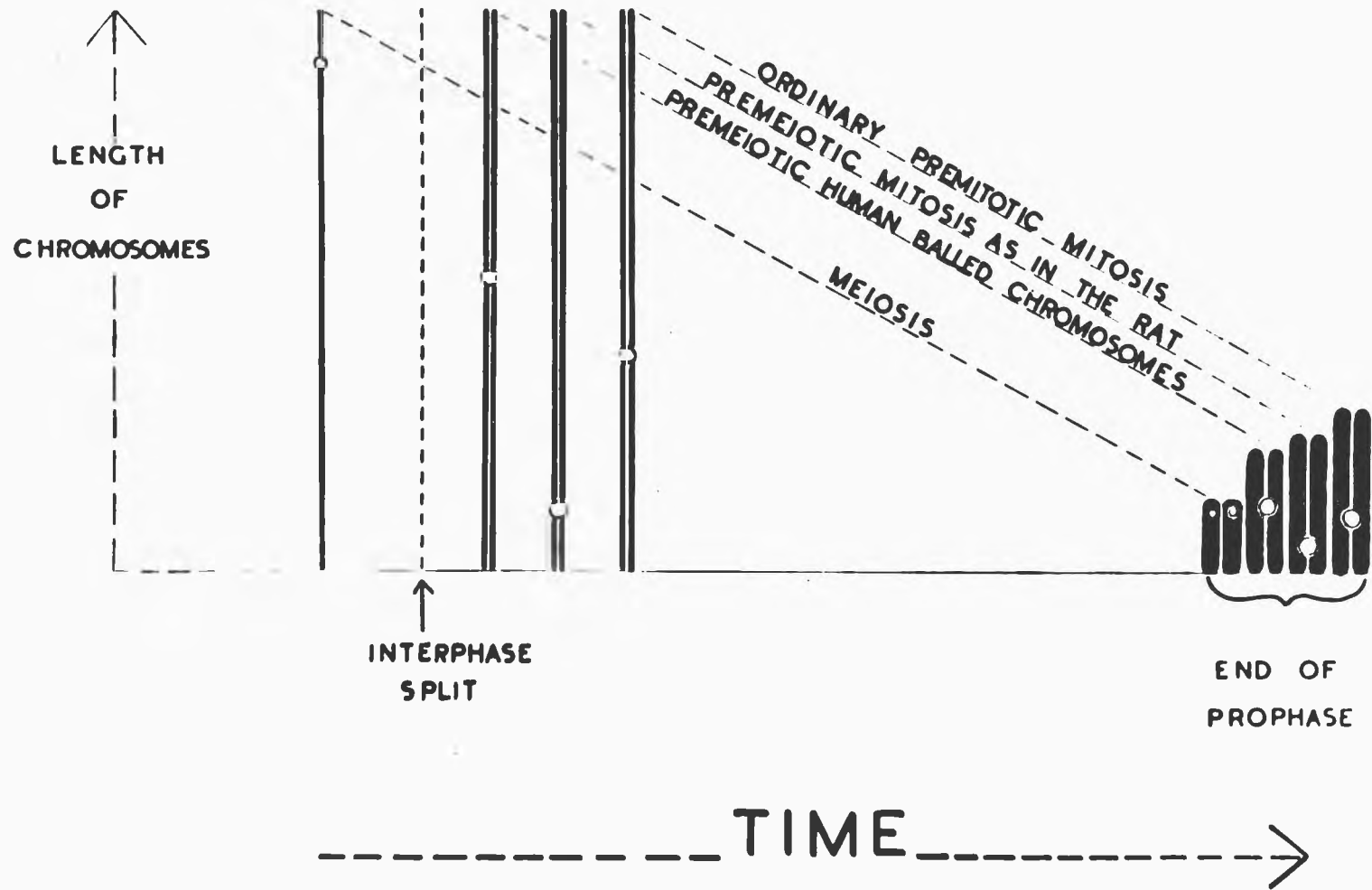
between the chromosomes of spermatogonia in young testes and those of spermatogonia in older testes. In the former, metaphasic chromosomes are longer and thinner than in the latter. A similar phenomenon was reported by Painter (1923) and confirmed by Koller (1937) in man: Koller has suggested that the extremely contracted or "balled" chromosomes are of premeiotic mitoses, the chromosomes being intermediate in their degree of contraction between typical mitotic and meiotic chromosomes. This greater contraction would suggest either a faster rate of contraction or a longer prophase; if the latter, the chromosomes begin to contract earlier, but not before their interphasic split has occurred. There is some indication, too, that the more contracted metaphase plates in the albino rat are of premeiotic mitoses. In this particular type of variation, it appears that the age factor is mediated through the approach to threshold value of the stimuli inducing the onset of meiosis. If this were so, the differences between typical mitosis and meiosis would be primarily quantitative: in meiosis, according to the most widely-accepted views, the prophasic contraction begins so early that the interphasic split of the chromosomes has not yet occurred.

Fig. 14 is a graphical representation of the prophasic contraction of chromosomes during ordinary - premitotic - mitosis, premeiotic mitosis (moderate degree in the rat, extreme degree in the human "balled" chromosomes) and meiosis. It has been assumed here that the rate of contraction is the same in

Figure 14. The varying duration of prophase in

- (a) ordinary(premitotic) mitosis;
- (b) premeiotic mitosis (albino rat);
- (c) premeiotic mitosis (man -
'balled chromosomes');
- (d) meiosis.

The diagram illustrates the effect of
varying durations of prophase on the
metaphasic lengths of chromosomes.



all four types of division and the curves are therefore parallel. This assumption is obviously false for the meiotic prophase, as there is a characteristic pattern of contraction up to pachytene, elongation at diplotene and, contraction again from diakinesis to metaphase; however, a parallel meiotic curve is included here simply to illustrate the apparent quantitative difference between meiosis and mitosis. As can be seen, the prophase of meiosis begins before the interphase split of the chromosomes has occurred. From this time-relation, it is possible that many of the other differences between meiotic prophase and mitotic prophase may result as secondary effects.

Further examples of age-variation in the size of chromosomes are provided by Bieseke (op. cit.) from his studies on somatic tissues in 44 albino rats of varying age. He concluded that there are three main patterns of age-variation, according to the tissues studied:

- (i) Epidermis and non-epithelial cells of lungs: little variation in the size of chromosomes from birth to old age;
- (ii) Epithelium of small intestine, lymph-node and spleen: increase in chromosomal volume at birth, fall during youth and gradual fall through maturity to old age;
- (iii) Kidney and liver: rise in chromosomal volume soon after birth, maintenance of level or slight increase, right through to old age.

In these tissues, therefore, the age-changes are intimately bound up with the physiological state. The fact

that in some tissues chromosomal volume becomes higher in old age, while in others it becomes lower, indicates that age is not a single common factor to be reckoned with in relation to chromosomal change. The old-age changes of the chromosomes in any senescent tissue are related in some as yet uncomprehended manner to the ageing changes in the particular tissue or cell-type, just as the volumes of the chromosomes vary with the tissue or cell-type (Bieseke 1946).

Variations of Chromosomal Length in the Mitotic Cycle.

Length and thickness are two features of the chromosomes which vary most markedly in the mitotic cycle. It would therefore not be out of place here to consider the chromosomal changes during mitosis in some detail.

Darlington has defined "mitosis" on the basis of certain essential properties, precisely those of genetic significance, which recent work has shown to be universal:

Mitosis is -

"the separation of the identical halves of the split chromosomes into two identical groups from which two daughter nuclei are reconstituted."

(Darlington 1937, p.22)

The events by which this separation and reconstitution are effected are divided arbitrarily into a series of phases: pro-, meta- ana- and telo-phase. Since observations are most commonly made on prophase and metaphase nuclei, these two

phases will receive most attention.

Prophase:

At this initial phase, the segmentation of the chromatin into discrete bodies or chromosomes becomes apparent. These bodies are at first long, thin structures which stain lightly with basic dyes. During the prophase, they shorten, thicken and stain progressively more intensely. Prophase ends with the disappearance of the stainable nuclear membrane, by which time the chromosomes are considerably shorter than they were at the outset.

Pro-Metaphase:

There are two possible sequences of development from the end of prophase: either (i) the chromosomes align themselves immediately in the equatorial plane of the division-spindle (i.e. the nucleus passes into metaphase); or (ii) they remain free in the cytoplasm undergoing further contraction for a variable period, but showing no active movement towards congression at the equator. This phase, intermediate between the end of prophase (demarcated by the disappearance of the nuclear membrane) and the onset of metaphase (characterised by equatorial congression of the chromosomes) is known as pro-metaphase. It is exemplified by the mitotic cycle in the marsupial, Sarcophilus ursinus (Koller 1936c), in which it is associated with a very slow rate of contraction of the chromosomes. The outcome of this tardiness is that the chromosomes are not yet

maximally contracted, nor do they undergo equatorial congression at the time of the disappearance of the nuclear membrane. It is clear that the incomplete state of chromosomal contraction at the disappearance of the nuclear membrane may also be explained on the basis of a more rapid rate of chromosomal contraction, but a shorter prophase. This will be discussed again below in relation to the findings in the gerbil.

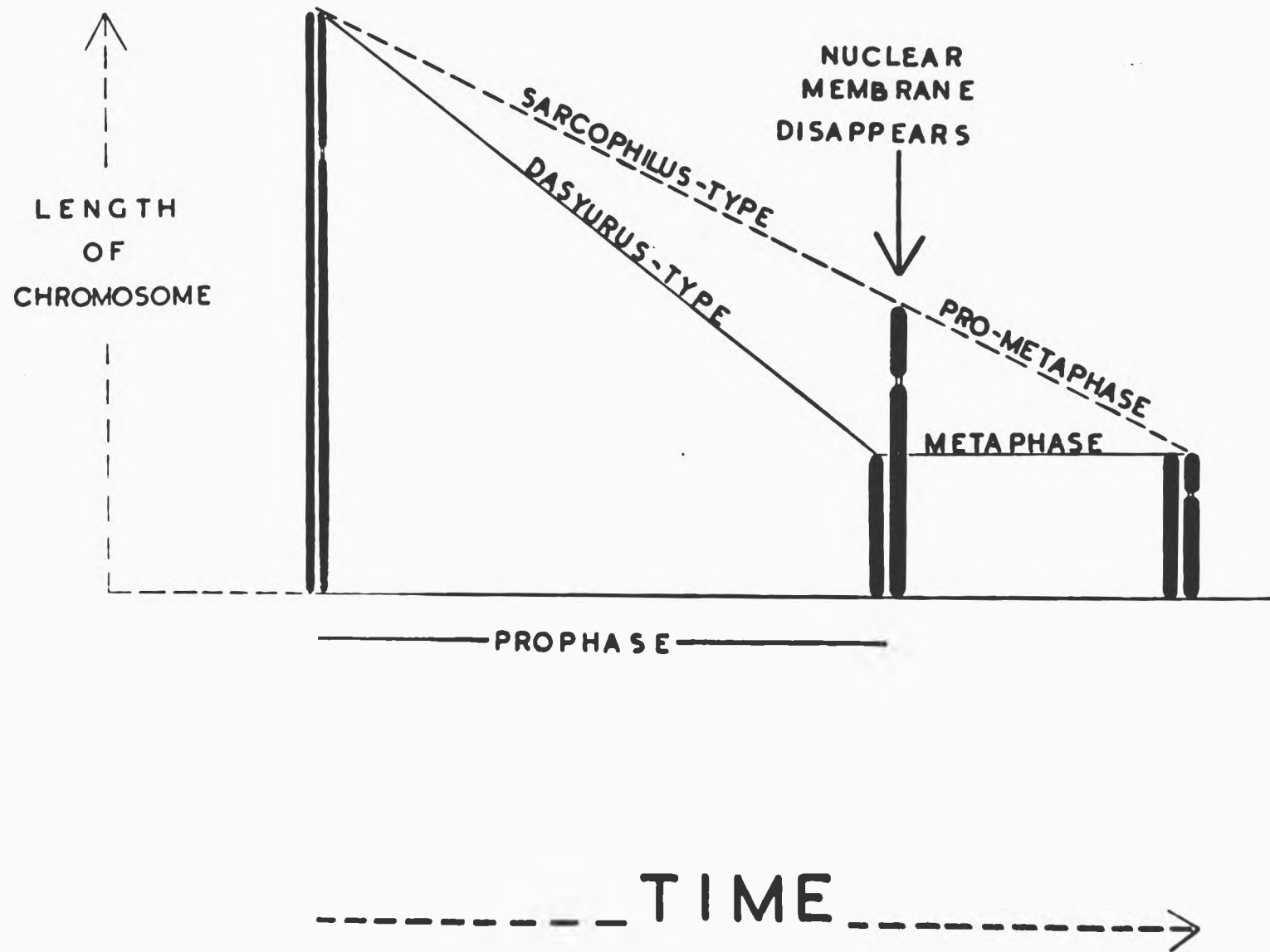
When the nuclear membrane disappears in Sarcophilus, the chromosomes are "still long thin threads and there follows a long pro-metaphase." That pro-metaphase is a stage of active contraction is illustrated by Koller's finding that, at the beginning of pro-metaphase, the chromosomes are about twice as long as in metaphase. Koller goes further and suggests that, just as Hollingshead had demonstrated genetic control of the degree of contraction (1930), the rate of chromosome contraction is regulated by the genotype. It has been found that a pro-metaphase occurs in the albino rat. In other mammals, e.g. Dasyurus maculatus, the first sequence mentioned above takes place: the prophasic contraction of the chromosomes is more rapid and is, in fact, complete by the time the nuclear membrane disappears. Thereafter, the fully contracted chromosomes immediately assemble at the equator, without the occurrence of an intervening pro-metaphase. This type of sequence would appear to be the commoner.

Fig. 15 represents the two types of sequence. For simplicity, a single pair of chromosomes has been drawn, that

Figure 15. The relationship between the rate of contraction of the chromosomes and the end of prophase.

The Sarcophilus-type is characterised by a long pro-metaphase;
the Dasyurus-type by no pro-metaphase.

Key: Sarcophilus-type chromosome identified in the diagram by its possession of a sub-terminal constriction.



of Sarcophilus being distinguished in the diagram by the presence of a sub-terminal constriction. It has also been assumed that there is only one variable, viz. the rate of contraction (indicated by the steepness of the curve), while the other possible variable, namely duration of prophase, has been assumed in the diagram to be the same in Sarcophilus as in the group of "typical" species.

Metaphase:

Metaphase is defined not in terms of the appearance of the chromosomes, but on the basis of their external mechanics. By definition, a cell is in metaphase when the centric constrictions of its chromosomes are aligned in the equatorial plane of the division spindle (Darlington 1937). Darlington offers a supplementary definition to the effect that "the momentary condition of 'full' metaphase is reached when the limbs of the chromosomes have come together as closely throughout their length as they were earlier at the centromere." Although we may assume that this definition is universally valid, it is difficult or impossible to apply it in all cases, since the double nature of the mitotic metaphasic chromosomes is often not apparent in mammals. Hence, the equatorial alignment of the chromosomal centromeres is the main visible distinguishing feature of metaphase in mammals and is so accepted in the present study.

With the meaning of metaphase elucidated, the problem

arises what relationship exists between the length, the thickness and the intensity of staining of the chromosomes, on the one hand, and their metaphasic alignment on the other. In the majority of instances, the onset of metaphase is regarded as being synchronous with the attainment by the chromosomes of their greatest contraction (Darlington 1937) and with their most intense basophilic staining capacity (Wilson 1937).^ø This means that no further change in the length and staining occurs during metaphase. The chromosomes remain unchanged and Wilson therefore concludes that the metaphase is a condition of relative stability. None the less, Darlington's supplementary definition does suggest at least some change during metaphase, namely the movement and approximation of the chromosomal limbs. The "momentary" duration of "full" metaphase conveys the impression that the chromosome remains constant in appearance for a very short space of time. Two questions spring to mind: What is the temporal duration of metaphase? If metaphase is more than a moment in time, do the chromosomes undergo any change other than the movement of their limbs during metaphase? In other words, do the chromosomes, in fact, attain their minimum length, their maximum thickness and their greatest staining capacity at the end of prophase or pro-metaphase?

^ø One recorded exception must be noted here: Ahmed (1941), working on three breeds of dogs, found that "later prophase and pro-metaphase chromosomes are thicker than those during the metaphase of mitosis". This was reported as a common characteristic of the chromosomes of the three types of dog studied.

In studies on the duration of the mitotic phases, an hypothesis first enunciated by Wright (1925) is often used: viz. that the relations between the durations of the various mitotic phases is identical with the relation between the frequencies of their occurrence in stained preparations. According to this hypothesis, it is possible to ascertain the time duration of all the mitotic phases if one possesses information relating to: (i) the duration of a single phase; and (ii) the relative frequencies of cells found in the various phases. Wright accepted 5 minutes as the average duration of telophase. This figure was based on the work of Levi (1916) and of Lewis and Lewis (1917), all of whom studied mitosis in chick fibroblasts cultivated in vitro and arrived at precisely the same duration of telophase. To ascertain the relative frequencies, Wright counted cells in the various mitotic phases in stained preparations from the same type of culture of chick fibroblasts, as that on which the time periods had been determined. He then calculated the duration of each phase, assuming telophase to be 5 minutes. Table 3 records his findings: (his "Early Prophase" and "Spireme" stages have been grouped as "Prophase"):

	<u>PROPHASE:</u>	<u>METAPHASE:</u>	<u>ANAPHASE:</u>	<u>TELOPHASE:</u>	<u>RECONSTRUC-</u> <u>TION.</u>
<u>Percentage:</u>	43%	15%	12%	15%	15%
<u>Time in</u> <u>Minutes:</u>	14½	5	4	5	5

TABLE 3.

Duration of the Phases of Mitosis (after Wright 1925)

Thus, the total duration of mitosis was estimated to be 34 minutes. This figure agrees well with the times of 35 minutes reported by Strangeways (1922) in living choroidal cells of the chick, of 25-45 minutes reported by Lambert and Hanes (1913) in connective tissue cells of the rat and of 48 minutes calculated by Roosen-Runge (1951) in spermatogonia of the rat; while it is not far removed from Levi's figure of 11-20 minutes for meta- and anaphase combined; nor from the figure of 5-17 minutes for meta-, ana- and the beginning of telophase, quoted by Lewis and Lewis. Apart from these figures in Vertebrates, Belar (quoted by Darlington) has given accurate estimations of the duration of metaphase in two Protozoa. In Ragostoma schüssleri, mitosis lasts for 32.5 minutes, of which 2 minutes (6%) are occupied in metaphase. In Euglypha sp., mitosis endures for 179 minutes, metaphase taking 18 minutes (10%). Observations of plant mitoses, in Tradescantia virginiana, reveal a mitotic duration of 340 minutes, of which only 4 minutes (1%) are spent in metaphase.

Apart from the observations in rat tissues (Lambert and Hanes 1913), no figures for other mammals have been found by me. There are indications, however, that the relative durations of metaphase and anaphase vary. For instance, in testis preparations of the South African shrew, Elephantulus myurus jamesoni, anaphase plates predominate (Brenner 1946), whereas I have found that in the testis of the albino rat, metaphase plates are much more numerous than anaphase figures.

It is clear that the few temporal observations recorded do not permit of any extrapolation about the conditions in mammals generally. Nevertheless, they may provide a clue as to the order of magnitude of the time spent in metaphase. The interval appears to be of relatively short duration, long enough only to allow the chromosomes to be in metaphase for several minutes.

As metaphase would appear to be, then, at least of several minutes' duration, the second question posed above now admits of discussion, viz. whether the chromosomes undergo any change, other than the movement of their arms, during metaphase.

Evidence has been found in man (Evans and Swezy 1929) and in the albino rat (Tobias 1947), that a size range exists for any pair of chromosomes in different cells at mitotic metaphase. Other factors being equal, this range may be attributed to (a) limitations of the technique, e.g. errors of measurement and (b) the "residual factors" mentioned earlier, associated with the functional state of individual cells. It is tempting and, indeed, plausible, to suggest that a size range has been found because of an inadequate demarcation of the mitotic phase in which the measured nuclei were fixed. In other words, it is just within the bounds of possibility that not all the chromosomes attain their minimal length at the onset of metaphase, but that some contract tardily, reaching their minimum length later than the others during metaphase. Thus differences might be expected according as to whether the nucleus was fixed

in early, intermediate or late metaphase.

In brief, it is conceivable that not all the chromosomes keep in step with one another during prophasic contraction. Just as there are factors which act in common on the entire complement e.g. causing rapid contraction in Dasyurus and slow contraction in Sarcophilus, so it is suggested that there are factors operating on individual chromosomes in the dividing cell which (a) determine the minimum length of any chromosome; and (b) determine the order in which the chromosomes reach their minimum lengths. The action of these forces will account for the varying results obtained according as the nucleus was fixed in early, intermediate or late metaphase.

The relation of length to thickness of chromosomes is approximately one of inverse proportionality. An exception to this generalisation has already been noted (Foot-note, page 55). Apart from the dog, we may apply what has been said with regard to length changes in the mitotic cycle mutatis mutandis to thickness.

When one considers staining capacity, the observer can detect no difference in the intensity of staining of chromosomes in early, intermediate or late mitotic metaphase. In rat testis preparations, for instance, I observed a number of metaphase plates on one slide. The same tubule contained both earlier and later stages i.e. nuclei in pro-metaphase and others in anaphase. Therefore, it is reasonable to assume that some of the plates

examined were early metaphase, some were in the intermediate stage of metaphase, while others were close to the end of metaphase. Yet, all the plates appeared alike in their staining capacity. If then the chromosomes are equally heavily stained throughout metaphase, this feature of maximal staining capacity may be regarded as a good indicator of metaphasic chromosomes.

In short, it is of the utmost importance to specify the stage at which a dividing nucleus is observed. Further, ranges of variation in length and thickness, but not necessarily in observable staining capacity, may be expected even within the definitive stage, as these are distinguished arbitrarily and are of some temporal duration. The finer sub-division of the arbitrary mitotic phases provides one clue as to the nature of the "residual factors" affecting variation.

To summarise:- it is clear from the afore-mentioned that while the dimensions of a chromosome may be limited by the ultimate size and nature of the chromosomal thread, the appearance of this thread may vary considerably. In other words, the chromosome is like a barometer, sensitive to the subtlest changes in its environment and, I have tried to analyse the major factors contributing to differences in this environment. The conclusion is that chromosomal length and width must be considered in relation to the species, the sex, the tissue, the age and the physiological state of the cells, the stage of mitosis and residual determinants of variability.

VARIATIONS IN CHROMOSOMAL SHAPE.

The shape of the chromosome is conditioned largely by the positions of constrictions and by the length of the limbs or segments in relation to their width. For instance, in a chromosome in which a sub-terminal constriction cuts off a trabant shorter than the diameter of the rest of the chromosome, this trabant assumes a spherical shape at metaphase (Darlington 1937, p.38).

At metaphase, the chromosomes often adopt configurations which are assumed to be constant. Berry (1941) has described V-shaped elements in the mitotic metaphase chromosomes of the sheep, but very often it can clearly be seen in his plates that the two limbs of these elements are separated by a wide angle. When in such dispositions, these chromosomes look more like slightly bent rods than V-shaped structures. These findings seem to be typical of the irregularity of the metaphasic conformations. Another example is found in Bryden's description of three pairs of curved elements in spermatogonial plates of the albino rat (1932). These elements are not apparent in Minouchi's plates (1928) and the observation was not consistently made in my own study of albino rat testis preparations. I am led to conclude that the outline of the chromosomes at metaphase is not as constant a feature as other chromosomal properties like number and dimensions. Certainly, it must be regarded as a dangerous procedure to infer the position of centric constrictions from the metaphasic configurations as

Matthey and Renaud have done (Matthey and Renaud 1936; Renaud 1938).

In mammals, constancies of shape emerge most clearly during the anaphase separation of the chromosomes. At this phase, a chromosome with a median or sub-median centric constriction appears V-shaped; a chromosome with a sub-terminal centric constriction appears J-shaped. These shapes are typical of each species and do not vary with the sex, the age, the cell-type or the functional state of the tissue.

VARIATIONS IN THE PATTERN OF INTERNAL DIFFERENTIATION OF THE CHROMOSOMES.

Any study of the nucleus is incomplete unless it takes into account the internal differentiation and the ultra-structure, through which, ultimately, all overt cytological behaviour, all cytochemical integrations and all genetical characteristics become manifest. Three sets of technical procedures have developed side by side in the approach to the internal differentiation of the chromosomes - the procedures of the geneticists, of the cytochemists and of the cytologists.

Experiments on Mendelian inheritance have led geneticists to the hypothesis that various loci on a chromosome are capable of mutating and, so, of changing the course of normal development. The phenomena of linkage and of genetic crossing-over and, the construction of linkage-maps, based on the cross-over frequencies between proximate loci, have given rise to the further hypothesis that such mutable loci are

situated in a characteristic linear order along the length of any chromosome.

The cytochemists have provided us with evidence for another sub-division of the chromosomes, namely into chemical constituents e.g. desoxyribose nucleic acid, histones, ribose nucleic acid and "residual" protein (Mirsky and Ris 1949).

Finally, the cytologists and the cytogeneticists have shown us specific and constant loci and regions which are landmarks indicative of the internal differentiation of a chromosome. The loci are:- the primary constrictions (points of attachment of spindle fibres), with the centromeres (small bodies situated in the primary constrictions), the secondary constrictions (indentations or achromatic gaps in the chromosome, at positions other than those of the primary constriction) and the nucleolar organisers (loci opposite which the true nucleoli regularly develop and remain attached for varying periods). The cytogeneticists have demonstrated that some chromosomes have trabants or satellites - small segments cut off from the rest of the chromosome by one or more secondary constrictions. Further, some chromosomes, especially the sex-chromosomes, are differentiated into regions characterised by a greater or lesser departure from the nucleic acid cycle of the other chromosomes. These regions are designated "heterochromatic".

It follows from the above remarks that an over-all

picture of the contents of the nucleus (the karyotype) must be painted by a variety of artists: while the contribution of the cytogeneticist is a vital one, it lacks colour and continuity of pattern for which it is necessary to look to the geneticist and to the cytochemist. Our primary concern here is with cytogenetical studies, the aim of which is to assess the morphological karyotype, as distinct from the physiological karyotype, the delineation of which is based on inference from genetical effects and from chemical composition. One would hesitate to carry this distinction too far, for the three investigators must work in phase with each other in order to establish the correlation that undoubtedly exists between the morphological and physiological karyotypes.

Direct observations on the morphological karyotypes have revealed that constrictions, trabants, nucleolar organisers and heterochromatic segments, are constant in number and in relative position within a species. Variations occur between species and, occasionally and exceptionally, in individual cells under the influence of residual factors (i.e. the determinants of translocations, inversions, deletions, reduplications etc.). The internal differentiation of the chromosomes has been shown not to vary with the sex, age, cell-type, functional state or mitotic phase.

COMMENT: THE INSTABILITY OF THE CHROMOSOMES.

In the foregoing account we have selected four properties which were determined in the characterisation of the

chromosomes of the albino rat and have used these properties as indicators of chromosomal variability.

In general, the variations discussed above are met with in mammals. In fact, many of the crucial observations mentioned were made on one or other mammal. In one respect, however, mammalian chromosomal studies have lagged behind researches on other species and, that is in the patterns of internal differentiation of the chromosomes. As the recorded observations here are sparse and, as it is important for us to know whether our conclusions apply equally to mammals, it would be wise to dwell on this aspect for a few moments.

From the genetical approach, little advance has been made in studies on mammals. Analyses of linkage have made it possible to assign some hereditary effects to loci within certain chromosomes. The most detailed localisation has been attempted on partially sex-linked characters; Haldane (1936), for instance, has mapped a number of mutant loci in their relative position on the human X-chromosomes, using linkage data and cross-over values. The accuracy of this provisional map of a human chromosome depends on the correctness of Haldane's assumption that the characters concerned are incompletely sex-linked. By and large, however, the difficulties of human and general mammalian genetical analyses have militated against any real advance in this sphere (Gates 1946, p.70).

If we turn to the attack by the cytogeneticist, we

see that only very few observations are available on the meristic sub-divisions of the germ-plasm in mammals. Primary and secondary constrictions are difficult to detect in the short mammalian chromosomes and little attempt has so far been made to study these morphological features. Matthey and Renaud (1937, 1938) inferred the existence of constrictions from the configurations of the chromosomes at metaphase, a procedure which, in my opinion, is unsatisfactory. Apart from their studies, the only instances known to me are in the mouse (Koller 1944), the elephant shrew, Elephantulus. (Brenner 1946, 1947), the albino rat and the gerbil (present study). In these four mammals, the relative positions of the constrictions along the chromosomes have been found constant for dividing spermatogonia, while Brenner found this constancy in female somatic cells as well.

From the few observations quoted and, by analogy with the chromosomes of other orders, it may be assumed that constrictions occur at constant points in the linearly-differentiated chromosomal thread and, as Darlington points out (1937, p.35), they give character to the individual chromosomes.

We may assume, then, that the variations of the chromosomes discussed above apply inter alia to the mammals. From our analysis, we have concluded that each indicator varies in accordance with a set of determinants: these results are summarised in Table 4. In the Table, a + signifies that the indicator in the left-hand column regularly varies with the

factor in the vertical column; a - denotes that the indicator does not vary with the modifying factor; a $\pm$ means an infrequent or irregular variation of the indicator with the particular determinant.

From Table 4, we see that our indicators possess differing degrees of instability. Most unstable (or variable) is the length of the chromosomes: this instability declares itself with every single one of our seven categories of determinants. Number is a more stable property of the chromosomes, varying regularly with one and, irregularly, with three modifying factors. Most stable are the shape and the internal differentiation of the chromosomes, varying consistently with one determinant only and, infrequently, with one or two modifying factors.

As a corollary, if we are to characterise the chromosomes by assessing our four indicators, it is necessary for us to be nicely discriminating in our choice and definition of the stable states of the chromosomal lengths; whereas a wider range of states would provide adequate stability to characterise the number, shape, and internal differentiation of the chromosomes.

THE MORE STABLE PHASES OF THE CHROMOSOMES.

Let us now define the states of stability within the ranges of variability we have established, for it is at these stable phases that the relatively constant properties of chromosomes are to be characterised. We shall consider each

TABLE 4.

VARIATIONS OF THE CHROMOSOMAL INDICATORS WITH
VARIOUS MODIFYING FACTORS.

<u>VARIABLE.</u>	<u>SPECIES.</u>	<u>SEX.</u>	<u>AGE.</u>	<u>CELL-TYPE.</u>	<u>FUNCTIONAL STATE.</u>	<u>MITOTIC STAGE.</u>	<u>RESIDUAL</u>
<u>Number</u>	+	-	-	+	+	-	+
<u>Length</u>	+	+	+	+	+	+	+
<u>Shape</u>	+	-	-	-	-	+	+
<u>Internal Differen- tiation.</u>	+	-	-	-	-	-	+

indicator in turn.

The chromosomal number regularly varies with the species or lower systematic group. But, within the taxonomic category, it is constant among all the members of the group. Exceptionally, variations such as polyploidy, arise in some tissue-cells under certain - undefined - functional states; but as these numerical variations are extremely rare in a group of cells, it is always possible to find a greater number of diploid nuclei. It is accepted that that number of mitotic metaphase chromosomes which is most often observed in a tissue is the type-number for the tissue-cells examined. This definition allows for the occasional numerical variations (e.g. monosomy, polysomy) which arise under the impact of residual factors as yet undetermined. All studies on mitoses occurring in healthy mammalian tissues (with the possible exception of human tissues - Timonen and Therman 1950) have shown that the type-number for the tissue is the same as the type-number in dividing spermatogonia in the testis. This is of practical importance because it is the latter number which is generally accepted as the type-number or, as it is usually called, the chromosomal number of the species. It has been found that this chromosomal number is constant

for all unhybridised members of a mammalian species.Ø
One may assume that the type-number for dividing spermatogonia is equal to the chromosomal number in most, if not all, mammals. Arising out of this point, it is a valid procedure to determine the chromosomal number of a new species by a study of spermatogonia alone. The spermatogonia will be studied under those conditions in which mitotic figures are most numerous i.e. in young or sexually mature, healthy animals and, at those stages in mitosis when the chromosomes are more easily counted i.e. late prophase, pro-metaphase, metaphase and early anaphase.

The lengths of the chromosomes are relatively constant under only a limited set of conditions. Whatever constancies exist are found among members of one species or lower group, not between members of different species. Since length variations occur between the two sexes, a set of chromosomal measurements should in theory be separated into those made on males and those on females. In practice, measurements are usually confined to male animals in line with the procedure of assessing chromosomal number only on spermatogonia. The age of the animal is important, whether one is considering somatic tissues or spermatogonia. A distinction exists between the

Ø One possible exception to this statement must be noted: Swezy (1928) has reported the existence of two types of spermatid in the grey Norway rat (Rattus norvegicus norvegicus). One contains 31 and the other 21 single chromosomes. These may have arisen by reduction division of two types of spermatogonia, containing 62 and 42 chromosomes respectively. Only spermatogonia containing 42 chromosomes were actually observed in the four animals examined. Swezy's observations await confirmation.

lengths of chromosomes in spermatogonia of young rats and those of older, sexually mature rats. Since the differences in chromosomal length between younger and older spermatogonia probably reflect two essentially different types of cell-division (pre-mitotic and pre-meiotic mitoses), it is as important to distinguish between spermatogonial chromosomes of younger and older rats as it is to distinguish the chromosomes of mitotic divisions from those of meiotic divisions!

Chromosomal length varies from cell-type to cell-type. Hence, separate observations should be made on germ-cells and on the various somatic cell-types. Again, in practice, studies are usually limited to one type of cell, the spermatogonium. The functional state of this cell is indicated by its biological age - whether it is a premitotic or a premeiotic mitosis. The changes of length with the mitotic cycle necessitate that measurements be taken at a defined phase in the division process. The onset of prophase is a time when the chromosomes as discrete, visible entities have their greatest length, but, for technical reasons, this phase is manifestly unsuitable for making chromosomal measurements. A much more suitable landmark in the chromosomal cycle is the attainment of the minimal length of the chromosomes at metaphase. Measurements are usually made at metaphase (or pro-metaphase). Under these circumstances, the range of variation of chromosomal length, though still present, is minimal; this range is ascribable to our inability to sub-divide metaphase by non-chromosomal

indicators and to other residual factors. Because of the constant existence of this range, chromosomal length is the only one of the four indicators in which stable states cannot be defined other than approximately.

The shape and the internal differentiation of the chromosomes are the most stable properties, their main variability being between species. Within a species, they occasionally vary from some unknown residual cause, while the shape may change during the phases of mitosis. Therefore, these properties may be studied over a wide range of stable states. In practice, however, since characterisation is a single procedure in which all four indicators are determined simultaneously, the shape and internal differentiation are studied under the same circumstances as is the length of the chromosomes i.e. in dividing spermatogonia at pro-metaphase or metaphase. In this instance, we select arbitrarily a single stable state out of many possible states of stability of chromosomal morphology.

From the above discussion, we conclude that each indicator has a set of stable states and that, in order to characterise the chromosomes (i.e. to assess all the indicators at the same time), we select the most restricted stable states of the most inconstant variable, chromosomal length. In this limited range of stabilities, we seek to determine the four properties about which discussion has centred. Further, since characterisation provides data to serve as a basis for comparisons between different species, not merely do we make our observations

on stable states, but we state explicitly what these states are, lest the criteria of characterisation vary from worker to worker.

CONCLUSION.

We have explored the limits of instability of the chromosomes and, from this flux, we have come to a knowledge of the more stable phases of the chromosomes. We know that, in the rat, we have determined a set of relative constancies (e.g. the chromosomal number and length) under certain conditions. To test whether these determinations permit a valid characterisation, we enquire under what conditions the relative constancies have been determined in the rat.

All observations were made on members of a single taxonomic group, Rattus norvegicus albinus. The animals selected were all males of the young adult age period. Studies were limited to one cell-type, the spermatogonium, and these cells were all at a similar functional stage, i.e. they showed the premitotic type of mitosis. The cells selected were those which had been fixed in the pro-metaphase or metaphase of mitosis.

It will be seen that the conditions under which the chromosomal properties have been determined in the rat correspond closely to what we have already concluded are the more stable phases of our chromosomes. We have, in fact, established relatively constant properties, typically associated with relatively stable states of the system: a procedure which

is the quintessence of characterisation. The conclusions we have reached by applying the procedure to the rat may be accepted as a valid characterisation of the chromosomes of this species.

CHAPTER IV.

THE CHROMOSOMAL COMPLEMENT
OF THE GERBIL
(TATERA BRANTSII DRACO)

INTRODUCTION.

In the present study, the methods of characterisation used on the albino rat are applied to the gerbil, Tatera brantsii draco.

According to Davis (1949), the geographical ranges of T. schinzi and T. brantsii overlap on the fringes of the Kalahari. The presence of these two related forms in the same locality directs attention to their evolutionary divergence. On current views, it must be assumed that, at some time in the past, the once actually or potentially interbreeding array of Tatera stock became segregated into groups of populations reproductively isolated from one another.

Reproductive isolation was probably preceded by geographical and ecological isolation, which thus enabled the various population groups to evolve further. Among the features of this divergent evolution, one at least apparently ensured reproductive isolation, even when spatial barriers were broken down and the ranges of the population groups once more overlapped. Such non-spatial isolation may have been effected by any one or any combination of the following features:-

- (i) Non-coincidence of the breeding seasons. Both T. brantsii and T. schinzi breed all the year round (Measroch 1948). T. brantsii shows periods of maximum and minimum breeding, but, according to Measroch, it is probable that T. schinzi does too. Thus, the reproductive isolation of these two species is not attributable to differences in the breeding season.
- (ii) Incompatibility of the genitalia. No evidence has been found on this point, nor on the next three points.
- (iii) The presence of group-distinctive characters e.g. the stimulating scent of the male and the specific mating reactions of the male and female.
- (iv) Non-correspondence of the chromosomal complements, resulting in the breakdown of meiosis and consequent hybrid sterility.
- (v) Psychological barriers to sexual union, imposed by the mating instinct.
- (vi) Differences of habitat. Some evidence suggests that at least this form of divergence has occurred in the speciation of Tatera. Ecological differences have been noted between T. brantsii and T. schinzi, especially in the choice of habitat, food and burrowing habits (Roberts 1936; Davis 1949). Davis summarises these differences by speaking of the T. brantsii forms as primarily "plains" gerbils and T. schinzi forms as "savanna and woodland" gerbils.

Whichever of these isolating mechanisms has become evolved in Tatera, it is clear that, where their geographical

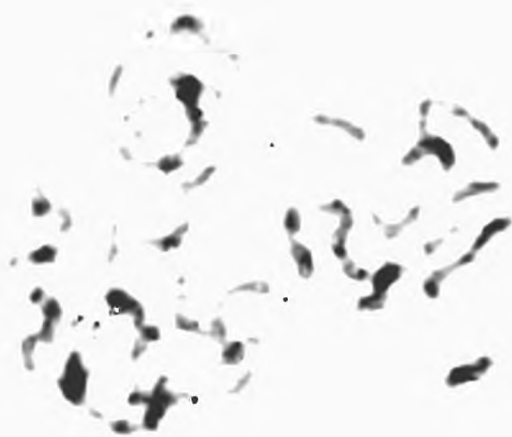
ranges overlap in the Kalahari, T. brantsii and T. schinzi remain distinct species without marginal intergrading. The barrier between them has its origins in the period of isolation and divergence and the features of particular significance in maintaining this barrier, whether chromosomal, genital, psycho-sexual, functional or ecological, must be regarded as part of the complex of differential features evolved in isolation.

It is hoped that the present study will pave the way for a comparison of the chromosomal complements of various species of Tatera. Such a comparison may, in turn, reveal whether there are differences in chromosomal properties, correlatable with the absence of a hybrid population. Chromosomal determinations in T. afra and T. schinzi, following the present study in T. brantsii, may throw new light or confirmatory evidence on the inter-relationships and line of descent of these South African gerbils.

Such an application of chromosomal studies to the fields of taxonomy and phylogeny is discussed more fully below.

SPERMATOGONIAL PLATES OF THE GERBIL.

Figures 16 to 27 are drawings and photomicrographs of selected metaphase and pro-metaphase plates. Although especially chosen for clarity and detail from several score of examined plates, the nine plates figured nevertheless include several (E,F,H,I) which show considerable overlaps and foreshortening of the chromosomes. In no single plate are all the



4μ

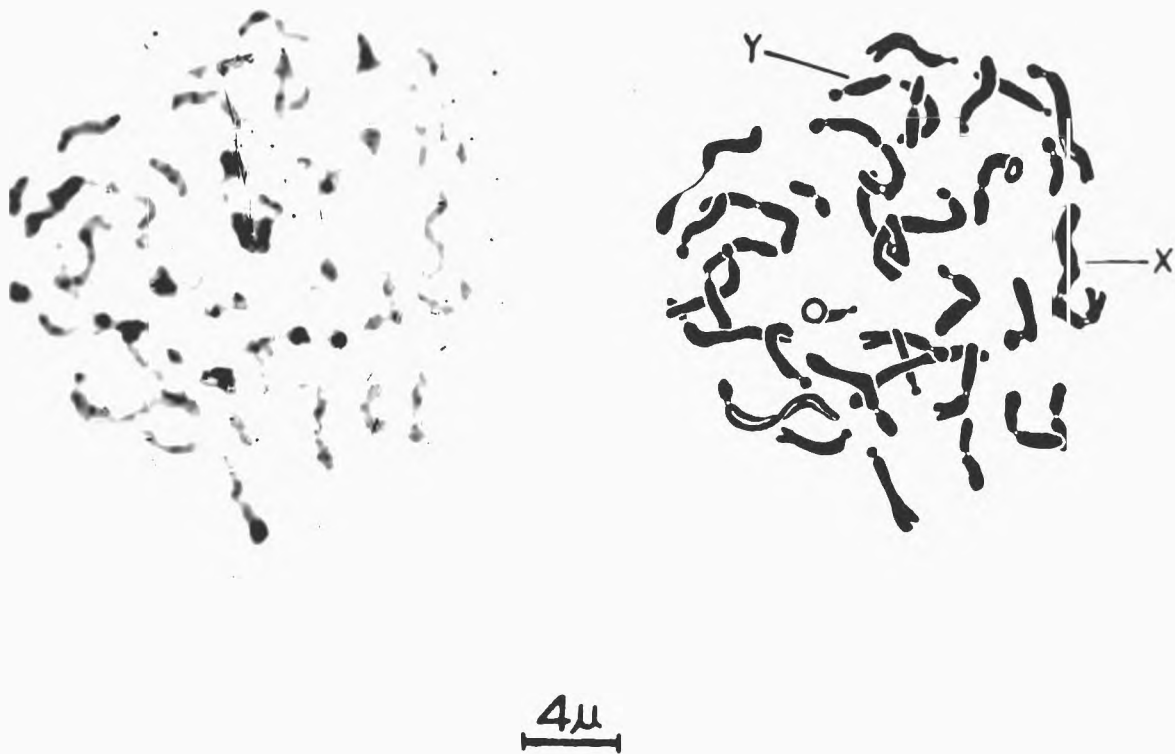


A

Figures 16 and 17. Photomicrograph and camera lucida drawing of mitotic late pro-metaphase plate of the gerbil (Plate A), showing the diploid complement of 34 chromosomes.

Acetic orcein squash preparation re-stained with Ehrlich's haematoxylin.

B



Figures 18 and 19. Photomicrograph and camera lucida drawing of mitotic late pro-metaphase plate of the gerbil (Plate B), showing the diploid complement of 34 chromosomes.

Acetic orcein squash preparation re-stained with Feulgen's leuco-basic fuchsin.

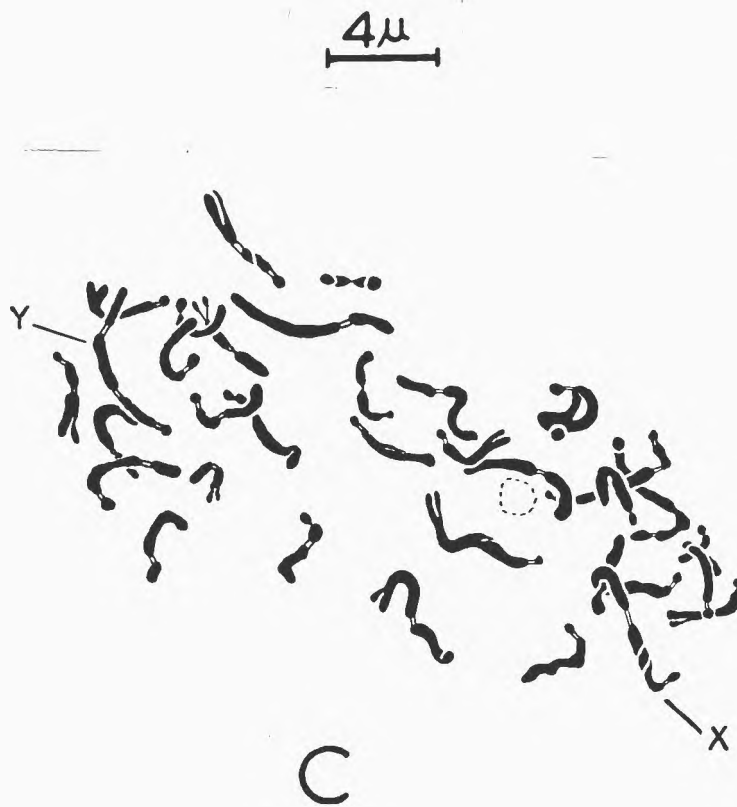


Figure 20. Camera lucida drawing of mitotic late pro-metaphase plate of the gerbil (Plate C), showing the diploid complement of 34 chromosomes.

Acetic orcein squash preparation
re-stained with Erlich's haematoxylin.



Figures 21 and 22. Photomicrograph and camera lucida drawing of mitotic pro-metaphase plate of the gerbil (Plate D), showing the diploid complement of 34 chromosomes.

Acetic orcein squash preparation re-stained with Feulgen's leuco-basic fuchsin.

Chromosome P belongs to a neighbouring plate.



Figure 23. Camera lucida drawing of mitotic metaphase plate of the gerbil (Plate E), showing the diploid complement of 34 chromosomes.

Acetic orcein squash preparation re-stained with Heidenhain's haematoxylin.

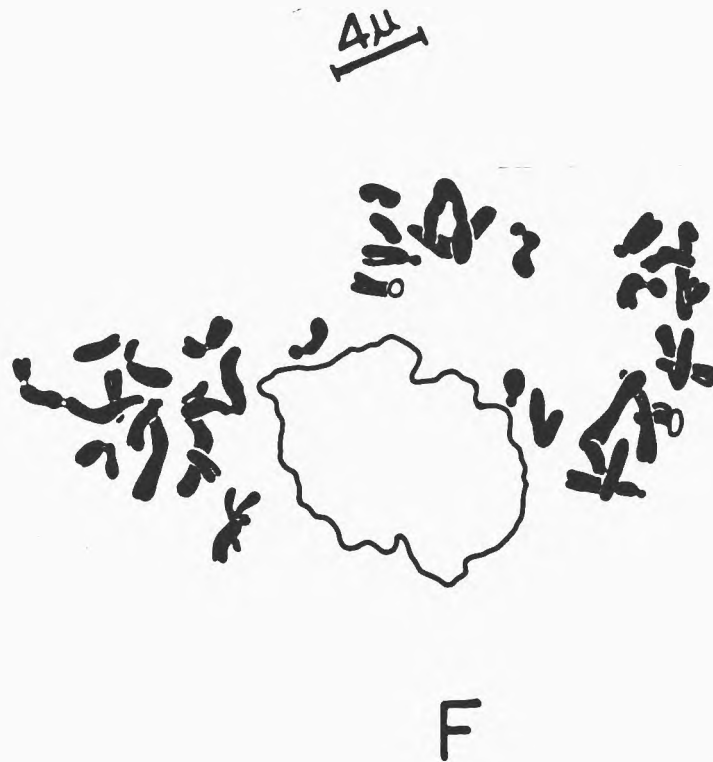


Figure 24. Camera lucida drawing of mitotic metaphase plate of the gerbil (Plate F), showing the diploid complement of 34 chromosomes.

Acetic orcein squash preparation re-stained with Heidenhain's haematoxylin.

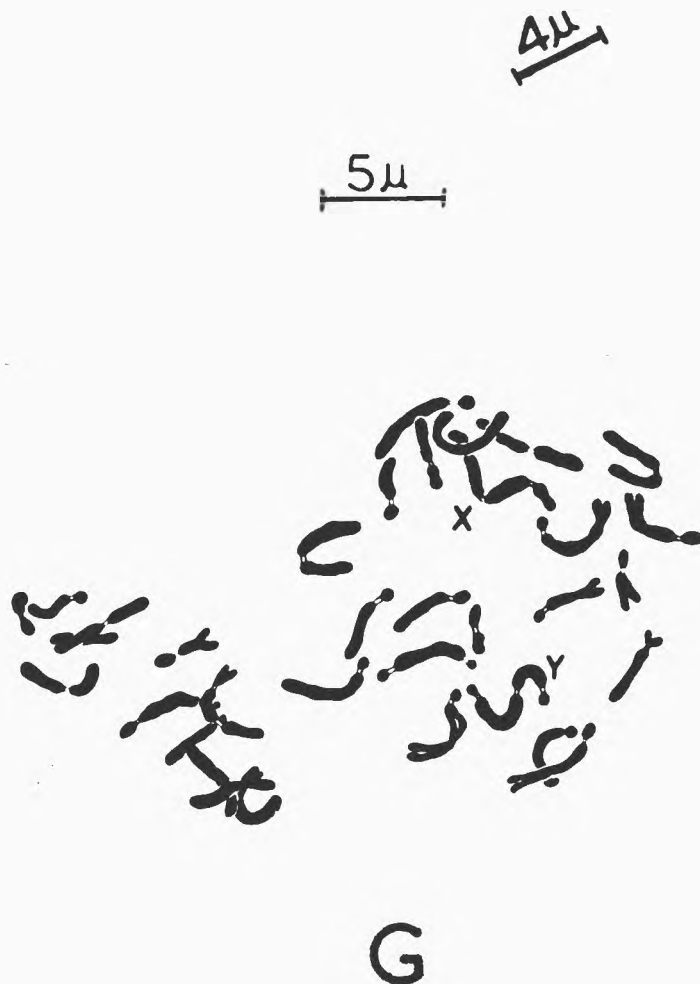


Figure 25. Camera lucida drawing of mitotic metaphase plate of the gerbil (Plate G), showing the diploid complement of 34 chromosomes.

Acetic orcein squash preparation re-stained with Erlich's haematoxylin.



Figure 26. Camera lucida drawing of mitotic metaphase plate of the gerbil (Plate H), showing the diploid complement of 34 chromosomes.

Acetic orcein squash preparation re-stained with Erlich's haematoxylin.



Figure 27. Camera lucida drawing of mitotic metaphase plate of the gerbil (Plate I), showing the diploid complement of 34 chromosomes.

Acetic orcein squash preparation re-stained with Erlich's haematoxylin.

chromosomes separated. The most clearly displayed chromosomes are those of B, C, D and G. With the exception of G, a metaphase plate, the most clearly-separated are those of pro-metaphase nuclei; while the plates showing most crowding of chromosomes are of metaphase nuclei. In the gerbil, we may conclude, it is easier to squash pro-metaphase than metaphase nuclei. While good pro-metaphase nuclei permit analysis of the number, length and morphology of the chromosomes, good metaphase plates often allow only an enumeration of the chromosomes, without providing details of chromosomal shape and length. For this reason, a detailed analysis of the chromosomes has been possible in only nine of the 15 plates, in which a count was made; plates in which discrete focussing failed to sort out the exact relations of overlapping segments were discarded.

The conditions in the gerbil's plates correspond fairly closely with those in the albino rat. Perhaps the gerbil's metaphase plates are slightly more difficult to open out than are those of the rat. One difference is that constrictions are much more clearly visible in metaphasic chromosomes of the gerbil than in metaphasic chromosomes of the rat. This may be due to longer constrictions or to a lesser degree of spiralisation in the gerbil. In both the gerbil and the rat, constrictions are most clearly seen in the pro-metaphasic chromosomes.

When the lengths of the chromosomes in spermatogonial plates are arranged in the manner described for the rat, we obtain a graph of chromosomal lengths in individual plates.

Figs. 28 to 32 are the graphs of the chromosomal lengths of plates A to I. Graphs B, C and D (Figs. 28 and 29) are of pro-metaphase plates; A is the graph of a plate almost at the beginning of metaphase (Fig. 28); while E, F, G, H and I are of metaphase plates (Figs. 30, 31 and 32).

As in the rat, the chromosomes are not paired off, nor is a heteromorphic pair identified in these graphs.

An examination of the graphs reveals that they correspond closely in pattern. Broadly speaking, there is an initial steep drop, a moderate degree of flattening in the intermediate region and a falling-off at the tail of the curve. On closer scrutiny, we see that the curves vary among themselves and that the varieties cluster about two main types of curve. The contrast between the two types of graph is brought out in Fig. 33 in which the length-curves of plates D and E are superimposed. In E, the first part of the graph shows a precipitous descent, while the terminal part slopes gently to the smallest chromosomal lengths. In D, the beginning of the curve, while highly elevated, descends in a less abrupt manner to the intermediate zone of slight flattening, while the terminal part of the graph drops more steeply to the shortest chromosomal lengths.

The E type of curve characterises the metaphase plates (E,F,G,H,I), while the D type of graph illustrates chromosomal length in pro-metaphase plates (A,B,C,D).

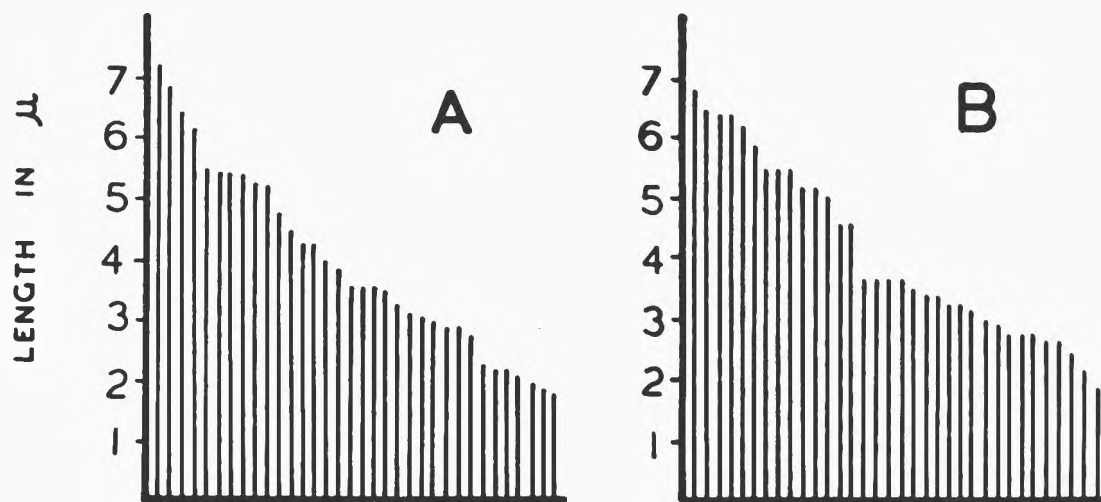


Figure 28. Graphs of the chromosomal complements of Plates A and B of the gerbil.

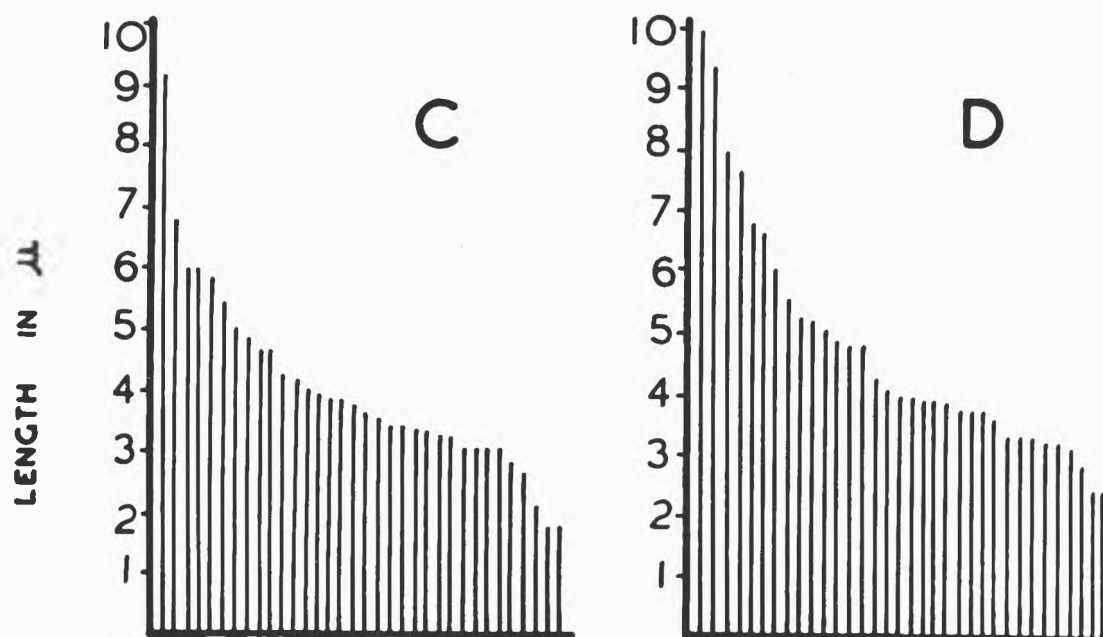


Figure 29. Graphs of the chromosomal complements of Plates C and D of the gerbil.

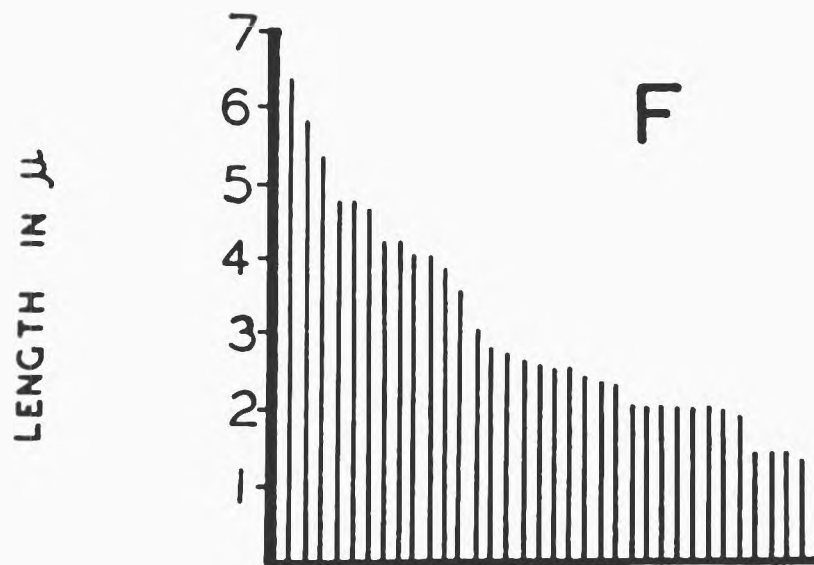
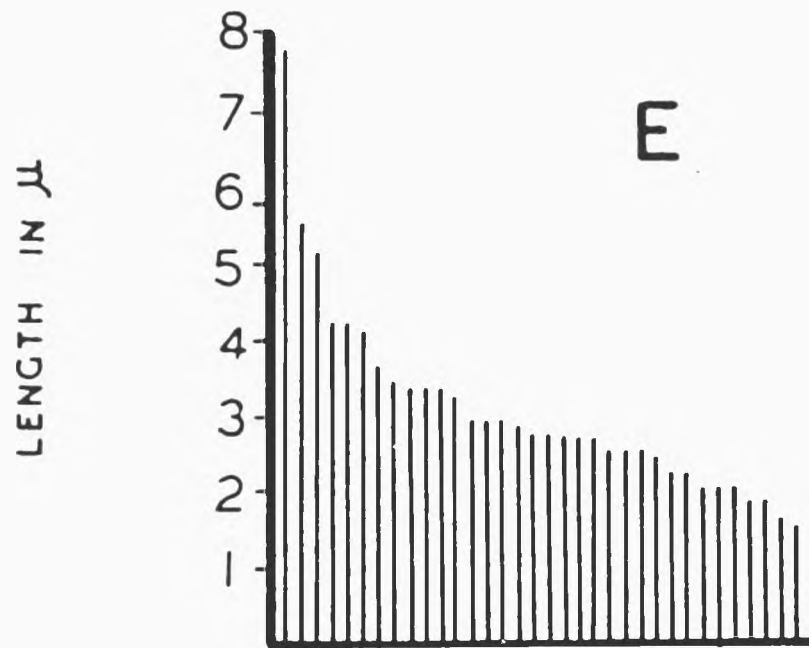


Figure 30. Graphs of the chromosomal complements of Plates E and F of the gerbil.

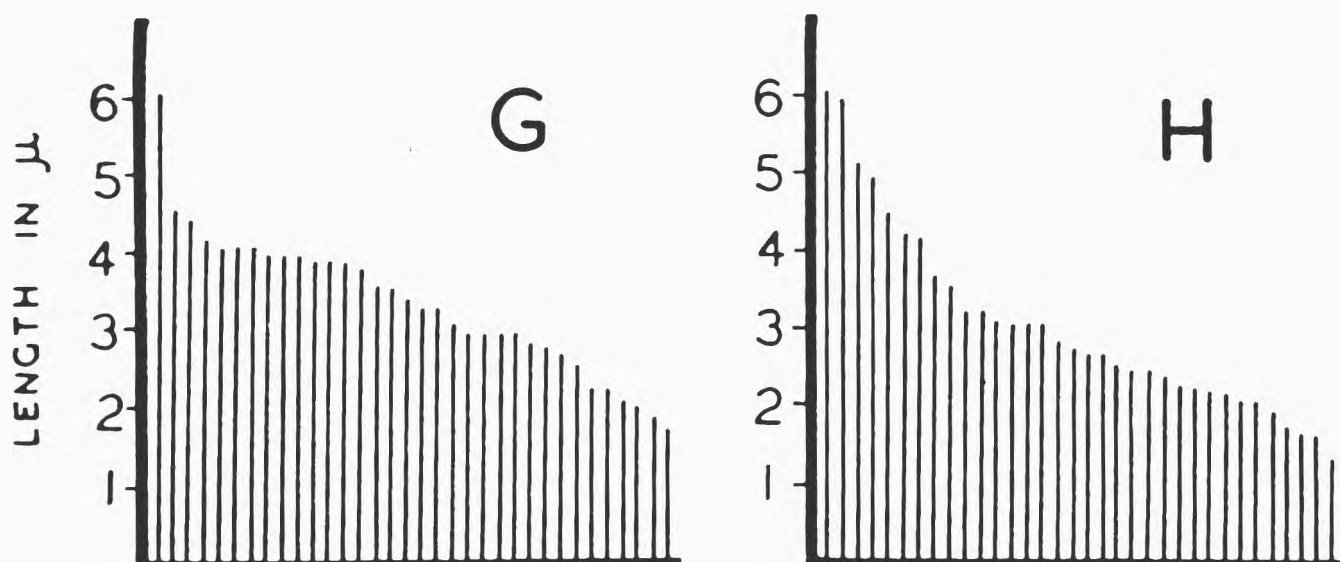


Figure 31. Graphs of the chromosomal complements of Plates G and H of the gerbil.

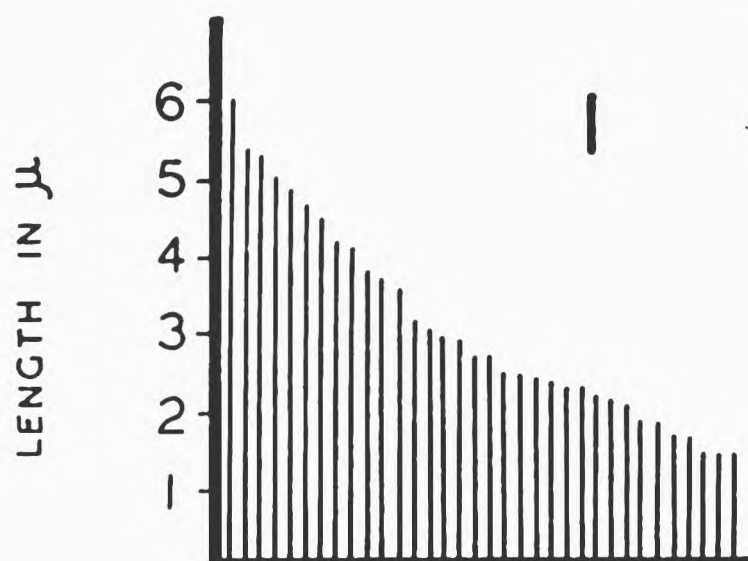


Figure 32. Graph of the chromosomal complement of Plate I of the gerbil.

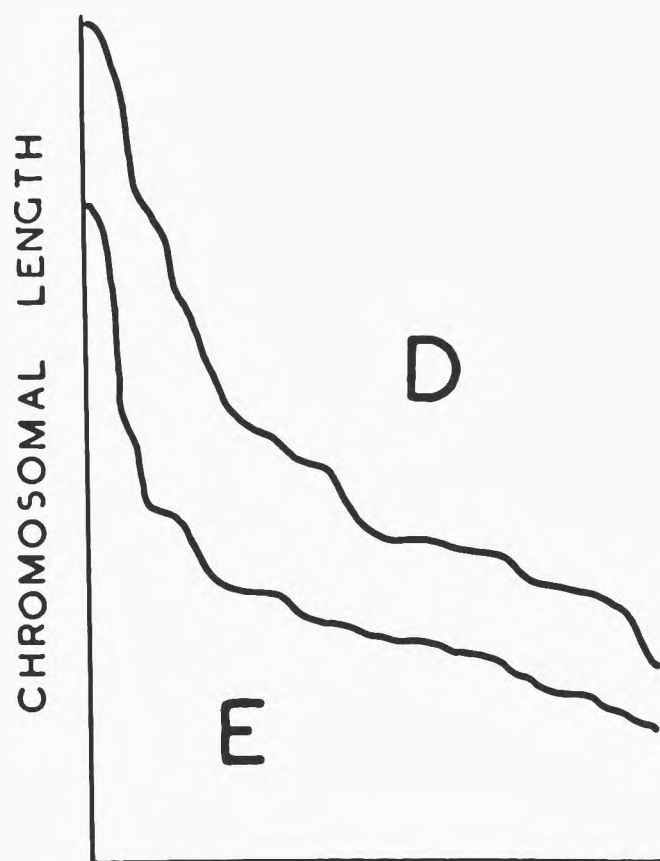


Figure 33. Superimposed length-curves of the chromosomes of Plates D and E.

When we address ourselves to the significance of the two styles of curve, we may interpret the steep, terminal descent in the pro-metaphasic curve (D) as an indication that the smaller chromosomes are ahead of the others in prophasic contraction. Probably, their rate of contraction is faster than that of the other chromosomes.

On the other hand, the intermediate group of chromosomes, while lagging behind the smaller elements, keeps pace with the larger chromosomes, as is shown by the gradual, continuous slope from the first to the intermediate parts of the curve(D).

In the metaphasic curve (E), we see that the intermediate group has contracted much more than the larger elements. In consequence, the abrupt descent from the intermediate to the smaller group has been flattened out, while the gradual curve from the larger to the intermediate groups has been replaced by a precipitous decline of the graph. The few largest chromosomes thus lag behind the intermediate group in their degree of contraction. In H and I, even the largest pair of chromosomes has 'caught up' in degree of contraction, although still remaining longer than the nearest intermediate pairs.

Earlier, I had occasion to suggest that not all the chromosomes gain their minimum length at the same time within metaphase, but I was unable to explain what factors influenced the order of contraction. Now, at least one indication of order in the sequence of contraction has come to light: the

shortest chromosomes tend to contract more rapidly and reach their minimum length first; the intermediate chromosomes next attain their shortest length, while the longest chromosomes are the last ones to contract fully and achieve their minimal length.

In staining capacity, the gerbil's chromosomes offer little difference from those of the albino rat. With most stains, it is impossible to distinguish between pro-metaphase, early, middle or late metaphase, on the staining of the chromosomes alone. The Feulgen reagent, however, gives a more discriminating stain than does haematoxylin and enables one to distinguish faint differences in intensity of staining between late prophase and metaphase and, sometimes, between pro-metaphase and metaphase. Even the Feulgen reagent does not permit visible differences in staining to emerge between early, middle and late metaphasic chromosomes.

CHROMOSOMAL NUMBER.

From my study of squashed, spermatogonial, pro-metaphase and metaphase nuclei, I am satisfied that the number of spermatogonial chromosomes in the gerbil is 34. Therefore, the diploid type-number of chromosomes in the species, T. brantsii draco, is 34.

Confirmation is afforded by the presence of 17 paired threads or tetrads in several squashed pachy-diplotene nuclei. Therefore, the haploid number of chromosomes in the gerbil is 17.

CHROMOSOMAL MORPHOLOGY.

The two chromatids of each chromosome are clearly seen in many cells. Often this feature shows best at one end of the chromosome, where the paired tips may be rather widely separated. Occasionally, the two chromatids of the pair appear coiled about each other one or more times (Figs. 20 and 22). This chromatid relational coiling is more easily seen in the gerbil than in the rat.

At pro-metaphase, a loose coiling is apparent in several chromosomes: according to Darlington (1935), such a "relic spiral" represents the uncoiling internal spiral developed during the previous mitotic division.

Most chromosomes possess only one distinct constriction, appearing as a short achromatic gap. In a few chromosomes, containing more than one constriction each, the anaphase configurations indicate which is the primary (centric) and which the secondary constriction. The positions of constrictions vary in different chromosomes, but, in general, constrictions may be grouped as subterminal or sub-median.

Of the sub-terminal constrictions, the two types described in the albino rat occur in the gerbil, namely those so close to the end of the chromosome that only a small chromatic granule is visible distal to the constriction and, those situated one-tenth to one-fifth of the chromosomal length away from the end. Intergrades occur between these two positions and, between

the sub-terminal constrictions furthest from the end and the sub-median constrictions. Thus, in borderline situations, classification of constrictions as sub-terminal or sub-median is arbitrary. Nevertheless, the numbers of sub-terminal and sub-median constrictions in different plates are fairly constant.

In the best plates, there are 22 chromosomes with sub-terminal and 12 with sub-median constrictions (e.g. Figs. 17, 19 and 20); in a few plates (e.g. Figs. 23 and 27), the numbers are 20 and 14 respectively, one pair presumably being subject to two interpretations.

CHROMOSOMAL LENGTH AND SIZE RANGE.

A comparison of graphs E, F, G, H and I (Figs. 30, 31 and 32) indicates the size range for metaphasic chromosomes in any part of the series. By such a comparison, a composite graph has been drawn of the maximum and minimum lengths of the chromosomes at corresponding positions in the series (Fig. 34).

The 34 chromosomes of the gerbil range in length from 1.4-1.9 μ , the size range of the smallest, to 6.0-7.9 μ , the length of the largest chromosome. The longest chromosome in the gerbil is thus appreciably longer than the longest pair in the rat (3.9-4.4 μ). On the other hand, the shortest chromosome in the gerbil is not as small as the smallest pair in the rat (0.8-0.9 μ). All the intermediate chromosomes (Nos. 3 - 32) in the gerbil are grouped between 1.5 μ and 5.3 μ , as against the rather narrower distribution of the rat's chromosomes (pairs 2 - 19) between

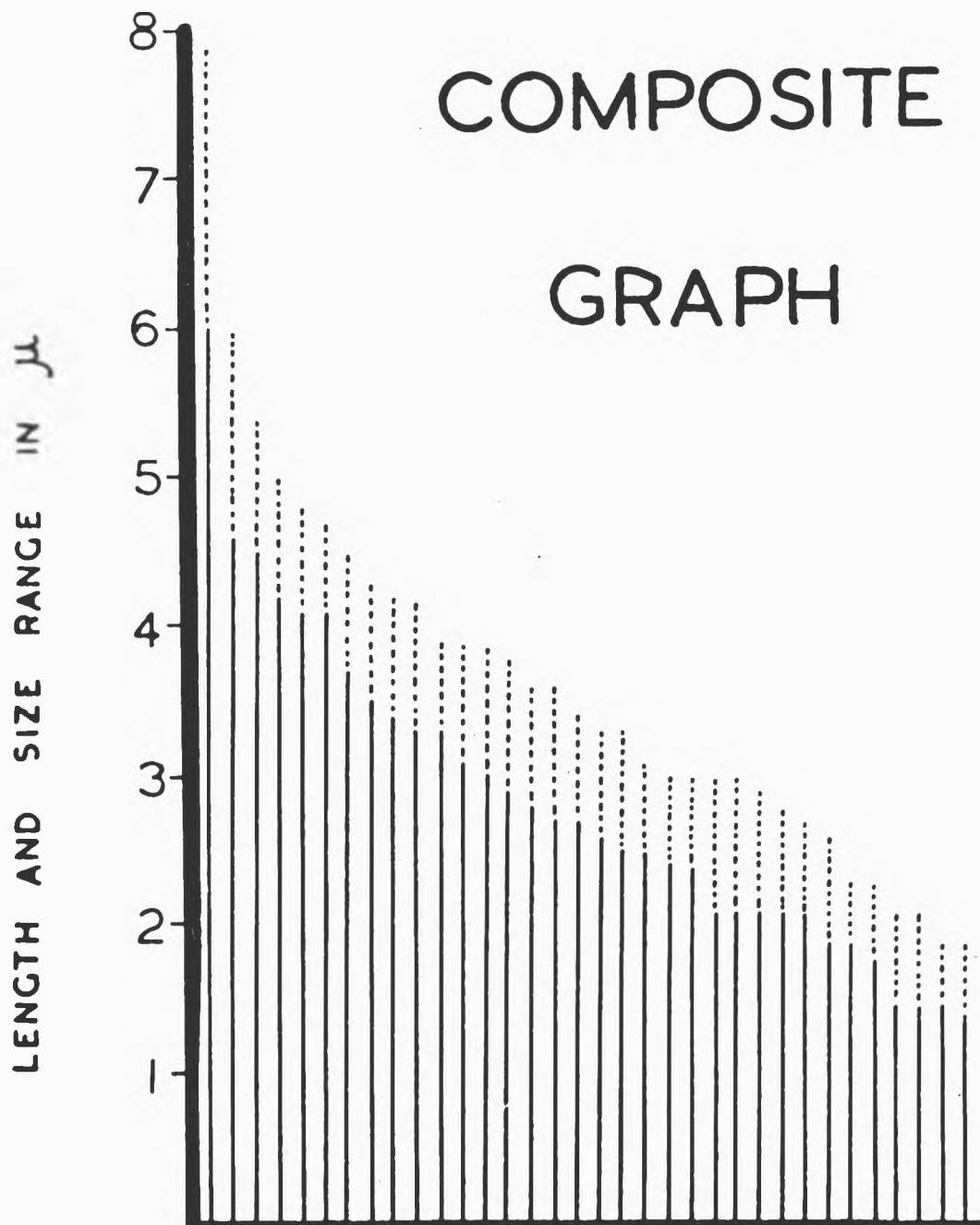


Figure 34. Type-graph of the chromosomal complement of the gerbil.

0.8 μ and 3.9 μ .

As in the composite graph of the albino rat, the graph of the gerbil's chromosomes must be interpreted with discrimination, in view of possible variations in the linear order of chromosomes. The main conclusion to be drawn from the graph is the magnitude of the size-range for any part of the linear series.

The ranges vary in the chromosomal series, much as they do in the albino rat. The absolute value of the range is smallest in the six shortest chromosomes (Nos. 29-34), where its average value is 0.5 μ . In the intermediate group (chromosomes 3-28), the average range is 0.7 μ , while in the longest pair (chromosomes 1-2), the size range is greatest, averaging 1.6 μ . There is no marked exception, unlike conditions in the albino rat, where the X-chromosome, though one of the longer elements, has a very small range.

When the relative range is calculated by expressing the average range as a percentage of the chromosomal length, the intermediate and smaller groups of chromosomes have more or less the same relative ranges (approximately 24% of minimal length or 19% of maximal length). On the other hand, the relative range of the largest pair is 33% of minimal and 25% of maximal length. The longest chromosomes therefore have greater size ranges, both absolutely and relatively, than the others; there is no distinction in this regard between the intermediate

and smaller groups.

The behaviour of the longest pair of chromosomes may be understood as the consequence of their tardy contraction; some metaphase plates have been fixed and stained before the complete contraction of the longest chromosomes, while others have been arrested after these chromosomes have attained their minimal length.

THE CHARACTERISATION OF THE GERBIL'S CHROMOSOMES.

We have considered several properties of the gerbil's chromosomes: their number, morphology, length and size ranges. It remains to correlate these sets of information in order to characterise the chromosomal complement. By a close study of camera lucida drawings and measurements of the chromosomes, controlled by discriminative microscopic study of the plates, I paired off the entire complement of each individual plate and thus characterised a number of isolated chromosomal complements (Table 5). By comparing these individual characterisations, as well as by a study of the composite graph, I have arrived at the following species characterisation.

The largest two chromosomes are readily distinguished on their size ^{and} morphology. They have a characteristic pattern of constrictions as illustrated in Fig. 35. One constriction, consistently present, is sub-median in position; from studies on anaphase figures, it is clearly the centric constriction. In addition, each arm is divided into a longer central segment

TABLE 5.

THE CHARACTERISATION OF THE CHROMOSOMES OF INDIVIDUAL
SPERMATOGONIA.

(Index letters of plates correspond with index letters of drawings
and graphs)

(Sm - sub-median constriction;
St - sub-terminal constriction.)

PLATE NO:	CONSTRICTIONS OF CHROMOSOMAL PAIRS																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
A	Sm	Sm	St	Sm	St	Sm	St	St	St	St	Sm	St	St	St	St	St	Sm
B	Sm	Sm	St	Sm	St	Sm	St	St	St	St	Sm	St	St	St	St	St	Sm
C	Sm	Sm	St	Sm	Sm	St	St	St	St	St	Sm	St	St	St	St	Sm	St
D	Sm	Sm	St	?	St	St	St	St	St	St	Sm	St	St	St	St	St	Sm
E	Sm	Sm	Sm	St	Sm	St	St	St	St	St	Sm	St	St	Sm	St	St	Sm
F	Sm	Sm	Sm	?St	Sm	?	St	St	St	Sm	St	St	St	St	St	Sm	St
G	Sm	Sm	Sm	St	St	?St	St	St	?	Sm	St	St	St	St	St	St	Sm
H	Sm	?Sm	St	?St	St	St	?	St	Sm	St	Sm	?	St	Sm	Sm	St	Sm
I	Sm	Sm	Sm	St	Sm	St	St	St	St	St	Sm	St	St	St	Sm	Sm	St

Figure 35. Schema of the sex-chromosomes in mitosis.

- Key: D - differential segment.
- D₁ - part of differential segment in longer arm.
- D₂ - part of differential segment in shorter arm.
- P₁ - longer plasmosome-forming, pairing segment.
- P₂ - shorter, second pairing segment.
- 1, 11 - secondary constrictions.
- 111 - primary (centric) constriction.

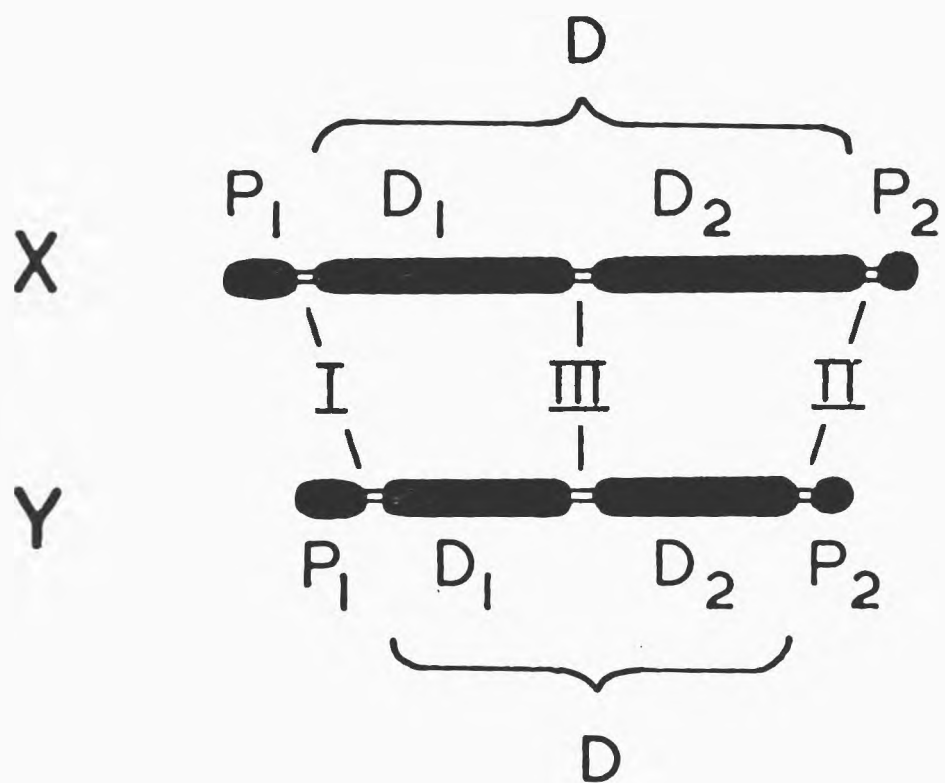


Figure 35. Schema of the sex-chromosomes in mitosis.

and a shorter distal or terminal segment by a secondary constriction. In one arm, this constriction is noticeably further from the extremity, thus cutting off a longer distal segment than in the other arm. We have then a chromosome divided into two arms and each arm sub-divided into two segments, making four segments in all, delimited by three constrictions. To simplify later discussion, the segments will be referred to as follows:

P_1 - the longer distal segment.

D_1 - the segment between P_1 and the centric constriction.

P_2 - the shorter distal segment.

D_2 - the segment between P_2 and the centric constriction.

Although the two largest chromosomes in the complement both possess this pattern of constrictions, there are generally significant differences in length between them. Their size-ranges do not even overlap, the range for the largest element being 6.0-7.9 μ , while that of the second largest is 4.6-6.0 μ .

On grounds of this inequality in length and, because the rest of the complement can be arranged in homomorphic pairs, it is inferred that the two largest chromosomes constitute a heteromorphic pair. It will be shown below that the two largest chromosomes are not completely homologous, each to each, and that, during meiosis, they exhibit many peculiar properties, characteristic of the sex-chromosomes of other mammals. It is concluded, therefore, that these large heteromorphic chromosomes

are the sex-chromosomes of the gerbil. It has not been possible to determine which of the two is the X-chromosome, i.e. the element which may be expected to occur twice in the diploid complement of female gerbils. However, in all mammals in which males and females have been studied so far, the X has been generally larger, occasionally equal to, but never smaller than the Y. By analogy with these other mammals, it may be accepted that the largest chromosome in the gerbil's complement is the presumptive X and the second largest - the presumptive Y.

We may now further elaborate the nomenclature suggested above by distinguishing P_1X , D_1X , P_2X and D_2X from P_1Y , D_1Y , P_2Y and D_2Y .

Both members of the second largest pair of chromosomes possess a sub-median centric constriction, while one arm usually contains a sub-terminal secondary constriction. The size-ranges for chromosomes 3 and 4 are $4.5-5.3\mu$ and $4.2-5.0\mu$. Because of the considerable overlap between the two ranges, the size range for the pair is best expressed as the combined total range i.e. $4.2-5.3\mu$.

At the other end of the linear series, the two smallest chromosomes generally have sub-median constrictions and may be coupled as pair 17. Their size-range is:

Chromosome No. 33: $1.5 - 1.9\mu$.

Chromosome No. 34: $1.4 - 1.9\mu$.

Pair No. 17
(Combined range): $1.4 - 1.9\mu$.

In several plates, this pair with the sub-median constriction is the second smallest in the complement, there being a pair with sub-terminal constrictions at the lower end of the series. Generally, however, the pair with sub-terminal constrictions is the 16th pair, having a combined size-range of 1.5-2.1 μ .

Thus, the two largest and the two smallest pairs are readily identified and characterised. Between these two extremes are 13 pairs of chromosomes. They lie in that part of the length-graph where there is a relative flattening, as we have seen above, and therefore it is more difficult to pair them off accurately.

The third biggest pair of chromosomes with sub-median constrictions may be recognised without much difficulty. It may lie 3rd or 4th in the linear series of pairs and its combined size-range in metaphase plates is 4.1-4.8 μ .

The preceding pair sometimes exchanges positions with the largest pair of chromosomes containing a sub-terminal constriction. The latter pair, No. 4, has a combined size-range in metaphase plates of 3.5-4.6 μ .

Pairs 5 and 6 again are interchangeable in the linear series; one pair has sub-terminal, the other sub-median, constrictions. The size-range of the pair with sub-terminal constrictions (the second biggest pair in the complement with constrictions in this position) is 3.3-4.0 μ ; while the range

for the other pair, namely that with sub-median constrictions, is 3.4-4.1 μ . The latter will therefore be designated pair 5 and the former pair 6.

Then follows a group of four successive pairs (Nos. 7 - 10) with sub-terminal constrictions. The combined size-range of these four pairs is 2.5-3.9 μ , and they are characterised in a group. The method of group characterisation, as tested on the rat, best overcomes such difficulties as the variable linear order of chromosomes and the arbitrary classification of constrictions as sub-terminal or sub-median.

The 11th pair of chromosomes possesses a sub-median constriction in almost every plate and a size range of 2.3-2.7 μ . Its members constitute the second smallest pair of chromosomes with sub-median constrictions.

The 12th to 15th pairs have sub-terminal constrictions. In one or two plates, one of these pairs (14 or 15) has been described as possessing a sub-median constriction, for reasons already indicated. The size range for this group of four pairs is 1.8-3.0 μ .

The 16th and 17th pairs have already been characterised.

CONCLUSION.

Arising from our composite graph and from characterisations of nine individual spermatogonial plates, we may now summarise the composite species-characterisation as follows:

<u>CHROMOSOMAL PAIR OR GROUP.</u>	<u>LENGTH IN μ</u>	<u>POSITION OF CENTRIC CONstriction.</u>
Pair 1 (X (Y	6.0 - 7.9 4.6 - 6.0	Sub-median
Pair 2	4.2 - 5.3	Sub-median
Pair 3	4.1 - 4.8	Sub-median
Pair 4	3.5 - 4.6	Sub-terminal
Pair 5	3.4 - 4.1	Sub-median
Pair 6	3.3 - 4.0	Sub-terminal
Pairs 7 - 10	2.5 - 3.9	Sub-terminal
Pair 11	2.3 - 2.7	Sub-median
Pairs 12 - 15	1.8 - 3.0	Sub-terminal
Pair 16	1.5 - 2.1	Sub-terminal
Pair 17	1.4 - 1.9	Sub-median

TABLE 6.

CHARACTERISATION OF THE CHROMOSOMES OF THE GERBIL.

In the above account of the chromosomal complement of the gerbil, attention has been drawn to a number of contrasts between the gerbil and the albino rat. The following table

summarises the salient differences between the two species:

<u>FEATURE</u>	<u>ALBINO RAT</u>	<u>GERBIL</u>
Chromosomal Number	42	34
Number of Chromosomes with Sub-terminal Constrictions	28 (or 66.7%)	22 (or 64.7%)
Number of Chromosomes with Sub-median Constrictions	14 (or 33.3%)	12 (or 35.3%)
Number of Chromosomal Arms	56	48 ϕ
Position of X in linear series	5th	1st
Position of Y in linear series	34th	2nd
Total size range	0.8 - 4.4 μ	1.4 - 7.9 μ
Size range: Pair 1	3.6 - 4.4 μ	4.6 - 7.9 μ
Size range: smallest pair	0.8 - 0.9 μ	1.4 - 1.9 μ

TABLE 7.

COMPARISON BETWEEN THE CHROMOSOMES OF THE GERBIL AND THE ALBINO RAT.

- ϕ One of the pairs of chromosomes with sub-terminal constrictions has a relatively long minor arm, giving two additional arms in the total, i.e.
 $(20 \times 1) + (2 \times 2) + (12 \times 2) = 48$

CHAPTER V.

CHROMOSOMAL EVOLUTION IN THE
RODENTS AND THE EVOLUTION OF
THE CHROMOSOMAL COMPLEMENT
OF THE GERBIL.

INTRODUCTION.

In the opening section of this thesis, I stated that all facts would be considered from several points of view. The now characterised chromosomal complement of the gerbil provides a set of facts the significance of which remains to be explored. In this chapter, attention will be directed to the historical or evolutionary aspect of the gerbil's chromosomes.

We may presume that the present karyotype of the gerbil is the end-product of a long line of evolutionary change from an ancestral karyotype. The problem, then, is to consider what deductions can be drawn about the direction of karyotypic evolution, from a study of the chromosomes of the gerbil, a living species. Considered alone, the chromosomal complement of the gerbil is practically meaningless; it acquires import, however, when we apply the technique of comparative anatomy. Let us turn aside for a moment to examine the procedure of the comparative anatomist.

From a wealth of anatomical detail, the comparative anatomist selects specific features as indicators of relationship

and descent. The manifestations of these features in various groups are compared. The information thus gleaned is supplemented by a study of anatomical vestiges, of embryonic traces and of fossil forms. From all these sources, the comparative anatomist comes to distinguish between recent acquisitions and ancient relics and he limns a common ancestral type for the group. By tracing many such lines, he can draw a phylogenetic tree which may be confirmed to a high degree by genetical, zoogeographical, physiological and serological data.

In applying now the procedures of the comparative anatomist to cytogenetics, one has to depend almost exclusively on comparison, since, from the nature of the medium in which we are operating, we are denied such evidence as fossils, vestiges and embryonic traces. As supplements to the comparative data, we can make use of generalisations about the way in which chromosomes may vary (although, in the last analysis, these generalisations are themselves often based on comparison!).

Before comparing the chromosomes of the gerbil with those of other animals, it is well to remember that this entire excursion into cyto-phylogeny is based on the idea that the chromosomal complement is a specific constant. It will not be out of place to digress briefly and consider the rise of the concept of the species-specificity of the chromosomal complement. Since the number of chromosomes is most easily determined, the ensuing discussion will concentrate on the origin of the idea that chromosomal number is a specific constant.

THE CHROMOSOMES AS SPECIFIC CONSTANTS.

The fundamental researches of the period 1840-1880 traced the broad outlines of cell-theory and the principles of genetic continuity. The cell-nucleus, which had been recognised and described by Robert Brown in 1831, was shown by the researches of Remak (1841) and Kolliker (1845) to take part in the mechanism of cell-division. Subsequently, the essential rôle of the nucleus became known and Oscar Hertwig was able to demonstrate in 1875 that the essence of fertilisation lay in the fusion of the nuclei of spermatozoon and ovum. W. Flemming first correctly established a specific number of chromosomes for a definite species, the salamander (1882). In the same paper, he reported an attempt to count the chromosomes of man.

It is important to note that Flemming's work came before the conception of the nucleus as the physical basis of heredity (1884-1885). The specificity of chromosomal complements, therefore, may be studied independently of any preconceptions about the relation of the chromosomes to the mechanism of inheritance.

The work of Flemming led to the view that each species possesses a type-number of chromosomes characteristic and constant in most of the cell-nuclei of the organism. This generalisation was found not to hold, however, for the germinal cells. In 1883, von Beneden discovered that the ovum-nucleus and the sperm-nucleus in Ascaris each contained half the number

of chromosomes present in nuclei of other body-cells. These germ-cell nuclei alone constantly departed from the number found in other body-cells of the species: and even this exception was soon successfully explained, when Hertwig and Boveri described the phenomena of meiosis.

Apart from this special mechanism, the idea gained ground that each species possessed a characteristic number of chromosomes, constant for all the non-meiotic cells of the organism. In this way, the study of the chromosomes was added to the armoury of the comparative anatomist, in time to take its place as a valid source of inference in taxonomy and evolution.

If, now, we wish to compare the chromosomes of the gerbil with those of other species, we need first enquire into the data available as a basis for the comparison.

THE CHROMOSOMAL NUMBERS OF RODENTS.

As the gerbil belongs to the Rodentia and, as this order is the only mammalian order in which a large number of species has been characterised karyologically, a brief review of the information pertaining to the chromosomes of rodents is not out of place here. Since chromosomal number is the most commonly determined feature of the complement, this aspect of the problem will receive prior attention.

Within the rodents, the chromosomes of 82 species

have been enumerated, according to the latest figures I have been able to compile. This total includes the gerbil. The 82 species are not, however, broadly representative of all rodent groups: some families are not represented at all, while, in others, many species have been studied.

The following Table shows the distribution among rodent families of the species whose chromosomes have been enumerated. The diploid number is given in the column headed ' $2n$ '. In the column headed 'N.F.', the fundamental number (number of chromosomal arms) is given; the fundamental numbers will be referred to later in the present chapter (page 128 et. seq.)

TABLE 8.

TAXONOMIC DISTRIBUTION OF CHROMOSOMAL NUMBERS AMONG THE RODENTIA.

<u>SUBORDER:</u>	<u>SUPERFAMILY:</u>	<u>FAMILY:</u>	<u>GENUS AND SPECIES:</u>	<u>2n</u>	<u>N.F.</u>
Sciuromorpha	Sciuroidea	<u>Sciuridae</u>	<u>Citellus columbianus columbianus</u>	32	?
			<u>Citellus tridecemlineatus</u>	50/54	?
			<u>Glaucomys volans volans</u>	52	54
			<u>Sciurus carolinensis carolinensis</u>	48	68-76
			<u>Sciurus carolinensis leucotus</u>	28	56
			<u>Sciurus niger limitus</u>	62	80
			<u>Sciurus niger rufiventer</u>	62	80
			<u>Sciurus vulgaris</u>	40	?
			<u>Tamiasciurus hudsonicus</u>	28	?
	Geomyoidea	<u>Geomyidae</u>	<u>Geomys breviceps breviceps</u>	784	100
		<u>Hetero- myidae</u>	<u>Dipodomys merriami merriami</u>	786	100
			<u>Perognathus fallax fallax</u>	44	60
	Aplodontoidea	<u>Aplodonti- dae</u>	--	-	-
	Castoroidea	<u>Castoridae</u>	--	-	-
	Anomaluroidea	<u>Anomaluri- dae</u>	--	-	-
		<u>Pedetidae</u>	--	-	-

TABLE 8 (CONTINUED)

<u>SUBORDER:</u>	<u>SUPERFAMILY:</u>	<u>FAMILY:</u>	<u>GENUS AND SPECIES:</u>	<u>2n</u>	<u>N.F.</u>
Myomorpha	Muroidea	<u>Muridae</u>	<u>Apodemus agrarius agrarius</u>	48	48
			<u>Apodemus agrarius ningpoensis</u>	48	48
			<u>Apodemus flavicollis</u>	48	48
			<u>Apodemus geisha</u>	46/48	46/48
			<u>Apodemus hebridensis</u>	48	48
			<u>Apodemus semotus</u>	48	48
			<u>Apodemus speciosus ainu</u>	48	48
			<u>Apodemus speciosus speciosus</u>	50	50
			<u>Apodemus sylvaticus</u>	48	48
			<u>Bandicota culturatus</u>	44	?
			<u>Bandicota nemorivaga</u>	46	68
			<u>Micromys minutus minutus</u>	68	70
			<u>Micromys minutus takasagoensis</u>	68	70
			<u>Mus caroli</u>	40	40
			<u>Mus formosanus</u>	40	40
			<u>Mus molossinus</u>	40	40
			<u>Mus musculus</u>	40	40
			<u>Mus wagneri albula</u>	40	40
			<u>Rattus confucianus culturatus</u>	46	46
			<u>Rattus fulvescens coxinga</u>	42/46	42/46
			<u>Rattus losea</u>	42	42
			<u>Rattus norvegicus albinus</u>	42	42/56
			<u>Rattus norvegicus norvegicus</u>	42	42
			<u>Rattus rattus alexandrinus</u>	42	42
			<u>Rattus rattus rattus</u>	42	42
			<u>Rattus rattus rufescens</u>	42	42

TABLE 8 (CONTINUED)

<u>SUBORDER:</u>	<u>SUPERFAMILY:</u>	<u>FAMILY:</u>	<u>GENUS AND SPECIES:</u>	<u>2n</u>	<u>N.F.</u>
Myomorpha (Contd.)	Muroidea (Contd.)	<u>Cricetidae</u>	<u>Cricetus auratus</u>	38/44	76
		<u>Sub-family</u>	<u>Cricetulus griseus</u>	14	?
		<u>Cricetinae</u>	<u>Neotoma floridana attwateri</u>	52	58
			<u>Peromyscus boylei attwateri</u>	48	±60
			<u>Peromyscus californicus</u>		
			<u>californicus</u>	48	±60
			<u>Peromyscus californicus insignis</u>	48	±60
			<u>Peromyscus eremicus eremicus</u>	60	±60
			<u>Peromyscus eremicus faterculus</u>	58	±60
			<u>Peromyscus gossypinus palmarius</u>	48	±60
			<u>Peromyscus leucopus</u>		
			<u>noveboracensis</u>	48	±60
			<u>Peromyscus leucopus texanus</u>	48	±60
			<u>Peromyscus maniculatus</u>		
			<u>artemisiae</u>	48	±60
			<u>Peromyscus maniculatus bairdii</u>	48	±60
			<u>Peromyscus maniculatus blandus</u>	48	±60
			<u>Peromyscus maniculatus gambeli</u>	48	±60
			<u>Peromyscus maniculatus hollesteri</u>	52	±60
			<u>Peromyscus maniculatus</u>		
			<u>maniculatus</u>	48	±60
			<u>Peromyscus maniculatus osgoodi</u>	48	±60
			<u>Peromyscus maniculatus</u>		
			<u>sonoriensis</u>	48	±60
			<u>Peromyscus polionotus</u>		
			<u>polionotus</u>	48	±60
			<u>Peromyscus truei truei</u>	48	±60
			<u>Sigmodon hispidus texianus</u>	54	54
			<u>Tscherskia triton</u>	30	?

TABLE 8 (CONTINUED)

<u>SUBORDER:</u>	<u>SUPERFAMILY:</u>	<u>FAMILY:</u>	<u>GENUS AND SPECIES:</u>	<u>2n</u>	<u>N.F.</u>
Myomorpha (Contd.)	Muroidea (Contd.)	<u>Cricetidae</u>	<u>Arvicola scherman</u>	36	62
		Sub-family	<u>Eothenomys melanogaster</u>	56	56
		<u>Microtinae</u>	<u>Evotomys (Clethrionomys)</u>		
			<u>bedfordiae</u>	56	56
			<u>Evotomys (Clethrionomys)</u>		
			<u>glareolus</u>	56	56
			<u>Evotomys (Clethrionomys)</u>		
			<u>rutilus mikado</u>	28	?
			<u>Microtus agrestis</u>	50	?
			<u>Microtus arvalis</u>	46	58
			<u>Microtus kikuchii</u>	28	56
			<u>Microtus montebelli</u>	32	54
			<u>Microtus nivalis</u>	56	56
			<u>Microtus orcadensis</u>	42	?
			<u>Microtus ratticeps</u>	30/46	?
			<u>Microtus townsendii</u>	50	50
		Sub-family			
		<u>Gerbillinae</u>	<u>Tatera brantsii draco</u>	34	48
		<u>Spalacidae</u>	--	-	-
		<u>Rhizomyidae</u>	--	-	-

TABLE 8 (CONTINUED)

<u>SUBORDER:</u>	<u>SUPERFAMILY:</u>	<u>FAMILY:</u>	<u>GENUS AND SPECIES:</u>	<u>2n</u>	<u>H.F.</u>	
Nyomorpha (Contd.)	Gliroidea	<u>Gliridae</u>	<u>Dryomys nitedula intermedius</u>	48	72/84	
			<u>Eliomys quercinus</u>	52	72/84	
			<u>Glis glis glis</u>	62	96	
			<u>Muscardinus avellanarius</u>	48	72/84	
			<u>Platacanthomyidae</u>	--	-	-
			<u>Seleviniidae</u>	--	-	-
	Dipodoidea	<u>Dipodidae</u>	--	-	-	
		<u>Zapodidae</u>	--	-	-	
	Hystricomorpha	Cavioidea	<u>Caviidae</u>	<u>Cavia cobaya</u>	64	66
			<u>Dasyproctidae</u>	--	-	-
<u>Dinomyidae</u>			--	-	-	
<u>Hydrochoeridae</u>			--	-	-	

TABLE 8 (CONTINUED)

<u>SUBORDER:</u>	<u>SUPERFAMILY:</u>	<u>FAMILY</u>	<u>GENUS AND SPECIES:</u>	<u>2n</u>	<u>N.F.</u>
Hystricomorpha (Contd.)	Erethizontoidea	<u>Erethizontidae</u>	--	-	-
		<u>Hystricidae</u>	<u>Hystrix cristata</u>	48	?
	Octodontoidea	<u>Capromyidae</u>	<u>Myocastor coypus</u>	42	?
		<u>Abrocomidae</u>	--	-	-
		<u>Ctenomyidae</u>	--	-	-
		<u>Echimyidae</u>	--	-	-
		<u>Octodontidae</u>	--	-	-
		<u>Petromyidae</u>	--	-	-
		<u>Thryonomyidae</u>	--	-	-
	Chinchilloidea	<u>Chinchillidae</u>	--	-	-

TABLE 8 (CONTINUED)

<u>SUBORDER:</u>	<u>SUPERFAMILY:</u>	<u>FAMILY:</u>	<u>GENUS AND SPECIES:</u>	<u>2n</u>	<u>N.F.</u>
Incertae sedis	Bathyerg- oidea	<u>Bathyerg-</u> <u>idae</u>	--	-	-
	Ctenodactyl- oidea	<u>Ctenodactyl-</u> <u>idae</u>	--	-	-

From Table 8 we see that of the three sub-orders of rodents, only two, the Sciuromorpha and the Myomorpha, have been substantially sampled. The Hystricomorpha still remain largely to be investigated.

Of the five superfamilies of sciuromorphs, only two - the Sciuroidea and the Geomyoidea - have been sampled.

Of the three superfamilies of the myomorphs, two - the Muroidea and the Gliroidea - have been studied cytogenetically and the Myomorpha is undoubtedly the best-documented group of the rodents and, incidentally, of the mammals. Yet, the 63 muroid species and sub-species are drawn from only 16 genera of two families.

Thus, only 9 of 32 extant families of rodents have been sampled.

The muroid superfamily includes four families, the Muridae, the Cricetidae, the Spalacidae and the Rhizomyidae: the last two are insignificant families, having 1 and 3 genera respectively, and are unexplored cytologically. The former two families, having 91 and 96 living genera respectively, have been studied in some detail.

The Muridae contains 6 sub-families, 5 of which are small, while one, the Murinae, is large. Only the Murinae have been studied cytologically: in this group, the diploid chromosomal numbers range from 40 to 68.

The Cricetidae contains five sub-families, of which the two largest, the Cricetinae and the Microtinae, have previously been sampled, while the gerbil is the first animal to have been studied in the third largest sub-family, the Gerbillinae.

In the Cricetinae, the diploid numbers range from 14 to 60. The diploid numbers in the Microtinae range from 28 to 56. In the Gerbillinae, the diploid chromosomal number of Tatera brantsii draco is 34 and thus falls in the lower part of the total Cricetid range of 14 to 60.

The sciuroid superfamily contains only a single family, the Sciuridae. The diploid chromosomal numbers in the Sciuridae range from 28 to 62.

Only one of the three gliroid families, namely the Gliridae, has been studied cytogenetically. Among four species, the chromosomes range in number from 48 to 62.

Both families of the Geomyoidea have been sampled: a species of the Geomyidae contains 784 chromosomes, while in two species of the Heteromyidae, the chromosomes number 44 and 786.

One species each in three Hystricomorph families has been studied: a caviid species contains 64, a hystricid - 48 and a capromyid - 42 chromosomes.

We may summarise all the rodent chromosomal numbers hitherto determined in the following Table (9), according to families and genera. (The figure in brackets after the name of

- 10 5 -

each genus is the number of species in that genus on which
the quoted chromosomal numbers are based.)

TABLE 9.

CHROMOSOMAL NUMBERS IN FAMILIES AND GENERA OF RODENTS.

<u>FAMILY</u>		<u>GENERA</u>	
<u>Sciuridae</u>	28 - 62	<u>Sciurus</u>	(5) 28 - 62
		<u>Tamasciurus</u>	(1) 28
		<u>Citellus</u>	(2) 32 - 50/54
		<u>Glaucornys</u>	(1) 52
<u>Geomyidae</u>	784	<u>Geomys</u>	(1) 784
<u>Heteromyidae</u>	44 - 786	<u>Perognathus</u>	(1) 44
		<u>Dipodomys</u>	(1) 786
<u>Muridae</u>	40 - 68	<u>Apodemus</u>	(9) 46/48 - 50
		<u>Mus</u>	(5) 40
		<u>Micromys</u>	(2) 68
		<u>Rattus</u>	(8) 42 - 46
		<u>Bandicota</u>	(2) 44 - 46
<u>Cricetidae</u>	14 - 60	<u>Peromyscus</u>	(18) 48 - 60
		<u>Sigmodon</u>	(1) 54
		<u>Tsherkia</u>	(1) 30
		<u>Neotoma</u>	(1) 52
		<u>Cricetus</u>	(1) 38
		<u>Cricetulus</u>	(1) 14
		<u>Microtus</u>	(8) 28 - 56
		<u>Evotomys</u>	(3) 28 - 56
		<u>Eothenomys</u>	(1) 56
		<u>Arvicola</u>	(1) 36
		<u>Tatera</u>	(1) 34
<u>Gliridae</u>	48 - 62	<u>Glis</u>	(1) 62
		<u>Eliomys</u>	(1) 52
		<u>Muscardinus</u>	(1) 48
		<u>Diromys</u>	(1) 48
<u>Caviidae</u>	64	<u>Cavia</u>	(1) 64
<u>Hystriidae</u>	48	<u>Hystrix</u>	(1) 48
<u>Capromyidae</u>	42	<u>Myocastor</u>	(1) 42

It is noteworthy that one family contains genera which have remarkably uniform chromosomal numbers in their species: in this family, the Muridae, 8 out of 9 Apodemus species probably have the same chromosomal number (48), 5 Mus species all have 40, 2 Micromys species both have 68, while 8 Rattus species vary within the narrow limits of 42 and 46, and 2 Bandicota species vary within the narrower limits of 44 and 46.

On the other hand, the Sciuridae contain genera which are multiform in respect of chromosomal number, e.g. in 5 Sciurus species, the number varies from 28 to 62, while in 2 Citellus species, number varies from 32 to 50/54. Likewise, the Cricetidae contain several genera possessing multiform chromosomal numbers - 18 Peromyscus species vary from 48 to 60, 8 Microtus and 3 Evotomys (Clethrionomys) species vary from 28 to 56.

Within the rodents, then, two types of families may be distinguished. In the first type, the genera contain species of which the chromosomal numbers fall within narrow limits. It is proposed that this type of family be spoken of as a 'uniform' family and that its genera be called 'uniform' genera. The second type of family (the 'multiform' family) contains 'multiform' genera, i.e. genera whose constituent species possess widely varying chromosomal numbers. It is clear that uniform genera must contain uniform species, i.e. species in

which the sub-species do not vary in chromosomal number; on the other hand, multiform genera are likely to contain multiform species (i.e. species in which the sub-species have varying chromosomal numbers), but might also contain uniform species. In point of fact, Table 10 demonstrates that the multiform genera (Sciurus, Peromyscus) possess some multiform species (Sciurus carolinensis, Peromyscus maniculatus, Peromyscus eremicus) and some uniform species (Sciurus niger, Peromyscus californicus, Peromyscus leucopus). Table 10 confirms that the uniform genera (Apodemus, Micromys, Rattus) contain only uniform species. Thus, it is true to say that, within the multiform genera, there is a higher proportion of multiform species and, within the uniform genera, there are more uniform species.

The number in brackets after the name of each species refers to the number of sub-species of which the chromosomal numbers have been determined.

TABLE 10.

CHROMOSOMAL NUMBERS I: POLYTYPIC SPECIES OF RODENTS.

<u>FAMILY</u>		<u>GENERA</u>		<u>SPECIES</u>	
<u>Sciuridae</u>	28-62	<u>Sciurus</u> (5)	28-62	<u>Sciurus</u>	
				<u>carolinensis</u> (2)	28-48
				<u>Sciurus niger</u> (2)	62
<u>Muridae</u>	40-68	<u>Apodemus</u> (9)	46/48-50	<u>A. agrarius</u> (2)	48
				<u>A. speciosus</u> (2)	48-50
		<u>Micromys</u> (2)	68	<u>M. minutus</u> (2)	68
<u>Cricetidae</u>	14-60	<u>Peromyscus</u> (18)	48-60	<u>P. californicus</u> (2)	48
				<u>P. maniculatus</u> (8)	48-52
				<u>P. eremicus</u> (2)	58-60
				<u>P. leucopus</u> (2)	48

Further, the range of variation of chromosomal numbers in the 'uniform' Muridae (40 - 68 i.e a range of 28) is lower than the ranges of either the 'multiform' Sciuridae (28 - 62 i.e. a range of 34) or the 'multiform' Cricetidae (14 - 60 i.e. a range of 46).

We now have three points of difference distinguishing 'multiform' from 'uniform' rodent families: the composition of their genera, the composition of their species and their range of chromosomal numbers. The gerbil thus belongs to the most

'multiform' family, the Cricetidae, and we might predict that later research will show many variations in the chromosomal numbers of the Gerbillinae, and, more specifically, within the genus Tatera, perhaps even among the sub-species of Tatera brantsii.

No other rodent families contain genera in which more than one species has been studied cytogenetically or species in which the chromosomal formula of more than one sub-species has been determined. However, if we consider families in which more than one genus has been studied, we see that numbers range in two genera of Heteromyidae from 44 to 786, a range of 42. This slender evidence suggests that the Heteromyidae is a 'multiform' family, its range being almost as great as that of the multiform Cricetidae. Four genera of Gliridae have from 48 - 62 chromosomes, a range of only 14, which is much lower than the range of 28 in the uniform Muridae. Perhaps, then, the Gliridae is a second rodent family in which chromosomal uniformity is more apparent than multiformity.

It appears that, in evolution, some rodent groups have been more prone to display chromosomal variability than others: this applies at all levels within the family, i.e. between genera, between species and between sub-species. In brief, morphological divergence in some rodent families has been accompanied by much divergence of chromosomal numbers; while, in others, there has been a divorce between morphological and chromosomal divergence, so that the former has proceeded without

the latter.

It may next be asked whether the tendencies to multiformity or to uniformity are peculiar to the rodents or whether they are manifested by other mammalian orders. The figures available for analysis are the chromosomal numbers of only 169 mammals, of which 82 are the rodent species already discussed. It would not be out of place to digress at this point in order to enquire why so few mammalian chromosomal numbers are available. This enquiry can be made by tracing the history of chromosomal enumeration.

CHROMOSOMAL ENUMERATION FROM 1900 TO 1950.

With the establishment of the species-specificity of chromosomal numbers, a few workers began to compile lists of enumerated species. Thus, in 1900, Wilson was able to publish a partial list of chromosomal numbers of about 50 species of animals and a few plants. This list included 5 mammals, man, guinea-pig, ox, rat and mouse. By later standards, all the results on mammals were inaccurate.

Montgomery's list in 1906, though nearly complete for that time, was marred by inaccuracies and omissions. In 1915, Tischler published a list of chromosomal numbers in plants and this was supplemented in 1917 by O. Winge with a more detailed list. Harvey published two very complete lists in 1916-17 and 1920. The first list embraced the Annelida, the Arthropoda and the Coelenterata; while the later list covered the Vertebrata.

Of the 88 vertebrates listed, 30 are Amphibia, 10 Aves, 16 Pisces, 5 Reptilia and 27 Mammalia.

From 1900 to 1925, then, the collection of data continued slowly; there was no flourishing and deliberate exploitation of this new technique in the analysis of species-differences. If we seek for the historical influences operating as limiting factors, we shall find them in the then current, dominant attitude towards the chromosomes. To the biologist of the first quarter of the twentieth century, the chromosomes were important only as the bearers of the genes and the behaviour of the chromosomes was significant only as it affected the contained groups of genes. Materially, biologists were therefore pre-occupied with chromosomal mechanics as a guide to genetic behaviour. At the same time, there was at first a failure to comprehend the possible significance of chromosomal numbers and their mode of changing in evolution.^Ø As long as the chromosomes were looked upon as strings of beads called genes and, as long as the character of these genes was considered the only determinant of heredity, it clearly was of little concern whether the total complement of genes was threaded on to a few or many chromosomes - provided the genes remained unchanged.

^Ø Two important exceptions to this generalisation were Montgomery and McClung, both of whom early supported the view that chromosomal number should be considered an important factor in taxonomy.

As Matthey (1949) points out:

"Le gene ayant longtemps paru seul important, l'étude de son substratum morphologique, le chromosome, se voit dédaignée."

(p. 169)

This is well illustrated by a quotation from Wilson: in the 3rd Edition (1925) of his monumental work, "The Cell in Development and Heredity", we find the following statement:

"...the number of chromosomes is per se a matter of secondary significance. Both cytological and genetic evidence proves that the chromosomes are compound bodies, containing many different components. So long as the sum-total of these remains the same, or nearly so, it seems to be immaterial whether they be grouped to form few or many aggregates."

(Wilson's italics)

(p. 866)

These views constituted definite limitations to biological thought, restraints which had to be outgrown before the evolutionary significance of chromosomes could be appreciated.

The turning-point came in 1925, when Sturtevant announced the discovery of a 'position-effect'. Now the geneticists began to think again in terms of chromosomes and genes and, gradually, the chromosome recovered its lost personality over the quarter-century from 1925 to 1950. The restoration of the status of the chromosome made it intellectually more acceptable to study chromosomal numbers and evolution. Thus, there occurred striking progress in chromosomal enumerations after 1925.

In that very year, we find Painter engaged in studying the chromosomes of mammals drawn from many different orders.

By 1932, it was possible for Oguma and Makino to include 63 different mammals in their list of chromosomal numbers of vertebrates. This number includes many earlier, inaccurate determinations and is therefore misleadingly high.

A complete and up-to-date list was published by Oguma and Makino in 1937, but it has not been possible to procure a copy of this work in South Africa.

McClung's inventory, published in 1940, includes earlier, unconfirmed and inaccurate studies and the figure of 99 mammals therefore exceeds the number of valid estimations made up to that time.

White's graphs (1945) show chromosomal determinations in 85 mammals (67 Eutherian and 18 Marsupial): this number may be accepted as accurate, the author having discarded earlier, incorrect or unconfirmed determinations.

As White does not enumerate these mammals, there is a definite need for a list of all the mammalian chromosomal estimations made in more recent times by modern cytogenetical techniques. Such a compilation was undertaken in 1946 by Brenner and myself and has been completed from 1947 to the present time by myself.^Ø Our list contains the chromosomal numbers of 169 mammalian species and sub-species. This is

^Ø After this section had been written, Matthey's new book on "Les Chromosomes des Vertebres" (1949) arrived in South Africa. In this work, Matthey records the names and chromosomal numbers of some 120 mammals.

the most complete list of mammalian chromosomal numbers so far compiled: and is sub-joined in its entirety as an Appendix to this thesis.Ø

The progress might have been greater still, but for the fact that mammalian chromosomal estimation is a difficult procedure. As evidence of this, we may cite the following examples of chromosomal determinations, all made recently by accepted, modern, cytogenetical techniques, yet showing conflicting results, which hark back to the disparities evident in the cytogenetical literature of 40 years ago.

Ø While the manuscript of this thesis was in course of preparation (1951), I received a copy of S. Makino's latest comprehensive "Atlas of the Chromosome Numbers in Animals" (1951a). The original Tokyo edition had appeared in 1949, but had not been available in South Africa. In 1951, the Iowa State College Press rendered cytologists a great service by printing a new edition of the Atlas, which has now also become available in South Africa. Makino lists chromosomal numbers of 3317 animals - 2754 invertebrates and 563 vertebrates. The best-documented vertebrate group is the Mammalia, of which Makino lists 176 different species and sub-species. This includes 10 forms which I have deliberately omitted from my list, either because they have been inadequately identified or because the chromosomal estimation is inaccurate or made by older techniques. Makino's list therefore contains 166 valid estimations on mammals. It includes recent results of Makino, Bovey, Matthey and Mudal on species studied by earlier workers. Several consequent modifications of chromosomal number have been incorporated in the list in the Appendix.

TABLE 11.

DISPARITIES IN CHROMOSOMAL COUNTS ON MAMMALS.

<u>SPECIES.</u>	<u>CHROMOSOMAL NUMBERS CLAIMED</u>	<u>REFERENCE.</u>
<u>Microtus ratciceps</u>	30 46	Makino 1949a, b. Muldal 1949
<u>Cricetus auratus</u>	38 44 38	Koller 1938a. Husted, Hopkins and Moore 1945 Muldal 1948
<u>Sigmodon hispidus texianus</u>	54 40	Cross 1931 Muldal 1948
<u>Talpa europea</u>	38 34 34	Koller 1936b Matthey 1949 Bovey 1949a
<u>Vulpes fulvus</u>	42 32	Woodsdalek 1931 Bishop 1942
<u>Vulpes vulpes</u>	34 38	Wipf and Shackelford 1942 Makino 1944
<u>Apodemus speciosus speciosus</u>	46 50	Tateishi 1934, 1935 Makino 1951
<u>Micromys minutus</u>	62 68	Muldal 1948 Matthey 1949

In this Table, only 8 examples have been selected from a total of 16 such species known to me. No onlooker could escape bewilderment when faced with Makino's and Muldal's results, obtained in the same year, 1949, on the same species, Microtus ratticeps: the former recorded 30 chromosomes, the latter 46! Whether we are confronted here with two different sub-species or with grave technical difficulties, the fact remains that such disparities are still occurring today. Small wonder that we have not more mammalian chromosomal numbers to use for comparison.

THE CHROMOSOMAL NUMBERS OF MAMMALS.

To return to our query whether multiformity and uniformity are evident among mammals other than rodents, the 169 mammals whose chromosomal numbers are known are distributed in orders as follows (the mammalian orders follow the classification by Simpson, 1945):-

TABLE 12.

DISTRIBUTION OF ENUMERATED SPECIES IN MAMMALIAN
ORDERS

<u>MAMMALIAN ORDER</u>	<u>NUMBER OF SPECIES WHOSE CHROMOSOMES HAVE BEEN COUNTED</u>
PROTOTHERIA	
Monotremata	1
METATHERIA	
Marsupialia	21
EUTHERIA	
Edentata	1
Sirenia	0
Cetacea	1
Ungulata: Artiodactyla	14
Ungulata: Perissodactyla	2
Ungulata: Proboscidea	0
Rodentia	82
Lagomorpha	4
Carnivora	14
Insectivora	8
Chiroptera	16
Primata	5

Apart from the Rodentia, the chromosomes of a fair number of species have been determined in the Marsupialia, the Artiodactyla, the Carnivora and the Chiroptera. Before we consider the mammals as a whole, let us first consider the chromosomal numbers in the mammalian orders (Table 13):-

TABLE 13.

DISTRIBUTION OF CHROMOSOMAL NUMBERS IN MAMMALIAN
ORDERS.

<u>ORDER</u>	<u>CHROMOSOMAL NUMBER</u>				<u>TOTAL RANGE</u>
	<u>Under 30</u>	<u>30-39</u>	<u>40-49</u>	<u>50 and over</u>	
Monotremata	-	-	-	1	70 $\pm$ 10
Marsupialia	21	-	-	-	12 - 28
Edentata	-	-	-	1	60
Cetacea	-	-	1	-	44
Artiodactyla	-	2	2	10	30 - 72
Perissodactyla	-	-	-	2	60 (766)
Rodentia	3	7 (78)	48 (747)	24	14 - 84
Lagomorpha	-	-	4	-	44 - 48
Carnivora	1	8	2	3	28 - 78
Insectivora	2	2	3	1	14 - 52
Chiroptera	-	3	9	4	22 - 58
Primata	-	-	4	1	42 - 54

The first point to observe is that in the better-documented groups, there is a great range between the lowest and highest chromosomal numbers. Thus, a range of 70 separates the rodents with the highest and lowest chromosomal numbers; in the carnivores, the range is 50; in the artiodactyls 42; in the insectivores 38; in the Chiroptera 36. The only

well-studied group which has a small range is the marsupials. Although second only to the rodents in the number of species studied, the marsupials possess a range of only 16. Thus, all well-studied eutherian orders are multiform as regards the chromosomal numbers of their species; whereas the marsupials are more uniform.

It is justifiable to conclude that, as a group, the marsupials have small chromosomal numbers, although whether this is a consequence of extreme 'specialisation', such as the marsupials are commonly held to have undergone, or whether the marsupials have retained an ancestral small number, we cannot yet say.

Of other groups, we can generalise less confidently. The ungulates tend to have higher chromosomal numbers. The rodents, despite their enormous range, tend towards higher numbers, the majority being in the class 40 - 49 and the second biggest group in the class '50 and over'. The carnivores have two peaks of distribution - a major one with lower numbers (30 - 39) and a minor one with high numbers. These peaks do not correspond with the division of the carnivores into Fissipedia and Pinnipedia. The bats tend to have higher numbers, mainly in the 40 - 49 group. The insectivores are evenly dispersed among higher and lower numbers; the few primates and lagomorphs each have higher numbers.

There are 28 eutherian mammals with numbers below 40 and 120 above 40. Since the preponderance of rodents may seemingly weight these figures on the side of the high numbers, let us exclude the rodents. There remain 17 eutherian mammals below 40 and 48 above 40. Hence, in general, most mammals (excluding the marsupials) have higher chromosomal numbers.

Table 14 shows how the non-rodent species are distributed in families and genera (only families and genera containing more than one enumerated species are tabulated). Numbers in brackets after generic names refer to the number of species whose karyotype has been determined in each genus.

TABLE 14.

DISTRIBUTION OF CHROMOSOMAL NUMBERS (EXCLUDING THOSE OF RODENTS) IN MAMMALIAN FAMILIES AND GENERA.

ORDER	FAMILY	MIN. AND MAX. NOS.	RANGE	GENUS	MIN. AND MAX. NOS.	RANGE
Marsupia- lia	<u>Phalanger- idae</u>	16 - 22	6	-	-	-
	<u>Macropod- idae</u>	12 - 28	16	<u>Macropus</u> <u>Bettongia</u>	(3) 12 - 22 (2) 22 - 28	10 6
	<u>Didelphidae</u>	22	0	<u>Didelphis</u>	(3) 22	0
	<u>Dasyuridae</u>	14	0	<u>Dasyurus</u>	(2) 14	0
Artiodac- tyla	<u>Suidae</u>	30 - 40	10	<u>Sus</u>	(2) 38 - 40	2
	<u>Camelidae</u>	70	0	<u>Camelus</u>	(2) 70	0
	<u>Bovidae</u>	48 - 60	12	<u>Bos</u> <u>Ovis</u>	(3) 60 (3) 54 - 60	0 6
Perisso- dactyla	<u>Equidae</u>	60/66	0	<u>Equus</u>	(2) 60/66	0
Lagomorpha	<u>Leporidae</u>	44 - 48	4	<u>Lepus</u>	(3) 44 - 48	4
Carnivora	<u>Canidae</u>	32 - 78	46	<u>Vulpes</u> <u>Canis</u>	(3) 32 - 52 (2) 78	20 0
	<u>Mustelidae</u>	28 - 38	10	<u>Mustela</u>	(2) 28 - 38	10
	<u>Felidae</u>	38	0	<u>Felis</u>	(3) 38	0
Insectivora	<u>Soricidae</u>	23 - 52	29	<u>Crocidura</u>	(2) 40 - 42	2
	<u>Talpidae</u>	32 - 34	2	-	-	-
Chiroptera	<u>Rhinoloph- idae</u>	54 - 58	4	<u>Rhinolophus</u>	(4) 54 - 58	4
	<u>Vespertili- onidae</u>	22 - 46	24	<u>Myotis</u> <u>Pipistrellus</u>	(4) 42 - 44 (2) 22 - 42	2 20

None of the genera listed contains more than four species, the chromosomes of which have been enumerated. Thus, only slight tendencies can be detected from an examination of the chromosomal numbers. We may say that the Canidae (range 46), the Soricidae (range 29) and the Vespertilionidae (range 24) incline to be multiform; the Didelphidae, the Dasyuridae, the Camelidae, the Equidae, the Felidae (all with a range of 0) and the Rhinolophidae (range 4) are apparently uniform families, while the remainder of the families are ranged between these limits, some approaching more to the uniform, others to the multiform.

Within the uniform families, the genera are all absolutely uniform, except Rhinolophus with a range of 4. Within the multiform families, as in those of the rodents, both multiform and uniform genera are encountered. In the Canidae, Vulpes is a multiform genus (range 20), Canis a uniform genus. The Soricidae contain a uniform genus, Crocidura; while the Vespertilionidae possess a uniform genus, Myotis (range 2), and a multiform genus, Pipistrellus (range 20).

It is concluded that the tendencies to multiformity and uniformity are evident in all adequately-studied mammalian orders. It may thus be proposed that, in the chromosomal evolution of the mammals, two main paths of development have been emphasized: along one path, leading to multiform groups, chromosomal numbers have changed as the general morphology altered; along the other, engendering uniform groups, chromosomal

numbers have altered but little, despite general morphological changes.

PRINCIPLES OF CHROMOSOMAL CHANGE.

The foregoing discussion has demonstrated that chromosomal numbers may be arranged in such a manner that broad trends of variation emerge. Even at this stage, the assembled facts do not warrant Makino's conclusion that "It is evident that our knowledge of the chromosomes of mammals is still too scanty to justify any generalisation." (1948). In particular, a trend towards uniformity or multiformity has been proposed for the mammals. But the history of cyto-phylogeny has thrown up other principles of change from the apparently meaningless welter of chromosomal numbers.

The modern era in chromosomal studies, as in biology as a whole, has been marked by a growing appreciation of the time factor. When cytogenetics arose, biology was still mainly a three-dimensional discipline. It was a period in the history of biology when emphasis centred around morphological description, when the concept of the species was circumscribed by a handful of museum specimens, when fossils were important structurally, rather than as events in an evolutionary stream. It was a phase markedly differing in its conceptual approach to the problems of biology from the present period.

In the modern conception of biology - which has grown with ever-increasing vigour since the publication of Darwin's

"Origin of Species by Natural Selection" (1859), since Wilhelm Roux founded the science of experimental embryology (1883-1886), and since Johamnsen formulated his concept of the interaction between nature and nurture (1911) - a consideration of the time dimension is a conditio sine qua non in all investigations.

If this is valid in the whole field of biology, it holds for the limited field of chromosomal studies. At first, the enumeration of chromosomal complements provided an interesting set of facts, which were held to be of no practical value: the results were looked upon as some mildly curious statistical data. The new mood in biology generated a new sphere of thought, cyto-phylogeny, the establishment of a sequence of past events which have led to the chromosomal patterns of to-day.

There began a search for principles of chromosomal change. The search took two forms: in the first, detailed comparative descriptions of chromosomal diversity were catalogued and the regularities revealed were formulated and generalised (the 'generalising induction'). In the second, experiments were conducted to ascertain what types of change the chromosomes were capable of undergoing, in order to establish the mechanisms for the trends revealed by the former type of investigation (the 'exact induction'). We shall consider the results of these two types of investigation separately.

(1) GENERALISATIONS BASED ON COMPARATIVE DATA.

Although a great number of generalising inductions

have been made on chromosomal diversity, we are concerned here only with those relevant to changes in the chromosomal number. Generalisations of two orders may be distinguished viz. those illustrating types and those showing trends of chromosomal change.

(A) Types of chromosomal numerical change.

Two broad categories of change exist: that affecting individual chromosomes in the set and that involving changes in whole sets.

(I) Changes affecting individual chromosomes.

(i) Numerical changes: Polysomy. This term refers to the duplication or loss of one or more chromosomes from the complement. It arises through two daughter chromosomes passing to the same pole in cell division. The commonest examples of polysomic races are found among plants, notably Datura, Zea, Crepis, Matthiola and Solanum (Saez 1949). Among animals, polysomic races of Drosophila exist. No instances of polysomy in mammals have hitherto been shown, but it is possible that the males of several mammals are monosomic in respect of their sex-chromosomes (e.g. Macropus ualabatus (X_1X_2-Y or $X-Y_1-Y_2$) - Agar 1923; Sorex araneus (X_1-X_2-Y or $X-Y_1-Y_2$) - Bovey 1949b; Potorous tridactyla ($X-Y_1-Y_2$) - Sharman, McIntosh and Barber 1950). The only rodent which may be monosomic in respect of its sex-chromosomes is Microtus montebelli

(Oguma 1937) for which an X-O formula in the male has been claimed.

(ii) Structural changes affecting the number of chromosomes: Fusion and Fragmentation. Robertson (1916) first suggested that V- or J-shaped (or metacentric) chromosomes might have arisen by the apical or centric fusion of two rod-shaped (or acrocentric) chromosomes and, conversely, that a V or a J might have fragmented into two rods. During his studies on insects, he had noted that many species had V- or J-shaped chromosomes and that other related species had fewer V's or J's but more rod-shaped chromosomes. If, instead of counting whole chromosomes, he counted the number of chromosomal arms, he obtained a nearly constant number in many groups. In short, a V or a J in one species might be equivalent to two rod-shaped, non-homologous chromosomes in a related species. This conception of fusion and its converse, fragmentation, is referred to by some workers as Robertson's law or Robertsonian variation. Fusion and fragmentation would explain the more obvious changes in chromosomal shape and number in many groups, especially insects and lizards.

Matthey has shown that centric fusions (possibly with the reverse process) account for a large part of the visible differences between the chromosome sets of allied species in the Lacertilian lizards. He has also applied Robertson's conception to many other groups and has

proposed a new term, 'nombre fondamental' (N.F.), for the total number of chromosomal arms in a complement.

Among mammals, Winiwarter (1934) thought he detected evidence of fragmentation in various races of the domestic cat. More recently, Matthey has searched for clear-cut lines of 'Robertsonian variation' among mammals without conspicuous success, except in the Chiroptera. In the rodents, in particular, his study of fundamental numbers, alongside diploid numbers, produced some results frankly contrary to the hypothesis of Robertsonian variation.

Matthey's results lead one to enquire whether all V-shaped chromosomes are necessarily the result of the fusion of rod-shaped elements. Clearly, other structural rearrangements can influence the number of chromosomal arms.

(iii) Other structural rearrangements affecting the fundamental number. We may note, with Darlington and Upcott (1941), that "within the limits of natural conditions unimagined possibilities occur for structural change in the chromosomes", whether under environmental stimulation, special genotypic circumstances or in abnormal tissue differentiation. Muller (1940), in summarising the bearings of the *Drosophila* work on systematics, tabulated no fewer than 27 different types of structural rearrangements of chromosomes. All of these structural changes have been found, either in natural populations, or spontaneously in

laboratory flies, or under irradiation. Of the 27 varieties, 22 are capable of altering the number of chromosomal arms! For example, a pericentric inversion may effectively transfer a sub-terminal spindle-fibre attachment to a median or sub-median position, thus changing an acrocentric into a metacentric chromosome. The fundamental number would thus increase without any decrease in the diploid number: in such an occurrence, the fundamental number would not be a true guide to the ancestral or generalised chromosomal number of the group. The converse process might alter a V into a rod, reducing the fundamental without amending the diploid number.

The occurrence of many sectional re-arrangements in a species would vitiate Robertsonian comparisons. Therefore, in groups in which the fundamental numbers show hardly more correspondence than do the diploid numbers, the Robertsonian type of variation may have played a less active evolutionary role than other types of structural variation or than polysomy.

There is little evidence on the occurrence of structural rearrangements in the mammals. Koller (1937) reported an inversion heterozygote in man, while Crew and Koller (1939) found inversion in the pig. In the rodents, inversion bridges have been seen in the squirrel (Sciurus carolinensis leucotus) (Koller 1936) and segmental interchange in the mouse under X-irradiation (Koller 1944). What part

structural changes may have played in the chromosomal evolution of the rodents can only be inferred on indirect evidence and by the elimination of other types of variation.

(II) Changes affecting sets of chromosomes.

Polyploidy. Reduplication of a whole chromosomal set, so that the nucleus contains three, four or more sets, is called polyploidy. The variation may arise by several mechanisms, the commonest of which is syndiploidy or the formation of gametes with a double number of chromosomes.

Polyploidy is one of the commonest forms of variation in plants. According to Muntzing (1936), more than half the higher plants have polyploid forms. The basic number for a polyploid series may be constant for a tribe, subfamily or family, or may even transgress the bounds of families. In some groups of plants, the basic number is unstable: and it is observed that the more this basic number changes, the less polyploidy occurs in the group (Darlington and Janaki Ammal 1945).

When we turn to the animal kingdom, we find that Painter (1921) early demonstrated syndiploidy in the male germ cells of lizards. Later he first attempted to generalise on the chromosomal formulae of vertebrates (Painter 1925). He studied at least one species from every mammalian order (except the Monotremata, the Sirenia and the Cetacea) and, on the basis of his results,

suggested that the modal number for the marsupials is 24 and that for the eutherian mammals 48. It was implied that the number 24 is ancestral and that the Eutheria had probably arisen as a polyploid group, by a doubling of the metatherian complement. But this process of doubling was not the only form of variation for, in some groups, the numbers were frequently much higher. Painter ascribed the higher numbers to a process of fragmentation.

Later work has served to confirm, with few exceptions, the jump between the chromosomal numbers of the marsupials and those of the eutherians. Even Painter's proposed modal numbers have stood the test of further estimations. The modal number for marsupials is 22, not 24, while that of the Eutheria remains 48. No fewer than 35 eutherian mammals have the number 48, the next biggest group being 13 eutherians with 42. But Painter's conception of chromosomal change in the mammals is not considered tenable by recent workers, especially Matthey, because it was not based on Robertsonian considerations, i.e. Painter failed to distinguish between the number of chromosomes and the number of arms. However, his emphasis on fragmentation in mammals was a useful advance.

An important paper by Vandel (1937) - which, incidentally, is not often quoted - crystallised the main trends of chromosomal evolution in the two biological kingdoms. In plants, polyploidy was the chief form of chromosomal

change, while, in animals, fragmentation had been the dominant type of change. At the same time, Vandel thought he could detect an apparent polyploidy in animals. Fragmentation, he suggested, had often occurred simultaneously in more than one chromosome. Where it affected every member of the complement, a doubling of the number would result. This extreme form of fragmentation resulted in a pseudo-polyploidy. True polyploidy involves a duplication of every chromosome in the set and a consequent doubling of the total volume of chromatin; whereas, in complete fragmentation, each chromosome is halved and the total volume of chromatin remains unchanged. The genetic consequences would also differ: polyploidy multiplies the number of mutable loci, thus augmenting the mutation rate; fragmentation alters the number of linkage groups and therefore increases the number of possible combinations at the time of gamete-formation.

More recently, Gates (1942) has revived Painter's idea that true polyploidy may have occurred in mammalian evolution. He stresses again that placental mammals have numbers approximately twice those of marsupials. He also emphasises that most ungulates have numbers appreciably higher than those of primates. The latest list of mammalian chromosomal numbers enables one to give the following table of modal numbers:-

TABLE 15.

MODAL NUMBERS IN MAMMALIAN ORDERS.

Marsupialia	22
Carnivora	38
Rodentia	48
Primata	48
Ungulata	60

This series of modal numbers is close to a series of multiples of 12, viz. 24, 36, 48, 60. However, these are the modes of chromosomal numbers, not of fundamental numbers, and it might be argued that any hypothesis of polyploidy in the mammals should take into account Robertsonian variation.

In animals with sex-chromosomes, there are serious a priori arguments against polyploidy, for considerable disturbances in bisexual reproduction may be occasioned by polyploidy. This is inferred because dioecious polyploids are rare in both plants and animals. The mechanism of the disturbance is thought to be threefold:-

- (1) An imbalance between autosomes and sex-chromosomes in unbalanced polyploids (e.g. triploids, pentaploids), resulting in intersexuality and sterility of the heterozygous sex. For example, male triploids of Drosophila are intersexual, as are female triploids of butterflies.
- (2) In triploids, the irregularities of segregation lead to few normal progeny.

- (3) In tetraploids of the heterogametic sex, the two X-chromosomes would tend to segregate from each other, with the resultant production of few or no gametes having a normal ratio of X-chromosomes to autosomes. (Muller 1940).

However, counter-evidence is adduced by Vandel (1937) in the form of two or three species in which triploids of the heterogametic sex are normal. In these cases, he suggests, there is a process of stabilisation or regulation to the new intra-cellular conditions. This regulative tendency might counteract the sex-disruption incurred by unbalanced polyploidy in nature. Further, Slack (quoted by Muller 1940) obtained evidence that, in the hemipteran sub-order, Heteroptera, polyploidy has occurred independently a number of times.

Additional evidence is required before these weighty objections to the occurrence of polyploidy in sexually-reproducing animals falls away. Meantime, most cytogeneticists (e.g. White 1945, p. 183; Saez 1949, p. 231) consider that polyploidy has not yet been established as a significant type of chromosomal variation in animals. In the words of Matthey,

"Si les faits suggèrent souvent la possibilité d'une évolution polyploïde, la preuve d'un tel processus n'a été apportée en aucun cas."

(1949, p. 181)

Fusion, fragmentation, other structural changes, polysomy and polyploidy are, then, the main types of chromosomal changes affecting chromosomal number. In general, polyploidy seems to have been the motif of evolution in plants, fusion and fragmentation that in animals. In addition, other structural variations and polysomy have probably occurred in both plants and animals. It is well to remember, as Muller (1940) has reminded us, that the relative frequency of various types of chromosomal change would not be the same for all groups, a fact confirmed by a study of comparative cytology. It is necessary to ascertain the main lines of variation for each group and our concern here is to find these trends in the rodents. There is then no evidence to favour, and strong a priori evidence against, the idea that polyploidy has played a part in the chromosomal evolution of the rodents.

(B) Trends in numerical change of the chromosomes.

(I) Multiformity and Uniformity.

Our study of mammalian chromosomal numbers has led us to postulate two main tendencies: according to one, evolution has rung the changes on chromosomal numbers, as general morphology has become modified; in other words, chromosomal numbers have undergone little alteration, despite general morphological changes. Various families of rodents have been influenced by one or other tendency: the gerbil belongs

to the most multiform family, the Cricetidae.

(II) Karyo-somatic dissociation.

In those groups where uniformity of chromosomal numbers is evident, a divorce is apparent between chromosomal and general morphological evolution: this divorce I propose to call karyo-somatic dissociation.

What are the karyo-somatic interrelations in the groups showing multiformity? Part of the answer we may glean from "The Chromosome Atlas of Cultivated Plants", recently published by Darlington and Janaki Ammal (1945). This work provides a "bird's eye view of the whole process of the evolution of genetic systems in the flowering plants." Based on the chromosomal numbers of no fewer than 10,000 plants, the authors have given a number of generalisations on the evolution of plant chromosomes. On the question of karyo-somatic interrelations, they make the point that polyploidy often arises within a single morphological species, splitting it into several potential new species. In these instances, since an efficient genetical isolation exists between the polyploid and the parent species, chromosomal change anticipates general morphological change (this is the converse of 'uniformity' where chromosomal change lags behind morphological change).

Another important point made by Darlington and Janaki Ammal is that, even in the multiform groups, the direction

of chromosomal change is not always the same as the apparent direction of morphological evolution. In some groups, forms classed on morphological grounds as 'primitive' do not have the lowest basic numbers. Numerical changes can occur tangential or, even, diametrically opposite to, the main line of chromosomal development for that group, without necessarily bringing in their wake any 'side-chain' developments in the organism's phenotype, nor any frank regression to a more 'primitive' or 'simpler' phenotype. In other words, even in multiform groups, a karyo-somatic dissociation may occur.

Both Darlington and Dobzhansky have referred to the existence of what I have called 'karyo-somatic dissociation'. Thus:-

"We.....find every relationship between structural and numerical and genotypic differences in the chromosomes on the one hand and systematic differences on the other."

(Darlington 1937, p.79)

"The extent of chromosome reconstruction is by no means necessarily proportional to the divergence in external morphology shown by the forms compared. Chromosomal differentiation is neither a precondition nor a consequence of morphological differentiation."

(Dobzhansky 1941, p.148)

An example of karyo-somatic dissociation in a multiform family of rodents is provided by the genus,

Peromyscus. Seven species (and 16 sub-species) of this genus have differentiated morphologically without a change in their chromosomal number of 48 (except one sub-species of P. maniculatus which has 52); while two sub-species of an eighth species, P. eremicus, have departed from the others and acquired 58 and 60 chromosomes respectively, although morphologically still belonging to the same genus.

Another example is found in Sciurus carolinensis: the carolinensis sub-species has 48, the leucotus sub-species 28 chromosomes. Yet, morphologically, the two groups have not diverged sufficiently to become classified as distinct species. As in the flowering plants, it is possible that such a change as that from 48 to 28 (or vice versa) in Sciurus carolinensis anticipates further morphological change, the recognition of which may one day promote the two sub-species to distinct species.

We see, then, that karyo-somatic dissociation is another broad trend manifest in the chromosomal evolution of the rodents.

(III) Homologous Change.

The idea of parallelism in comparative chromosomal studies was suggested in an unpublished essay by myself early in 1946:

"Still another possibility to be considered is that of parallelism in the evolution of the germ-plasm, as it is now generally held to have occurred in phenotypical evolution (e.g. Weidenreich's theory of parallel hominid evolution, 1939; also le Gros Clark, 1934). As early as 1912, Ruggles Gates described the first instance of parallel mutation and, subsequently pointed out numerous similar cases in animals and plants (1920). More recently, Gates (1944) has stated:

'As evolutionary factors in man and anthropoids have to be considered not only the fundamental types of change which presumably depend upon larger and smaller mutations, but also parallel mutations, longer parallel lineages, and crossing between lineages.'

The established existence of parallel mutations is not very surprising because, as Simpson has put it, 'homologous genes tend to mutate in the same way' (1945). The possibility of the occurrence of parallelism in chromosomal change is one serious deterrent in the application of cytological findings to phylogeny."

Again, in the same essay, I stated:-

"The possibility of chance parallelism in sectional re-arrangements as well cannot be dismissed. If identical sectional re-arrangements are apparent in two of a group of species, this may or may not indicate that these two species are more closely related to each other than either is to the other species in the group. The aberration may have been a parallel phenomenon; or it may have originated from a common ancestry of the two species.

"What are the tactics of the comparative anatomist when faced with the possibility of parallelism in the evolution of a character? It is usually possible for him to ascertain whether the character in question has arisen by parallel evolution, from a consideration of the evidence provided by fossils, by embryonic development and by the condition in other closely-related species. The position is assessed on the basis of other phylogenetic indicators: and we might apply the same technique to analogous situations in

cytological characters. The evolutionary past of the germ-plasm can only be inferred from existing forms and, the essence of the study is a comparative one. Where the possibility of a parallel structural change arises, it must be considered in relation to the general phylogeny of the group, arrived at on the basis of morphology, embryology, palaeontology, etc. In the hypothetical example cited in the previous paragraph, the phylogeny of the group of species concerned might indicate that the two species exhibiting a particular chromosomal aberration were, in fact, more closely related to some other species in the group (not possessing the aberration) than to each other. If, on the other hand, the balance of evidence indicates a close relationship and a near common ancestry of the two species, the cytological findings would serve to confirm this...."

At the time unknown to me, White (1945) independently put forward and elaborated the same principle of homologous change:

"It is obvious that the lengths and shapes of chromosomes in a set are not at random. In species after species we find that the chromosome set consists of members which are alike in size and in the position of the centromere. Alternatively, there may be two size-classes, each with definite characteristics. Such conditions would not be encountered if the structural changes which become established in phylogeny were of all possible types. It seems, rather, that in many groups chromosome after chromosome has undergone the same types of structural change, so that they have all retained a similar morphology.....The structural changes which are successful in the face of selection seem to obey what we may call the principle of homologous change (i.e. one chromosome after another undergoes the same type of change in the same phyletic line). This would be unintelligible if the crude mechanical views (of chromosomal structure)... were entirely true. But we must now consider chromosomes not as mere linear assemblages of genes, but as organised bodies, whose sequence of centromere, hetero- and eu-chromatic regions determines the mechanical relations of the chromosome to the spindle, the amount and position of crossing-over, and a variety of position effects. It is thus natural

that the structural re-arrangements which become established in evolution should tend to be of one or two main types in all the chromosomes of a particular group, thus leading to the very usual situation where all the chromosomes or chromosome limbs are about the same length and have a similar distribution of heterochromatic segments."

(White 1945: p. 173-174)

This conception served to place the chromosomes back into their cellular environment, for, as White stated, "it is probable that the whole architecture of the cell imposes a certain degree of limitation and canalisation on the types of structural chromosomal changes which can be expected to survive and establish themselves" (op.cit. p.174). It was not enough for a structural change not to upset the genic balance of the animal nor to be incompatible with a normal meiosis; but it had also not to diminish the efficiency of the mitotic or meiotic processes in a mechanical way, e.g. by an excessively long fusion-chromosome gyrating on a small or weak spindle.

As cells of related species might be alike in size, spindle properties, cytoplasmic density, etc., they would be expected to impose similar restraints on the possible pathways of visible chromosomal change.

That an upward limit to chromosome numbers is set by the size of the cell in relation to that of the chromosomes was mentioned by Darlington and Janaki

Ammal (1945).

As yet the principle of parallelism has not been tested on the mammals. In the rodents, we have already seen evidence of homologous change in that the theme of multiformity runs through groups of genera, species and even sub-species. Whatever polysomic and structural changes of the chromosomes have led to the multiformity seem to have recurred again and again in related groups. Conversely, the absence of such changes in the speciation of uniform groups again illustrates the principle of parallelism among all the species and sub-species of the uniform families and genera. A more precise characterisation of the homologous changes leading to multiformity will be attempted below, when the types of chromosomal alteration in multiform groups will be examined.

(IV) Progressive Reduction of Chromosomal Numbers.

Perhaps the most comprehensive attempt to detect trends in the evolution of animal chromosomes has been that of Matthey (1949). To assess the direction of chromosomal change in the mammals, he has examined the position in other vertebrate classes.

In the fishes, amphibians and birds, the N.F. (fundamental number) is very high (70 arms). In the reptiles, the Chelonians are similar in make-up to the

afore-mentioned three groups, whereas the Ophidian and Lacertilian orders of reptiles have, as their commonest N.F., 48 arms.

Of the mammals, the few monotremes studied closely resemble the Chelonians and, especially, the birds, in their chromosomal complements. There is a large number of arms and a clear division into macro- and micro-chromosomes ('H'- and 'm'-chromosomes). The marsupials, on the other hand, have a very low N.F., between 22 and 36. The problem in the eutherian mammals is complicated because of the big range of chromosomal numbers. Matthey shows that a good case could be made out for either a high or a low ancestral N.F. in this group.

If the N.F. had been initially low, Matthey argues, marsupials would occupy an ancestral position, while the monotremes with high chromosomal numbers would not occupy an ancient position in the mammalian lineage.

On the other hand, if the ancestral N.F. were high, the monotremes would occupy a position of great antiquity, while the marsupials would be regarded as a 'specialised' group, not ancestral to the main mammalian lineage. The close relation of monotreme chromosomal formulae and general morphology to those of birds and reptiles favours an ancestral position for the high N.F. of the

monotremes.

Matthey therefore proposes the hypothesis that the primitive, ancestral N.F. of the vertebrates as a whole and, the mammals specifically, was high. From this starting-point, evolution has taken the course of a reduction in the primitive number by fusion to form metacentrics (Robertson's hypothesis) and by elimination of the m-chromosomes. These processes have advanced to varying degrees in different vertebrates:

In the Amphibia, they are most advanced in the most 'highly-evolved' families, the Salamandridae and the Bufonidae; they are least advanced in the more 'primitive' families, Hynobiidae and Pipidae.

In the Reptilia, the Chelonians are at an early stage in reduction, while, in the Ophidians and Lacertilians, the N.F. has been much reduced, although there are still some inert m-chromosomes present; the process in these two groups has been accomplished chiefly by centric fusions.

The birds and the monotremes have retained a high N.F. and the sub-division into M- and m- chromosomes.

In the marsupials, again, the process of elimination is far advanced and the chromosomal numbers are small.

The eutherians, however, present a bewildering array of diploid and fundamental numbers. It seems as if, says Matthey, the processes of decrease or increase have occurred independently of the degree of morphological differentiation. He contrasts the ancient and almost extinct Amphibia, in which species formation seems to have ceased, with the more recent, still flourishing Mammalia, in which the role of chromosomal change remains considerable. Matthey concludes that, since chromosomal formulae vary in their adaptive value, by the time the mammals reach the end of their evolution, many formulae will have disappeared: chromosomal homogeneity will have replaced heterogeneity.

Matthey thus attributes the disorder and irregularity of eutherian chromosomal complements to the state of evolutionary flux through which the placental mammals are now passing. In this age of mammals, the tremendous efflorescence of new species, experimenting with countless new modes of life, testing innumerable new sets of phenotypic equipment, evincing manifold variations in basic genetic and chromosomal complements, militates against the finding of any clear-cut paths of karyotypic development.

Matthey's conclusion is unnecessarily pessimistic.

We know that relatively few chromosomal estimations have been made on mammals. Of the recorded chromosomal numbers, only a proportion are accompanied by the information necessary to determine the fundamental numbers. May we not conclude that, to read order into the apparent heterogeneity of mammalian chromosomes, we should wait, not until the mammals have reached the end of their evolution, but until more facts are available or until newer concepts emerge and find application?

(2) GENERALISATIONS BASED ON EXPERIMENTAL DATA.

The above discussion has been based on comparative data, from which types and trends of chromosomal change have been inferred. Let us now enquire what changes it has been possible to induce in the chromosomes under experimental conditions. Again, we shall be concerned with chromosomal changes relevant to alterations in number.

(A) Re-arrangements induced by Irradiation.

All of the two-break and most types of the three-break re-arrangements tabulated by Muller (1940) have been found in irradiation experiments on *Drosophila*. A series of generalisations has been made by Muller on the results of these experiments. The broadest principle is that "changes of gene-arrangement which survive do not
hromosome threads,

but always an exchange of connections of the threads, i.e. breakage, with re-attachment at each surviving point of breakage."

Secondary principles follow:

- (1) "For a re-arrangement to occur, breakage must take place at at least two points (either in the same or different chromosomes)."
- (2) "...Union of the resulting pieces can occur only between broken ends."
- (3) "At any given point of re-attachment, one broken end can unite with only one other."

Implicit in No. (2) is the idea put forward by Darlington, by Haldane and by Muller that the ends of every chromosome are marked by permanent chromosomal structures ('telomeres') distinguished by their constantly monopolar character. This concept is not fully accepted, Stadler and McClintock having obtained ~~terminal~~ deficiencies of chromosomes in Zea mays under ultra-violet irradiation. If the telomere concept is correct and, if we accept that there is a single centromere in all chromosomes, simple fusion of two rods to form a V cannot be assumed to have occurred. Instead, the mechanism underlying Robertsonian variation would be reciprocal translocations between two acrocentric chromosomes. The break in the first chromosome would be in the short arm,

that in the second in the long arm. Such changes have been found experimentally (Muller 1940), so it may be concluded that chromosomes do possess the potentiality for Robertsonian variation. It is important to note that reciprocal translocation has been induced by X-irradiation in mice (Koller and Auerbach 1941, Koller 1944). Thus, even in the Rodentia, the mechanism of Robertsonian variation is known to occur.

It should be mentioned that Matthey (1951) has queried whether the mechanism underlying Robertsonian variation is indeed the reciprocal translocation as just described. This type of segmental interchange, he argues, involving a break in the tiny shorter arm of an acrocentric, would be extremely rare, whereas comparative cytological studies have demonstrated that V-to-rod or rod-to-V changes occur very frequently. Matthey is tempted to suggest that a V is the result of the apical fusion of two telocentric chromosomes. However, he appreciates the difficulties inherent in this hypothesis, namely that the existence of two centromeres in a metacentric chromosome (a necessary consequence if two telo-centromeres were to unite) has never been demonstrated and secondly, that even the existence of strictly telocentric chromosomes is denied. He concludes that the mechanism of Robertsonian variation has not been elucidated. Our own conclusion is that the special type of reciprocal translocation

described above is the only possible explanation of Robertsonian variation consistent with present views on chromosomal change and the only mechanism which has been experimentally demonstrated.

(B) Experimental Induction of Polysomy.

Several examples of artificially induced polysomy in plants are known. Collins, Hollingshead and Avery (1929) were able to produce a polysomic species, Crepis artificialis, by crossing C. biennis and C. setosa and, by continued selfing of the progeny. Darlington (1937, p. 241-242) has quoted other instances of artificially induced polysomy.

(C) Experimental Induction of Polyploidy.

Various stimuli - colchicine, extreme temperatures and species-crossing - have produced polyploids in plants (Waddington 1939).

(D) Experimental Confirmation of Comparative Chromosomal Differences.

Differences between Drosophiline chromosomes found by genetic or comparative methods have, in all instances, been confirmed when it has been possible to obtain hybrids between the species compared. This is true of D. melanogaster and D. simulans; D. virilis and D. americana; D. pseudo-obscura and D. miranda; D. athabasca and D. axteca (Muller 1940).

We may conclude that the chromosomes have been shown experimentally to be capable of all the types of chromosomal change postulated on comparative studies.

CHROMOSOMAL EVOLUTION IN THE RODENTS.

The types and trends of chromosomal change have been delineated and it has been seen that some of these apply to the rodents. Evidence has been adduced for the existence of polysomy and of structural re-arrangements in rodents; evidence so far collected for Robertsonian variation in rodents is slender, while, for polyploidy there is none at all. Multiformity and uniformity, karyo-somatic dissociation and homologous change have all been demonstrated in rodents, while there is no evidence from rodent material to support Matthey's notion of progressive reduction in chromosomal number.

It remains now to apply the concepts of chromosomal change to a more detailed analysis of chromosomal variation in rodents and, in particular, in the gerbil.

In the first place, let us address ourselves to the fundamental numbers of the rodents in order to ascertain whether or not Robertsonian variation has indeed been a factor in their diversification. In the majority of chromosomal studies, little or no mention is made of V- or J-shaped chromosomes and one is forced to rely on the illustrations

of metaphase plates - not always a reliable procedure, as was pointed out in Chapter III. Matthey (1949) has given his interpretation of the published data and, on this basis, I have compiled in the last column of Table 8 (pages 96-102), a list of fundamental numbers of species in the sub-orders, super-families and families of rodents.

The figures for the gerbil are included in the Table. There are generally 12 chromosomes with sub-median constrictions and 2 chromosomes which are sometimes interpreted as having sub-terminal and sometimes sub-median constrictions. The latter pair of chromosomes have rather a long minor arm, in other words are J-shaped. The fundamental number of the gerbil has therefore been recorded as $34 + 14 = 48$ arms.

The information in Table 8 may be summarised as follows (families containing only one species are omitted):-

TABLE 16.

DISTRIBUTION OF CHROMOSOMAL AND FUNDAMENTAL NUMBERS
IN RODENT FAMILIES.

<u>FAMILY</u>	<u>2n</u>	<u>N.F.</u>
<u>Sciuridae</u>	28 - 62 (range 34)	54 - 80 (range 26)
<u>Heteromyidae</u>	44 - 86 (range 42)	60 - 100 (range 40)
<u>Muridae</u>	40 - 68 (range 28)	40 - 70 (range 30)
<u>Cricetidae</u>	14 - 60 (range 46)	48 - 76 (range 28)
<u>Gliridae</u>	48 - 62 (range 14)	72 - 96 (range 24)

In interpreting the ranges of diploid and fundamental numbers in Table 16, we rely on the assumption that the formation of a V from two rods reduces the chromosomal number without altering the fundamental number. If, then, all the species in a family had undergone only Robertsonian changes, we should expect that, though possessing a wide range of diploid numbers, all the species would have the same fundamental number. In other words, the range of diploid numbers would be reduced from some variable whole number to nil, as the range of fundamental numbers. If any other form of structural change or polysomy had played a small rôle in the chromosomal evolution of a family of species, it would be expected that the N.F. range would not be nil but some small whole number. Thus, if a V in one species had been created by a pericentric inversion instead of by a fusion of two rods, the N.F. would be increased by the addition of two extra arms not represented in the other species of the family: the species concerned would therefore have an N.F. equal to two arms more than the N.F. of the other species. The range of fundamental numbers would then be 2 instead of 0. Of course, not all structural changes would work in the same direction: thus a pericentric inversion converting a V to two rods would liquidate one arm and give an N.F. equal to two arms lower than the N.F. of the other species. But, this would still give a range of 2 for the fundamental

numbers of all the species in the family. Whether structural changes remove or add arms, they will have the effect of increasing the range of the N.F. in the family of species.

An interesting illustration of the reduction of the diploid range to an N.F. range of 2 is provided by Bovey's results in a study of Chiroptera (1949a). In two families of bats, he obtained the following diploid and fundamental numbers (ranges only are given):-

	<u>2n</u>	<u>N.F.</u>
<u>Rhinolophidae</u>	54 - 58 (range 4)	60 - 62 (range 2)
<u>Vespertilionidae</u>	32 - 46 (range 14)	52 - 54 (range 2)

When diploid numbers are converted to fundamental numbers, there is an appreciable reduction in the range. Therefore, in these two families, we may conclude that Robertsonian variation has seemingly been the most important method of chromosomal divergence.

However, non-Robertsonian structural re-arrangements are not the only class of changes which can influence the size of the N.F. range. Polysomy will function in a similar manner to structural changes. If a chromosome is lost, the diploid chromosomal number will be smaller by two, while the N.F. will be smaller by 2 or 4 according as the chromosome lost was acrocentric or metacentric. Thus the N.F. range will be greater by 2 or 4 for every chromosome lost from the haploid complement. Conversely, the gaining

of an extra chromosome will increase the N.F. by 2 or 4 and thus increase the N.F. range from 0 to 2 or 4.

Whether polysomy adds or removes chromosomes, it will increase the range of the N.F. in the family of species.

We see then that both polysomy and non-Robertsonian structural changes will act against the reduction of the range to nil, when fundamental numbers are compared with diploid numbers. If, then, there is any reduction in range whatever, on changing diploid into fundamental numbers, the reduction is to be attributed to Robertsonian variation; to the extent that the reduced range exceeds nil, polysomy and/or non-Robertsonian structural changes have occurred. Should the N.F. range exceed the $2n$ range, either

(i) polysomic and/or non-Robertsonian structural changes have been the only types of visible chromosomal variation to occur in the speciation of that family, or (ii) any Robertsonian variation which has occurred has been more than counter-balanced by the non-Robertsonian and polysomic changes.

Reverting to Table 16, we see that in three families, the Sciuridae, the Cricetidae and the Heteromyidae, the N.F. has a smaller range than the $2n$ number. From the foregoing argument, this can only mean that Robertsonian variation has been an appreciable factor in the chromosomal evolution of these families. That the ranges have been reduced only from 34 to 26, 46 to 28 and 42 to 40, and not to nil, indicates

that, as well as Robertsonian variation, non-Robertsonian and/or polysomic changes have also been factors in the chromosomal divergence of the species in each of these three families.

In two other families, however, namely the Muridae and the Gliridae, the N.F. is higher than the $2n$ number. This suggests that Robertsonian variation has been relatively less important in the evolution of these groups, while non-Robertsonian and/or polysomic changes have been relatively more significant.

If we assume that both Robertsonian and non-Robertsonian (and/or polysomic) variations have operated in all cases, we may arbitrarily assign scores to the relative contributions of Robertsonian and non-Robertsonian variation. A range of N.F. equal to nil connotes 100% operation of Robertsonian and 0% of non-Robertsonian variation. If the N.F. range is equal to the $2n$ range, the non-Robertsonian exactly counterbalances the Robertsonian variation, i.e. each scores 50%. Arbitrarily, we may extrapolate the relationship in the direction of an increased N.F. range so that when the N.F. range is double the diploid range, non-Robertsonian variation may be said to contribute 100% and Robertsonian 0% to the chromosomal diversity. Intermediate degrees of reduction or increase of the N.F. range can be graded on a simple proportional basis.

The following scores are then obtained:-

<u>Family</u>	<u>Robertsonian Variation</u>	<u>Non-Robertsonian (and/or polysomic) variation.</u>
<u>Sciuridae</u>	61.8%	38.2%
<u>Cricetidae</u>	69.5%	30.5%
<u>Heteromyidae</u>	52.4%	47.6%
<u>Muridae</u>	46.4%	53.6%
<u>Gliridae</u>	14.3%	85.7%

The three families showing over 50% Robertsonian variation are the three multiform groups; the two families showing under 50% Robertsonian changes are the two uniform groups. In other words, it is probable that the very multiformity of the Sciuridae, the Cricetidae and the Heteromyidae is due largely to the great amount of Robertsonian change to which they have been subject; conversely, the slighter occurrence of Robertsonian variation in the Muridae and the Gliridae seems to have been a contributory factor to, if not the main cause of, their uniformity.

We may now define more exactly the nature of the homologous changes in the five rodent families. In the multiform families, species after species seems to have undergone rods-to-V or V-to-rods changes, while being subject to lesser degrees of polysomy and/or non-Robertsonian structural re-arrangements. In the uniform families, the chromosomes of one species after another seem to have proved resistant

to Robertsonian changes, but to have been subject to greater degrees of polysomy and/or non-Robertsonian structural variations. Special conditions would seemingly prevail in the cells of the latter group of families, militating against V-to-rods or rods-to-V changes, unlike the former group of families, where the cells have apparently proved susceptible to the stimuli evoking Robertsonian changes.

If now we consider the relation between N.F. and $2n$ numbers of individual species, we see that two types of species exist: that in which the N.F. and the $2n$ number are equal and that in which the N.F. is higher than the $2n$. The former type of species shows no sign of V- or J-formation having played a part in its evolution; the latter shows evidence of V- or J-formation.

The two types of species are distributed in families as follows:

TABLE 17.

<u>FAMILY</u>	<u>NUMBER OF SPECIES IN WHICH</u>	
	<u>N.F. = 2n</u>	<u>N.F. > 2n</u>
<u>Sciuridae</u>	0	5 (100%)
<u>Heteromyidae</u>	0	2 (100%)
<u>Muridae</u>	22 (88%)	3 (12%)
<u>Cricetidae</u>	6 (19.4%)	25 (80.6%)
<u>Gliridae</u>	0	4 (100%)

When we considered the range of N.F. and 2n numbers above, we concluded that Robertsonian variation had played a bigger rôle in the multiform than in the uniform families. Now, by analysing the number of species in each family showing N.F. = 2n and N.F. > 2n, we are partly able to corroborate and partly to extend our earlier conclusions. From Table 17, we see that in the three multiform families, Sciuridae, Cricetidae and Heteromyidae, the vast majority of the species possess N.F. > 2n. This suggests that a process has been at work creating V-or J-shaped chromosomes in these species: such a process may be either Robertsonian 'fusion' or non-Robertsonian structural re-arrangements, e.g. pericentric inversion. In view of the relatively greater frequency of the former than of the latter type of variation in these 3 families, we may accept that Robertsonian 'fusions'

have been the more important producer of V's and J's in the chromosomal evolution of the multiform groups.

In the uniform Muridae, 88% of species seem to have been uninfluenced by V- or J-formation and therefore by the process leading to it, namely Robertsonian variation and non-Robertsonian structural rearrangements. It should be stressed here that this does not mean non-Robertsonian structural changes have not occurred to any appreciable extent, but only non-Robertsonian changes in those positions which would lead to a change in the number of arms. On the other hand, all four species of the uniform Gliridae show that V- and J-formation have been important in this group. Since we have already adduced evidence above that Robertsonian variations have accounted for only a minor part of chromosomal evolution in the Gliridae, we may conclude that the V- and J-formation in this family has been mainly effected by non-Robertsonian structural changes e.g. pericentric inversions.

The evidence for this type of rearrangement in four species of Gliridae provides us with another example of homologous chromosomal change in related species.

Another consideration to arise from Table 8 is that we have evidence for complete fragmentation (pseudo-polyploidy) or complete fusion as postulated by Vandel. The best example is in the sub-family, Microtinae. Most members of this group

have a chromosomal number of 56 and a N.F. of 56.

Microtus kikuchii, however, has $2n = 28$ and N.F. = 56.

Clethrionomys rutilus mikado also has $2n = 28$ and probably N.F. = 56. The chromosomal formulae of these two species

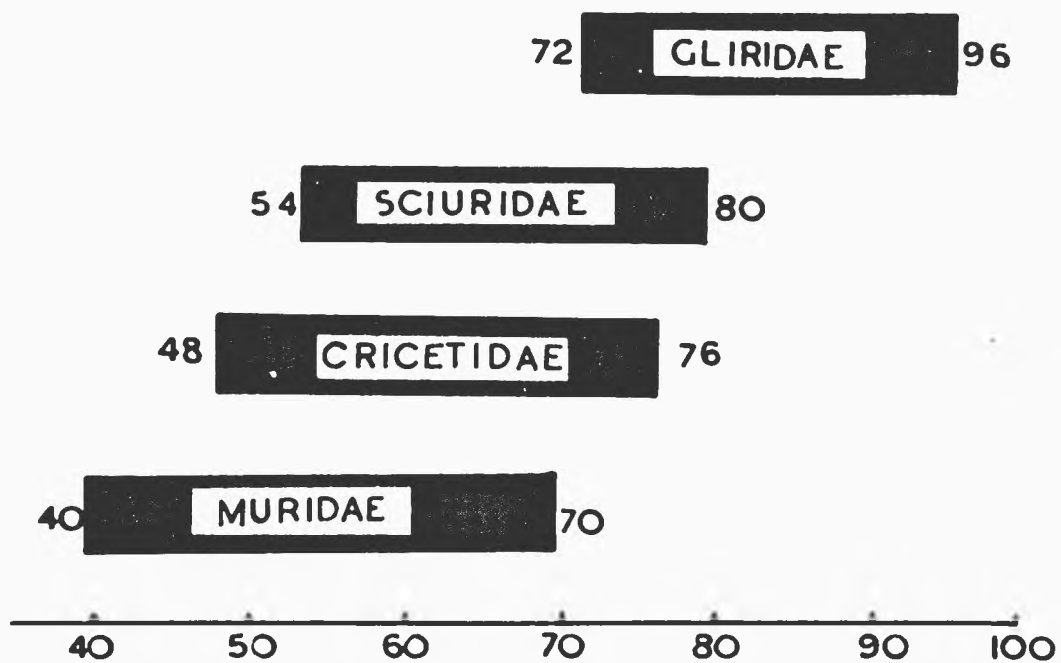
have presumably arisen by complete Robertsonian fusion affecting all the 56 chromosomes of the N.F. and giving a chromosomal number just half that of most species in the group. Other possible examples, not as clearly defined,

are Sciurus carolinensis carolinensis and Sciurus carolinensis leucotus; Sciurus and Tamasciurus; Perognathus fallax and Dipodomys merriami.

THE EVOLUTION OF THE GERBIL'S CHROMOSOMAL FORMULA..

It is now possible to see the gerbil's position in mammalian cyto-phylogeny more clearly. From its classificatory status, we may make certain predictions.

As a member of the Mammalia and, more specifically, of the Rodentia, the gerbil might be expected to have a higher chromosomal or fundamental number. However, the diploid number, 34, falls in the 'below 40' group of mammals, while the fundamental number is 48. In view of this low number, we may next enquire whether the family to which the gerbil belongs is characterised by numbers rather lower than the modal number for rodents. Fig. 36 shows the scheme of fundamental numbers in rodent families. It is clear that only two families contain species with fundamental numbers as low



SCHEME OF FUNDAMENTAL NUMBERS IN
FOUR FAMILIES OF RODENTS

Figure 36. Scheme of fundamental numbers in four families of rodents.

as that of the gerbil, viz. the Cricetidae and the Muridae. So, in a certain measure, the low $2n$ and N.F. of the gerbil reflects its classificatory position, even although the gerbil's N.F. falls at the lower end of the range of Cricetid fundamental numbers.

If we were to accept Matthey's hypothesis of progressive reduction of numbers, we might conclude that the Gliridae have retained their primitive high numbers while the other groups have progressively reduced their fundamental numbers. Viewed in this light, the gerbil, close to the extreme lower end of the entire range, would have reached an advanced stage in this process of reduction of the N.F. However, while Matthey's conception may have some validity when applied to whole vertebrate classes, its application within a limited group like the rodents is less clearly indicated and, certainly, of restricted value. We have already referred above to the pessimistic conclusion to which this conception led Matthey. More useful results may be obtained by interpreting the gerbil's chromosomal number in terms of such concepts as multiformity and uniformity, Robertsonian variation, polysomy, karyo-somatic dissociation and homologous change.

The gerbil belongs to a multiform family, the Cricetidae. From this fact, we might make several predictions: it is likely that variances will be found (a) between the chromosomal numbers of different Gerbilline genera, (b) between

those of various species of Tatera and, (c) perhaps, even between those of different sub-species of Tatera brantsii. All these predictions are capable of empirical verification. Further, we may predict that Robertsonian variation has played an appreciable part in the chromosomal evolution of Tatera and other gerbils; as one evidence hereof, we would expect T. brantsii draco to have a greater N.F. than 2n number.

When we now look at the facts, we see that the gerbil has, indeed, a higher N.F. than 2n number - the difference being 14. This verifies, in part, our prediction that Robertsonian variation would prove to have been important in the evolution of the gerbil.

The diploid and fundamental numbers of Tatera are compared below with the diploid and fundamental numbers in the Cricetidae as a whole:

<u>T. brantsii draco</u>	2n = 34	N.F. = 48
<u>Cricetidae</u> (range)	2n = 14 - 60	N.F. = 48 - 76
<u>Cricetidae</u> (modes)	2n = 48	N.F. = 56

In its chromosomal number, the gerbil falls comfortably within the Cricetid range but well below the mode of 48. The small value of the gerbil's number suggests that the complement may contain many metacentric chromosomes.

However, from our characterisation of the gerbil's

chromosomes, we know that there are only 14 clearly metacentric chromosomes, giving a fundamental number of 48. The latter figure is smaller than the previous lower limit of Cricetid fundamental numbers (50). This suggests that the 14 metacentrics in the gerbil's set are the result of Robertsonian fusion of 28 acrocentric chromosomes, rather than the consequence of, say, pericentric inversion which would make the true N.F. still lower. Conditions in the gerbil's cells, then, parallel those in other multiform groups by imposing no restraint on the occurrence of Robertsonian fusions.

Even allowing for these Robertsonian changes, the number of arms in the gerbil is very low - lower than any other estimated Cricetid. We may conclude that the gerbil has probably lost a number of chromosomes or chromosomal arms either by polysomy or by non-Robertsonian structural change. If the modal N.F. of the Cricetidae, 56, is also the ancestral N.F. for this group, the gerbil with its N.F. of 48 must have lost at least 8 chromosomal arms in the course of its evolution.

Thus, both Robertsonian and non-Robertsonian variations seem to have moulded the chromosomal pattern of the gerbil from an ancestral formula to its present composition. But, while the Robertsonian changes have paralleled those in other Cricetid species, the degree of loss of arms is peculiar to the gerbil's cells. It is difficult to determine whether karyo-somatic dissociation is evident in the evolution

of the gerbils, until we have more evidence from related species. The only indication of homologous change is the accordant Robertsonian variation in the gerbil along with other members of the multiform Cricetidae. Those cellular conditions which encourage or evoke fusion or fragmentation, are shared by the gerbil with other Cricetid species.

At some stage in the evolution of the gerbil, then, we must assume either that an increase in the establishment of polysomy occurred, or that a series of other changes led to the loss of chromosomal arms. Whatever the mechanism, the gerbil differs in respect of the resulting changes from most other explored species of Cricetidae: the loss of chromosomes or arms may be confined to the speciation of Tatera brantsii, or further estimations may show it to be common to all the Gerbillinae. Thus, further studies on gerbillines may indicate whether an increased loss of chromosomes or arms occurred at more ancient or more recent times in this part of the rodent lineage; while the possibility of arm or chromosome loss being a parallel change in various gerbils will have to be faced.

In sum, the gerbil has departed from the postulated ancestral chromosomal number of the Cricetidae by diminishing its chromosomal number. This has apparently been effected by two mechanisms:

(i) by Robertsonian change of at least 28 rod-shaped chromo-

somes to form metacentrics, in parallel with similar variations in other multiform species; and

- (ii) by polysomic loss of a number of chromosomes and/or structural changes leading to loss of chromosomal arms.

From the chromosomal equipment of the gerbil, we have been able to reconstruct an historical context, which reveals one aspect of the intricate mechanism of speciation. Essentially the same principles could be applied to throw light on the grand process of phylogenesis. It is well to recall, however, that this chromosomal approach provides only one sketch of past history. Other sketches could be drawn from a study of almost every aspect of the organism and its life. For any one such sketch to be valid, it must blend with the others in an harmonious panorama. There must be no single part of the scene which jars the intellect, as an injudicious stroke of the brush jars the sensitive eye. Our picture must be coherent and consistent.

This sketch of the gerbil's cyto-phylogeny is one picture of the past: its ultimate validity will depend upon its ability to be integrated into the broader sweep of rodent evolution.

SUMMARY.

(1) The rise of the concept of species-specificity of the chromosomal number has been traced, since cyto-phylogeny

is based on the idea that the chromosomal complement is a specific constant.

(2) From the literature, the chromosomal numbers of 82 rodents have been compiled and analysed; the survey shows a very unequal distribution of the animals characterised - only 9 of 32 extant families of rodents have been sampled.

(3) Two types of rodent family may be distinguished on the patterns of their chromosomal numbers: in a 'uniform' family (e.g. the Muridae), the genera are uniform, i.e. contain species of which the chromosomal numbers fall within narrow limits, the species are uniform as regards the chromosomal numbers of their sub-species and, the total range of chromosomal numbers in the family is small. The 'multiform' family (e.g. Sciuridae, Cricetidae) contains multiform genera, while the latter comprise multiform species and the total range of chromosomal numbers in the family is large.

(4) The gerbil belongs to the most multiform family, the Cricetidae.

(5) It seems that evolutionary morphological divergence in some rodent families has been accompanied by much divergence of chromosomal numbers; while, in others, there has been a dissociation between morphological and chromosomal divergence, so that the former has proceeded without the latter.

(6) The history of chromosome enumeration is traced and it is demonstrated that the earlier dominant attitude towards the chromosomes profoundly limited the collection of data on chromosomal numbers.

(7) From a tabulation and analysis of 169 mammalian chromosomal numbers, it is demonstrated that, in all adequately studied mammalian orders, the tendencies to multiformity and uniformity are manifest.

(8) Other trends manifest in chromosomal evolution are those of karyo-somatic dissociation and homologous change; both trends are exemplified by the rodents.

(9) The principles of chromosomal change are discussed as they have emerged from both comparative and experimental data and, it is concluded that all types of chromosomal change postulated on comparative studies (e.g. polysomy, fusion and fragmentation, other structural re-arrangements and polyploidy) have been produced under experimental conditions.

(10) In applying the generalisations on chromosomal change to the rodents, it is demonstrated that multiform families owe their multiformity largely to the great amount of Robertsonian (rods-to-V or V-to-rods) variation which they have undergone; whereas, in the uniform families, species after species has apparently resisted Robertsonian variation, but has been subject to greater degrees of polysomy and/or non-Robertsonian structural variations.

(11) Evidence is adduced that complete fragmentation (pseudo-polyploidy) or complete fusion has occurred in the evolution of some rodent genera.

(12) The gerbil has apparently departed from the postulated, ancestral chromosomal number of the Cricetidae by diminishing its chromosomal number, partly by Robertsonian fusions and partly by non-Robertsonian changes (polysomy or structural modifications eliminating chromosomal arms).

END OF PART 2.

PART 3.

DESCRIPTIVE ACCOUNT OF SPERMATOGENESIS

C H A P T E R VI.

M I T O S I S. M E I O S I S A N D
S P E R M I O G E N E S I S.

INTRODUCTION.

In this chapter, mitosis, meiosis and spermiogenesis are described. The events in the seminiferous tubule provide a temporal and spatial setting for the modifications of chromosomal morphology, to which the preceding part of this thesis has been devoted. However, not only do we view spermatogenetic happenings as they influence the chromosomes; but, on their own merits, mitosis, meiosis and spermiogenesis warrant detailed study for the light they throw on the pattern of differentiation in the tubule.

The sequence of events in the cytoplasm is traced in parallel with that of the nucleus, as far as the techniques permitted. Bouin-fixed, iron-haematoxylin-stained sections reveal much of interest in the cytoplasm. The cell organoids studied are the following:

- (i) the centrioles;
- (ii) the archoplasm (idiosome or chromophobic part of the Golgi apparatus);
- (iii) the division-spindle, including the phragmoplast and the mid-body;

and (iv) the chromatoid bodies, including the manchette and the neck rod.

The four sets of structures receive detailed attention in the description, since the cytoplasmic events are subsequently treated as modulations in the integral seminiferous tubule.

TERMINOLOGY AND DEFINITIONS.

Since most of the present chapter treats of spermatogonia and spermatocytes, it is necessary at the outset to define these cell-types. However, Chapter XII, in considering the lineage of the cells in the seminiferous tubule, discusses at length the recognition of spermatogonia and spermatocytes. Suffice it, here, to quote the definitions (modified after Hoffman, 1947) and to defer the more detailed discussion to Chapter XII.

The class 'spermatogonia' consists of (a) those germ-cells undergoing their last mitotic division before the onset of meiosis and (b) those germ-cells still capable of undergoing mitotic division, before proceeding to meiosis.

The class 'primary spermatocytes' comprises those germ-cells which arise after the last mitotic division of the spermatogonia and become transformed after the first meiotic division into secondary spermatocytes.

Several terms applying to cytoplasmic inclusions

require clarification.

(1) Centriolar apparatus: Among the terms used in relation to the centrioles are microcentrum, centrosome, centrosphere, central bodies, centropiasm, centrotheca, idiozome or idiosome, centrum, cytocentrum.

Some of these terms overlap; some have been superseded; some apply only in certain groups. Sifting through these names, one concludes from the study on the gerbil that only three terms are necessary:-

- (i) Centriole: This is the smallest structure associated with the division-centre. The centriole is a minute granule, staining deeply with iron haematoxylin and, often not much larger or more distinct than some of the other iron-haematoxylin-stained, cytoplasmic granules.
- (ii) Centrosome: This is a rounded, lightly-staining body, surrounding the centrioles. Often it resembles nothing more than a halo or contraction-space such as one may find encompassing dense cytoplasmic structures like the chromatoid bodies.
- (iii) Archoplasm: The centrosome, with its contained centriole, may lie within or to one side of a large area of modified cytoplasm, the archoplasm. This will be discussed in the next section. The term 'archoplasm' is preferred to such other terms as 'centrosphere', 'attraction

sphere', 'centrotheca', which stress only the relation to the centrioles, whereas this archoplasm is known to play an important role in the germ-cells apart from its relation to the centrioles (vide infra).

As far as can be judged, the following terms are used synonymously (based on Gatenby and Woodger 1921, Wilson 1925, Gresson 1948, De Robertis 1949):

TABLE 18.

SYNONYMOUS TERMS RELATING TO THE CENTRAL APPARATUS
AND THE ARCHOPLASM.

<u>ALTERNATIVE OR OLDER TERMS.</u>	<u>RECOMMENDED TERMS.</u>
Central body ? Centrum ?	Centriole
Central body ? Centroplasm Centrum Microcentrum Cytocentrum	Centrosome
Centrosphere Centrotheca Idiozome Idiosome Sphere Attraction sphere Accessory corpuscle Archiplasm	Archoplasm

(2) Archoplasm: Boveri (1888) first coined the term 'archiplasm' referring to the material of the spindle-fibres and the astral rays. Gatenby and Woodger (1920-21) have listed the following terms which have been applied to the archoplasm:-

TABLE 19.

EARLIER NAMES APPLIED TO THE ARCHOPLASM.

<u>TERM</u>	<u>AUTHOR</u>
Nebenkern	Hermann 1891
Sphare	(Niessing 1896 (Meves 1899
Archiplasm	Benda 1896-97
Idiozome	Meves 1899
Archoplasm	Moore 1906
Accessory Corpuscle	Brown 1910
Idiosome	Papanicolaou and Stockard 1918

Gatenby and Woodger suggest the adoption of Moore's term, archoplasm, for use by English-speaking workers. Later, Gatenby appears to have dropped the term in favour of "the chromophobic part of the Golgi apparatus" (Gatenby and Beams 1935). It is not certain whether it can validly be assumed that the two cell-inclusions are identical. Moreover, it is convenient to have a shorter term to refer to the structure and most workers have continued to use

'archoplasm' (e.g. Gresson 1948, 1951). This usage is maintained in the present study.

(3) Chromatoid body: This body was originally called by Benda (1891) the chromatoider Nebenkörper or chromatoider Körper; Duesberg (1908) adopted this term in French - corps chromatode - and, it is generally known among English-speaking workers by the English equivalent. Gresson (1948) has named it the 'accessory body' but this term is unsatisfactory, as it may lead to confusion with 'accessory bodies' described as products of the Golgi apparatus in spermatogenesis (Gatenby and Beams 1935). Hence, in this study, we shall use the term 'chromatoid body'.

SECTION I : MITOSIS.

THE SPERMATOGONIUM: INTERPHASE.

(a) Nucleus.

Fig. 37 shows the oval nucleus of the spermatogonium, lying with its long axis parallel to the basement membrane. One surface of the nucleus is often flattened along the limiting membrane. During the interphase, there is a thin but distinct, Feulgen-positive nuclear membrane. All the chromatin is finely dispersed as strands or granules, except three or four small, deeply-staining chromatic masses, often fused together and closely applied, as an incomplete ring, to the surface of the plasmosome. All the stainable

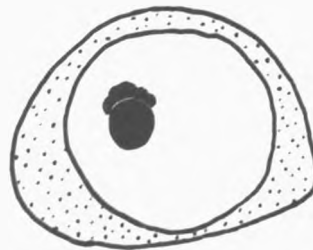
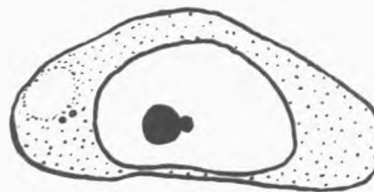
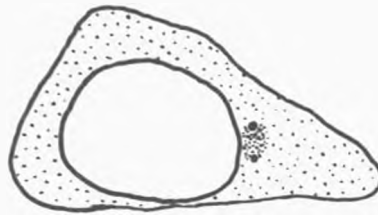


Figure 37. Camera lucida drawings of three interphasic spermatogonia.

- Note:- (i) plasmosome and karyosome;
(ii) suggestion of archoplasm in upper cell (rare at this stage);
(iii) centrioles and centrosome in middle cell.

16 μ section stained with Heidenhain's haematoxylin.

Magnification: upper two cells x3250
lower cell x3500

material in the single, large, spherical plasmosome is evenly distributed, unlike the Sertoli cell-plasmosomes which have a lightly-staining core. The significance of these two forms of staining is not known. The spermatogonia differ, too, from the spermatocytes, in that the latter have more than one plasmosome. We thus have evidence of an alteration in plasmosomal physiology as the germ-cells pass from the mitotic to the meiotic stages of spermatogenesis. These plasmosomal variations will be discussed more fully later.

(b) Cytoplasm.

Although abundant, the cytoplasm in the spermatogonium stains poorly in fixed, haematoxylin-stained preparations. It is attenuated into strips of faintly granular material, irregularly arranged and separated by clear spaces. With Bouin's picro-formol-acetic fixative, the cytoplasm collapses easily and is difficult to fix. In human spermatogonia, Gatenby and Beams (1935) have noted a similar phenomenon, which they have ascribed to an abundance of water in the cytoplasm.

Cytoplasmic inclusions are not regularly seen in spermatogonia. In some cells (Fig. 37), centrioles are identifiable as a pair of deeply-staining granules. Occasionally, the archoplasm is visible; the size, shape and appearance corresponding closely with similar features of the archoplasm in spermatocytes. Generally, however, the archoplasm is not seen before the pachytene stage of the primary spermatocyte.

apart from the few exceptional cells containing an archoplasm, it is true to say that the cytoplasm of the spermatogonia is free from any extraordinary inclusions, unlike the highly-specialised cytoplasm of the spermatocyte.

THE SPERMATOGONIUM: PROPHASE.

A study of prophasic events in the gerbil's spermatogonia provides clear evidence that (a) the nuclear membrane consists of at least two layers and (b) a part at least of the chromatin of mobilising, prophasic chromosomes is provided by the chromatin of the chromatic membrane.

Evidence has for many years been accumulating to show that the nuclear membrane is a compound structure, consisting in most cells of a non-staining outer layer, the true membrane or 'karyotheca' and a staining, inner layer of chromatin or 'chromatic membrane'. Nevertheless, while cytologists have long emphasised the double structure of the nuclear membrane, cytogeneticists have apparently not adequately appreciated that the chromosomal threads of mitosis or meiosis become resolved from the chromatic nuclear membrane of the interphase.

The gerbil has provided particularly favourable material for observations on the intimate relation between the chromatic membrane and the chromosomes of dividing cells. Clearly, this relation could best be demonstrated in cells

at that stage when the chromatic membrane disappears and the chromosomes make their appearance as discrete entities (i.e. prophase) or at that stage when the chromatic membrane re-appears and the chromosomes lose their discrete identity (i.e. telophase).

Accordingly, a detailed description will be given of the prophasic mobilisation of the chromosomes in the gerbil.

Fig. 38 shows a spermatogonium in early prophase. Fine, Feulgen-positive threads fill the large, oval nuclei. The chromatic nuclear membrane is Feulgen-positive and, at this stage, is discontinuous. Close focussing reveals a fine chromosomal thread hanging from each stained segment of the nuclear membrane. Prophasic nuclei in the gerbil possess this characteristic appearance of chromosomes disposed about the surface of a hollow ellipsoid and peeling off the nucleocytoplasmic interface.

In the interstices between the segments of chromatic membrane, a distinct interface is seen between the clear nucleoplasm and the granular cytoplasm. The presence of this clear linear interface and the absence of a visible blending between the apposed nucleoplasm and cytoplasm provide evidence for the existence of the karyotheca or non-staining nuclear membrane.

Some of the chromosomal threads seem to be situated

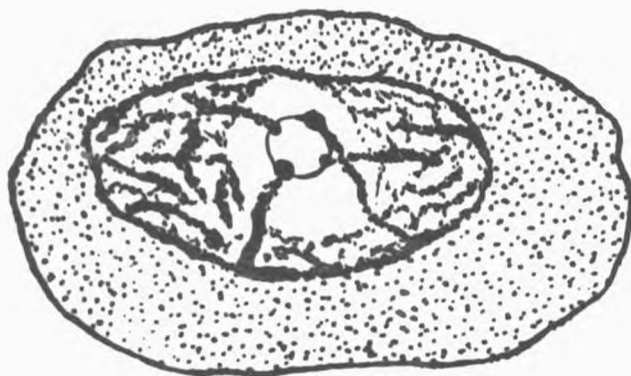


Figure 38. Early mitotic prophase of dust-like spermatogonium. The presumptive sex-chromosomes and a pair of autosomes are related to the plasmosome.

10 μ section stained with Feulgen's leuco-basic fuchsin and light green.

x7000

within the body of the nucleus. However, when allowance is made for the thickness of the sections permitting both upper and lower surfaces of the nucleus to be included within one section, the threads within the body of the nucleus can be traced into the nuclear membrane, with which they merge.

The presence of chromatic threads and peripheral segments of chromatin in the nucleus recalls the structure of a special type of nucleus characterising spermatogonia which are about to undergo either the penultimate or the last mitotic division before meiosis ('premeiotic spermatogonia'). Table 22 on page 254 summarises the main distinctions.

Fig. 39 shows later stages in prophase. All the chromosomal threads are thicker and stain more intensely. The place of the chromatic membrane has been taken by the chromosomes. The Feulgen-positive segments of the chromatic membrane of early prophase have become resolved into the definitive chromosomes of later prophase.

Relic coiling and chromatid relational coiling of the chromosomes have been described in Chapter IV. From Figs. 38 and 39, it is seen that the plasmosome is generally associated with three or four separate chromosomes in prophase. With the resolution of the chromosomes into discrete entities, it thus becomes clear that each of the

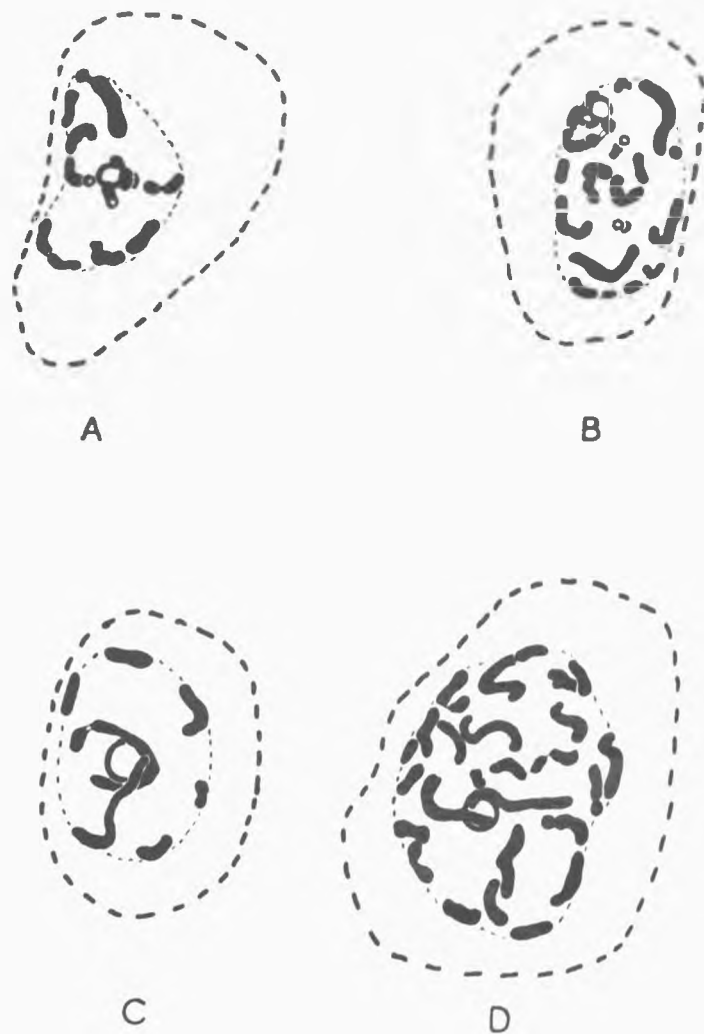


Figure 39. Four views of late mitotic prophase of dust-like sporogonium. Most of the chromosomes are aligned along the inner periphery of the karyotheca. Note relation between the plasma membrane and several chromosomes, two longer elements (the presumptive sex-chromosomes)

10 μ section stained with leuco-basic fuchsin and 1

3 or 4 chromatin bodies present on the interphasic plasmosome is actually a chromomere on a separate chromosome. This observation suggests that the single spermatogonial plasmosome may be a compound nucleolus, related to at least three or four plasmosomal organisers. Evidence will presently be adduced to support the view that the spermatogonial plasmosome is a compound plasmosome. Later in prophase, the plasmosome may appear to lose all connection with any chromosomal segments and to lie freely, often in a clear space, in the nucleus. Finally, shortly before metaphase, the plasmosome disappears, not to return until late in the following telophase. The dot-like karyosomes present during the interphase seem to vanish in prophase, once the rest of the nucleolar chromosomes come to stain as intensely as the karyosomes.

After the continuous nuclear membrane disappears, the chromosomes do not immediately align themselves on a division-spindle. Instead, they continue to shorten and thicken. A pro-metaphase, as was defined in Chapter III, may therefore be delimited (Fig. 40).

THE SPERMATOGONIUM: METAPHASE.

With the ending of pro-metaphasic contraction, the chromosomes align themselves on the equatorial plate (Fig. 41). In some plates, the larger chromosomes lie on the periphery, while the smaller ones occupy a central position. During



Figure 40. Mitotic pro-metaphase of dust-like spermatogonium. The diploid complement of 34 chromosomes may be counted in this plate.

16 μ section stained with Heidenhain's haematoxylin.

X 3500

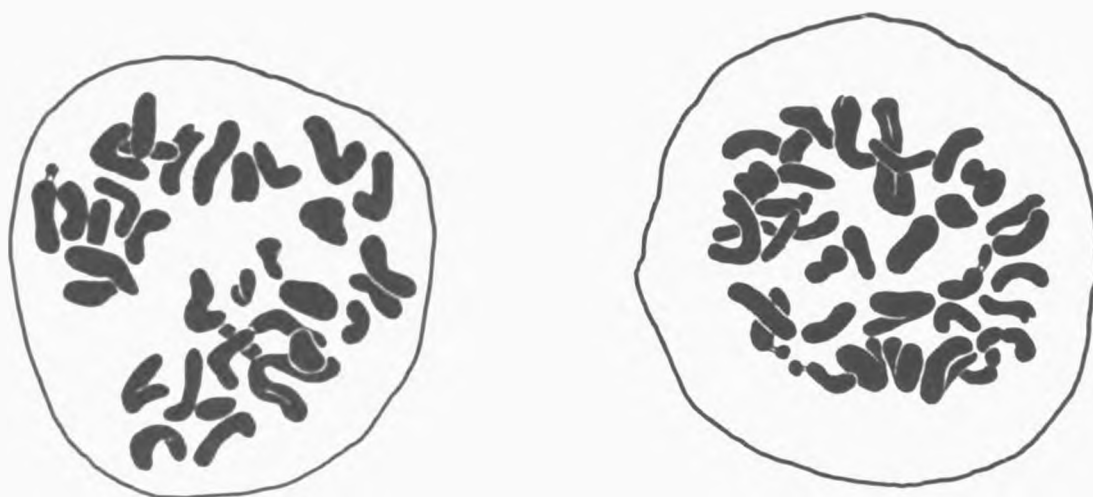


Figure 41. Polar views of mitotic metaphase plates, each containing 34 chromosomes.

16 μ section stained with Ehrlich's haematoxylin.

X 3500

mitotic metaphase, there is no partial somatic pairing, as described in several marsupials (Koller 1936c). Further, all the chromosomes stain equally, in contrast with the peculiar staining properties of the sex-chromosomes in meiotic metaphase, to be set forth below.

Careful note was taken of the spindle-fibres and the centrioles, because of the special properties which these structures manifest during meiosis in the gerbil. The fine, lightly-staining mitotic spindle-fibres connect at each pole of the spindle with a single centriole, a little larger and more faintly-staining than in the dividing spermatocyte.

THE SPERMATOGONIUM: ANAPHASE.

Several long chromosomes, including V-shaped elements, are often delayed in separating on the spindle (Fig. 42). The length of these chromosomes might appear to account for the chromosomal arms lying on the spindle after the majority of elements have reached the centriole; but the fact that even the laggards are eventually entirely incorporated into the ball of daughter chromosomes suggests that other factors e.g. variable centromere-centriolar relations, are at play.

The slender, mitotic, interchromosomal spindle-fibres stain very poorly, as compared with the meiotic interchromosomal fibres. At anaphase, a typical barrel-shaped spindle separates the two groups of chromosomes of the



Figure 42. Late mitotic anaphase with laggings. Note the large pair of V-shaped elements (right member foreshortened), the presumptive sex-chromosomes.

Acetic orcein squash preparation
re-stained with Heidenhain's haema-
toxylin.

X 3500

prospective daughter-cells. Similar barrel-shaped spindles, called 'phragmoplasts', occur in plant cells (Wilson 1925, Saez 1949). This term, phragmoplast, will be retained in the present study.

The centrioles, still unpaired bodies, remain clearly visible at the extremities of the spindle. One or two chromatic structures sometimes lie on the phragmoplast as seen in Fig. 43. They are more lightly-staining and of more irregular outline than the chromatoid bodies of later stages. It is concluded that these chromatic structures are not true chromatoid bodies and that, as in other mammals, the chromatoid body does not reveal itself at the spermatogonial stage in the gerbil.

THE SPERMATOGONIUM: TELOPHASE.

During the telophasic reconstruction of the nucleus, the chromosomes become longer, thinner and more lightly-staining and, they develop an irregular coil. Once more we see evidence of the close affinity between nuclear membrane and chromatin, as the chromatic membrane seems to be re-formed by the apposition of the chromosomes to the nucleo-cytoplasmic interface. While the chromosomes are once more aligning themselves preipherally, the chromatin ball becomes clearly segregated from the granular cytoplasm; this observation suggests the re-constitution of the karyotheca in telophase. It seems that both layers of the nuclear membrane, viz. the



Figure 43. Anaphase of spermatogonium.
Note the chromatic structures
on the phragmoplast.

16 μ section stained with
Heidenhain's haematoxylin. X 3250

chromatic membrane and the karyotheca, are re-formed in telophase.

The reversal of the prophasic changes in the chromosomes continues until, by the unravelling, the loss of staining and the diffuse orientation of the chromosomes, the nucleus assumes the typical interphasic appearance of 'dust-like' spermatogonial nuclei. But the loss of staining is not uniform. In each of three or four chromosomes, one segment remains deeply-staining. These condensed chromomeres lie near one another and, presently, in close propinquity, a single plasmosome makes its appearance. There is no earlier stage at which three or four separate plasmosomes arise, each next to one of the chromomeres. The three or four nucleolar chromosomes unravel and disappear as discrete structures, save for the chromomeres which persist as discrete or fused, condensed, deeply-staining karyosomes, throughout the ensuing interphase.

It may be concluded from these observations that there is more than one distinct chromosomal region ('plasmosomal organiser') associated with the production of the spermatogonial plasmosome. In many cells, four associated chromosomal regions are found; in some, only three. In the latter, a fourth may be present, lying above or below the plasmosome.

Meantime, the cytoplasm has been the seat of a

dramatic transformation (Fig. 44). A constriction-furrow looms on the surface of the cell about the equator of the phragmoplast. The furrow progresses rapidly across the cell, cleaving the cytoplasm into daughter cytoplasts. The phragmoplast collapses into a narrow figure connecting the two cells. Each spindle-fibre develops a localised thickening at its centre and, as the fibres concentrate in a smaller region, the thickenings are apposed to one another, forming a small, deeply-staining 'mid-body' (Zwischenkörper) across the equator of the collapsed spindle. Two stages in the development of the mid-body are shown in Fig. 44 (a and b). From the mid-body, the spindle-fibres fan out towards each daughter-nucleus, but fade from sight before reaching it. The mitotic spindle-remnant or 'mitosome' is an ephemeral structure like the gossamer spindle-fibres of mitosis; soon the mitosome vanishes and leaves no trace in the interphase that follows. This fleeting behaviour contrasts sharply with the hardy and more durable meiotic spindle-fibres.

The centrioles, too, have been affected by the cytoplasmic transfiguration. While the latest anaphase figures all contain only a single centriole at each pole of the spindle (Fig. 43), the centrioles double themselves during the telophase (Fig. 44). Again, the spermatogonium differs from the spermatocyte in the time at which centrioles divide.

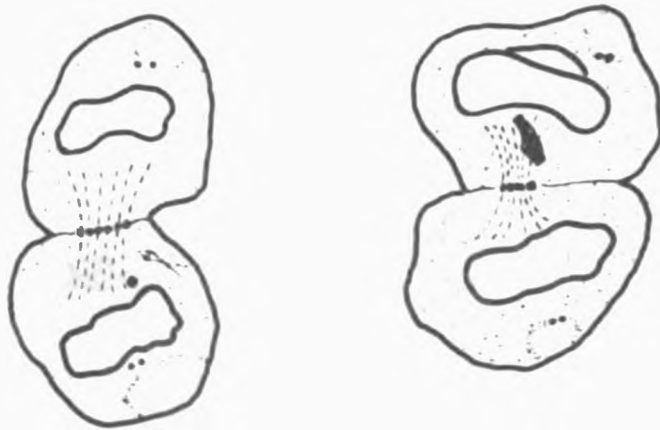


Figure 44. Telophase of spermatogonium.
The figures show the relic spindle
and the constriction furrow.
Note the centrioles and centrosome.
16 μ section stained with Heidenhain's
haematoxylin.
X 3250.

THE PREMEIOTIC SPERMATOGONIUM: MITOSIS.

In Fig. 45, we see a number of outlined spermatogonia with excessively-contracted chromosomes, packed closely on to diminutive equatorial plates. Such mitoses, we shall show in Chapter XII, are peculiar to the penultimate and the last mitotic divisions before the onset of meiosis: they may therefore be styled 'premeiotic mitoses'.

Very few premeiotic cells are found showing a typical prophasic appearance, i.e. of mobilising chromosomes, contracting and becoming more heavily-condensed. From this observation, it seems that the definitive prophase of the premeiotic mitoses is short. On the other hand, prior to the onset of obvious prophasic changes, premeiotic nuclei possess special features - the chromosomes are already partly resolved into discrete threads and partly condensed into peripheral blocks. Subsequently, the remaining threads rapidly condense while the chromocentres unravel. The small metaphasic and anaphasic figures consist of short, closely-packed chromosomes. The disparity in size between the figures of premeiotic and those of 'premitotic' mitoses is strikingly brought out in Fig. 46 and provokes thought on the mechanism by which this difference arises. It is later propounded that premeiotic chromosomes are already in a 'prophasic' condition before the onset of obvious prophasic changes and that the consequent lengthy persistence of the chromosomes in a prophasic state is connected with the excessive contraction at metaphase.



Figure 45. Equatorial view of metaphase plates
in compact (premeiotic) mitoses.

10 μ section stained with Feulgen's
leuco-basic fuchsin.

x 3250

Figure 46. Comparison of premeiotic and premitotic mitoses.
A. Metaphase, polar view.
B. Metaphase, equatorial view.
C. Anaphase.
D. Telophase.

Premeiotic mitoses on left, premitotic mitoses on right.

x 3250



D



C



B



A



SECTION II : MEIOSIS.

THE PRIMARY SPERMATOCYTE: PRELEPTOTENE.

After the last mitotic division of a premeiotic spermatogonium, the cell enters the preleptotene stage of the primary spermatocyte. It is shown below that this stage represents the earliest sign of the first meiotic prophase. As in the premeiotic divisions, the nucleus is initially 'prophasic', since the chromosomes have remained partially unravelled at the end of the last mitotic telophase. The tenuous chromosomal threads are dispersed amid a number of small, darkly-staining, chromatic blocks (Fig. 47). The Feulgen-positive nuclear membrane consists of short, deeply-staining segments which dip inwards as fine threads among the rest of the unravelling chromosomes: hence, again, we have the effect of chromosomes peeling off the membrane.

THE PRIMARY SPERMATOCYTE: LEPTOTENE.

Fig. 48 demonstrates that the chromosomes of leptotene nuclei have resolved completely from the chromatic blocks of preleptotene nuclei. The leptotene nucleus therefore contains a ball of intensely-staining, long, thin, single threads, making analysis exceedingly difficult. A number of fine, chromosomal ends protrude from the surface of the mass of threads: the fact that these projecting ends are single permits a diagnosis of leptotene. Typically, the ball of threads is shrunken or retracted from the nucleocytoplasmic



Figure 47. Primary spermatocytes in the pre-leptotene stage.

16 μ section stained with Heidenhain's haematoxylin.

x 3500



Figure 48. Primary spermatocytes in the leptotene stage. All the arms protruding from the ball of threads are single.

16 μ section stained with Heidenhain's haematoxylin.

X3500

interface. At this stage no stainable nuclear membrane is seen. This observation underscores again that the chromatic membrane is visible only when chromosomes are present at the nucleo-cytoplasmic interface. Here, as in mitosis, the change of emphasis occurs from nuclear membrane to chromosomes; but, whereas in mitosis, the chromosomes retain their peripheral orientation, in meiosis, they retract from the periphery to form a compact ball in the centre or to one side of the nucleus. This observation makes it clear that once the chromosomes of meiotic prophase have become resolved and have withdrawn from the surface of the nucleus, no stainable nuclear membrane is left. Surrounding the ball of threads, there is a clear halo limited by a distinct interface with the cytoplasm - evidence that the karyotheca is still intact.

Unlike the early prophase of the spermatogonium, preleptotene and leptotene nuclei contain no plasmosomes: the latter have not been re-formed in the telophase of the last premeiotic division. This accords with Allen's observation (1918) that plasmosomes are absent early in meiosis in the rat.

Up to the end of leptotene, the cytoplasm remains devoid of archoplasm and chromatoid bodies. The nuclear differentiation, marking the beginnings of meiosis, as yet, has no counterpart in overt specialisation of the cytoplasm.

THE PRIMARY SPERMATOCYTE: ZYGOTENE.

In Fig. 49, both paired and single ends protrude from the ball of chromosomes. On this evidence, it is concluded that zygotene has begun in such nuclei. The chromosomal pairs are differentiated longitudinally into thicker (chromomeric) regions alternating with thinner (inter-chromomeric) regions.

In many nuclei, like those in Fig. 50, the mass of chromosomes has gravitated towards one pole of the nucleus: a phenomenon known as synizesis (e.g. Waddington 1939). White (1945) believes that, in some instances, synizesis is an exaggeration of the shrinkage observed in leptotene nuclei; as such, it would be a constant artefact, rather than a polarising movement of the chromosomes. In the gerbil, synizesis is manifestly due to both a collapsing and a polarising effect. The evidence is as follows:

Careful study of lightly-stained preparations reveals that the chromosomes have not merely collapsed in a disordered mass to one pole, but that the ends of several bivalents have definitely become polarised to a narrow area of the nuclear membrane. Such polarisation of the ends of bivalents has long been known as a feature of zygotene in many species. Some bivalents have both ends polarised, so that the bivalent forms a loop in the nucleus. Others have only one end polarised; while the remainder are entirely unpolarised. Yet,

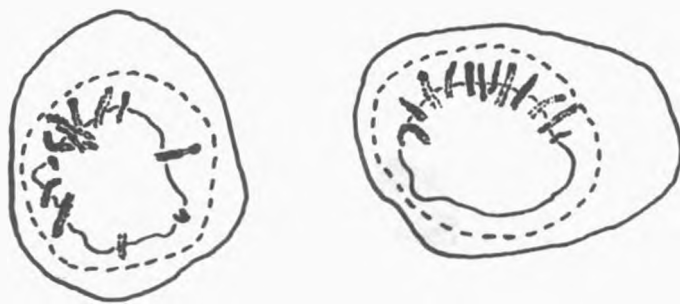


Figure 49. Primary spermatocytes in the zygotene stage. Some protruding arms are paired, others are single.

16 μ section stained with
Heidenhain's haematoxylin.

x 3500

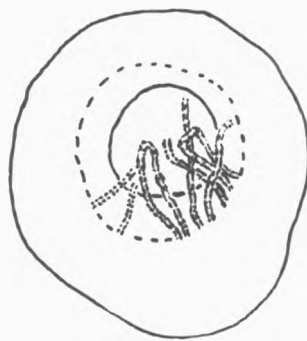


Figure 50. Primary spermatocyte in the zygotene stage. Note the polarisation of chromosomal ends to one area of the nuclear membrane.

Dotted outline: nucleocytoplasmic interface (karyotheca).
Solid outline: ball of chromosomes shrunken from the nucleocytoplasmic interface.

16 μ section stained with Heidenhain's haematoxylin.

x 3500

the bivalents with unpolarised ends are not randomly distributed through the nucleus but are orientated amidst the polarised threads. The resultant appearance is that of a bouquet.

We may conclude that, with the onset of zygotene, one or both ends of some threads undergo a definite polarisation in a small area on the nuclear surface. At the same time, the whole mass of threads collapses to the same pole of the nucleus as that to which the ends have become polarised. This collapse may be an exaggeration of the shrinkage observed in leptotene nuclei; or, it may be secondary to the polarisation of the ends, the chromosomes, drawn together as a cohesive ball, being attracted towards the locus of polarisation.

In only a proportion of nuclei is this polarising effect clearly observed, since the position of the loci of polarisation is apparently random. Many nuclei are therefore not cut in a plane suitable to demonstrate the synizesis.

During zygotene, a large, deeply-staining body reveals itself close to the polarised ends of the chromosomes (see Fig. 99 p.329). This is the first time the sex-karyosome becomes evident during meiosis. The sex-chromosomes unravel from this body later. A plasmosome makes its appearance at this time, associated with the sex-karyosome. A second plasmosome is seen among the autosomes. Allen (1918) found that plasmosomes did not become visible in the rat until

pachytene.

It is at zygotene, too, that the first cytoplasmic inclusion of the spermatocyte occasionally arises in the gerbil: the archoplasm may be seen towards one pole of the cell, as a denser region of the cytoplasm, staining grey with Heidenhain's haematoxylin and, limited by a rim of deeply-staining cytoplasmic granules (Fig. 51). No chromatoid body is visible yet. In the majority of zygotene spermatocytes, not even the archoplasm is present to betray a specialisation of the cytoplasm.

THE PRIMARY SPERMATOCYTE: PACHYTENE.

During the pachytene stage, the primary spermatocyte increases greatly in size. Because of this growth phase, the term 'auxocyte' is commonly applied to the pachytene spermatocyte. It follows that the appearance of pachytene nuclei are very variable; we may distinguish two distinct appearances of pachytene nuclei (Fig. 52), representing the extremes before and after the growth phase, while all intergrades between these limits are encountered.

The earliest pachytene figure ('compact pachytene') closely resembles the zygotene conformation. The thick paired threads still lie in a mass towards one nuclear pole; all their projecting ends are now clearly double, the threads looking thicker in consequence. The threads have also become shorter and present a woolly contour, which masks the individual chromomeres.

The sex-karyosome lies at the nucleocytoplasmic interface and is now separated from the autosomes by a clear space. In the compact pachytene nucleus depicted in Fig. 53, another, smaller, heavily-staining body may be seen among the autosomes, distinct and separate from the sex-karyosome. This smaller body is Feulgen-positive and may therefore be spoken of as an autosomal karyosome. The latter karyosome declares its presence as part of the nuclear specialisation of the pachytene stage. The plasmosome seen in zygotene among the autosomes is now clearly associated with the autosomal karyosome.

At the same time, the cytoplasm becomes visibly differentiated; in Fig. 54, three distinct classes of inclusions present themselves:-

- (i) Especially from the growing period of pachytene, the archoplasm is a large body, constantly present, and lying characteristically close to one pole of the nucleus. It is larger than the archoplasm sometimes seen in the spermatogonium.
- (ii) A pair of centrioles can be recognised, usually lying on or close to the nuclear surface of the archoplasm, i.e. in the small space between the nuclear membrane and the archoplasm. Early in pachytene, the centrioles sometimes lie far out in the peripheral cytoplasm, where they remain for a time, before assuming their para-nuclear

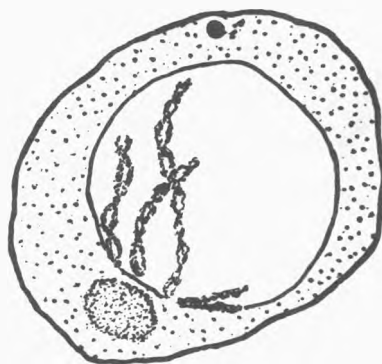


Figure 51. Primary spermatocyte in the zygotene stage. Several chromosomal ends are polarised to one area of the nuclear membrane. The archoplasm may be seen in the cytoplasm.

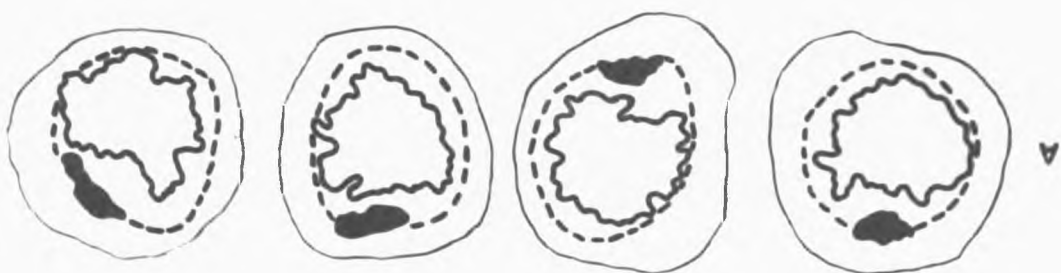
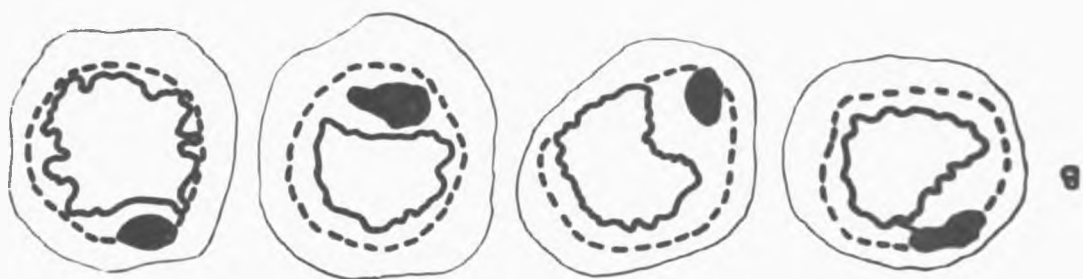
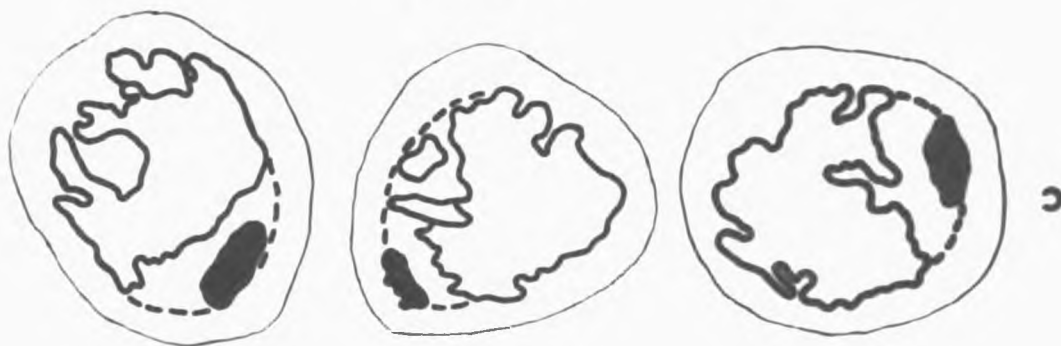
16 μ section stained with Heidenhain's haematoxylin.

x 3500

Figure 52. Pachytene spermatocytes before (A and B) and after (C and D) the growth period. Note the size and position of the sex-karyosomal complex.

16 μ section stained with Heidenhain's haematoxylin.

x 3500



100

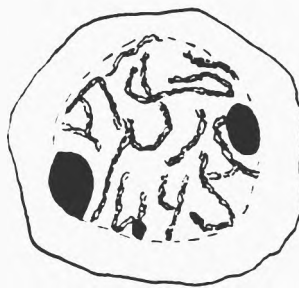


Figure 53. Primary spermatocyte with compact pachytene nucleus. Note the larger sex-karyosome and the smaller autosomal karyosome.

16 μ section stained with Heidenhain's haematoxylin.

x 3500

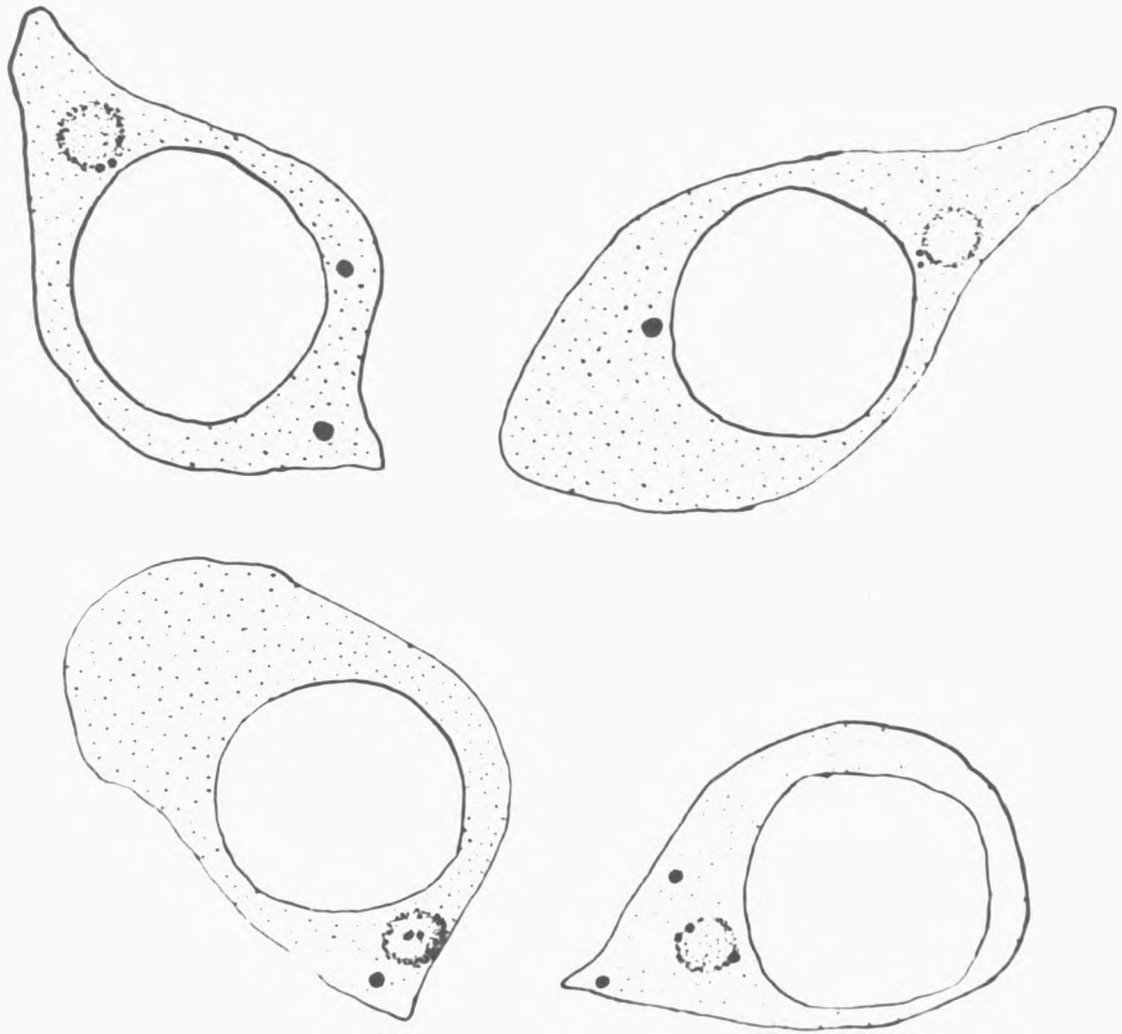


Figure 54. Primary spermatocytes in the pachytene stage, after the growth phase. Centrioles, archoplasm and chromatoid bodies are seen in the cytoplasm.

16 μ section stained with Heidenhain's haematoxylin.

x 3500

(iii) One or two chromatoid bodies are seen lying in the cytoplasm for the first time. Typically, the chromatoid body in the spermatocytes of the gerbil is a spheroidal structure of regular outline. There may be a single body, 0.8u in diameter, or two smaller bodies, 0.4 - 0.65u in diameter. When two are present, they generally lie on the same side of the nucleus, not far removed from each other. In some cells, the chromatoid bodies are situated close to the archoplasm; in others, at the opposite pole of the cell; sometimes near the nucleus; sometimes close to the cell-membrane. There is no evidence that they regularly arise in relation to the archoplasm, the centrioles, the nuclear membrane or the cell-surface, a fact which will assume importance when we consider below the genesis of the chromatoid body.

The chromatoid body stains vividly with Heidenhain's iron haematoxylin and with Newton's crystal violet, but not with Feulgen's leuco-basic fuchsin. Thus, in haematoxylin or crystal violet preparations, it may resemble a mass of extra-nuclear chromatin, especially as it is often seen in metaphasic and anaphasic spermatocytes, in which the chromosomes are free in the cytoplasm. Notwithstanding, even when Feulgen's reagent is not used, the chromatoid body may be distinguished from meiotic tetrads because it is more opaque and dense than chromatin material, an observation commented upon also by Koller and Darlington in the rat (1934).

In the gerbil, the chromatoid body usually has a non-staining or lightly-staining core, giving the body the cast of a thick ring, no matter from what aspect it is viewed.

The emergence of archoplasm and chromatoid bodies, which are destined to play their part in spermatogenesis, marks the visible meiotic specialisation of the cytoplasm, in much the same way as the influence of an impending meiosis becomes manifest in the nucleus as early as the penultimate premeiotic mitosis.

THE GROWTH PHASE.

After the compact pachytene phase, the primary spermatocyte enlarges. This growth phase is characteristic of first meiotic prophase in spermatogenesis and is characterised by:-

- (i) growth of nucleus and cell;
- (ii) changes in the size, staining, form and distribution of (a) the autosomes, and (b) the sex-karyosomal complex.

Growth of nucleus and cell.

Fig. 52 demonstrates that the nucleus comes almost to fill the cell. Eventually, the nucleus attains a diameter of 10 - 12 μ , which is half as great again as the diameter of the compact pachytene nuclei. That the cell itself grows is equally clear from Fig. 52. This drawing shows that the whole spermatocyte at the compact pachytene

stage is of about the same size as, and often smaller than, the nucleus alone of the enlarged or 'diffuse' pachytene cell.

Changes in the autosomes.

The nuclear volume increases pari passu with a dispersion of the paired threads. There is a tendency for the lepto-zygotene clumping of the chromosomes to be reversed and for the individual chromosomes to assume once more a peripheral position. As they become apposed to the nucleocytoplasmic interface, the superficial appearance of a reconstituted nuclear membrane is seen. The tetrads retain this centrifugal arrangement right up to diakinesis. Their more diffuse distribution discloses that their fine structure is internally differentiated into a succession of deeply- and lightly-staining segments. This segmental pattern typifies the prophasic bivalents of meiosis and has led cytologists to liken the bivalents to strings of beads. Each 'bead' or chromomere is a short, round, highly-condensed region. Between the chromomeres are more faintly-staining, under-condensed regions. The chromomeres differ among themselves in their size and intensity of staining. It is at this diffuse pachytene stage that it becomes possible, especially in squash preparations, to count the full complement of 17 bivalents (including the unravelling sex-karyosomal complex).

The autosomal karyosome is now seen to be waisted or

bipartite (Fig. 55). One of the two parts of this body, or perhaps the constriction between them, is closely associated with a plasmosome, often characterised by its elongated shape. Two additional minute plasmosomes among the autosomes disappear during the growth period, probably by fusion with the large autosomal plasmosome. On fusion, the faint chromatic rim surrounding each plasmosome may persist, enabling the product of fusion to be 'dissected' into its components. We thus see further sign of nucleolar chromosomes in the gerbil.

Changes in the sex-karyosomal complex.

The sex-karyosomal complex remains separated from the autosomes by a clear space, perhaps due to an increasing repulsion between the complex and the autosomes. Both the complex and the space become augmented during the growth phase, so that the dispersed autosomes occupy not more than two-thirds to three-quarters of the nucleus (Fig. 52). Considerable variability is shown by the sex-karyosomal complex (Fig. 56). Generally, it is asymmetrically lenticular in side view, the outer convexity conforming to the curved nucleo-cytoplasmic interface, while the inner convexity bulges into the nucleus and is slightly irregular. In surface view, the complex is unevenly ovoidal in outline. Internally, a regional differentiation discloses itself. In haematoxylin-stained preparations, one or two large, pale, spherical bodies (plasmosome I) are present within the complex.



Figure 55. The autosomal karyosome with associated bivalent (I, II, IV, V) and associated plasmosome (III) from diffuse pachytene and diplotene nuclei of primary spermatocytes during the prophase of the primary spermatocyte.

10 μ and 16 μ sections stained with Heidenhain's haematoxylin.

x 3500

Figure 56. The evolution of the sex-chromosomes from the sex-karyosomal complex.

- A. The sex-karyosomal complex during zygotene and pachytene.
- B. Earliest beginnings of the unravelling process; the presumptive X-chromosome appears (pachytene).
- C. Later stage in the unravelling of the presumptive X.
- D. First signs of the unravelling of the presumptive Y.
- E and F. The sex-chromosomes have almost completely unravelled. Their association in the condensed sex-karyosome is clearly shown. The plasmosome associated with the condensed sex-karyosome appears in several figures.

Key: Stippled body - diffuse sex-karyosome.
Solid body - condensed sex-karyosome.
Clearly outlined bodies - plasmosome.

From squash preparations and sections, stained variously with Ehrlich's and with Heidenhain's haematoxylin and with Feulgen's leuco-basic fuchsin.



5μ

These bodies stain with light green while the rest of the complex is Feulgen-positive. They are seemingly embedded in the complex and, at early pachytene, can be clearly detected only when the plane of section has passed through the complex. Thus, the sex-karyosomal complex may be analysed into a Feulgen-positive and a Feulgen-negative moiety, or a karyosomal and a plasmosomal component. It is now clear that both the sex-chromosomes and at least one pair of autosomes influence the formation of plasmosomes.

During late pachytene, the complex, seen from the surface, contains up to a dozen small, light-green-staining bodies, suggesting that plasmosome I is disintegrating progressively, finally to disappear altogether. At the same time, another, larger plasmosome (II) arises alongside the sex-karyosome. It is possible that plasmosome I gives rise to plasmosome II by disintegration of plasmosome I, migration from the diffuse to the condensed karyosome, followed by re-integration of the plasmosomal fragments into a single plasmosome (II). The IInd plasmosome does not persist longer than diplotene.

It is further obvious in lightly-stained preparations that the sex-karyosome is itself bipartite, consisting of a smaller, heavily-staining portion ('condensed sex-karyosome') in contact with a larger, faintly-stained component ('diffuse sex-karyosome') (Fig. 56). During early pachytene, large segments of the sex-chromosome lie embedded in the periphery

of the diffuse karyosome. They begin to emerge from this position later in pachytene; at first, from the surface of the diffuse karyosome, a small double thread projects. It takes the form of a terminal chromomere, connected by two fine filaments with the complex. Occasionally, a chromosomal thread is seen running round the periphery of the diffuse segment and connecting the protuberant end to the condensed karyosome. This thread represents one sex-chromosome secluded in the diffuse karyosome. It is therefore in the later stages of pachytene that the sex-chromosomes proper first begin to unravel from the sex-karyosomal complex.

THE PRIMARY SPERMATOCYTE: PACHY-DIPILOTENE.

The end of pachytene is marked by the falling apart of the paired chromosomes and the manifestation of diplotene rings between chiasmata. In the gerbil, chromosomal repulsion is very slight so that the rings are ill-defined. In consequence of this, the onset of diplotene is difficult to detect. A number of nuclei which had completed the growth phase were therefore classified as pachy-diplotene, it being implied that the autosomal pairs were beginning or had already begun to fall apart. The most marked shifts at this period are the transformations of the sex-karyosomal complex. It undergoes a series of changes which continue throughout the definitive diplotene period and will accordingly be described under that head.

THE PRIMARY SPERMATOCYTE: DIPLLOTENE.

There is a pause and a temporary reversal of the direction of meiotic unfolding during the growth phase. Chromosomal contraction and condensation are suspended and even reversed for a time, while the bivalents lose much of their staining capacity. But, in the gerbil, the chromosomes do not lose their staining capacity completely nor temporarily resume a quasi-interphase appearance, such as occurs in the 'diffuse stage' of marsupials.

After the post-pachytene repulsion of chromosomes, the sixteen autosomal bivalents are seen as clearly double threads connected at intervals by chiasmata. The exceedingly slight repulsion already mentioned precludes any attempt to count the chiasmata. The chromosomes are more lightly-staining than they were during the leptotene, zygotene and pachytene stages; this modification in the staining of the chromosomes will be discussed later in connection with the nucleic acid cycle. The loss of staining is accompanied by a lengthening of the chromosomes. Each chromosome of a bivalent is now double, the longitudinal split having presumably occurred in pachytene. The resulting tetrads are dispersed throughout the body of the nucleus.

Before the other plasmosomes disappear at diplotene, the autosomal plasmosome traverses the repulsion-space and often fuses with the sex-karyosomal plasmosome. The resulting

large plasmosome lies in close relation to the condensed sex-karyosome. When the diplotene stage is reached, no trace of a plasmosome can be seen within the diffuse sex-karyosome.

During the diplotene stage, the repulsion between the sex-karyosomal complex and the autosomes lapses; the repulsion space disappears and the autosomal pairs again come in close proximity to the complex. With the falling apart of homologous chromosomes and the further contraction to diakinesis, the chromatic membrane again passes from view, as the tetrads on the nucleo-cytoplasmic interface develop markedly irregular outlines.

Significant developments of the sex-karyosomal complex have meanwhile been occurring (Fig. 56). At the end of the pachytene stage, we have seen, the sex-chromosomes had begun to project from the complex. During diplotene, the original protruding structure unravels further from the diffuse moiety of the complex and it is clearly a loop of internally differentiated chromosome. There are no chiasmata between the two limbs of the loop. In some instances, an apparently interwoven arrangement exists, but careful focussing invariably reveals that the two limbs are merely twisted about each other (Fig. 101 p. 329). The limbs join together at the end distal to the complex. After this loop of chromosome has emerged from the sex-karyosomal complex for 3 - 4u, a second loop starts to unravel from the diffuse karyosome. Its

internal morphology is similar to that described for the first element.

The two chromosomes thus laid bare draw out their length from the complex, as two large double loops. The proximal ends of the loops are not free but pass into the small, deeply-staining karyosome. It follows that the condensed karyosome represents part of the sex-chromosomes in a state of precocious condensation. The rest of the sex-chromosomes are equally condensed with the autosomes. Attached to the condensed segment, the diffuse karyosome remains, to all seeming, a discrete structure and not an integral part of the sex-chromosomes proper, despite an affinity of the diffuse karyosome for leuco-basic fuchsin.

From this sequence of events, it is seen that the condensed karyosome is formed by the two ends of one sex-chromosome coming into close contact with the two ends of the other sex-chromosome. It is clear that the sex-chromosomes of the gerbil have two pairing segments apiece, separated by a long, loop-shaped, non-pairing or differential segment.

Further, although both X and Y differential segments are lodged within the diffuse karyosome, they are never paired, as far as can be judged from their appearance on unravelling. Each loop is distinct from the other and the two loops are situated on opposite sides of the condensed pairing-segments.

Simultaneous with these far-reaching nuclear events during diplotene, the two centrioles separate and begin to migrate in opposite directions around the periphery of the nucleus. Situated close to the nucleus, the archoplasm begins to manifest changes in its internal morphology. Several clear spaces - archoplasmic vacuoles - arise within its substance. Similar archoplasmic vacuoles have been described in other species (e.g. the 'idiogranulothecae' of Papanicolaou and Stockard (1918) in the guinea-pig; Gatenby and Beams (1935) in man); and, in suitable preparations, (e.g. Champy fixation, followed by Benda's alizarin and crystal violet), it has been demonstrated that the pro-acrosomic granules arise in these vacuoles. However, no pro-acrosomic granules were found inside the archoplasmic vacuoles in the present material, fixed in Bouin's picro-formol-acetic and stained with iron haematoxylin or Newton's crystal violet.

The chromatoid bodies persist through the later stages of the 1st meiotic prophase, sometimes apparently growing and dividing.

THE PRIMARY SPERMATOCYTE: DIAKINESIS.

Having elongated and become more lightly-staining in diplotene, the bivalents now undergo the reverse processes - they shorten considerably, thicken and stain more intensely. The bivalents are still arranged around the surface of a hollow sphere, against the intact, non-staining interface. The

differentiated segments decrease in length at diakinesis, but they remain lightly-staining, exhibiting negative heteropycnosis. In diakinetic nuclei, no plasmosomes are visible, either alongside the condensed karyosome or in the diffuse karyosome.

The autosomal karyosome has disappeared. The diffuse sex-karyosome becomes smaller but remains uncondensed. Occasionally, at this stage, the diffuse karyosome is apparently detached completely from the chromosomes. Between diakinesis and metaphase, it disappears, leaving no obvious trace. The fate of the diffuse karyosome is unknown and provides a problem to be discussed in the chapter on the sex-chromosomes.

Where they can be found in diakinetic spermatocytes, the centrioles are situated at opposite nuclear poles. Each centriole has doubled itself, giving two minute granules lying close together. Thus, centriolar division occurs in the late prophase of the spermatocyte, but in telophase of the spermatogonium. The archoplasm is present up to the end of diakinesis and, at that time, possesses a more intensely-staining granular rim. No spindle-fibres have been detected in this study, running from the centrioles towards the intact karyotheca in late prophase. Nor are astral rays present, emanating in radial fashion from the centrioles. In other words, no fibres of the division-spindle have been found before metaphase.

THE PRIMARY SPERMATOCYTE: METAPHASE.

With the appearance of the spindle, the bivalents orientate themselves around the equator. The resulting 'hollow spindle' is clearly seen in the polar views of metaphase plates shown in Fig. 57 (c.f. mitotic metaphase in man, Koller 1937). Occasionally, a bivalent fails to undergo congression on the equatorial plate (Fig. 58). In favourable preparations, seventeen bivalents can be counted (Figs. 57 and 59a). They vary considerably in size and shape and include bivalents showing the 'Derby hat' configuration so often found in meiotic metaphase plates in other species (e.g. Painter 1924a, 1924b, 1924c; Bryden 1932). The sex-chromosomal pair - a very large asymmetrical bivalent - usually lies in the equatorial position, to one side of the plate (Fig. 59). Often, indeed, - as in Fig. 60 - the two spindle-fibres attaching to the sex-chromosomes seem to sag outwards, away from the other bivalents and from the rest of the spindle, suggesting that the heavy sex-chromosomal pair is being repelled by the autosomes. Apart from the sex-bivalent, it is impossible to identify all the bivalents individually; there is, however, a general correspondence in size between the bivalents and the characterised pairs of mitotic chromosomes.

It will assist this description to anticipate part of the later discussion on the sex-chromosomes. If we accept that the central condensed portions of the sex-chromosomes are

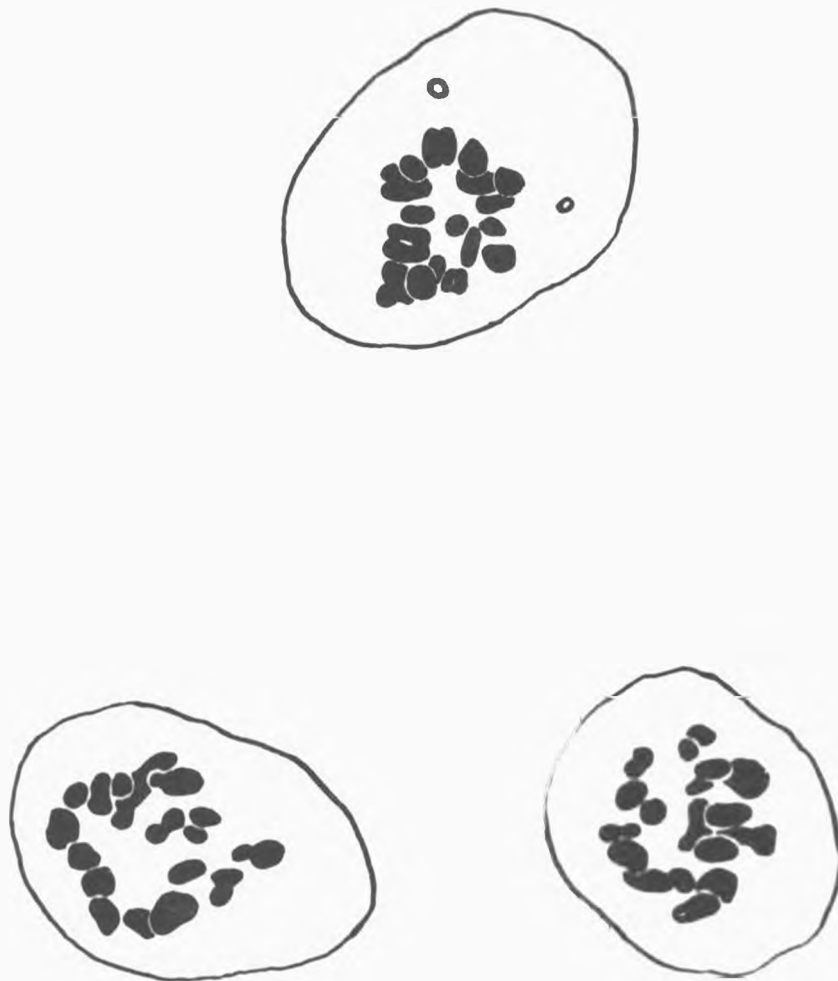


Figure 57. First meiotic metaphase plates in polar view. 17 tetrads are present on each plate.
16 μ section stained with iron haematoxylin. x 3500



Figure 58. Photomicrograph of primary spermatocyte in first meiotic metaphase, showing non-congression of one bivalent.

Note the thick spindle fibres and the suggestion of a double centriole at one pole of the spindle.

16 μ section stained with Heidenhain's haematoxylin.

$\times \pm 4900$

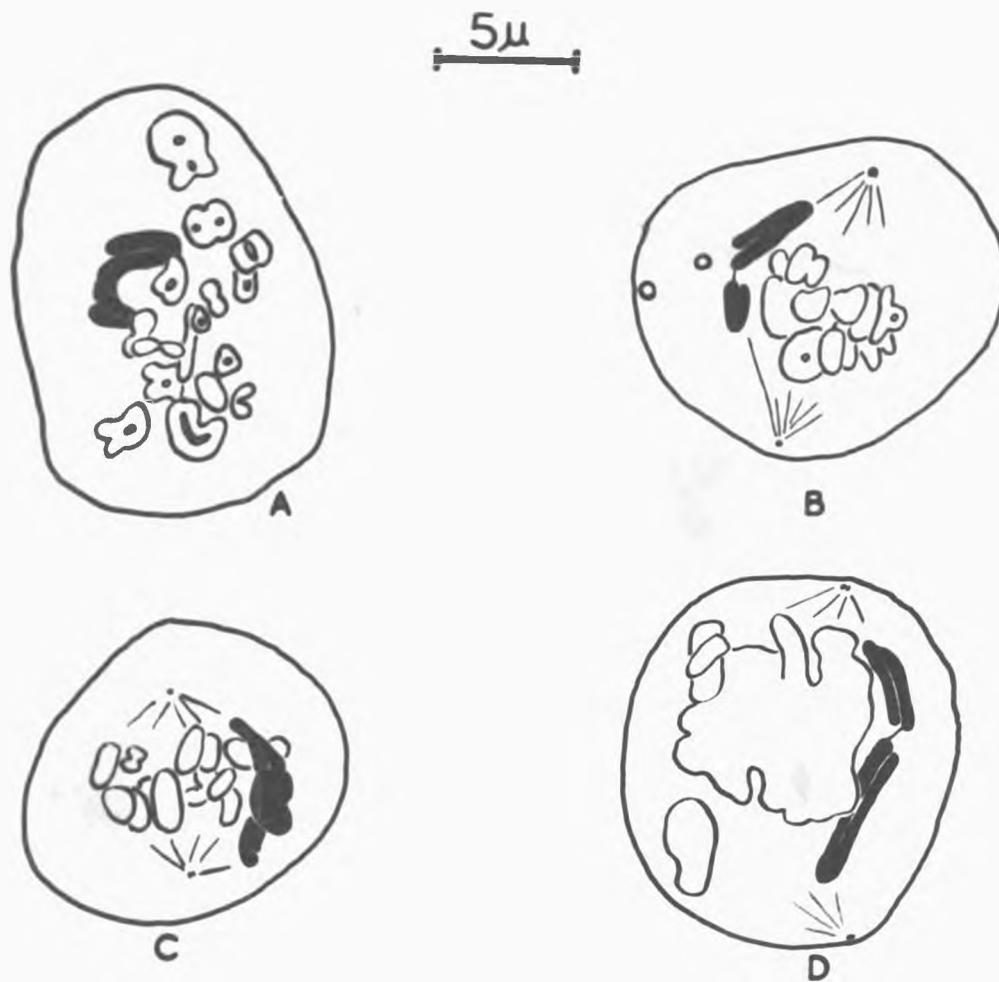


Figure 59. First meiotic metaphase, showing various forms of the sex-bivalent, orientated typically outside the periphery of the equatorial plate. In A, 17 bivalents can be counted. 16μ sections with various stains. x 3500.



Figure 60. First meiotic metaphase in equatorial view. In each cell, one pair of spindle-fibres seems to sag under the weight of a large bivalent, the presumptive X-Y.

16 μ section stained with Heidenhain's haematoxylin.

X 3250

the pairing segments, while the peripheral unravelled threads are the differential segments, we may say that the varying appearances of the sex-bivalent in different nuclei resolve themselves into:-

(a) various modes of association of the pairing segments, and (b) differences in the degree of condensation of the pairing and differential segments.

In no spermatocyte is the diffuse karyosome visible during the metaphase; the disappearance of the diffuse karyosome after diakinesis suggests that this body does not participate in the metaphasic structure of the sex-bivalent: in fact, as we earlier suggested, it does not seem to be an integral part of the sex-chromosomes at all.

In the following description, we shall classify the varieties of sex-bivalent primarily on their mode of association: secondarily, we shall discuss the condensation-states found in each category.

(1) Association in one arm only.

In Fig. 61, several plates are illustrated in which only one of the arms of the presumptive X is associated terminally by a fine thread with an arm of the presumptive Y. Both chromosomes are under-condensed in the associated pairing and differential segments.

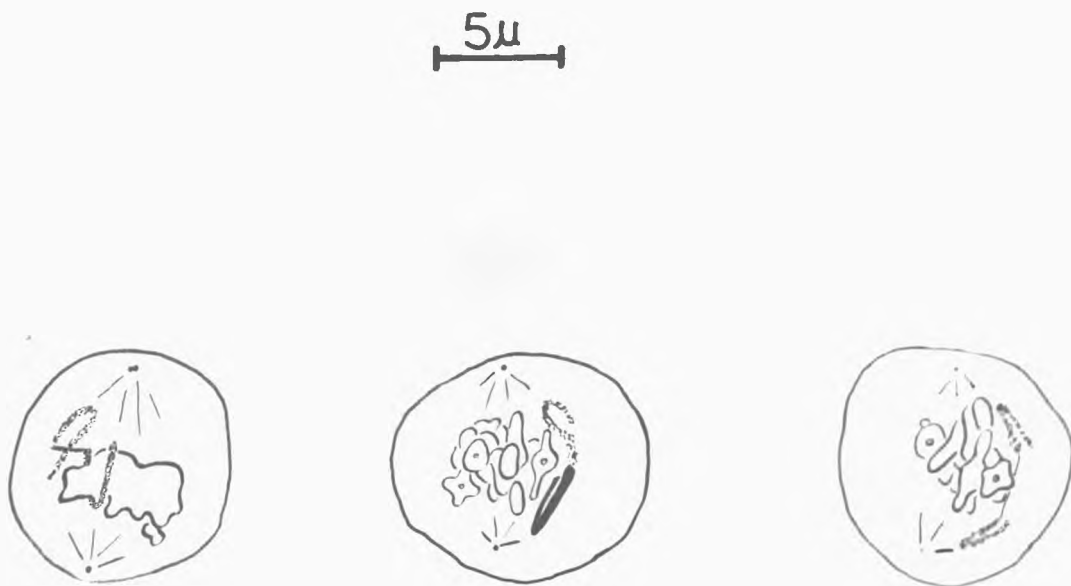


Figure 61. First meiotic metaphase showing type 1 sex-bivalent. The X-Y is uniformly undercharged in the first and the third cells, while, in the second cell, the X is undercharged and the Y is charged. 16μ sections with various stains.

x 3500

(2) Terminal association in both arms (Fig. 62).

In a proportion of plates, a pair of fine threads connects the respective ends of the sex-chromosomes. The two limbs of each chromosome lie close together and one is slightly longer than the other. About half of the bivalents of this type are evenly stained and condensed to the same extent as the autosomes (Fig. 62 A and B). Of the others, most are under-condensed throughout their length (Fig. 62 C and D), while a few have the pairing segments under-condensed and the differential segment fully condensed.

(3) Sub-terminal association in one arm.

A large proportion of plates show a highly-condensed central region when viewed from the correct angle (Fig. 63). This condensed segment is often bipartite and it represents the sub-terminally paired ends of one pair of corresponding arms, the other pair being connected by a thin thread. In no case is there an indication that a thickly-condensed and deeply-staining body can form on both homologous pairs of arms at the same time.

In bivalents with sub-terminal association of one pair of arms, it is usual for the differential segment and the pairing segment in one arm to be undercondensed and for the pairing segment in the other arm to be fully condensed. A few plates are found in which the large central body is present but in an undercondensed state (Fig. 63 D).

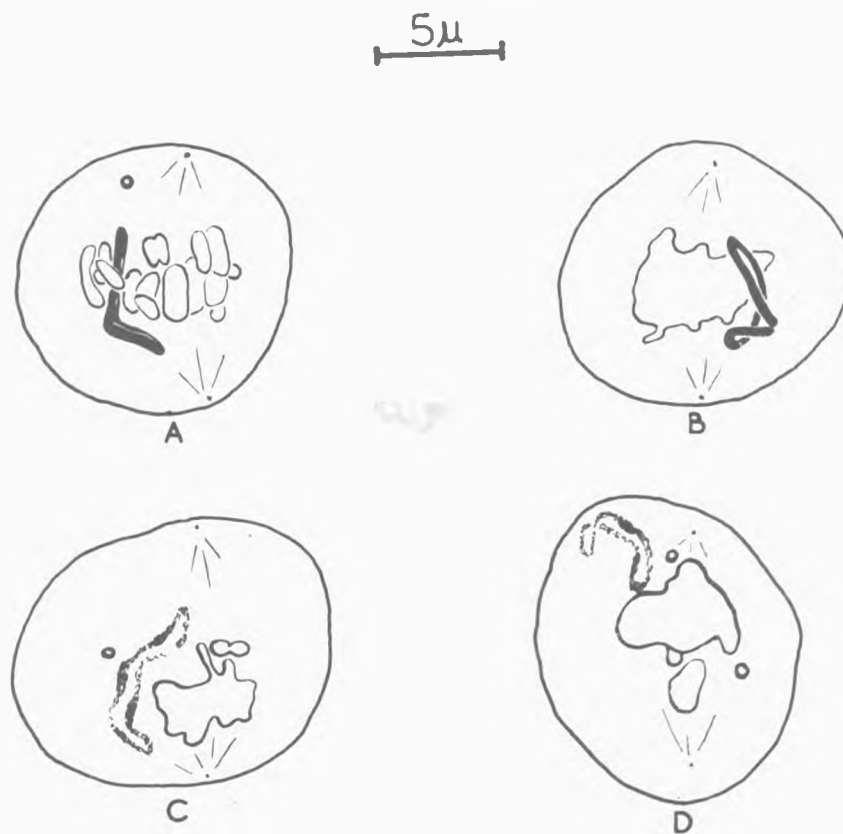


Figure 62. First meiotic metaphase showing type 2 sex-bivalent (terminal association in both arms), fully charged in A and B and uniformly undercharged in C and D.
16μ sections with various stains.
x 3500

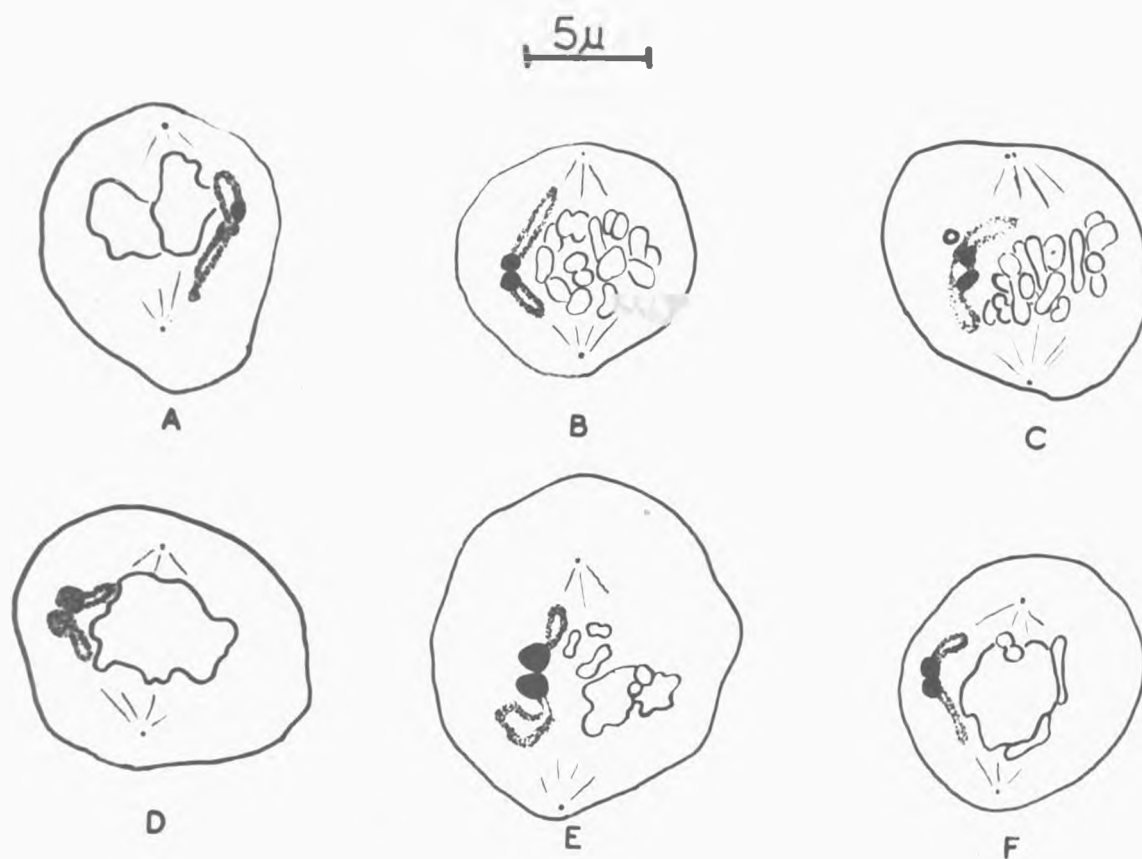


Figure 63. First meiotic metaphase showing type 3 sex-bivalent.
 In A, B, C, E and F, segments D and P₂ are undercharged and segment P₁ fully charged;
 In D, the sex-chromosomes are uniformly undercharged.
 16μ sections with various stains.
 x 3500

In relation to the autosomes and to the spindle as a whole, the longer member of the heteromorphic pair is usually orientated with the other bivalents, i.e. perpendicular to the equatorial plane. The presumptive Y-chromosome, on the other hand, often lies at an obtuse angle to the presumptive X, i.e. almost parallel, or diagonal, to the plane of the equator. The points about which the two arms of each sex-chromosome hinge, the centromeres, are far apart, projecting from the plate towards the spindle poles. The latter observation focuses attention on the interrelationships between centromere and centriole, to which point special consideration is given in the chapter on the sex-chromosomes.

(4) No association.

In a very few plates (e.g. Fig. 64), the two members of the heteromorphic pair lie unpaired on either side of the equatorial plate, towards the spindle poles. In such cells, the presumptive Y is fully condensed; the presumptive X may be either fully or under-condensed.

Having classified the protean forms of the sex-bivalent into the above four categories, we have yet to explain the varieties of association and of condensation in terms of several processes operating in all bivalents, viz. (i) the nucleic acid cycle and (ii) the formation of chiasmata. The play of these processes will become clearer after we have considered the forms of the sex-bivalent as seen at anaphase.

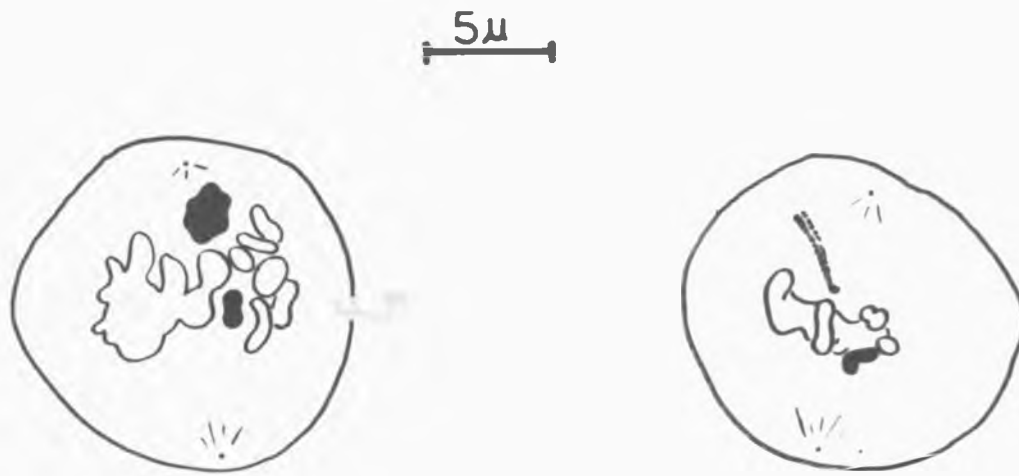


Figure 64. First meiotic metaphase
showing unpaired sex-chromosomes.

16μ section stained with Heiden-
hain's haematoxylin.

x 3500

Apart from the chromosomes, the cytoplasmic inclusions are clearly modified at metaphase. A comparison of the meiotic and the mitotic spindles reveals two striking differences. Firstly, in the spermatocyte, the spindle-fibres are thicker and more deeply-staining, than in the spermatogonium. Secondly, in the first meiotic division spindle only, not one but two centrioles lie at each extremity of the spindle. In both of these respects, the spindle of the second meiotic division resembles the mitotic rather than the first meiotic spindle. No reference has been found to such a distinction in the literature and we shall refer to this phenomenon again later.

As may be seen from many of the figured metaphasic spermatocytes, a large round chromatoid body is usually present lying off the spindle, between the equatorial plate and the spindle pole. Sometimes, it is accompanied by a second chromatoid body. The archoplasm, which can be recognised adjacent to the spindle at the end of diakinesis, fades from sight in metaphase and remains absent for the rest of the first meiotic division. In its behaviour, the archoplasm of the gerbil thus resembles the archoplasm of man (Gatenby and Beams 1935), but differs from that of many other mammals.

THE PRIMARY SPERMATOCYTE: ANAPHASE.

The two complements, in separating, maintain the 'hollow spindle', although, as they approach the paired

centrioles, the ring of diads decreases in diameter. Fine, inter-chromosomal fibres are spun out between the separating homologues. The staining properties of these inter-chromosomal fibres are similar to those of the metaphasic spindle-fibres. Towards late anaphase, the inter-chromosomal part of the spindle assumes a barrel-shape; this is the phragmoplast, well shown in Fig. 65 (A, B, C). Individual fibres may be traced from the diads at one pole far out into the cytoplasm before they gradually veer in the direction of the diads at the other pole. This spindle conformation is maintained throughout anaphase, i.e. as long as chromosomes persist on the spindle.

One or more chromatoid bodies is usually situated off the spindle and towards one or both spindle-poles. Often, as many as three or four chromatoid bodies occur in the cell at anaphase. It is apparent that the chromatoids have divided and are distributing themselves to the daughter-cells. The archoplasm remains unseen.

When all the autosomes have completed disjunction and arrived at the poles, the sex-chromosomes are usually still present on the spindle, undergoing delayed separation - a phenomenon called 'lagging'. In Fig. 66 is depicted two sets of daughter-chromosomes, each 16 in number, with the lagging sex-bivalent displaced. Delayed separation facilitates the study of the sex-chromosomes. As at metaphase, a variety of

Figure 65. First meiotic anaphase, showing various types of sex-bivalent.

Type 1, with varying charges of D.N.A., - B,C,G, H and I.

Type 2, - D and E.

Type 3, - A,F,H and I.

L6 μ sections with various stains.

x 3500

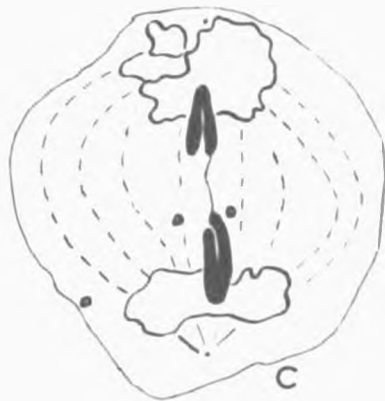
5 μ



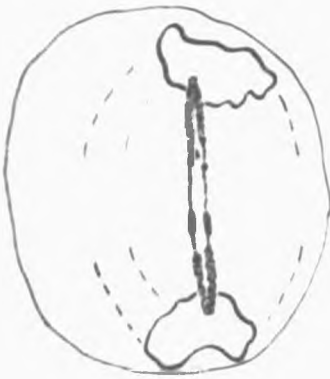
A



B



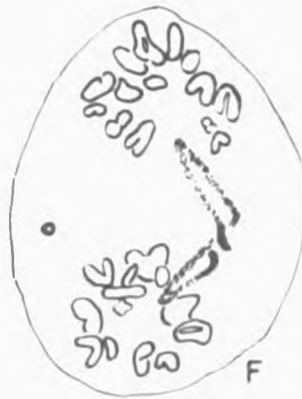
C



D



E



F



G



H



I



Figure 66. Photomicrograph and camera lucida drawing of bivalents in mid-anaphase of the first meiotic division. 16 bivalents may be counted in each daughter group, while the lagging sex-bivalent lies displaced to one side. Note the variation in charge between the differential segments of the two sex-chromosomes.

Acetic orcein squash preparation re-stained with Ehrlich's haematoxylin.

x 3500

configurations is observed; Fig. 65 illustrates some typical appearances. The anaphasic figures can be analysed into three main groups. In the following table, each of these anaphasic configurations is placed beside the appropriate metaphasic conformation.

TABLE 20.

THE CORRESPONDENCE BETWEEN THE METAPHASIC AND THE ANAPHASIC CONFIGURATIONS OF THE SEX-CHROMOSOMES.

<u>TYPE</u>	<u>METAPHASIC ALIGNMENT</u>	<u>ANAPHASIC ALIGNMENT</u>
1.	Terminal association in one arm.	A long fine thread is drawn out between one pair of arms.
2.	Terminal association in both arms.	Two long fine threads are drawn out between both pairs of arms.
3.	Sub-terminal association of first pairing segments and terminal association of second pairing segments.	First pairing segments remain fully condensed and associated with each other, while second pairing segments are free.

The fine threads mentioned in Table 20 are Feulgen-positive 'anaphase bridges' and are, indeed, part of the chromosomal pairs. These anaphase bridges are readily distinguishable from the more lightly-staining, Feulgen-negative, interchromosomal spindle-fibres (See Fig. 106 following page 351).

Each mode of association tabulated above seems to be characterised by a particular pattern of condensation. Thus, a type 3 bivalent at anaphase is most commonly uniformly undercondensed, except for the terminal pairing segment of one arm which is fully condensed (Fig. 65 A, F). There are two variations of this anaphase type 3 bivalent; in Fig. 66, one sex-chromosome is normally condensed, the other under-condensed, while the pairing segments in one arm are fully condensed. In Fig. 65 I, the pairing segments of one arm form two large central bodies, but these are under-condensed: the configuration is seemingly the anaphase counterpart of the metaphasic sex-bivalent in Fig. 63 D.

In yet other nuclei, with types 1 and 2 bivalents, the entire sex-chromosomes are uniformly under-condensed (e.g. Fig. 65 E, G) or uniformly fully condensed (Fig. 65 C).

The general impression is that definite forms of association (i.e. patterns of chiasmata) are coupled with specific states of condensation (or, as we shall soon paraphrase the latter term, with specific charges of nucleic acid). The nature of this correlation between chiasmata and charge will be explored in the chapter on the sex-chromosomes.

THE PRIMARY SPERMATOCYTE: TELOPHASE.

The diads of each daughter-nucleus are arranged in a ring (Fig. 67), the remnant of the hollow spindle of metaphase.

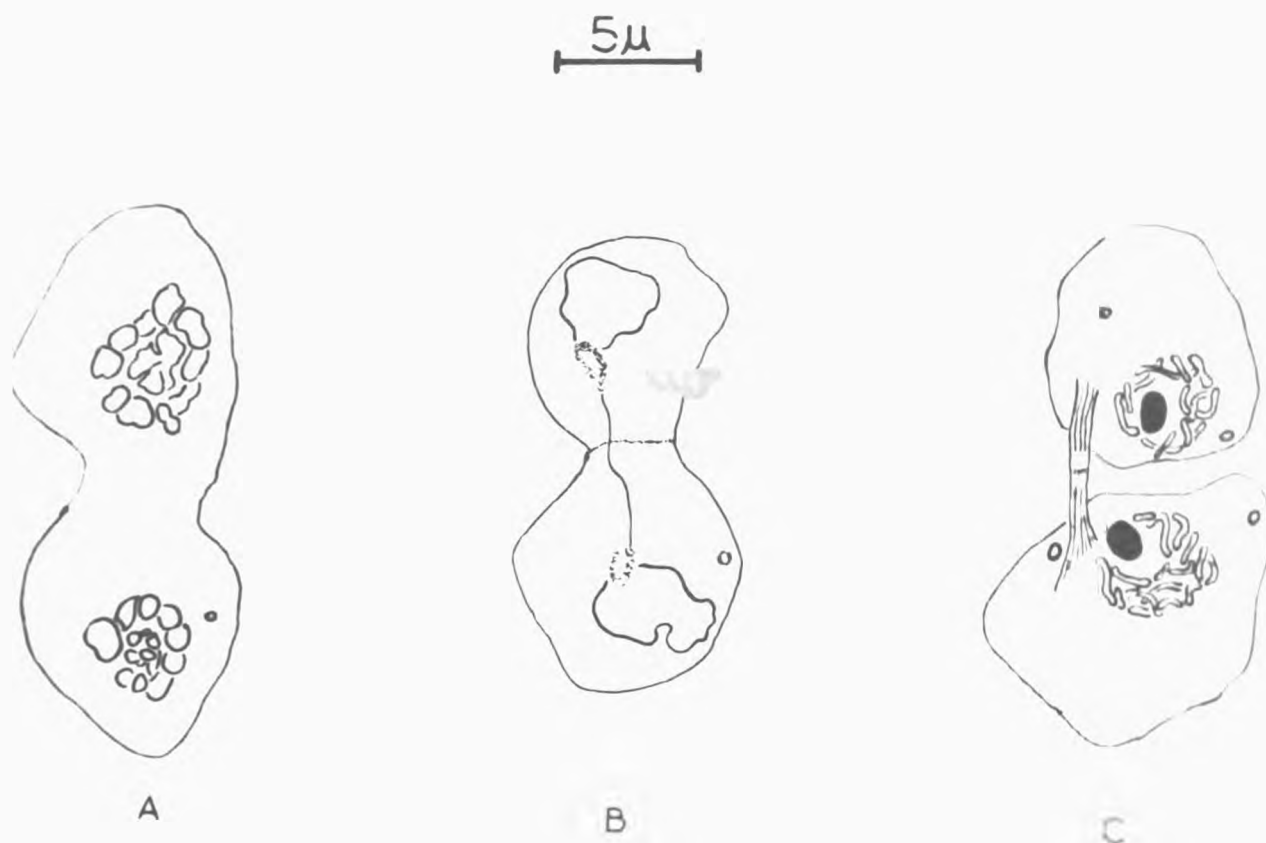


Figure 67. First meiotic telophase.

B is exceptional in possessing a persistent bridge between the sex-chromosomes. In C, note that the sex-karyosome has already developed in each daughter nucleus. The relic spindle (mitosome) is shown in C.

16 μ sections with various stains.

x 3500

The autosomes then unravel rapidly, becoming a tangled mass of fine, rather lightly-staining threads, and retaining their annular arrangement, as in Fig. 67 (C). Segments of the chromosomes align themselves along the newly-restored nucleocytoplasmic interface (karyotheca) to re-constitute a discontinuous, Feulgen-positive, chromatic membrane.

The sex-chromosome does not undergo changes similar to those of the autosomes. It is situated in the centre of the ring of chromatic threads, but is polarised above or below the level of the ring. If below, the sex-chromosome presumably passes through the centre of the ring to reach this position. Sometimes, by careful focussing, the passage through the centre of the ring can be traced. There seems to be a strong repulsion between the sex-chromosome and the autosomes, indicated by the clear space separating the sex-chromosome from the others. From the apex of the V-shaped sex-chromosome, a large, Feulgen-positive karyosome develops. This sex-karyosome grows in size, gradually taking in the remainder of the sex-chromosomal threads, until nothing is left but a short double thread, projecting from the surface of the large sex-karyosome.

Fig. 67 B illustrates a connecting thread between the sex-karyosomes of two neighbouring spermatocytes which have just divided. This thread is distinct from the inter-chromosomal spindle and is presumably a persistent chromosomal bridge which has remained from anaphase.

One autosome does not parallel the behaviour of the others: this autosome remains strongly condensed and rounds itself off as a karyosome lodged in a clear space among the autosomes.

Thus, in meiotic telophase, both the karyotheca and the chromatic membrane are restored and both an autosomal- and a sex-karyosome re-appear as in the preceding prophase.

Important cytokinetic processes accompany the above-described nuclear changes. The first sign of cytokinesis is the appearance of a cleft or constriction furrow in the cell-wall, in the region of the spindle-equator (Fig. 68). This furrow leads to deformative changes in the phragmoplast (Figs. 67C, 68 A, B). In the cell depicted in Fig. 68A, the constriction furrow appears to indent the peripheral fibres. This indentation precipitates the collapse of the phragmoplast and, the equator of the inter-chromosomal spindle, which has been the widest part of the spindle since metaphase, becomes progressively narrower.

The collapse of the spindle is accompanied by lengthwise shrinking of the fibres themselves. The indentations in the peripheral fibres become ironed out and there is no slackness as the fibres come in from their arched phragmoplastic position to their almost rectilinear conformation in

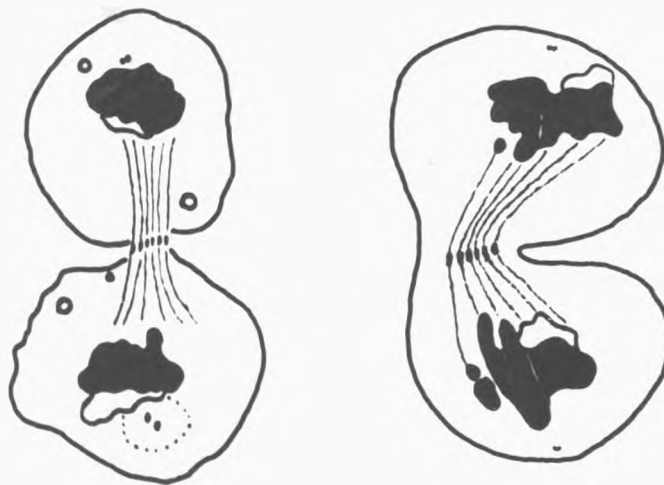


Figure 68. First meiotic telophase. The constriction furrow is seen impinging upon the mitosome. Note the divided centrioles and the chromatoid bodies.

16 μ section stained with Heidenhain's haematoxylin.

x 3500

the collapsed spindle.

While the phragmoplast is collapsing and the fibres are shortening, a granular thickening develops about the middle of each fibre, as though the slackness were being taken up in a knot. There is formed in this way a plate of discrete granules across the equator of the spindle-remnant, the mid-body (Zwischenkörper) (See Fig. 118 p.486).

The mid-body of the spermatocyte is a more prominent structure than that of the spermatogonium; unlike that of the spermatogonium, too, the spindle-remnant of the spermatocyte persists after the completion of the telophase. These observations testify to the greater development and durability of the entire spindle-apparatus in the primary spermatocyte. Table 21 summarises the differences between the two types of spindle.

TABLE 21.

DIFFERENCES BETWEEN MITOTIC AND MEIOTIC SPINDLE-
CENTRIOLE BEHAVIOUR.

<u>PROPERTY</u>	<u>MITOSIS</u>	<u>1ST MEIOTIC</u>	<u>2ND MEIOTIC</u>
<u>Doubling of centrioles</u> (for next cell-division)	During telophase	During prophase (diakinesis)	During telophase
<u>No. of centrioles at each spindle-pole in metaphase-anaphase.</u>	One	Two	One
<u>Morphology of spindle-fibre.</u>	Thinner, more lightly-staining.	Thicker, more intensely-staining.	Thinner, more lightly-staining
<u>Durability of spindle-fibre.</u>	Mitosome seldom present beyond telophase.	Mitosome usually persists until the end of the ensuing 2nd meiotic division.	Mitosome usually persists beyond telophase for a short period.
<u>Mid-body.</u>	Slight, ephemeral.	Prominent, durable	Slight, ephemeral
<u>Number of chromosomal threads attached to each pair of spindle-fibres.</u>	Two	Four	Two

As the phragmoplast collapses asymmetrically before the advancing constriction-furrow, the spindle is moved slightly towards one side of the cell. This movement, together with a migration of the nucleus from its original position opposite the end of the spindle towards one side of the spindle, alters the relation of the spindle-remnant to the adjacent surface of the daughter-cells. While originally running into each cell at right angles to the cell-surface, the spindle-remnant now runs tangentially from the peripheral cytoplasm of one cell to that of the other.

One or more chromatoid bodies may be present in the cytoplasm. The archoplasm cannot be identified.

Finally, the cytoplasmic segregation into two daughter-cells is complete.

Telophase is normally the phase during which an interphasic nucleus is re-constituted. In the gerbil, it is accepted that the 1st meiotic telophase ends when (a) all the autosomes have become longer and more lightly-staining, (b) the sex-karyosome has segregated to one side of the nucleus, (c) the chromatic nuclear membrane has been re-constituted, and (d) the cytoplasmic division is completed. These four criteria have been selected arbitrarily because, after the 1st meiotic division, the nucleus of a spermatocyte does not assume a true interphasic or 'resting' appearance. In its failure to

restore the nucleus to the replica of its pre-divisional structure, the 1st meiotic division resembles the last mitotic division of the premeiotic spermatogonium and the 2nd meiotic division of a spermatocyte. Each of these three exceptional telophases produces a post-divisional appearance markedly different from the pre-divisional aspect.

THE SECONDARY SPERMATOCYTE.

Any germ-cell which has completed the 1st meiotic division but has not yet negotiated the 2nd meiotic division is described as a 'secondary spermatocyte'. The class of secondary spermatocytes includes non-dividing and dividing cells.

- (a) Non-dividing secondary spermatocytes are those secondary spermatocytes in the interkinesis between the end of the 1st meiotic telophase and the onset of the 2nd meiotic prophase.
- (b) Dividing secondary spermatocytes are those secondary spermatocytes which are undergoing the 2nd meiotic division, i.e. have passed the onset of the 2nd meiotic prophase, but have not reached the end of the 2nd meiotic telophase.

THE NON-DIVIDING SECONDARY SPERMATOCYTE: INTERKINESIS.

Interkinesis is a clearly-recognisable stage of short duration, as adjudged by the paucity of cells in interkinesis. It may best be studied in a tubule cut longitudinally;

in such a tubule, the topographical sequence of cell morphologies from the 1st to the 2nd meiotic figures indicates the temporal sequence from the 1st meiotic to the 2nd meiotic divisions. Where a longitudinal section of a tubule contains metaphase figures of both the 1st and the 2nd meiotic divisions, several dozen cells usually intervene spatially between the two metaphasic groups. From the 1st to the 2nd meiotic metaphasic groups, the gradations of nuclear structure can be classified into:-

- (i) 1st meiotic anaphase and telophase;
- (ii) interkinesis of secondary spermatocyte;
- and (iii) prophase of secondary spermatocyte.

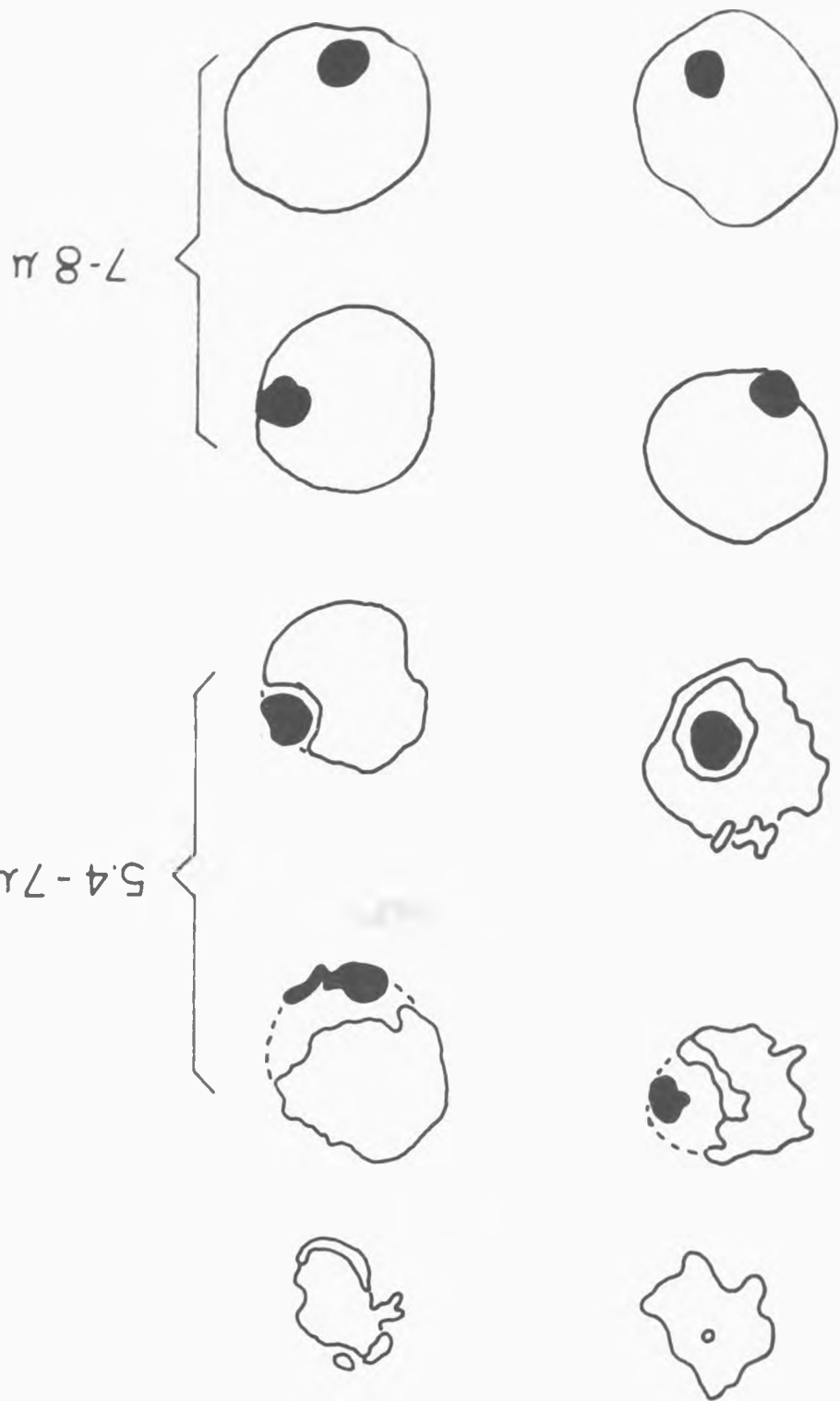
It may be inferred from this topographical sequence that, in the gerbil, a definite interkinesis occurs between the two meiotic divisions. Interkinesis is a feature also in the mole and the ferret (Koller 1936b), the marsupial, Pseudochirus (Koller 1936c), the cat (Koller (1941b) and the rat (Allen 1918). In contrast, Koller reports that no interkinesis separates the 1st meiotic anaphase from the 2nd meiotic metaphase in three marsupials, Dasyurus, Sarcophilus and Phascolarctus (Koller 1936c), the grey squirrel (Koller 1936a) and the golden hamster (Koller 1938a).

In the gerbil, both nuclear and cytoplasmic unfoldments occur during the interkinesis. Some of the nuclear changes are conveyed in the series of drawings in Fig. 69 and in the photomicrographs in Fig. 70.

Figure 69. Size changes in the nuclei of
secondary spermatocytes from
the first meiotic telophase.

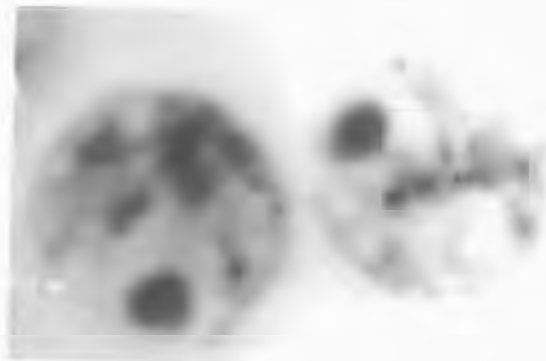
16 μ sections with various
stains.

x 3500



- Figure 70.
1. Nuclei of secondary spermatocytes in early interkinesis. Note the karyosome developing from the sex-chromosomes.
 2. A later stage in interkinesis. the sex-chromosome has now passed over completely into the sex-karyosome, which still lies within a clear space.
 3. After the growth period of interkinesis: the clear space surrounding the sex-karyosome is reduced.
- 16 μ sections stained with Heidenhain's haematoxylin.

x $\pm$ 4000



Nuclear events.

(1) Progressively during interkinesis, the diameter of the nucleus increases from a mode of about 5μ to a mode of $7 - 8\mu$.

(2) The autosomes elongate and lose much of their initial marked affinity for stain, so that, by the time the nucleus has enlarged, the threads are rather faintly-staining. From their annular dispersion, the threads assume a more diffuse distribution throughout the nucleus. The threads now consist of thinner, more lightly-staining and thicker, more deeply-staining regions and may often be seen to be double. The thicker regions resemble a number of minute granules, which recall to some extent the structure of the typical 'resting' nucleus.

(3) The autosomal- and the sex-karyosomes remain deeply-staining and become progressively more condensed. No plasmosome develops in interkinesis.

(4) At first, the sex-karyosome is located in a clear space at the centre of the ring of autosomes; but, when the ring collapses and the unravelling chromosomes come to form a compact mass, the repulsion-space between karyosome and autosomes persists. As the karyosome becomes more condensed, the repulsion lapses, the clear space being reduced to a narrow halo around the karyosome (Fig. 70, 71).

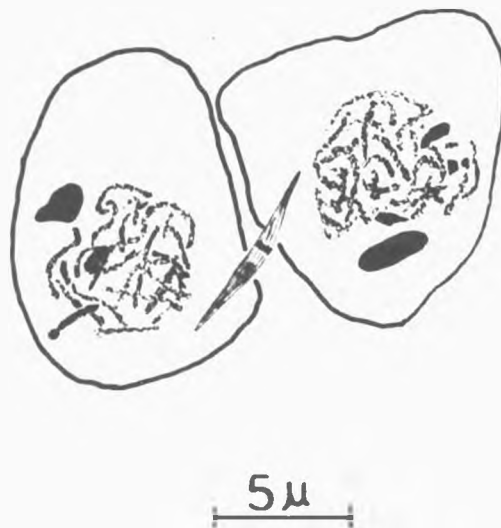


Figure 71. Early interkinesis of secondary spermatocytes, showing the relic spindle of the first meiotic division. Note the fully-charged sex-chromosomes (sex-karyosome).

16μ section stained with Heidenhain's haematoxylin.

Cytoplasmic events.

(5) Immediately after the first meiotic telophase, the spindle consists of a mass of fibres stretched between the two spermatocytes. Its central (or intercellular) portion, the mid-body, remains distinct for some time. The fan-like terminal (or intracellular) organisation of the spindle soon collapses, however, and the bundle of spindle-fibres withers to a tapering end, as seen in Fig. 71. The whole spindle-remnant once more becomes fusiform. Subsequently, the mitosome becomes much thinner, persisting as a deeply-staining line between two neighbouring cytoplasts. The fusiform arrangement, the last that one can observe of the 1st meiotic spindle, persists during the 2nd meiotic division and may still be seen at the end of the 2nd meiotic telophase. The lasting character of the 1st meiotic spindle contrasts sharply with those of the mitotic and of the 2nd meiotic spindles.

(6) The archoplasm, not evident from the 1st meiotic metaphase to telophase, re-appears in interkinesis. From Fig. 72, it is apparent that the archoplasm has much the same form as it manifested during the previous prophase. In a small proportion of cells, centrioles are identified in relation to the archoplasm.

(7) Chromatoid bodies are present in the cytoplasm of the interkinetic spermatocyte, but they have a varied morphology. Instead of smoothly-outlined, spherical bodies, they are

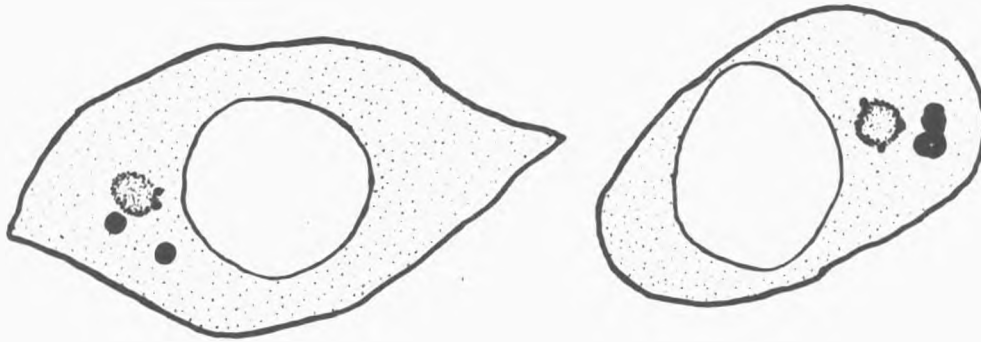


Figure 72. Secondary spermatocytes with cytoplasm inclusions.

Note the minute centrioles, the archoplasm (stippled) and the chromatoid bodies (solid).

16 μ section stained with Heidenhain's haematoxylin.

x 3500

sometimes elongated in shape, not infrequently irregular in outline and generally larger than the chromatoid bodies of the primary spermatocyte. Apparently, two or more chromatoid bodies are in process of fusing. Fusion is followed by a regulation to a spherical form. The staining properties are, however, unchanged. The presence of chromatoid bodies in every secondary spermatocyte suggests that the chromatoids have divided and segregated themselves to the daughter-cells, although it is impossible to determine whether an equal division occurs.

THE DIVIDING SECONDARY SPERMATOCYTE: PROPHASE.

The second meiotic prophase is marked by the transition from lightly-staining, interkinetic nuclear threads to deeply-staining, heavily-condensed, metaphasic chromosomes. In a longitudinal section of a tubule, there is a characteristic topographical sequence from interkinetic cells to those in the 2nd meiotic metaphase. The nuclear criteria of the transition are these:- The chromatic membrane becomes markedly discontinuous, as small peripheral chromatic blocks develop from the chromosomal threads. At least one chromatic block is so large as to justify calling it a third karyosome. The further contraction and condensation of the chromosomes must be extremely rapid, to judge by the small number of cells in the 2nd meiotic prophase. In fact, if we accept that the number of cells fixed in a specific division-phase is proportional to the duration of that phase, the entire 2nd

meiotic division is very rapid.

THE DIVIDING SECONDARY SPERMATOCYTE: METAPHASE.

Few secondary spermatocytes were found in the metaphase stage. In one of these, depicted in Fig. 73, a chromosomal pair has two under-charged segments, probably the differential segments of the chromatids of a sex-diad. Chromatoid bodies lie on or off the spindle, but, as at metaphase in the 1st meiosis, the archoplasm cannot be located. Conspicuously differing from the 1st meiosis, the centrioles have not doubled by the 2nd meiotic metaphase and the spindle-fibres are thinner and stain feebly; the secondary spermatocyte resembles the spermatogonium in these features. As if to underline the contrast, the 1st meiotic spindle-remnant can still be located in heavily-stained secondary spermatocytes undergoing the 2nd meiotic division (see Table 21).

THE DIVIDING SECONDARY SPERMATOCYTE: ANAPHASE.

The separating anaphase groups of chromosomes are orientated on the surface of the spindle, thus forming two rings. Commonly, a pair of undercharged V-shaped elements lags on the spindle (Fig. 74). From their anomalous behaviour and undercharged state, these laggings presumably represent the X- or the Y-chromosome.

Anaphase bridges are much commoner in the 2nd than in the 1st meiotic division. Generally, the big V-shaped



Figure 73. Second meiotic metaphase
with partly undercharged
sex-chromosome.

16 μ section stained with
Heidenhain's haematoxylin.

x 3500

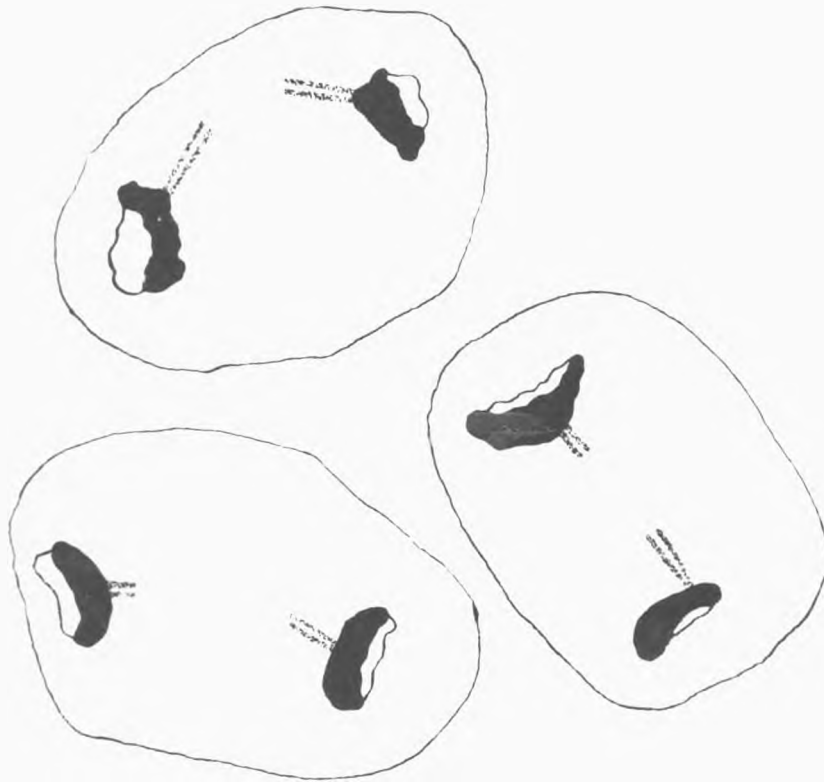


Figure 74. Secondary spermatocytes in the second meiotic anaphase. Note the large undercharged V-shaped laggings, presumably the X or the Y.

10 μ section stained with Feulgen's leuco-basic fuchsin.

x 3500

element (X or Y) is involved and there may be one or two bridges.

The cytoplasm, like that of the anaphasic primary spermatocyte, contains chromatoid bodies, but no detectable archoplasm. The spindle has the phragmoplast conformation at anaphase, but its fibres stain less intensely than do those of 1st meiotic figures.

THE DIVIDING SECONDARY SPERMATOCYTE: TELOPHASE.

The separated monads are very much smaller than 1st meiotic diads (Fig. 75A). In two or three secondary spermatocytes, 17 monads may be counted, but it is impossible to identify the sex-chromosomes. The monads group closely together into a fairly compact mass in each daughter-cell.

A large chromatoid body is lodged in the cytoplasm, usually of each daughter-cell (Fig. 75).

During the rest of telophase, the modifications which occur in the secondary spermatocyte proceed in the direction of (a) cytokinesis - or cytoplasmic division and (b) the re-constitution of an interphasic nucleus.

The nuclear transformation is similar to that following the 1st meiotic telophase. From discrete monads, fine threads unravel, so that the nucleus becomes filled with a skeinwork of thick chromatin bodies and thin threads. Two chromosomes, however, remain as condensed bodies or karyosomes, after all

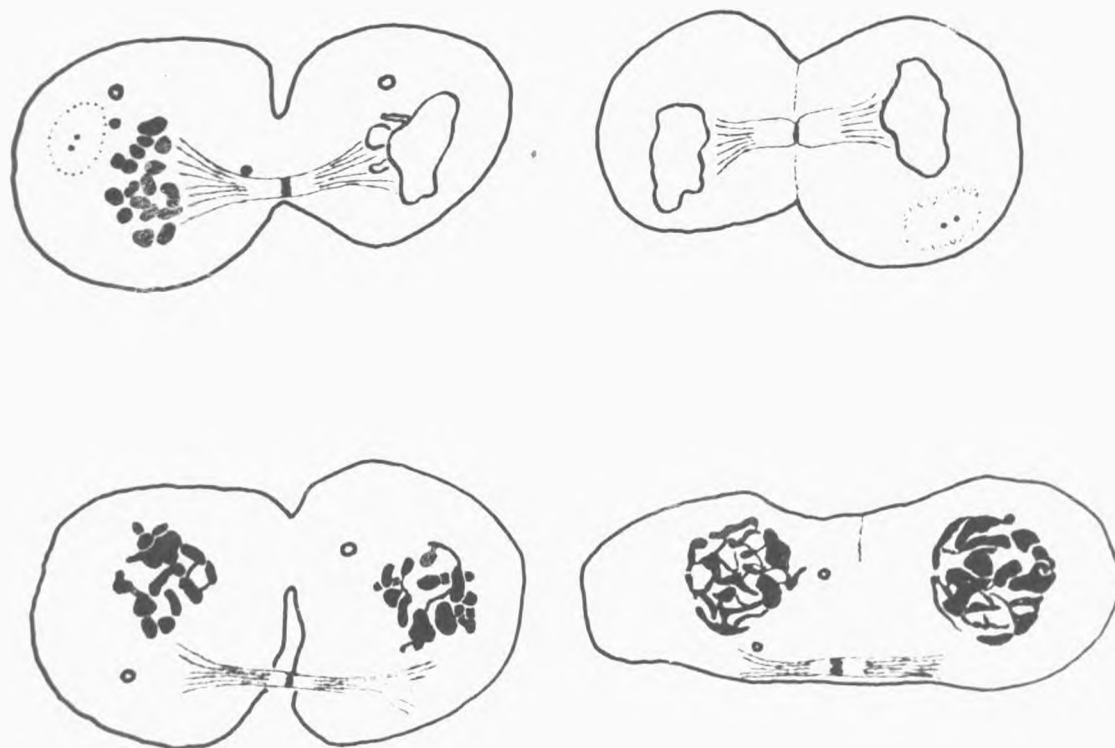


Figure 75. Telophase of the second meiotic division. In the upper left-hand cell, 17 chromosomes may be counted.

Various forms of the mitosome are shown. Note, too, the centrioles, centrosome and chromatoid bodies.

16 μ section stained with Heidenhain's haematoxylin.

x 3500.

the others have unravelled more or less completely. The larger of these karyosomes is presumably the sex-karyosome; the smaller is an autosomal karyosome like those of 1st meiotic prophase and interkinesis.

The cytokinetic shifts are similar to those already described for the 1st meiotic division. The spindle, constricted by the cleavage-furrow, is reduced to a thin sheath of fibres splaying out towards the new daughter-nuclei. During the telophase, the centrioles double themselves once more, the timing of centriolar division thus corresponding with that of mitosis (see Table 21). On this occasion, centriolar division is unequal, one centriole being large and ellipsoid, the other, small and dot-like, lying a short distance off one face of the ellipsoid. The two bodies, maintaining this relation to each other, rapidly move away from the nuclear surface towards the peripheral cytoplasm.

While these centriolar movements are taking place, the archoplasm re-appears in the cytoplasm, rather smaller than on its previous appearance and, unrelated in position to the centrioles. The chromatoid body or bodies, too, occupies a different part of the cytoplasm. The spindle-remnant, now a thin collapsed structure with a persistent mid-body, lies on the edge of the cytoplasm, running tangentially off the surface towards another cell.

When cytoplasmic division is complete, each daughter

cell is called a spermatid. The ensuing changes in the cell, known collectively as spermiogenesis or spermioteleosis, transform the spermatid into a mature spermatozoon. These spermiogenic events, like the preceding stages of spermatogenesis, must be regarded as drastic modifications in the entire micromorphology and economy of the cells. It is inconceivable that any one part of the cell is not vitally affected by the metamorphosis. In the next section of the chapter, then, attention is focussed in the main on nuclear events, but also on cytoplasmic organoids, including the accessory structures associated with the sperm-head.

SECTION III: SPERMIOGENESIS.

THE SPERMATID.

The class of 'spermatid' comprises all germ-cells which have passed the telophase of the 2nd meiotic division, but have not yet completed the metamorphosis into spermatozoa. We may distinguish two types of spermatid: (a) the post-meiotic spermatid, which has just completed meiosis; and (b) the metamorphosing spermatid, which is in process of transforming into a spermatozoon and may have advanced any distance along the differentiative pathway to the almost mature spermatozoon.

THE POST-MEIOTIC SPERMATID: THE NUCLEUS.

Diagrams of a series of spermatid nuclei are given in Fig. 76. From these, it is clear that the nucleus is round and small, its diameter varying between 3.4 μ and 6.2 μ . The chromatin is in the form of (i) fine threads and granules and (ii) several large aggregates. One of the aggregates, a round and constant karyosome associated with a plasmosome, is probably the sex-karyosome. Other aggregates are less constant, but there is usually a smaller karyosome associated with a second plasmosome: this represents the autosomal karyosome. A careful study was made of these karyosomes, to determine whether the spermatids fell into two classes, according as to whether the nucleus contained an X- or a Y-chromosome. However, no significant differences were observed between spermatids; a result which we might have predicted, since both X and Y have evinced identical karyosomal behaviour at all earlier stages. Thus, although we may assume that there are two kinds of spermatid, one female-determining, containing an X-chromosome and, the other, male-determining, containing a Y-chromosome, these two varieties of spermatid cannot be distinguished cytologically.

The chromatic nuclear membrane has been re-constituted, when the unravelled telophasic threads become apposed to the nucleo-cytoplasmic interface. The staining membrane is interrupted at intervals, exactly like that of the

Figure 76. Series of post-meiotic spermatids.

The sex-karyosomal structure is visible in each nucleus. Note the archoplasm and various forms of chromatic bodies in the cytoplasm.

16 μ section stained with Heidenhain's haematoxylin.

x 3500



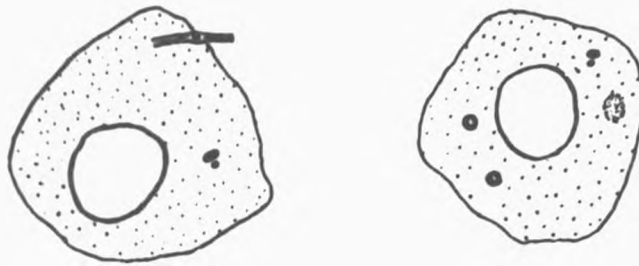
interkinetic secondary spermatocyte. But, whereas the membrane of the latter becomes perfectly continuous, that of the spermatid remains discontinuous. In fact, subsequent development of the spermatid reveals that the breaks in the membrane are arranged in a pattern, which is one expression of a polarity assumed by the nucleus and even the whole cell in its metamorphosis.

THE POST-MEIOTIC SPERMATID: THE CYTOPLASM.

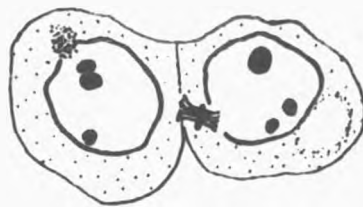
Shortly after the telophase of the 2nd meiotic division, the cytoplasm contains the following organoids - mitosome, archoplasm, centrioles, proximal portion of the tail-filament and chromatoid bodies.

The mitosome: Immediately after the telophase, the spindle is a long, slender, tapering structure with a broader, more deeply-staining body on its equator (Fig. 77 A). Subsequently, its ends, having already regressed from a broad fan to a spike of concentrated fibres, undergo a further collapse; the ends contract longitudinally towards the mid-body which becomes stout and squat, not quite 2μ in length. The whole mitosome now resembles a sheaf of corn (Fig. 78). No later appearance of the spindle has been observed, since, after a time, the mid-body and the mitosome disappear; apparently, they leave no relic nor make any contribution to the developing spermium.

The archoplasm at first shows its usual morphology. It



77



78

Figure 77. Post-meiotic spermatids, showing the unequally-sized centrioles in a peripheral position.

Note the mitosome in the cell on the left and the archoplasm in the cell on the right.

Figure 78. Post-meiotic spermatids. The mitosome is shaped like a sheaf of corn.

16 μ sections stained with Heidenhain's haematoxylin.

x 3250

rapidly comes to occupy a position against one pole of the nucleus; as it will be shown that this pole eventually forms the cranial end of the sperm-head, the position of the archoplasm will be designated the anterior pole of the nucleus. The archoplasm flattens itself upon the nuclear membrane forming a crescentic organoid.

The centrioles: During the preceding telophase, it was noted that both centrioles are duplicated and migrate to the peripheral cytoplasm. These features are seen in Fig. 77. The tail-filament becomes manifest, leading from the peripherally-situated centrioles, beyond the cytoplasm for a short distance towards the lumen of the seminiferous tubule. In the gerbil, the tail-filament seems to arise from both centrioles, as has been described in man (Gatenby and Beams 1935), the guinea-pig (Gatenby and Woodger 1921) and other species. Presently, the centrioles begin to migrate towards the posterior pole of the nucleus, carrying the proximal part of the filament with them (Fig. 79).

The chromatoid bodies: Each spermatid usually contains two chromatoid bodies, suggesting a division and segregation of chromatoids during the 2nd meiosis. The chromatoid bodies in the very young spermatid grow larger and fuse. They are generally spherical, but occasionally more lightly-staining and elongate. Such an appearance is followed by the curling-up and condensation of the bodies to form the usual spherical



Figure 79. Early metamorphosing spermatids. The centrioles have returned to the post-nuclear position and the tail-filament has appeared. Note the fusing chromatoid bodies, migrating towards the caudal pole of the nucleus and, in the right-hand cell, the migrating archoplasm.

16 μ section stained with
Heidenhain's haematoxylin.
x 3250

organoids with deeply-staining cortex and faintly-staining or achromatic core. The two chromatoid bodies of each spermatid then coalesce (Fig. 79 A and B), the compound structure being for a time elongated and surrounded by a halo of cytoplasm devoid of stainable granules. Although never at any time in visible contact with the archoplasm, the fusion-chromatoid lies to one side of the archoplasm at the anterior pole of the nucleus. In this position, the fusion-chromatoid may be confused with the spindle-remnant, often still seen in the peripheral cytoplasm.

THE METAMORPHOSING SPERMATID.

It will be convenient to consider the transformations of the spermatid under a series of arbitrary stages, some of which emphasise nuclear, others cytoplasmic changes.

(a) External polarisation of the nucleus.

The movements of the cytoplasmic organoids impose a polarity on the nucleus from without: the archoplasm defines the prospective anterior pole of the nucleus, while the centrioles and emergent axial filament anticipate the posterior pole. The longitudinal axis of the spermatid lies perpendicular to the basement membrane of the tubule, the anterior pole towards the lining membrane, the posterior towards the lumen.

The archoplasm flattens, then indents, the anterior

pole of the nucleus, giving the nucleus the shape of a pear (Figs. 79 A and 80). The lining of the anterior depression stains very deeply but no strongly-staining pro-acrosomal granule can be seen occupying the indentation. It is assumed by analogy with other mammals that the depression is due partly to the pro-acrosome being deposited and, partly to the pressure of the archoplasmic vacuole. There is a movement of chromatin away from the anterior pole since the depression coincides with the largest area of interrupted nuclear membrane, behind which is a clear, vacuolar, intra-nuclear space.

Meantime, the centrioles have reached the posterior pole of the nucleus, coming into such close contact with it that they produce a tiny local indentation. The proximal centriole often overlaps the chromatin of the nucleus; while the distal centriole is a somewhat larger, ellipsoidal or spheroidal granule close to the edge of the nucleus.

During these cytoplasmic manoeuvres, the nucleus has changed its morphology; it has undergone anterior flattening, the movement of chromatin and the posterior indentation.

(b) Caudal migration of the cytoplasm.

In Fig. 79 A, B and C, the chromatoid body (or still fusing chromatoid bodies) is migrating round one side of the nucleus, approaching the posterior pole. In this process,

the archoplasm rounds itself off once more on the anterior pole and then, also, travels down the side of the nucleus, usually on the opposite side to that on which the chromatoid travels (e.g. Fig. 79 C). The caudal migration of chromatoid body and archoplasm is seemingly part of a general movement of the cytoplasm around or over the nucleus to form a posterior cytoplasmic mass. The cytoplasmic movement leaves a clear anterior rim of non-staining cytoplasm in which, presumably, the acrosome is developing across the anterior pole of the nucleus. The migrating cytoplasm has not been seen to bud off a bead which passes to the junction of the neck and mid-piece, as has been described for the Golgi-archoplasm complex in the guinea-pig (Gatenby and Woodger 1921) and the mouse (Gresson 1942).

(c) The formation of the manchette.

While the chromatoid is located close to the posterior pole of the nucleus, a cylindrical manchette or caudal sheath grows back from the region about the equator of the nucleus. It is a thin, diaphanous structure, having a few delicate, longitudinal venules or filaments. Within the manchette, the cytoplasm is devoid of fine granules. It is traversed by the tail-filament which emerges freely into the cytoplasm at the posterior end of the manchette. At the first appearance of the manchette, the fusion-chromatoid, now much reduced in size and again regulated to a spherical shape, lies astride or to one side of the caudal free edge of the manchette (Fig.

80 B and C). Whether the chromatoid body has become smaller through condensation, or through the loss of some material, could not be established. In respect of the first possibility, there is no change in staining as the chromatoid migrates and it retains its lightly- or non-staining core; while, as for the second possibility, no chromatoid granules or other breakdown products are seen escaping from the body. The emergence of a new structure, the manchette, in the vicinity of the diminishing chromatoid body, suggests a relationship between the chromatoid body and the outgrowth of the manchette; but no further observational evidence is available to support this speculation.

(d) The establishment of the chromatoid-centriolar complex in the prospective neck region.

After the formation of the manchette, the chromatoid ceases to follow the posterior flow of the cytoplasm, but is seemingly attracted forwards to the centriolar area. Thus, it migrates within the manchette, close to the axial filament, and occurs consistently in this position as a spherical, deeply-staining body with a lighter core. A thread makes its appearance, connecting the chromatoid with the centriolar region (Fig. 81). From this moment, the history of the chromatoid body is intimately linked with the fate of the centrioles.

Figure 80. Metamorphosing spermatids (11).
The following features may be observed:

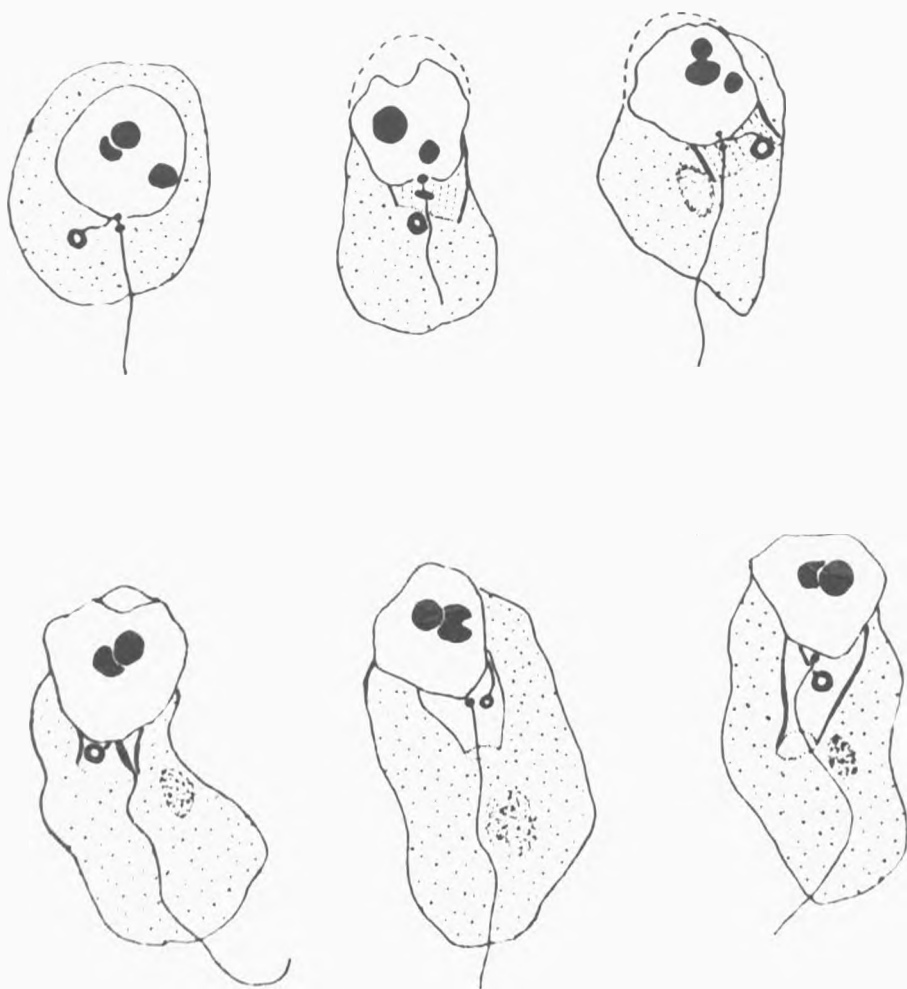
- (i) the indented cranial pole of the nucleus.
- (ii) the newly-arisen manchette.
- (iii) the chromatoid body, regulated once more to a spherical shape, and lying astride the caudal free edge of the manchette.

Figure 81. Metamorphosing spermatids (iii).
The chromatoid body has migrated in a cranial direction within the manchette and is now connected by a fine chromatic thread to the centriolar region.

Note that the manchette has elongated and that the archoplasm has attained a position in the caudal cytoplasmic mass.

16 μ sections stained with Heidenhain's haematoxylin.

x 3250



(e) Internal polarisation of the nucleus.

The modifications in nuclear structure, affecting its size, shape, membrane and contents, are summarised in Fig. 82. The nucleus decreases markedly in size, assuming an ovoidal shape, in contrast with its early spheroidal appearance. The anterior indentation disappears and the anterior pole of the nucleus resumes a smooth, rounded contour. The posterior pole of the nucleus is clothed by a Feulgen-positive nuclear membrane, forming an intact and continuous sheath around the posterior segment of the nucleus. At the anterior pole, the Feulgen-positive nuclear membrane is defective, permitting a seemingly naked interface (karyotheca) with the cytoplasm. Thus, the metamorphosing spermatid develops a peculiar polarisation of the chromatic nuclear membrane, reflected in the continuous cup of chromatic membrane at the posterior pole and the absence of a chromatic membrane at the anterior pole.

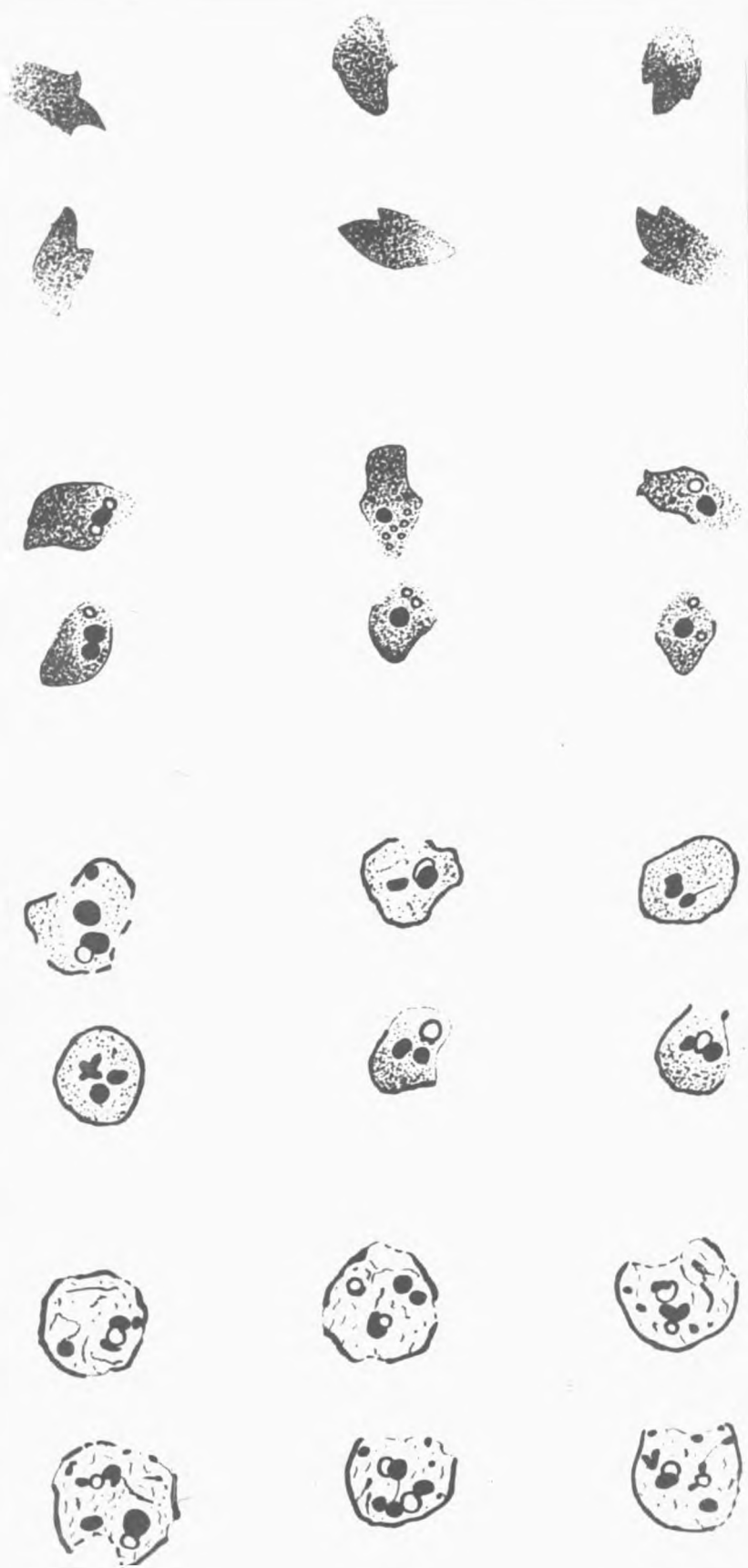
The non-aggregated portions of chromatin become punctate, there being no longer any trace of chromatic filaments. Most of the granules accumulate at the posterior pole of the nucleus, within the cup formed by the chromatic membrane, while the anterior pole contains very few granules and is consequently more lightly-staining. Thus, the chromatin granules may be regarded as distributing themselves according to the same co-ordinates of polarity as the nuclear membrane.

Figure 82. The transformation of the spermatid nucleus. Summary of the modifications in nuclear structure, affecting size, shape, membrane and contents, from the post-meiotic stage to the mature sperm-head.

Key: Outlined bodies -plasmosomes
 Solid bodies - karyosomes.

Drawn from sections stained with Feulgen's leuco-basic fuchsin and light green.

x 3250



In the nucleus, the junction of the two polar regions is marked by a fairly distinct transitional zone in the plane of the equator, i.e. at right angles to the axis of polarity and, in such a position that about two-thirds of the nucleus is deeply-staining (i.e. filled with granules) while only about one-third at the anterior pole is lightly-staining. In short, the nucleus has assumed a structural polarity affecting its shape and the distribution of the chromatin granules and membrane.

Unravelling and fusion reduce the main chromatin aggregates to a single large karyosome at the junction of the anterior and posterior regions. The two large plasmosomes may fuse to form a single plasmosome, which decreases in size. Late in the metamorphosis of the spermatid, we find either a single plasmosome or two plasmosomes associated with a karyosome. Even when the spermium has attained almost complete maturity, a large darkly-staining body is still detectable at the junction of the two zones.

(f) Development of the chromatoid-centriolar complex in the prospective neck region.

Figs. 83 and 84 demonstrate that the above nuclear transformations are paralleled by further cytoplasmic changes, notwithstanding that the thickening of the membrane over the posterior pole adds to the difficulty of analysing events immediately caudal to the nucleus. The centrioles assume

a new appearance: the proximal centriole remains a single small granule, while the distal centriole becomes a flattened, disc-like structure with a hollow-centre traversed by the axial filament. Such a structure has been called a 'ring-centriole'. At first, it lies on the edge of the nucleus (Fig. 83 A and B). Subsequently, it moves to a more caudal position, a short distance (about $1/5$ to $\frac{1}{2} \mu$) down the filament (Fig. 83 C, D, Fig. 84, in that order).

The caudal migration of the ring-centriole reveals that the thread running from the chromatoid body to the centriolar region is composed of, or splits into, two fibres; one connects with the edge of the ring-centriole, the other with the region of the proximal centriole (Fig. 82 C and D). The latter thread grows beyond the chromatoid body towards the manchette, retaining only a slender, filamentous connection with the chromatoid. This new neck filament, stretching almost from nucleus to manchette, thickens progressively. The appearance strongly suggests a flow of chromatoid material into the filament to form the neck rod.

By this stage in the metamorphosis, the chromatoid body is much reduced in size, though it maintains its lighter core. It lies very close to one edge of the ring-centriole, to which it is still joined by a fine thread; the second thread leaving the chromatoid body, approximately at right

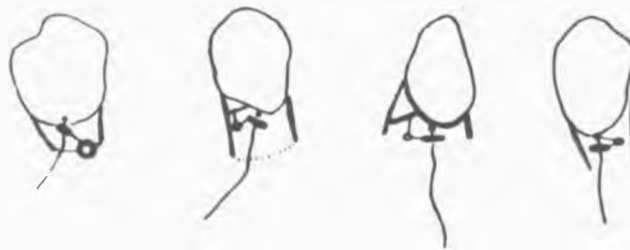


Figure 83. Metamorphosing spermatids (1V).
Differentiation in the post-
nuclear region.

The neck-rod is present and the
chromatoid is connected by
filaments to both the neck-rod
and the ring-centriole.

16 μ section stained with
Heidenhain's haematoxylin.

x 3250

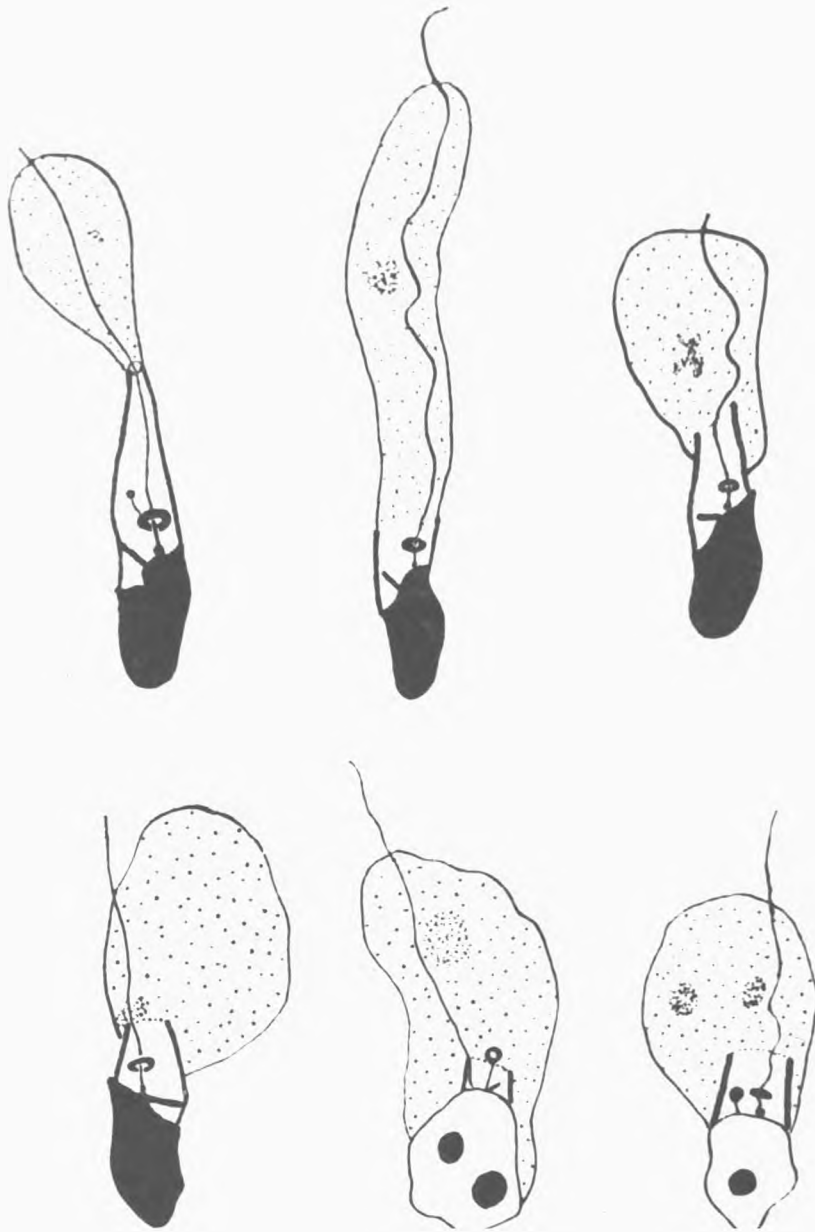


Figure 84. Metamorphosing spermatids (V).

The ring-centriole has migrated in a caudal direction. Note the caudal migration of the cytoplasmic mass until it remains connected with the nucleus only by the manchette. The chromatoid body is much reduced in size and now connects only with the ring-centriole by a fine filament.

16 μ section stained with Heidenhain's haematoxylin.

x 3250

angles to the first, is intercepted by the new structure, the neck-rod (Fig. 83 B and C). Further migration of the ring-centriole down the axial filament brings it into a position not quite 1μ from the nucleus; the filament connecting the chromatoid to the ring-centriole travels caudally as well. There follows an advancing enlargement of the ring-centriole, apparently at the expense of the chromatoid body. The latter, in consequence, becomes minute in size. The connection of the chromatoid body with the neck rod disappears, but that with the ring-centriole persists for long as a fine thread to which the tiny chromatoid remains attached. As the chromatoid becomes smaller and more faintly-staining, it is plastered on to the ring-centriole, after which it can no longer be seen as a discrete entity.

(g) The maturation of the sperm-head.

The final stages in the metamorphosis of the nucleus are a further reduction in size and heavy clumping of the chromatin of the caudal zone into a compact mass (Fig. 82). This solid, homogeneous mass encroaches forwards, filling most of the lighter, cranial pole save a faint rim. At the caudal nuclear pole, the indentation from which the tail-filament emerges, deepens into a definite cleft separating the caudal sheathed portion of the nucleus from a spur pointing backwards in front of the cleft. On the opposite side of

the caudal nuclear cup to the spur, a faint suggestion of a second cleft or depression becomes apparent. This second notch does not usually deepen and merely separates the caudal part of the nucleus from a prominence cranially. Very rarely and, depending on the angle of vision, this latter prominence is so well developed as to form a second spur. The formation of the two clefts cuts off a definite cup of chromatin caudally; from this membranous cup, the more massed chromatin becomes, as it were, squeezed out and crowds forward into the cranial, previously more lightly-staining region. In this way, there arises the characteristic morphology of the mature sperm-head.

(h) The end of spermiogenesis.

During the last stages of spermiogenesis, the cytoplasm becomes drawn out and apparently attaches to the nucleus solely by the manchette, now a protracted, slender funnel (Fig. 84 F). The axial filament lengthens enormously and traverses the long axis of the manchette. Still recognisable are the remains of the archoplasm, sometimes split into two and showing signs of granular degeneration. At its full development, the neck-rod is a thick, deeply-staining structure, jutting out from the proximal centriolar region, to meet the manchette at an angle of about 70° (Figs. 85 and 86).

In old crystal violet preparations, sperm-heads are

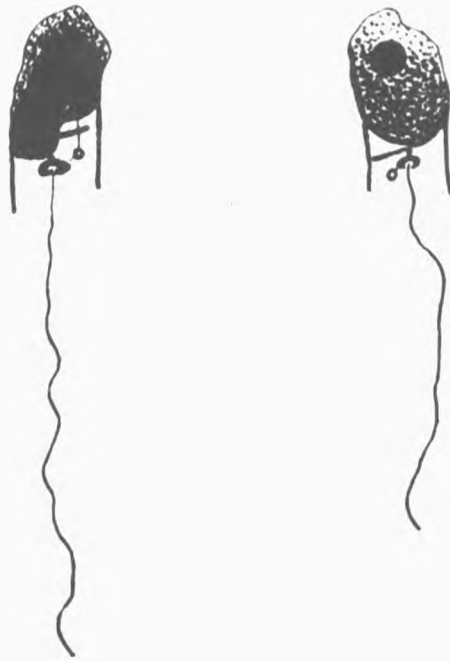


Figure 85. Almost mature spermatozoa. The neck rod is seen at its full development, while the chromatoid body has not yet become plastered on to the ring centriole.

Within the nucleus, the chromatin is crowding forward from the post-nuclear cup and the thickened post-nuclear segment of the nuclear membrane may be observed in the cell on the right.



Figure 86. Photomicrograph of sperm-head at about 4000 diameters. Note the characteristic shape of the sperm-head. The manchette, the neck-rod and the ring centriole may be seen.

16 μ section stained with Heidenhain's haematoxylin.

$\times \pm 4000$

clothed by an acrosome, loosely overlying the cranial tip of the nucleus. The acrosome is shaped like a helmet and often has two or three fine granules over its cranial surface and along its caudal free edge (Fig. 87).

At the latest stage seen in the testis, the cytoplasm has become reduced to a few blobs of homogeneously-staining material, lying some distance down the axial filament.

When mature, the spermia lie in groups, with their heads lining the lumen of the tubule, while their tails hang in sheaves into the lumen.

SUMMARY.

(1) A detailed description is given of nuclear and cytoplasmic events during spermatogenesis in the gerbil. In broad outline, the processes of mitosis and meiosis in the gerbil accord with the well-established sequence of mitotic and meiotic events in other mammals.

(2) Special attention is devoted to an atypical mitotic figure, distinguished by excessively-contracted chromosomes on a small equatorial plate.

(3) Synizesis in the gerbil is attributed both to a polarisation of chromosomal ends and to a collapse of the ball of chromosomes.



Figure 87. Camera lucida drawing of
spermatozoa showing the
acrosome.
10 μ section stained with
Newton's crystal violet.
x 3250

(4) The close connection between the chromosomes and the chromatic nuclear membrane has been demonstrated; a cytogenetical account has been given of the mobilisation of prophasic chromosomes from the chromatic membrane and of the telophasic re-constitution of a chromatic membrane by the peripheral alignment of the chromosomes.

(5) Peculiar features of the behaviour of the heteromorphic or sex-chromosomes are set forth and analysed in terms of (a) varying modes of association or pairing and (b) differing degrees of condensation. It has been shown that each of the sex-chromosomes has two terminal pairing segments, separated by a long loop of differential segment. Attention has been directed to the history of the 'diffuse sex-karyosome' which, it would seem, is not an integral part of the sex-chromosomes proper.

(6) Parts of the chapter are concerned with the relation between the sex-chromosomes and one autosomal pair, on the one hand, and the formation of plasmosomes, on the other. There are at least four plasmosomal chromosomes in the diploid chromosomal complement of the gerbil.

(7) The material has proved favourable for the study of secondary spermatocytes. Between the 1st and 2nd meiotic divisions, an interkinesis occurs in the gerbil and, this stage, as well as the 2nd meiotic division, have received attention.

(8) The history of the division-spindle is imparted in detail. Several unusual differences between the spindle-centriolar behaviour of the 1st meiotic and that of mitotic division have been placed on record.

(9) The cytoplasmic organoids typical of the germ-cells are traced from their first appearance to their ultimate fate in spermiogenesis. Their interrelated contribution to spermatogenesis is considered in concert with nuclear phenomena. Special stress is laid on the chromatoid body and a novel version is given of its participation in the formative events in the prospective neck region of the metamorphosing spermatid.

END OF PART 3.