CYTOLOGY

FOR

MEDICAL STUDENTS

CYTOLOGY

FOR

MEDICAL STUDENTS

By

Dr. AHMAD KABARITY

Head of Cytogenetic Unit
Faculty of Science
Ain Shams University

1974

DAR AL-MAAREF

Published by : DAR AL-MAAREF, 1119 Corniche, El Nil, Cairo.

PREFACE

This book presents the outline of modern cytology. The first part of this book deals with the cell and its constituents. The fine structure of its organs under the electron microscope is referred to in detail. Special care is given to the study of the chromosome as a carrier of genetic material from cell to cell and from one generation to the other. The structural and numerical aberrations of chromosoms are described in a whole part.

As this book was written for medical students an approach was made to discuss the recent findings which appeared since Levan and Hsu in 1959 were able to demonstrate the human chromosome with a simple but definite method. Since that year a new branch of cytology was borne and in a few years many findings were made, concerning hereditary diseases in man, and the role of chromosomes in producing them.

Finally the different types of cell division were briefly described which is sufficient for a student who studies the principles of cytology.

It is hoped that the contents of the book reflect more than just the author's interests, taking into consideration to convey to the reader the exciting and stimulating discoveries that have come to light in recent years.

A. Kabarity

CONTENTS

INTRODUCTION

A human being begins his existence when a spermatozoon fertilizes an egg cell. A fertilized human egg cell is just large enough to be visible to a naked eye. Its weight is estimated as about one twenty of an ounce (slightly more than one millionth of a gram). Starting from this insignificant piece of matter, the body grows until it attains the adult size and weight of say 75 to 80 kilogram. This is, then an approximately 50 billion-fold increase.

The source of this material for this growth is the food consumed first by the mother in whose body the embryo coming from the fertilized egg develops, and then by the growing individual himself. Now food is derived ultimately from the environment in which the organism lives. For green plants this is water and certain mineral salts taken in from the soil, oxygen and carbon dioxide from the atmosphere, and the energy of a part of the sunlight spectrum. All animals, and almost of the chlorophyll-less plants, require for food some of the organic substances found in the bodies of other animals or plants. All life is derived from the energy of sunlight bound by the chlorophyll of green plants.

When a crystal grows in a supersaturated solution, the growth occurs by addition of the substance from the solution to the surface of the crystal. The growth of living bodies is a different story. The food undergoes a series of chemical changes before it is assimilated that is becomes a component part of the organism. Thus animals break down the proteins from these constituents. Now it is surely a most significant fact that the body reproduces itself from the food which it consumes. In the process of assimilation and growth the food is transformed into a likeness of the assimilating body and of its ancestors. Indeed, the human body, whether an embryo or an adult, transforms food not merely into human flesh but into an individual who resembles his parents and relatives more or less closly. The outcome of growth and development depends very largely on the nature, the **heredity,** of the body and only secondarily on the food which it assimilates. The same kind of diet can maintain life not only of different men but also of other species of animals, for example, of dogs and cats kept as domestic animals. It is the heredity which causes the process of assimilation to result in self-reproduction.

The production of a likeness of the assimilating body and of its ancestors is most strikingly apparent when the organism grows or gives rise to a progeny. Owing to heredity, to self-reproduction, every form of life tends to transform the materials in the environment capable of serving as food into copies of itself. Self-reproduction continues in adult bodies which no longer grow in size or in weight, it continues as long as life itself. Every organism not only assimilates foods but also breaks down the assimilated products and reconstruct them again. This continuous build up and break down of living matter is the **metabolism.**

The cell theory : -

The now familiar idea that the cell is the basic unit of life is known as the **cell theory.** We should recognize that the cell theory is more a statement of fact than it is a theory in the usual sense i.e. it states that organisms are cellular in structure. Two german scientests in 1838 - 1839, M.I. Schleiden and T. Schwann, the former is a botanist and the latter is a zoologist, announced it. The cell theory represented a decisive consolidation and synthesis of biological thought that now ranks with Charles Darwin's **evolution theory** as one of the foundation stones of modern biology. Indeed we understand life itself only to the extent that we understand the structure and function of cells.

Schleiden and Schwann were not the first to believe in, or advance, the idea that plants and animals are made of cells and cell products. During the 18th century many workers in Europe described cells and discussed their significance, and by 1800 good microscopes were becoming available so that more refined observations were possible. There was in fact, by 1800 a rather general acceptance of the idea that organisms are cellular, but here was a much confusion over the definition of the cells, their mode of origin, and their significance in development.

Cytoplasmic structures in the cell and their function

We shall briefly describe some of the structures of the cell as seen by the light microscope and the electron microscope and their function.

A) The endoplasmic reticulum, Ribosomes and Lysosomes : -

The ground substance of almost all cells is traversed by a membrane-bound more or less continuous vesicular system, which has been named the endoplasmic reticulum (ER). The lipoprotein membranes of the system separate the material inside the cavities of the ER from the surrounding cytoplasmic matrix. The ER is associated with the nuclear enve-

lope, which consists of two membranes and a space. The outer of the two membranes of the nuclear envelope has been shown to be continuous with the membranes of the ER and the nuclear envelope is apparently a part of the ER. Elements of the ER extend to the cell surface. In plant cells, the ER-elements may even extend through the cell wall into neighboring cells.

The ER varies from cell to cell. In some cases the vesicles of the ER are few, whereas in other cases they are numerous. As a rule, the ER is not a prominent strncture in undifferentiated cells; but when the cell begins to differentiate the ER also develops. The morphological pattern of the system also tends to vary with the type of cell in which it occurs. The ER appears to be similar in cells with similar function.

The main types of ER has been distinguished. These are :

(*a*) The granular or rough form.

(*b*) The agranular or smooth form.

The smooth form is found in cells such as the mature leukocyte spermatocyte. The rough form which is easier to recognize is associated with a particulate component of the ground substance. The particles are attached to the outer surface of the ER-elements. Chemically, they consist mainly of ribonucleic acid (RNA) and protein are particularly found in protein producing cells. These particles, which are called **ribosomes,** are known to be the structures on which the cytoplasmic protein sythesis of the cell occurs. The ribosomes are not necessarily localized on the surface of the ER, but may also be freely distributed within the ground substance.

In cell division, the nuclear envelope and the other elements of the ER are broken down into fragments, some of which associate with the separating chromosome groups at anaphase. At telophase, when the daughter nuclei are formed, the fragments of the ER grow and fuse to form the new nuclear membrane. In plant cells some of the fragments which do not participate in the formation of the nuclear envelope migrate toward the interzonal region of the spindle, where they form a lattice of microtubules along the equator of this region. It is within this network that the cell plate first appears.

The function of the ER is not known for certain. We do know that its rough form is involved in protein synthesis, but this is probably a function of the ribosomes rather than of the membrane system. It has also been suggested that the ER functions in intracellular transport and that it carries enzymes and metabolites important in physisological events taking place within the localized regions where it is found. Finally, it may

have a function as a conductor of intracelullar impulses.

The **lysosomes** represent a class of cytoplasmic particles which have centrifugal properties between mitochondria and ribosomes. They are surrounded by a membrane and contain enzymes which are primarily hydrolytic in function. Among the lysosomal enzymes may be mentioned acid phosphatase, acid desoxyribonuclease (DNase) acid ribonuclease (RNase), cathepsin and d-glucosidase.

B) Golgi Apparatus : -

The treatment of animal cells by osmium or silver in the form of dark precipitate generally brings out a series of lobed bodies or a continuous network of strands. These are called golgi material. The golgi material is chemically lipoprotein in structure. Sjcstrand and Hanzen 1954 have shwon that the golgi metrial consisting of membranes, ground substances and granules which were demonstrated by electron microscope (Fig. 1j.

In plant cells it seems that there is no golgi material in this form. Some investigators started that the golgi material associate with the formation of vacuoles in plants.

Vitamin C is found associated in fairly high concentrations within the golgi apparatus in active embryonic cells. It has been proposed that this storage of vitamin C is possible as the golgi material provides in the cytoplasm a segregated area of high reducing capacity, which prevents the oxidation of the synthetic materials when the metabolism of the cells is reduced.

In electron micrographs, the golgi apparatus appears as a stack of membrane bound cisternae, the membranes being of the same general thickness as those of the endoplasmic reticulum (Fig. 1 b). In plant cells, the golgi apparatus produces membrane-bound vesicles which secrete their content though the plasma membrane of the cell.

C) Mitochondria : -

Mitochondria occur in the cytoplasm of nearly all aerobic plant and animal cells. They may be either rod-shaped or spherical and they may change from one shape to another. The diameter of spherical mitochondria varies between 0.5 and 1 U and the long diameter of rod-shaped mitochondria may be greater than 10 U. The number of mitochondria is different in different types of cells, in the rat liver cell there are 1000 to 2500 mitochondria. For identifying mitochondria in the living cells, staining with Janus Green B or with tetrazolium is the easiest and most commenly used method. Chemical analysis of mitochondria have shown that lipids and protein are the main constituents of dry mitochondria. The

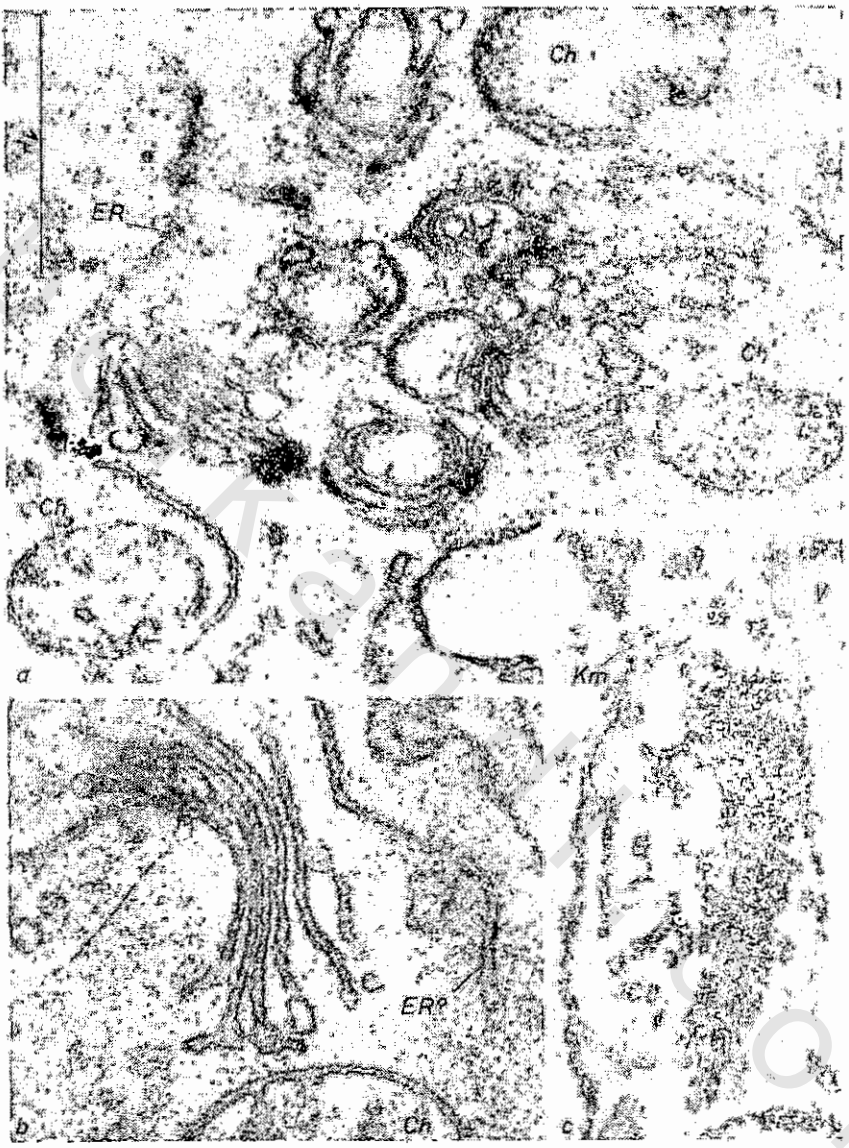

Fig. 1 — Golgi apparatus in plant cells a-Meristematic cells of Allium cepa root tips. Ch = Mitochondria ER = endoplasmic reticulum. b-Golgi materials in Zea Mays root c-Golgi app. in chlamydomonas reinherditi V—Vacoule Km nuclear membrane (Sager u. Palade 1957).

lipid content amounts to about 30 per cent of the dry weight and the protein content to 65 to 70 per cent. The lipids appear to be mostly of the phosphatide type. A small amount of RNA is generally found in mitochondria, but it is possibly derived from ribosomes contaminating the mitochondrial fraction. Good evidence has recently been obtained for DNA's being a mitochondrial component. The structure of mitochondria has been revealed in great detail by electron microscopic studies (Fig. 2). The electron micrographs show that the mitochondrion is surrounded by two membranes, each of which is about 60 A thick and separated from the other by a clear space. The inner membrane is folded to form the so-called **cristae,** which may be described as sac like extensions from the inner membrane into the interior of the mitochondrion. The membranes are believed to be built on the same principles as the plasma membrane. They contain lipids and proteins, but there is some disagreement about the molecular structure of the membranes. According to one theory, the membranes consist of double layers of lipid molecules sandwiched between protein molecules according to another, the membranes are networks of alternating protein and lipid units linked by hydrophobic bonds.

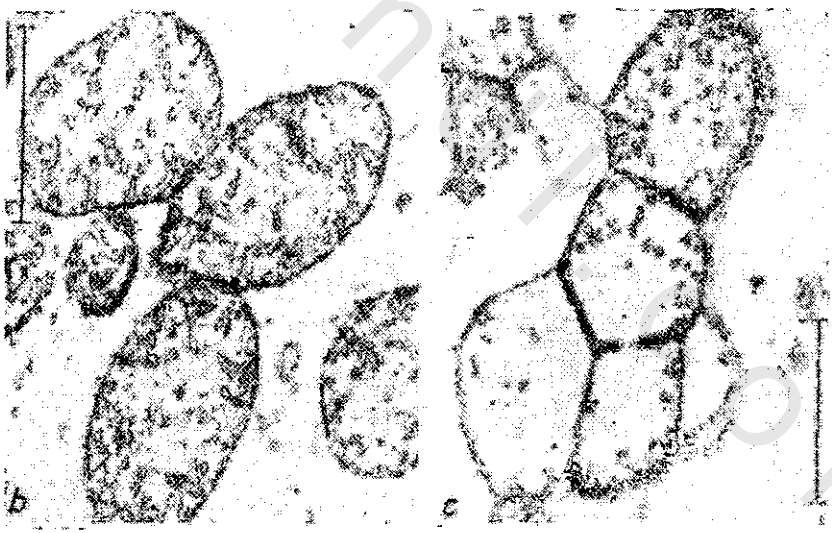

Fig. 2 — Mitochondria in plant cells (Elodea canadensis).

Function of Mitochondria :

It has been known for some time that the mitochondria function as centers for the release of chemical energy by aerobic oxidation and the

conversion of this energy into the energy rich phosphate bonds of adenosine tri-phosphate (ATP). This coupling of oxidation to syntheses of ATP has been called oxidative phosphorylation, and is the universal function of all mitochondria. In the energy rich bond of ATP, the energy released by oxidation of foodstuff is stored in a form that can be utilized by the cell. Most of the energy required by the cell is provided in this way, and since most of the ATP is produced in mitochondria, they have been called "the powerhouse of the cell".

D) **The plasma membrane : -**

The plasma membrane is more difficult to demonstrate than the membranes of the endoplasmic reticulum or the membrane surrounding the cell nucleus and the mitochondria; however, by the use of particular fixation procedures, considerable information has been obtained about the structure of the plasma membrane. By using fixation procedures which caused the plasma membrane to swell, it was found that the plasma membrane of maize root cells appeared as two thin dark lines separated by a light region. The thickness of the plasma membrane in both plant and animal cells has been estimated to be about 75 A, each of the dark lines being about 20A thick.

The fundamental permeability studies indicated that the ability of a molecule to penetrate the plasma membrane was dependent on its solubility in lipids in such a way that the greater the solubility of the molecule, the greater also its ability to penetrate into the living cell. On the basis of these results, it was concluded that the hypothetical cell or plasma membrane was impregnated with fat like substances i.e. with lipids. In support of this hypothesis, chemical analyses of red blood cell membranes have indicated the presence of lipids and proteins.

The molecular structure of the plasma membrane is not known. According to a hypothesis by Danielli (1954), the membrane consists essentially of two layers of lipid molecules arranged with their hydrophilic or polar groups extending outwardly toward the surfaces of the structure. The double layer of lipid molecules would be sandwiched between two layers of protein molecules. The protein consist of polypeptide chains, or meshworks of such chains, which are believed to be oriented in such a way that the hydrocarbon positions of the amino acid residues are dissolved in the lipid layer and the hydrophilic groups extend into the aqueous phase. The permeability properites of the membrane require that in some areas a polar structure extend right through the membrane.

It is possible to distinguish between two different types of movements

of substances through the membrane (1) movement in response to a concentration gradient (diffusion) and (2) active transport. When the first type of movement is discussed it is, both from a theoretical and a practical point of view, convenient to distinguish between the premeability to :

(*a*) non-electrolytes

(*b*) weak acids and bases

(*c*) strong electrolytes

Each type of these molecules has its own diffusion rate and the discussion of this problem is out the aim of this book.

The movement of molecules through the cell membrane is more increased by temperature than in free diffusion. A 10 degree rise of temperature may result in a several fold increase of the rate of penetration.

Active transport does not apply to very large molecules such as DNA molecules which may be taken up by a mechanism called **cytosis.** The process may vary in structural appearance and dimensions, but its essential feature appears to be that "a certain area of the surface membrane of the cell encloses a surrounding medium, separates from the surface and migrates into the cell.

Of particular interest from a genetic point of view is the observation that mammalian cells in tissue culture are able to take up whole desoxyribonucleic acid (DNA) molecules from the intercellular environment by cytosis, because it indicates that the phenomenon of DNA-mediated genetic transformation, now well established in bacteria, may also be possible in mammalian cells. During the last few years conclusive evidence has been presented for the incorporatin of extracellular high molecular weight DNA into the nuclear DNA of mouse and human cells in tissue culture. That this incorporation may indeed result in stransformation of genetic charateristics has been demonstrated by many authors. They treated a human cell line which lacked the enzyme inosinic acid pyrophosphorylase (IMP Pase) with DNA isolated from IMP Pase positive cells. The treatment resulted in the appearance of IMP Pase positive, genetically transformed cells. The transforming activity was abolished by desoxyribonuclease, but not by ribonuclease. DNA isolated from IMP pase-negative cells or from cells of other mammals had no transforming-activity.

STRUCTURES OF THE INTERPHASE NUCLEUS

1. The nuclear envelope : -

The first detailed description of the sturcutre of the nuclear envelope are those of Callan and Tomlin (1950). These authors succeeded in isolating nuclear membranes from amphibian oocyte nuclei. The isolated membranes were then studied both chemically and with the aid of the electron microscope. Callan and Tomlin found that the nuclear envelope consisted of a double membrane. The outer membrane contained pores with a diameter of 300 A, the inner was continuous and lacked visible structure. The elasticity and considerable strength of the nuclear envelope depend on the inner membrane.

Since the paper of Callan and Tomlin, appeared, a great number of electron microscopic studies on the structure of the nuclear envelope have been published. Nuclear membranes have been studied from different types of organisms. The envelops have proved to be of the same structure i.e. they are double and contain pores, the size of which are 200 to 400 A. In amoeba it is the inner membrane which contains pores, more recent electron micrography show both membranes to be penetrated by the pores. It is now clear that the nuclear envelope is continuous with and a component of the endoplasmic reticulum. The membranes of the nuclear envelope are distinguished from those of the reticulum only by the pores. The membrane system in interphase cells extends from the nucleus to the cell surface and sometimes even into the cytoplasm of adjacent cells. During the cell division the system is fragmented. In telophase the fragments grow and fuse to form the envelope surrounding the daughter nuclei. The pores probably represent structures through which exchanges of substances may take place. However, they are not simple openings but contain a diffuse material of different nature and density than that of the cytoplasm and nucleoplasm.

Callan and Tomlin (1950) also studied the chemical composition of nuclear envelope. They found that the cell nucleus is surrounded by a lipid layer has been demonstrated by many authors. The available information about the permeability properties of the nuclear membrane is contradictory. Some authors found that the nuclear membrane is semipermeable. it penetrates water, simple electrolytes, various sugars and

nucleic acid. The membrane is impermeable towards high molecular compounds such as egg albumen, glycogen and synthetic soluble celluloses.

Another authors conclude that the nuclear envelope is permeable to all kinds of molecules and does not function as a permeability barrier. However most of the studies supporting this conclusion have been performed with isolated nuclei. Since the nuclear envelope is a part of the ER, the membrane structure in the isolated nuclei is likely to have been damaged. Therefore, these results are of doubtful value. The findings by recent authors that the nuclear envelope is permeable at least to DNA. However it is conceivable that molecules which cannot penetrate the nuclear membrane are transported into the nucleus by means of an energy requiring mechanism.

2. **The nuclear sap** :-

The amount of nuclear sap varies considerably. In the compact nuclei of sperm cells there is hardly any nuclear sap, whereas in the oocyte neucleus the nuclear sap constitutes the main part of the nuclear mass. The nuclear sap of the oocyte nucleus is in the nuclei of somatic cells concentrated in the nucleoli. Chemically the nuclear sap contains two colloid phases which can be distinguished from one another, one of these phases is a disperse fluid, and the other is a structural colloid with mechanical rigidity. The analyses further indicated that the nuclear sap contains chiefly proteins and amino acids. According to recent research, the greater portion of the acid soluble nuclear proteins occur in the nuclear sap (Busch et al 1963). In the studies of Brown et al, nucleic acid and their hydrolytic products were not found in the nuclear sap. However, it was found recently that the nucleoplasm of the new oocyte contains as much DNA as the chromosomes. Part of the nucleoplasmic DNA is located in the numerous small nucleoli, which in this material are not attached to the chromosomes.

Evidence indicating the presence of ribosomes in the nuclear sap has also been presented.

3. **The nucleolus** :-

Nucleoli occur in almost all cell nuclei. They are usually spherical but may also be rod shaped or irregular. The nucleoli are formed during telophase at the so called nucleolar organizer region in the nucleolar chromosomes. The number of nucleoli are to begin with equal to the number of nucleolar chromosomes, of which there is one for each haploid set of chromosomes in most plant species. However, the nucleoli have a

tendency to fuse so that in most meristematic interphase cells of diploid plants there is only one nucleolus.

The density of the nucleoli is usually higher than that of the rest of the nucleus.

The existence of two or more phases in nucleoli has also been demonstrated by electron microscopic studies. Thus early electron micrographs of animal cells indicated the presence of two phases, one the nucleoloplasm, was structureless, whereas the other consisted of a fibrous highly spiralized material called **nuclealonema.** However, although recent electron micrographic studies confirm the presence of different structures within nucleoli, they do not support the nucleolonema nucleoloplasm concept.

The most detailed light and electron microscopic studies of plant cells have been made in **Allium cepa** and **Vicia faba.** Under the light microscope the preprophase nucleolus of **Vicia faba** consists of a densly stained material in which vacuole-like structures are embedded.

The nucleoli contain ribonucleic acid RNA. The absoption in ultra violet light indicated the presence of nucleic acid, and since the nucleoli were Feulgen negative. At least a part of the RNA in nucleoli appears to be combined with proteins. Chemical and electron microscopic analyses have shown that their chemical composition and structure is very similar to cytoplasmic ribosomes.

The results of the recent studies suggest that the nucleolus contains a DNA fraction, which is Feulgen negative and which is not easily detected by radioautographic technique. The possibility that this DNA was derived from accompanying chromosomal material was excluded. The relationship between DNA & RNA content of the nucleclus was found to be 2 : 1. It was suggested that the function of the DNA fraction in the nucleolus is to act as a primer in nucleolar RNA sythesis.

THE CHROMOSOMES

If the unit of genetics is the gene, then the unit of cytogentic is the chromosome, for the major contribution of parent to offspring is a set of chromosomes. In those organisms having an organized nucleus, the chromosome is a complex organelle, the structural details of which are still only partly known despite nearly a century of observation and experiment.

Chromosome shape : -

It is usual to ccmpare the shape of chromosomes by their morphology as seen in somatic metaphase or anaphase. In these two stages the chromosomes have reached their maximum contraction, attaining a length that under normal environmental conditions remains constant and use has been made of them in identifying the various chromosomes within and between species (Fig. 3).

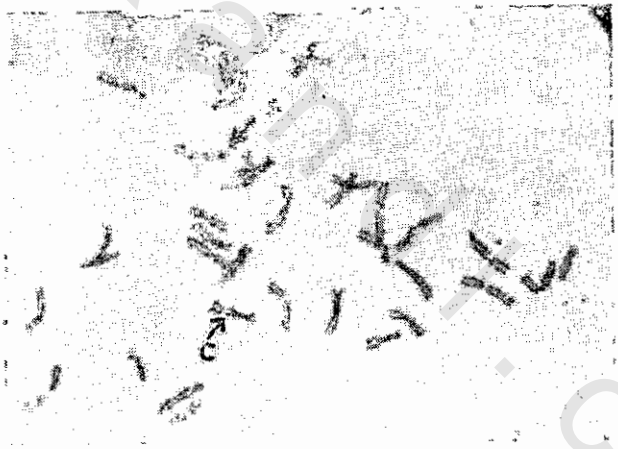

Fig 3 — Chromosome set of Triticum asturien (hexaploid wheat) S = satellite chromosome c= centromere (Kabarity 1966)

The shape of a chromosome is largely determined by the type & position of its centromere. The centromere is generally not visible as a definable entity (structure), but its structure creates a constriction in the chromosome. This constrictions may be located at a terminal, subterminal, or median position causing the chromosome to assume respectively, a rod,

J or V shape. Ring chromosomes are known in Drosophila & Maize. Very small chromosomes, of the order of 1-2 U or less in length, may appear elongated in somatic metaphases, with distinct centric constrictions, but in meiotic cells as spherical dots. It can be assumed that the contraction of such chromosomes has been so great as to cause the centromere to disappear in the body of the chromosomes thus obscuring details that would be visible in larger ones.

The localized centromere produces the primary constriction. In addition, secondary constrictions in certain chromosomes provide characteristic structure. When occuring in the distal position of an arm, they cut off a portion of the end which remains attached to the main body by a fine thread of chromatin. These terminal bodies have been termed **trabants** or **satellites** & such chromosomes bearing them are often referred to as S A T - Chromosomes. (Fig.3)

Chromosome size :

The size of chromosomes, as determined from metaphase configurations, is a relatively constant feature within a species, although some size variation may be detected in the chromosomes of different tissues even within a single organism. The diameter & length of a chromosome vary inversely, the chromosome becoming thicker the more it is contracted.

One might suspect that the length of a chromosome is principally, a function of the number of genes it contains. The genetic evidence from D. melanogaster indicates the number of genes in each of the 3 major chromosomes, the X, II & III, is rougly proportional to length. On the other hand the Y chromosome, which in somatic cells is larger than the X, is practically devoid of genes in the usual sense. It is known that the Y chromosome is almost totally made up of a kind of chromatin (heterochromatin) that generally lacks genes, so that size does not provide a realy guide to genetic content.

Variations in size of chromosomes within a species can be readily induced by a number of environmental agents. Cell dividing at low temperatures have in general shorter, more compact chromosomes than at higher temperatures. Colchicine, an alkaloid drug which interferes with spindle formation & cell division, also tends to shorten the chromosomes.

The size of chromosomes varies within wide limits. The Fungi, in general possess minute mitotic chromosomes, the entire nucleus in the mycelial strands being seen only with difficulty. Some Ascomycetes such

as Neurospora the meiotic chromosomes are of sufficient length to be usable in cytogenetic studies. (Mc-Clintock 1945, Singleton 1953).

Among the higher plants, the monocots generally possess larger chromosomes than the dicots. Trillium has somatic metaphase chromosome as long as 30 U., while these of Lilium & Allium & as the whole group of spring flowering bulbs range from 10 - 20 U in length.

In the animal kingdom, only the Orthoptera & the Amphibians are characterised by their large chromosomes. Those of human are about 4 - 6 U in length. The mitotic chromosomes of the 2 most thoroughly studied organisms, Drosophila & Maize average 3.5-8-10 U respectively at metaphase.

Chromosome number :

The number of chromosomes in the somatic cells of a higher organism can be expressed as the diploid or somatic number (2n), while those in the reduced egg or sperm are the gametic or haploid number (n). Any indiv. which possesses in its regular cells a number of chromosomes higher than the diploid number is c. a **polyploid.** Polyploids are of many different type & they are giving names corresponding the number of the repetition of basic number. Triploid (3 x) tetraploid (4 x) etc. The basic number is the number of chromosomes found in the gametes of a diploid ancestor of a polyploid & represent with X.e.g.

In the genus Triticum x = 7. There are diploid species (Tr. monocoecum 2 n = 14 thus n = 7. Tr. durum. is tetraploid 4 x = 28 (2 n). Tr. vulagre hexaploid 2 n = 42 6 x = (28) & x = 7 only by diploid sp. that x & n are equal in this case. By Tr. nonoccum x = 7, n = 7.

Chromosome number varies widely in both plants & animals, being to over several hundred in other organisms (**Equisetum** 108 **Ophioglossum** 256). Belar (1926) has recovered that **Aulcantha.** has as a diploid number approximately 1600 chromosomes. **Ascaris** has a single pair of chromosomes in the cell of the germ line, but, since in the diploid soma the 2 chromes fragment into numerous small chromosomes. The single haploid chromosome has to be considered as **aggregate** chromosome which for reasons unknown maintains its unity under the conditions imposed by the cells of the germ line.

The study of chromosomal organization has been conducted on plants

& animals possessing a few large chromosomes. **Trillium** n = 5, **Tradescantia** n = 6, **Allium** n = 8, Various members of Diptera possess giant salivary gland chromosomes (**Drosophila**).

Structure of the chromosome :

The chromosome appears as an elongiated body consisting of 2 filaments called **chromatides** which are spirally coiled. The chromatids which are seen in the mitotic prometaphase stage are still further subdivided into 2 **chromonemata.**

These are surrounded by a filling substance called **matrix** or sheath. The external part which is a limiting membrane of this matrix is c. **pellicle.** Along the length of the chromonemata are distributed granules which are densly stained and are known as **chromomeres.** They are clearly observed in the prophase especially that of meiosis & appears as accumilation of the chromatic substances. (Fig. 4)

At the point where the arms of the chromosomes are joined there lies the primary constriction. In the middle of this constriction is found a clear & achromatic region which is known as **centromere** & has a functional relation to the movement of the chromosome during nuclear division. The structure of the centromere is difficult to demonstrate & various interpretations have been made. In the somatic chromosomes it appears simply as a non-staining constriction with no morphological evidence in structures. (Fig. 5)

(*a*) The centromere may be a modified portion of the chromonemata.

(*b*) The centromere may be a spherule connected to the chromonomata.

(*c*) A number of oriented micelles, these micells may be arranged in such a way as to facilitate misdivision in a transverse instead of a longitidinal plane.

At certain stages or under special conditions a chromosome may have other differentiated regions.

Some chromosomes during division show constrictions similar to that indicating the position of the centromere. These regions are known as secondary constrictions and are frequently associated with nucleolus formation, though whether as sites of synthesis or accumulation is not altogether clear, but the latter seems likely. Not all nucleolus forming regions are marked by an obvious constriction are nucleolus-forming regions (Kabarity 1967). In many cases secondary constrictions are constant, readily observable markers, but in some cases they may be so short

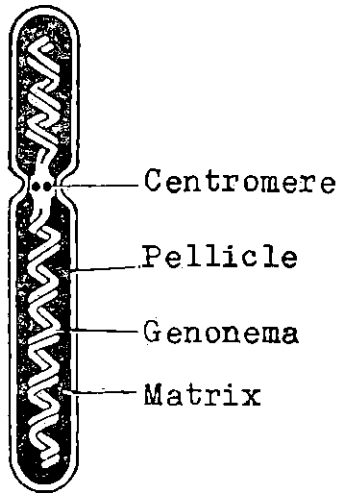

Fig. 4 — Diagram of the morphology of an anaphase chromosome in mitosis with the minimal number of strands indicated.

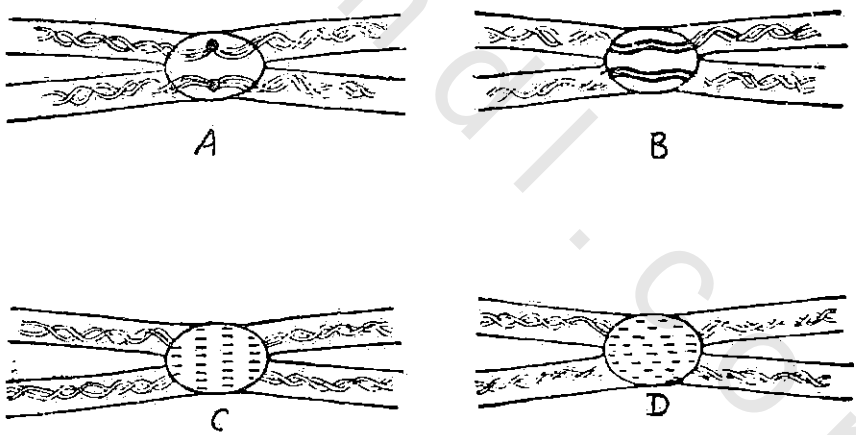

Fig. 5 — Hypothetical structure of the centromere in a chromosome with two pairs of chromonemata. The centromere is pictured as an ellipsoidal structure with the essential organ of movement in the form of A, a regionally modified portion of the chromonemata, B, a spindle spherule connected to the chromonemata C, a number of oriented micelles and D. a number of oriented micelles arranged in such a way as to facilitate misdivision in a transverse instead of a longitudinal plate (Schrader 1953).

as to be detectable only with difficulty or under special conditions. For example the long M chromosome of **Vicia faba** (broad been) has an obvious secondary constriction in one arm. Two of the short chromosomes also have more median secondary constrictions that are visible occasionally and then only when the chromosomes are over-contracted and well spread. Similary the chromosome set of Human being may appear with satellites upon the acrocentric chromosomes of group D & G. In most preparations of human chromosomes, the satellite structures are lacking.

The genetic importance of the chromosome lies in the chromonemata since they are the physiologically active parts. It is supposed that genes are located in them.

NORMAL HUMAN CHROMOSOMES

Attempts at electron microscopic study of human chromosomes run into great difficulties. The first results obtained, improved the resolving power as compared with the ordinary microscope, however with the development of new techniques, it may in future be possible to use the electron microscope. The detection of the fine structure of the chromatids is for the fine structure of the chromatids is for the time being at the limits of posibility. The previous studies about the chromosomes permit us to conclude that the number and morphology of the various elements of the karyotype of normal individuals are constant. Therefore it is essential to use an international classification to permit exchange of information.

A group of researchers meeting in Denever 1960 and in Chicago 1966 worked out a numerical classification used at the present time and representing a reference code. This is based on two principles :

1. The chromosomes are classed in order of diminishing size, The largest one bearing the number 1 and the smallest the number 22, recognition of each of them is based on the relative length of the element on the one hand and the position of the centromere on the other hand.

2. Within each group the chromosomes are arranged in descending order of size. The two sex chromosomes X & Y keep their classical lettering to avoid confusion. The Denever document defines seven groups which we shall briefly mention. The interest of the groups, numbered from the first and the last number of the sequence (e.g. group 1-3 for large chromosomes lies in the fact that any normal chromosomes may be formally assigned to a given group. On the other hand, even within a given group, difficulties of identification may arise which are made even worse if the quality of the preparation studied is not optimum. (Fig. 6)

(*a*) group 1-3 large chromosomes with approximately median centromeres. The 3 chromosomes are readily distinguished from each other by their size and centromere position.

(*b*) group 4-5 large chromosomes with distal centromeres. These two chromosomes are difficult to distinguish, but chromosome 4 is slightly longer.

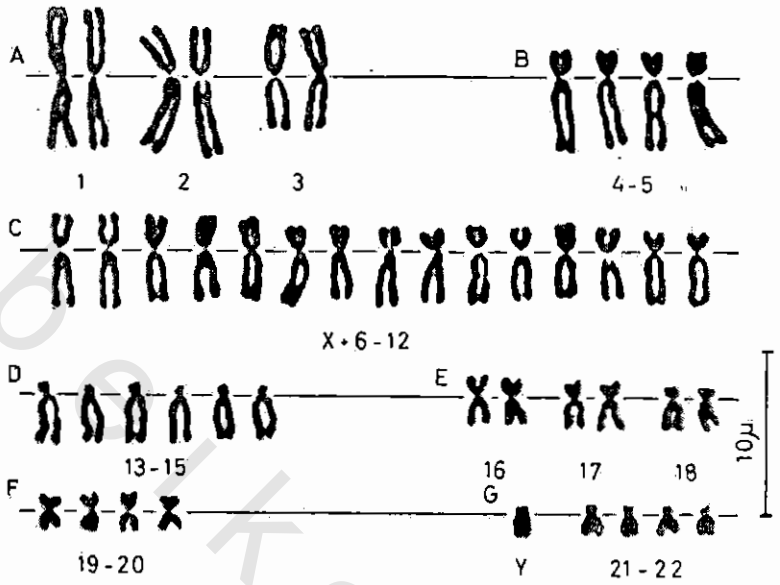

Fig. 6 — The 46 chromosomes of a normal male arranged according to the Denever-London classification System (original).

(*c*) group 6 - 12 medium sized chromosomes with submedian centromeres. The X chromosome resembles the longer chromosomes in the group especially chromosome 6 from which it is difficult to distinguish it. This important group is the one which presents major difficulty in the identification of individual chromosomes.

(*d*) group 13 - 15 medium-sized chromosomes with nearly terminal centromeres (acrocentric chromosomes). Chromosome 13 has prominent satellites on the short arms. Chromosome 14 has small satellites on the short arms. No satellites have been detected on the chrom. 15 or almost none.

(*e*) group (16 - 18) rather short chromosomes with approximately median centromeres (in chromosomes 16) or sub-median ones.

(*f*) group (19 - 20) - short chromosomes with approximately median centromeres.

(*g*) group (21 - 22) very short chromosomes, acrocentric. Chromosome 21 has satellites on its short arms. The Y chromosome is similar to these chromosomes.

This analysis of the human karyotype is based on the fact that one may define a chromosome by two parameters, its length and the position of the centromere. These parameters related to the physical structure of the chromosomes are theoretically fixed but show an important variance arising from 2 main causes : the technique of preparation and the physiological state of the cells.

Since each chromosome pair consists of a chromosome coming from the father and a chromosome coming from the mother therefore the two homologous chromosomes should show a greater resemblence between themselves than 2 nonhomologous chromosomes. It is thus possible to assemble two by two the 46 element of the karyotype to form 23 pairs (in the female) each of which will be composed of two elements the length and position of the centromere of which will be as similar as possible. These pairs will be arranged in groups and distributed according to their length within the groups.

This simple method gives very faithful results and it is quite remarkable to note the highly significant results in the hands of very different workers using different techniques on tissues of the most diverse origin.

The second stage is to describe by one number the length of each chromosome and by a second, the position of its centromere. The accumulation of many karyotypes can then be used to calculate a mean value for these two parameters and to say for example, chromosome 1 has such a length and its centromere is found is such a position.

After cutting up the photograph into individual chromosomes they are systematically arranged in the 7 groups and then the homologous chromosomes are paired within the group. It may be possible to use a computer coupled with television camera to analyse the mitotic set automamatically. However analysis of the karyotype described below is likely to remain the method of choice for quite a long time to come.

(*a* - *b*) **The 10 large chromosomes :**

Among these it is easy to recongize chromosome 1 the largest with median centromere, chromosome 2 a little shorter than 1 and with a more distal centromere and chromosome 3 considerably shorter than 1 but with a similar median centromere.

Pairing off hamologous chromosomes for these 3 types raises no difficulty. The remaining 4 chromosomes can then be paired two by two, using the following criterion : pair 4 is a little longer and has a more distal centromere than pair 5.

(*c*) The 15 or 16 medium sized chromosomes

The classification of these chromosomes would appear to be a very long and uncertain procedure. Select the six medium sized chromosomes with a distal centromere, that is, situated at least one third along the length of the element. In excellent cells such selection presents no difficulty.

These 6 chromosomes are then paired two by two according to their similarity (size & position of centromere), the largest of the 3 pairs is numbered 8, the second 10 & the third 12.

Then the remaining nine or ten (according to whether it is a male or female) are paired two by two, the determination of the X chromosome being made following the rule that it is the longest in the group (although its size may be very close to 6) and has a centromere a little more median than that of 6. In the male diagnosis by exclusion (X is the unpaired chromosomes) will confirm this choice. Finally the remaining 4 pairs are best arranged in order of diminishing size, giving them numbers. 6 7, 8, 9 and 11.

(*d*) Group 13-15 :

These six acrocentric medium sized chromosomes are best paired two by two and numbered according to the following rule. The pair carrying the larger satellites is 13, that with smaller satellites 14 and the third, without or with very small satellites is 15.

(*e*) Group 16 - 18 :

Easily separated from the proceeding groups these 6 chromosomes are paired two by two, chromosomes 16 being the largest with a paramedian centromere. In pairing off 17 and 18 it is necessary to remember that 18 is smaller than 17 and has a more distal centromere.

(*f*) Group 19 - 20

These small chromosomes in the form of crosses are best paired two by two, the largest pair being numbered 19. There is no formal criterion for differentiating between these pairs.

(*g*) group 21 - 22 - Y

Among the 5 small V shaped acrocentric one should first distinguish (for a male) the Y chromosome. In over half of very good cells the strict parallelism of the two chromatids, without constriction at the level of the centromere permits visual identification. This characteristic aspect very probably deriving from early separation of the centromere of the Y chro-

mosome is however not constant and the Y chrom. then appears identical with the 22 although a little longer.

To our knowledge, the normal Y chromosome is never satellited.

In the female, only the 4 remaining acrocentrics are separated according to the already outlined criteria-the 21 is smaller, has short almost undetectable arms and is satellited, while the 22 is longer, has short distinct arms and no (or few) satellites.

CHROMOSOME ABERRATIONS

We have noted that the karytypes of different species are often characterstic to provide the cytologist with morphological criteriá that can be used for purposes of identification much in the man that the taxonmist may employ the floral structure of plants as guide to systematic relationships.

The constancy of the karyotype and its usefulness in comparative studies originates from the fact that at a given stage of cell division and in a given tissue, each cell of an organism has a constant number of chromosome of definite length, volume and shape. The chromosomal constants are determined by a fixed number of genes per chromosome arranged in a definite serial or with a centromere occupying a fixed position. The constancy of the karyotype from one cell to another and from one generation to the next, lies in its capacity to duplicate itself exactly at each cell division.

Nevertheless, changes may occur in the chromosomes about by accidents which disturb their regularity and produce disarrangements in the structure of their parts.

Although such changes are sometimes found in nature most of the knowledge of these changes has been produced by means of experimental methods which increase their frequency.

In organisms subjected to the action of x-rays, and the influence of chemical substances or to rapid changes in temperature the frequency of alternation is increased. Thus providing available means for the analysis of the genetic and structural organization of the chromosome in relation to the mechanism of mutation and the evolution of the living organisms.

The changes in the chromosomes can be summarized in the following classification :

(*a*) Sub microscopic changes at the molecular level - **genic mutations.**

(*b*) changes in the number. or visible structure of the chromosome. **chromosomal mutations.**

1. **Numerical changes** :
   (*a*) increase by one or more complete complements (**polyploidy**)
   (*b*) loss of one of the complements of the diploid (**haploidy**).
   (*c*) increase in one or more chromosome (**Aneuploidy**)
   (*d*) loss of one or more chromosome (**aneuploidy**).

2. **Structural changes** :
   (*a*) Intrachromosomal rearrangement (**inversion**)
   (*b*) Inter chromosomal rearrangement (**translocation**)
   (*c*) Loss of segment of a chromosome (**deletion**)
   (*d*) Increase of chromosomal segments (**duplication**).

1. NUMERICAL CHANGES (ABERRATIONS)

A) **Polyploidy** :

Polypolidy are of general occurance in nature and many of cultivated crops such as wheat and cotton are at their best form in the polyploid condition. Polyploidy seems to be lethal in man. Many authors have found triploidy in man but only among spontaneous abortions.

Polyploids originate in more than one way :

1. Doubling the chromosomes in somatic tissues, it is called the **Somatic doubling.** This method has been applied artificially with success in the production of many tetraploid species from diploid species. If a plant is subjected to cold treatment or certain chemicals as colchicine, phenols etc. which inhibit the formation of spindle fibres resulting in a doubling of the chromosome number, thus the resulting plant will be tetraploid and every chromosome will be represented 4 times.

AB	CD	EF		AB	CD	EF
AB	CD	EF	→	AB	CD	EF
				AB	CD	EF
				AB	CD	EF

2. The second method is by **doubling in gametogensis** :

It happens sometimes that during the process of meiosis failure of the separation of the bivalents takes place. Thus a non-reduced ovule or pollen grains will be produced. This would be a diploid sexual cell and if fertilisation takes place between a normal haploid sexual cell and this abnormal diploid cell, the resulting seed will give a triploid individual. Similarly if by change 2 such unreduced gametes one male and one female fertilise each other the resulting seed will give us a tetraploid plant.

B) **Haploidy**

It sometimes happens that an egg with the haploid number of chromosomes, develops without fertilisation and produces an individual with a single set of chromosomes. This happens normally in the reproduction of some animals (such as bees) in which the unfertilised eggs develop into haploid males. In some animals and plants the egg may be artificially induced to develop parthenogenetically. In lower plants one normal part of the life cycle (the gametophyte) is regularly haploid, while in higher plants this haploid generation is reduced to few cells (in pollen tube and embryosac) which are unable to lead an independent existence. In higher

plants haploids can be produced in a variety of ways. An unfertilised egg. can be stimulated to develop by pollen which takes no further part in the developmental processes. Haploids may be induced by cold, radiation, chemicals or other external changes. Haploids have been found in several species for example Datura, Nicotiana wheat, Maize and tomato. In each case the haploid plant showed close resemblance to the diploid parent type except for smaller size and its sterility.

In meiosis they have very irregular division because of the absence of homologous chromosomes which could pair together.

In these individuals there are only univalents which give rise to gametes with a varying number of chromosomes. No zygotene pairing takes place.

Haploids are of intrerest in that they cannot be heterozygous. The chromosome number can be doubled by colchicine, or they may produce seeds by self fertilisation. Such offspring would be completely homozygous for all genes and would achieve in one step a condition that would require many generations to approach through close inbreeding.

C) **Aneuploidy** :

In general, the two homologous chromosomes regularly separate during meiosis in a normal diploid cell to give a haploid set of chromosomes in each gamete or spores or to give, in mitosis two cells of similar chromosome sets. Exceptions in the form of accident occur, however to give products deficient or duplicated for a particular chromosome. Bridges (1916) recognized the phenomenon, and utilized it in his classical study of exceptional individuals in Drosophila. He referred to the process as one of **non-disjunction.** i.e. the two homologous chromosomes failed to disjoin, both passing into the same anaphase nucleus. It is now recognized that the occurrence of aneuploid gametes or spores results rather from a failure to pair initially and with a random passage to one or the other of the two poles, both chromosomes will pass into the same nucleus. therefore the 2 gametes one n—1 and the other n + 1 will, on union with a normal gamete, give individuals which are 2n+1 or 2n—1. such individuals are commenly referred to as trisomics and monosmomics respectively.

Trisomic types 2n+1 types occasionally are found among the offspring of diploid organisms, as described above. Trisomics have been studied most extensively in Datura, maize, tomato, Nicotiana, Drosophila and man. Since the discovery of the new method of analysing the chomosome set in man by Levan and Hsu (1959) as described before many triosomies

in human chromosome set were found. Till now the following trisomics types were discovered.

Trisomy 21, "Mongolism" Down Syndrome or G - Trisomy

The curious mental disease which was later be termed "mongolism" was noticed for the first time by Esquirol 1838. Langdon Down in 1866 described the disease in the following. "This is so marked that placed side by side it is difficult to believe that the specimens thus compared are not the children of the same parents". He described further their idiocy as mongolian idiocy and placed it after negroid and Malysian idiocy. The term Down's syndrome rejected by some (Spalding 1961), can hardly be supported since it would compound a double error, historical and aetiological; historical since Seguin (1848) was the first to describe the disorder, aetiological since the disease has absolutely no connection with the racial hypothesis of Langdon Down.

The typical faces of children and especially of very young children permits a very early clinical diagnosis. The clincical description of the disturbances both morphological from shortness to epicanthus which is a fold of skin obscuring the inner corner of the eye - and functional from hypotonia (low tension) to psychomotor retardation and eye bone and heart malformations, stubby hands and feet (Fig. 7)

The mental debility of subjects with trisomy 21 though often studied has so far revealed no symptomatic peculiarities apart from limitation of the faculties of abstraction which prohibits an I.Q. not higher than 70 and progressive deteriotration with age. The existence of subjects with trisomy 21 of normal intellegence as mentioned by certain authors (webster 1963) has never been demonstrated apart from cases of mosaicism (existence of normal cells and abnormal cells together).

One of the most characterstic morphological property is the anormaly of the epidermal structures of the fingers, the palms of the hands & the sales of the feet, and without going into a detailed description, it is possible to establish the diagnosis from the following 4 criteria : -

1. Transverse orientation of the papillary ridges of the distal part of the hand instead of the normal oblique orientation.
2. Presence of a loop with an ulnar opening on the hypothenar eminence.
3. Elevation of the axial triradius to a mediopalmar position.
4. Presence of only one single flexion creases of the hand resulting from

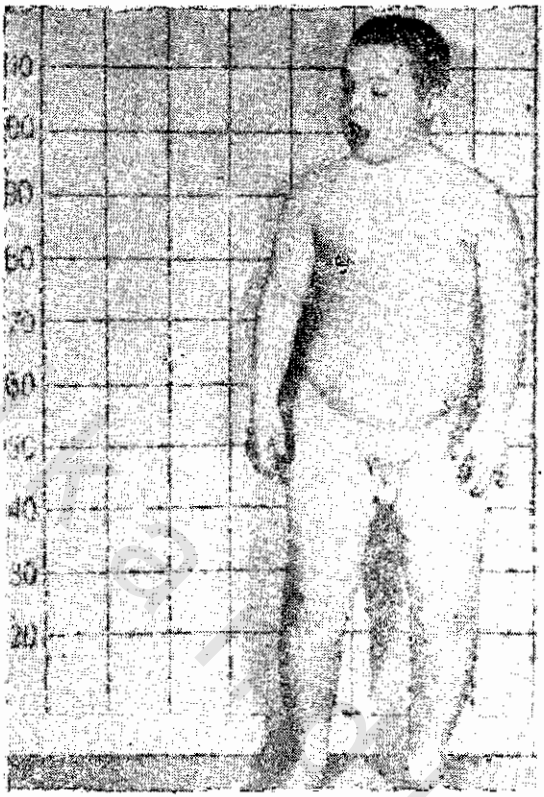

Fig. 7 — Down Syndrome or G - Trisomie (Mosby 1961)

coalescence of the two normal flexion creases popularly known as head line and heart line.

A statistical study shows that a print classification by these 4 categories permits diagnosis of trisomy 21 in 95% of the cases.

Frequency of G Trisomy

Trisomy G is very probably the most frequent hereditary disease. In different populations permitting a correct statistical study the frequency of the disease is established between 1 in 600 & 1 in 700. The frequency of G -Trisomy in coloured races has for a long time been considered as lower than in the white race. However, according to the findings of schull and Neel (1962) in Japan and Wagner (1962) in Honolulu the frequency of the disease is quite comparable with that of the populations of European origin.

Effect of maternal age.

As early as 1895 Shuttelworth observed that nearly haf the subjects affected belonged to large family of which they were the last born. In 1909 he demonstrated that mothers of affected children were much older at the birth of the abnormal children than mothers of normal children usually are. Tenkins 1933 & Penrose 1933 then showed that the age of the father does'nt seem to have any influence. A possible effect of the age of the father has been discussed in the exceptional cases of the transmission of a 21 - 21 translocation.

The graph drawn from the data of Penrose (1961) clearly brings out the advanced age of the mothers at the birth of G-Trisomie children from these cases and takirg into consideration the overall incidence of the disorder it is possible to establish the risk of appearance of a G-trisomy as a function of the age of the mother at the birth. The recent estimates of Cater etal. (1961) are in full agreement with other authors and it may be estimated that before the age of 30 the risk is of the order of 1 in 2000. After the age of 35 it rises to 4 in 1000 to reach a value of the order of 2 % after the age of 45. In a study of children born in London to mothers of over 45 years revealed the existence of 10 cases of G-Trisomy in the 543 births examined. Evaluation of risk as a function of maternal age permits obvious conclusions since about 1 G-Trisomy in three is born to a mother over 40 years old. As we shall see later, this effect of maternal age doesn't appear to be confined to G-Trisomy alone but is important in the two others Trisomies 18 and 13 and even extends to all so called congenital anomalies. Under certain experimental conditions it may be found in Mice (Bodmer 1961).

Chromosomal Determination

Systematic study of the chromosomes of children with this disease was undertaken for the first time by Mittwoch (1952) but the technical imperfections did not allow this author to reach any conclusion. The first patient observed in Paris 1958 revealed the existence of an extra chromosome (47 instead of 46). In Jan. 1959 study of two other cases provided evidence of the existence of the extrachromosome (**Lejeune et al**) which was confirmed in February 1959 in a total of 9 patients and the hypophesis of a trisomy was proposed.

Confirmation of these findings was rapidly supplied by C.E. Ford et al (1959) in a case of trisomy 21 with Kline-felter's Syndrome, by Jacobs et al (1959) in a case of trisomy 12, then by Book et al in 1959 in cases. (Fig. 8)

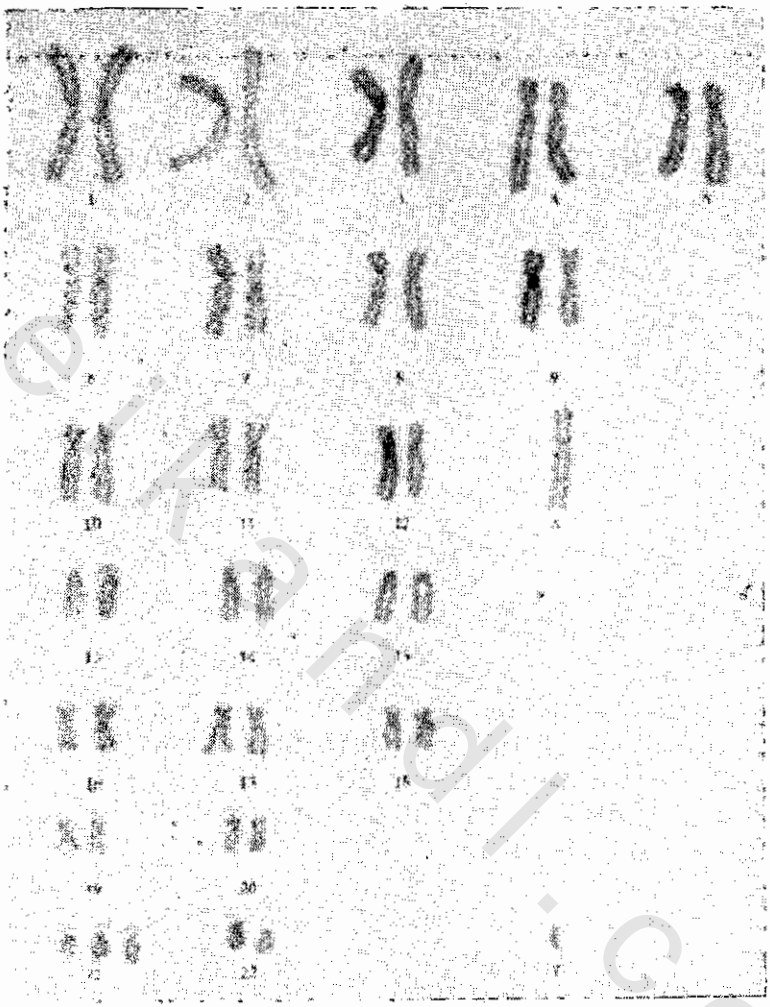

Fig. 8 — Karyotype with 47 chromosome of a boy with G - trisomy

At present the existence of a trisomy for a small acrocentric has been recognized in all cytological laboratories and in subjects of all races. Precise classification of the extra chromosomes can only be done morphologically in excellent preparations. The photographs, which show that in these abnormal children three chromosomes of type 21 and two of type 22 can be found.

Identification of chromosome 21 by its satellites attached to a very

small short arm has raised and still raises difficulties owing to the possible presence of satellites on one and exceptionally on the two 22 chromosomes. Some workers discussing this difficulty have suggested calling the disease trisomy 21 or 22. or G Trisomy.

We therefore propose that the term mongolism be definitely replaced by G-trisomy in order to avoid use of such unsuitable term.

The classical G-Trisomy Karyotype with 47 chromosomes is not the only possible expression of this disorder and many different karyotypes are known. For clarity we shall arrange them under 4 main headings :

1. Trisomies by translocation
2. partial trisomies.
3. mosaics
4. mosaics & Trisomies associated with normal cells.

(*a*) Trisomies by translocation :

Translocations mainly concern acrocentric chromosomes having undergone "centromeric fusion". As pointed out by Hamerton and Steinberg

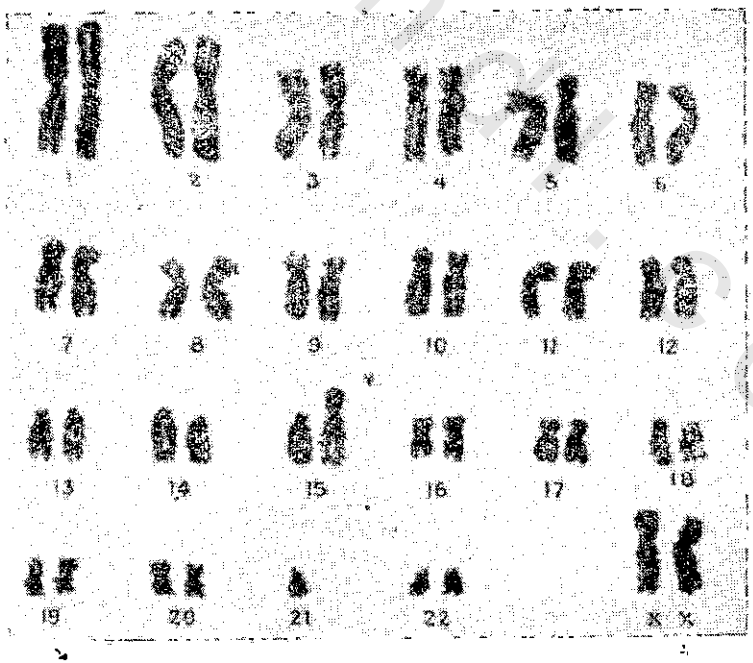

Fig. 9 — a Karyolype with G/D translocation)

(1962). Translocations of the type 21 / D produce a trisomy in one third of the children if the mother is the transmitter. On the other hand, in the cases of transmission by the father, trisomies appear exceptional (Fig. 9)

The same tendency is also found in cases of translocation between small acrocentrics of the type 21/G but the situation here is more difficult to define because of the difficulties of cytological diagnosis of the 3 possible types 21/21, 21/22 and 22/22.

Mosaics :

Mosaics are individuals who carry side by side two or even three different cell clones (sorts) overlapping to form a mixed population one with trisomic 21 cells, the other with normal diploid 21 cells.

It is difficult to draw precise conclusions from the published findings since it is doubtful whether a percentage (between normal and trisomy 21 cells) calculated from a biopsy is representative of the tissue examined. Moreover the distribution of the mosaic in different tissues may vary greatly and the relation between the relative incidence of triplo 21 cells & the malformations of tissue which contains them is actually impossible to establish quantitatively. We would simply note that individuals, those who show almost normal development are diplo 21/Triplo 21 mosaics with a clear predominance of diplo 21.

On the other hand the cases of very severe debility show in addition to diplo 21 and triplo 21 tetraple 21 cells or even pentaple 21.

However, it must be pointed out that clark et al (1963) reported that the relative frequency of the two populations diplo 21 and triplo 21 may vary in time. Within 3 years the child described by clark et al showed 17% triplo 21 against 32% in 1960.

(*c*) Associations :

The chromosome anomaly, trisomy 21 is sometimes associated with other anomalies. Trisomy by Translocation 21 ~ 21 or 21 ~ 13 has been mentioned previously and we shall examine the other associations essentially sex aneuploidies in addition to G trisomy.

(*g*) Trisomy and Klinefelter's XXX syndrome :

In 1959 Ford etal described the first case of association of chromosome aberrations is a subject presenting a typical Klinefelter's syndrome coupled with the classical trisomy 21 syndrome. The subject born to a 40-year

old mother had a karyotype of 48 with three 21, two X and one Y chromosomes. Monozygotic twins born to a 43-year old mother were identical boys both carrying 48 chromosomes with the formula 44 AA + 21 + XXY.

G-Trisomy and Triplo X :

A 2.5 year old child with a retino-blastoma showed in addition G trisomy and three XXX chromosomes (Karyotype 48 chromosomes). Here too the combination of the three characters G Trisomy, retinoblastoma and Triplo X was highly improbable.

G. Trisomy plus Trisomy 18 :

One case of double trisomy has been reported by Gagnon et al 1962 in a child born in the seventh month of pregnancy and who lived only 8 hr. The complex deformities seemed to be the sum of the specific signs of trisomy 21 on the one hand & of trisomy 18 on the other.

4. **Partial trisomies and allied Syndromes**

Some patients presenting a clinical syndrome similar to that of G. trisomy but incomplete have been studied as an example for this type we shall compare the two case of Zellweger (1962), two trisomic 21 Girls with a slightly a typical clinical picture with a patient of Hall (1962) with 46 normal chromosomes. At first described as a trisomy 21 with normal chromosomes. This patient was diagnosed as suffering from a clinical syndrome districtly different from that of trisomy 21. These two examples will illustrate well the difficutlies encountered in clinical diagnosis in certain "border line cases'.

D — TRISOMY

Discovered in 1960 by Patau et al. this trisomy for a medium sized acroceatric of the 13-15 group was rapidly confirmed at first by the same authors, then by many other laboratories. This syndrome has been described in 28 cases in available Publications.

External Abnormalities :

D-Trisomy children are undeveloped children (average weight 2.3 Kg with a standard deviation of 0.4 kg) with multiple malformations and great mental defeciency. Deafness is often mentioned. The skull is small mainly through abnormality of the frontal and partial regions. The eyes are very small. The ears are malformed. Low implantation of the

lobes and the smallness of the mandible (lower jaw bone) are often noted. Finally, very frequently there is a harelip, often bilateral with sometimes almost complete cleft (wolf-mouth). The trunk is generally not very deformed. In the extremities one observes flexion of the hands & fingers which often cannot be straightened. Polydactyly of the hands and feets is an almost regular feature, the extra fingers or toes may be completely developed (hexadactyly) or be reduced to a simple side stump fused fingers and toes are frequent. Deformity of the feet is very characterstic. Finally the nails are narrow. (Fig. 10)

Internal Abnormalities :

Heart defects are common (77%). The kidney's may be malformed and the intestine developed abnormally (segmental douplication of the small intestine. Malformation in uterus and penis. In girls, the uterus is often bicornuate (double uterus) or even entirely partitioned and in boys hidden testis and abnormal extension of the scrotum (the pouch in the male which contains the testicles) on the lower aspect of the penis are common. The cerebral malformations appear to be very extensive. The most constant is aplasia of the olfactory bulbs (nerve supplying the nose) and the trigone giving typical arhinencephalia.

Dermatoglyphic abnormalities : Uchida et al (1961) were the first to report the existence of 2 dermatoglyphic signs almost constant in this condition : the transverse palmar crease and the mediopalmar position of the axial triradius. Of 20 cases covered in the present statistics 16 showed a unilateral or more often bilateral transcase crease. In 10 cases, for which the palmar prints were fully analysed, the axial Triradius was always in position "t". The dermatoglyphic patterns of the hypothenar eminence are still not very clear. It should be noted that these two disorders illustrate the difficulties of using malformation symptoms in evaluating the gene contents of the chromosomes. Finally Uchida et al (1962) described on the sale of the foot presence of an arch involving all the the toes. This formations is thought to be very typical of trisomy 13.

Effect of maternal age : The Effect of maternal age reported as early as 1961 by smith et al is difficult to demonstrate in this trisomy. The majority of children with trisomy 13 were born to young mothers under 35 years but it is difficult to specify at the present moment the statistical significance of the excess of mothers over 40 years of age observed.

Fig. 10 — boy with trisomy 13 (D - Trisomy)

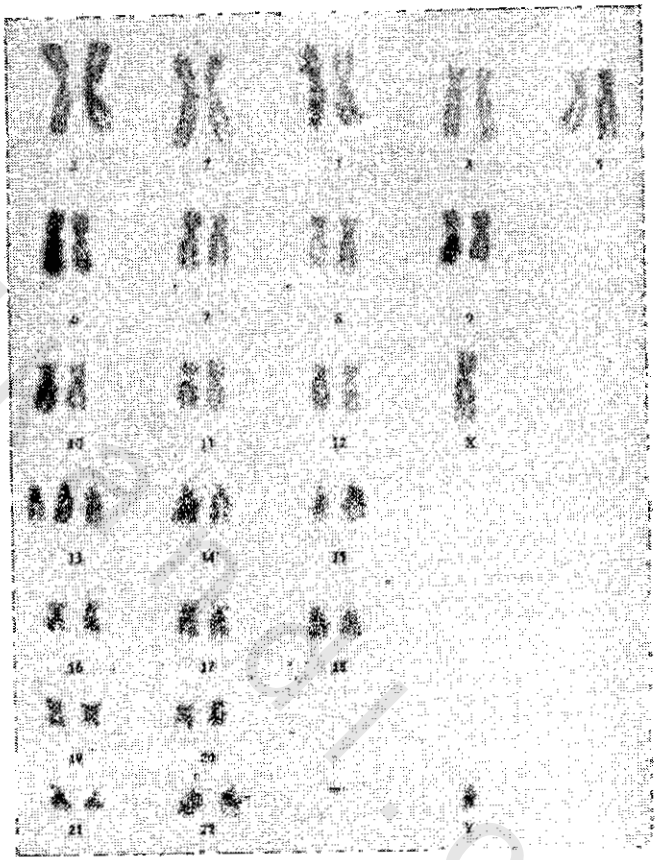

Fig. 11 — Karytype of a boy with trisomy 13 (D - Trisomy)

Cytology of D-Trisomy :

As was recognized from the finding of Patau et al (1960), the additional chromosome is a medium sized acrocentric of group 13-15. From our personal experience it seems that the extra chromosome is the carrier of small well developed euchromatic arms and has no satellites. To be more exact, we find among these subjects three acrocentric chromosomes instead of two, corresponding to these characterstics. (Fig. 11)

E. TRISOMY (EDWARDS SYNDROME)

Discovered in 1960 by Edwards, Hardon, Cameron, Crosse and Wolff. E-trisomy appears to be more frequent than D-Trisomy. The clinical diagnosis of the condition is relatively easy for the experienced clinician. These are small infants born after a pregnancy often complicated by hydramnios (excess of water in amniotic fluid) and a small placenta. The weight at birth is low (2.41 + 0.51 kg on average in 35 cases). The skull shows an anterio-posterior elongation and a relative lateral flattening. The bridge of the nose is sometimes broad and flat but more often the nose is sharp or even prominant, the ears are very low set and often the pinna is poorly shaped or frankly malformed. In the chest, relative aplasia of the sternum can be seen. The pelvis is narrow, often associated with congenital dislocation of the hips. Other features, webbed neck, harelip upper links, very typical lesions exist in nearly all cases. The fingers are pressed together, which may cause weakness of the palm. Marked deviation of the index & little fingers towards the median line is noted, these two fingers overlapping respectively the middle and the ring finger. This arrangement appears to be particularly typical. The children hold their arms raised on each side of the head in a supplicant position. In the lower limbs it is often possible to note an anomaly of the hips and the existence of the club foot of quite varied type is noted in half the cases. Likewise, the first toe is very often short and retracted and syndactly of the second and third toes is very frequent, sometimes also seen in the fingers.

Finally, two major signs complete the picture, the children are puny, do not gain weight and present signs of very profound mental debility. Their viability is extremely low with the average age at death 2.9 ± 1.2 months for 11 boys and 4.2 ± 0.8 months for 31 girls. The oldest child was 23 months at the time of examination (Fig. 12).

Internal abnormalities : The majority of the deaths are due to

Fig. 12 — a - Karyotype of a boy with trisomy 18
b - boy with trisomy 18.

aspiration pneumonia or acute heart failure (from 45 cases 39 presented great heart defects). Thus extreme frequency of heart conditions very probably accounts for the poor viability of the children affected and suggests that amongst stillborn, the frequency of trisomy 18 is perhaps higher than we at present suspect. Malposition of the colon is frequent as are renal malformations (Polycystic kidney, horseshoe kidney) sex anormalies in girls (hypertrophy of the clitoris, abnormal ovaries).

Dermatoglyphic abnormalities : Reported in 1961 by Uchida et al 1961 and confirmed by other authors, the dermatoglyphic syndrome in Trisomy 18 appears to be very characteristic. On the fingers we note an extra ordinary frequency of arches, that is instead of forming a whorl or a loop the ridges regularly arrange themsleves on each other Moreover, clinodactyly of the fifth digit and the presence of a single flexion crease at the level of this finger also seems to be very frequent. Finally, a transverse palmar crease usually unilateral, is sometimes encountered without it at present being possible to estimate its frequency.

The frequency of the disease is not yet known with precision, it appears that the disorder may be just as frequent as G-Trisomy (2 cases in 999 & 2 cases in 617). A provisional estimate of one to two per thousand may be considered reasonable.

The appearance of the disease seems to be influenced by the age of the mother. In 50 recorded cases the mean age of the mothers at birth was 34.7 years with a standard denation 8.4 and a mean error of 1.1 However, it should be noted that there may exist a considerable number of cases of E-trisomy independent of maternal age as noted by Hecht et al (1964).

Sex ratio of patients :

Ferguson-Smith (1962) noted in 31 cases clear predominance of girls (22 ♀ against 9 ♂). The distribution of the patients as a function of the age of the mother shows a very curious irregularity. Before the mother has reached 35 years the sex ratio is already abnormal 14 ♀ against 8♂) but after 35 years predominance of girls becomes enormous (25 ♀ as against 6♂). A possible explanation of this phenomenon may be found in the notion of supplementation by the mother of the abnormal metabolism of the trisomic child. Maternal age would lower this faculty and the male sex more sensitive than the female would be preferentially eliminated.

In other words, two contradictory factors would be at work because of age : —

(*a*) increases in the frequency of chromosome anomalies.

(*b*) reduction in the probability of survival of the foetus; the male sex being primarily affected.

Cytology of E-Trisomic :

Described by Edwards et al (1960) as a trisomy for chromosome 17 this condition which no doubt represents a trisomy for the same chromosomes in all cases is considered as a trisomy 18 by Smith et al (1962), Patau et al 1961) and many other authors. In the personal cases we have studied the most plausible diagnosis appears to be that of trisomy 18. This discussion reflects the difficulties of diagnosis encountered is the individualization of chromosomes 17 and 18. It appears advisable for the time being to name the disorder E-Trisomy awaiting final cytological precision.

2. STRUCTURAL ABERRATIONS

A) **Deletion** :

A deletion involves the detachment and loss of a part of an arm from the chromosome. This portion will not survive if it lacks a centromere since it will have no power of movement in anaphase.

Defeciencies can be either terminal or interstitial. The terminal can arise by a single break in a chromosome followed by a healing of the broken end. The interstitial results from 2 breaks followed by the reunion of the broken ends.

Each type, if large enough can be recognised in pachytene or salivary gland chromosomes by the manner in which pairing takes place with a normal homologue. The location of defeciencies can be determined in the salivary gland chromosomes. by comparing the band structures of the defecient and normal chromosomes, but such a procedure is not always possible in plants and animals lacking giant chromosomes.

Defeciencies or deletions occur spontaneously or they can be readily induced by radiation. In maize ultra violet rays appears to give rise chiefly to terminal deletions. x-rays give rise only to interstitial defeciencies. since a defeciency involves the less of genic material, it would be expected that defeciency would have a loss effect on an organism. The effect depends upon the amount of the lost genic material and its quality. Homozygous viable defenciencies would be expected to be rare. In maize such homozygous defeciency was observed. In Drosophila homozygeus losses involving the tip of the X-chromosome are viable because they are very minute.

Heterozygous deficiencies are common. In **Drosophila melanogaster** a loss of more than 50 bands in generally considered to be lethal even when the homologous chromosomes is intact.

Since a defeciency results from loss of genes, it is not surprising that give rise to certain genetic effects. Deletions were observed in chromosome 18 and chromosome 5 in man causing mental defeciency and some other bad effects described below.

Deletions in human chromosomes :

Two types of isolated delation among human chromosome set are now known.

Deletion of the short arm of 18.

Grouchy et al (1963) described a particular dysmorphic syndrome in a boy with several mental retardation. They noted the following signs : hypertelorism with incomplete convergence in the right eye low set ears and deformed hand and feet. The karyotype with 46 chromosome revealed the absence of a chromosome 18 replaced by a telocentric the arm of which was identical in size to that of the long arm of 18. The most plausible explanation for such an anomaly is loss of the short arm of 18 just above the centromere. A very similar case with palpebral ptosis, low set ears, micrognathia and anomalus articulation of the thumb is briefly mentioned by Lewis et al (1963). The karyotype showed the same anormaly, complete deletion of the short arm of one of the 18 chromosomes.

2. Deletion of half the short arm of 5 (Cri du chat Syndrome)

A new clincial entity attributed to an isolated deletion was recently observed (Lejeune et al 1963). (Fig 13 a)

Four patients presented an absolutely concordant clinical Syndrome and identical chromosme aberration characterized by loss of about half the length of the short arm of one of the chromosome. (Fig. 13 b)

The difficulty of diagnosis between 4 & 5 has already been discussed and it was only from an impression (based on many karyotypes) that the abnormal chromosomes was considered as replacing one of the 5 chromosomes. At any rate, in accordance with the rule already proposed for the trisomies 21;18 and 13 Lejeune suggested that the number 5 be definitily given to the chromosome, partial deletion of the short arm of which determines the clinical syndrome observed which we can briefly described as follows : multiple malformations including microcephaly, hypertelorism in the epicanthus, normal ear lobes.

The dermatograyphs reveal three features : axial triradius in position transverse palmar crease (or equivalent) and absence of the triradius of the fourth finger.

The children are characterised with severe mental deficiency. A particular sound of crying simulating by its special quality of the sound to the miaowing of the cat. This latter sign, extremely typical in the

Fig. 13 — a - boy with "Cri-du-chat' Syndrome.
b - Karyotype from the same boy showing deletio in chrom 5.

first month of life, seems to be related to that part of the larynx associated with voice production.

It is possible that the clinical picture is more complex the larger the fragment lost and there is no formal reason for postulating that the deletion must always concern the same segment.

B) **Duplication** :

An extra piece of a chromosome, whether attached in some manner to one of the members of the regular complement, or existing as a fragment chromosome is known as duplication. As might be expected, duplications are more frequent and they are also less lethal than defeciency to the individual.

The term repeat is also applied when a repeated duplication of small segment of a chromosome producing for example abcd abcd abcd. It occurs in Drosophila and causes mutation formed by repitition of one zone of chromosome which when repeated again gives rise to the variety of double narrow eyes instead of single narrow eyes.

C) **Inversion** :

If the central segment of a chromosome which has borken into three parts is inverted and becomes fused in this reversed position, having effected a rotation of 180° degrees, we say that an inversion, has occured.

Inversion can be long or short. The shortest inversion detectable cytolojgcally is that in Drosophila and involves one or two bands. Whether there are inversions at submicroscopic levels remain to be determined. The longer inversion have been studied most deeply and as length increases the possibilities of multiple crossing over similarly increase.

When an inversion occurs in one of the chromosome of a pair of homologous, In order to conjugate, the normal chromosome must then make a turn in order to adapt itself to the inverted homologous chromosome.

If the crossing over is carried out between a pair of heterozygotic chromosomes an inversion may cause important changes particularly if the interchange produces a chiasmata within the inverted segment. Under these circumstances after the interchange between the chromatids has been produced, there results at the anaphase what is called a dicentric chromatid bridge. This bridge consists of one chromatid with 2 centromeres. The bridge is drawn out until it raptures when the chromosomes separate towards the poles, at the same time an acentric chromosome forms.

Inversions are called paracentric if the centromere is contained outside the inverted segment and pericentric if the centromere is contained within the inverted segment.

D) **Translocation** :

Translocation is the fragmentation of two chromosomes and its transposition (exchange) between non-homologous chromosomes. The two new chromosomes will function normally if each possesses a single centromore. If the reunion produce centromere, this will be lost during the cell division because of mechanical irregularities. Other type of translocations are also known. Simple translocation which involve the transfer of the end of one chromosome to the end of another, occurs rarely.

In general translocations are produced by the rupture of two non-homologous chromosomes followed by a transference and fusion of the reciprocal segments. For this reason it is called **reciprocal translocation.** The rupture may occur in any section along the length of the chromosome which causes variations in the resulting chromosomes. In this case, the individual has two complete chromosomes with the genes arranged as before and two translocated chromosomes which have a different arrangement of their genes. The chromosomes at the time of pairing constitute a bivalent is the form of a cross which at the end of this stage opens and forms an annular element. The segregation of the 4 chromosomes gives 6 classes of gametes.

An interesting result occurs when the translocation is very close to the centromere and there originates a V-shaped chromosome with two arms in the form of V-shape. This is usually known as B-chromosome and a small fragment which tends to be eliminated because it is practically inactive, even though it has a centromere. Fusion of this type is called centric fusion. In this way a new chromosome is formed (B-chromosome and at the same time the somatic number of the species is decreased. In many animals and plants a chromosomal mutation seems to have been produced in this way, by decreesing the number of chromosomes and giving rise to a new type of chromosomes & thus to a new race or species.

Kabarity and Schade (1969) described for the first time in man a new type of translocation in a child with multiple malformations. Successful examination of 40 metaphases showed a set of 46 chromosomes including two X-chromosomes. But on chromosome 2 of the A-group an extra segment was seen. It was inserted laterally into each short arm, near the centromere, by a chromatid fragment. The same chromosomal anamaly was found is the child's mother, who was affected by the same malformation syndrome. (Fig. 14).

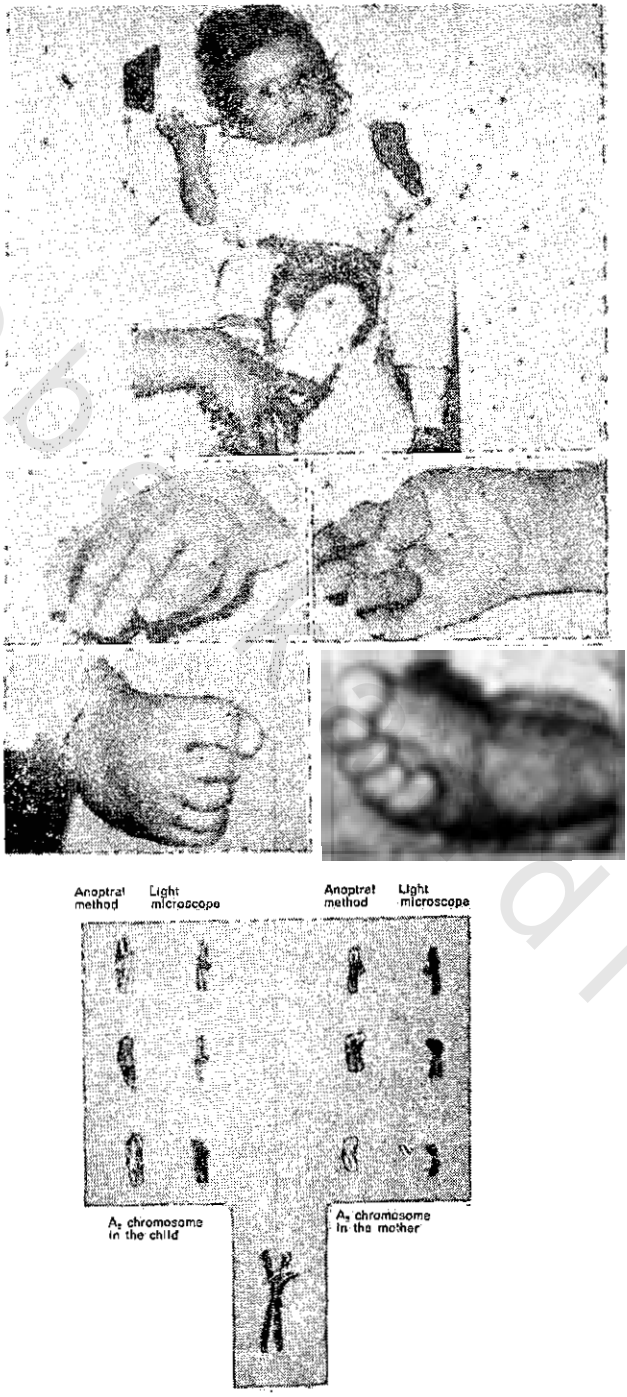

Fig. 14 — a - Fingers and toes of the child with lateral translocation.
b - Lateral translocation of the chrom. A_2 incells from the child and its mother viewed by the anoptral and light microscope (Kabarity 1969).

Translocation 21 D :

This type found in many cases of familial trisomy 21 is characterized by translocation of a 21 onto a D without producing particular anomalies in the heterozygous carriers with balanced karyotypes.

The particular importance of this structural aberration lies in the fact that during chromatic reduction the translocated chromosome 21/D is found in half of the gametes. The fate of the free 21 may be irregular and if this chromosome moves to the same pole translocated chromosome, a diplo 21 gamete is produced. Fertilization by a normal haploid gamete will lead to masked G-trisomy with apparently 46 chromosomes. The other gamete without chromosome 21 would after fertilisation give a haplo 21 zygote which has still not been observed.

Kabarity et al (1972) discovered a new type of translocation in man in a case of a child of multiple malformation. Successful examination of metaphases from blood and fibroblasts cultures showed a set of 45 chromosomes including two XX chromosomes. A D chromosome is translocated onto an E chromosome .In this way a new chromosome is formed longer than any chromosome of the C-group. This Translocation and the insertion of the two chromosomes onto another was confirmed by the most famous human cytologist the world Lejeune in Paris using a very recent method in determining the region of the translocation. and this discovery was published under the name Kabarity, Lejeune, Puschel, Schade (1972). (Fig. 15 & 16)

E) Philadelphia or Ph Chromosome by chronic myeloid leukaemia :

Ford (1960) noted the existence of a chromosome fragment in a case of chronic myeloid leukaemia. It is due to Nowell and Hungerford (1960) that credit goes for having detected the presence of a very small acrocentric in the blood cells of two patients with chronic myeloid leukaemia, then in 5 others. Shortely afterwards many authors confirmed this finding in so many cases of chronic meyloid Leukaemia. Among 147 cases of chronic Leukaemia the small acrocentric is found 127 times. The designation of the small acrocentric encountered in chronic framalocytic leukaemias, chromosome Ph was proposed by British workers in the line with the recommendation in the Denever document of naming the abnormal chromosomes after the initial letters of the town of discovery (in the present case, philadelphia).

The Philadelphia chromosome is a small acrocentric of the group

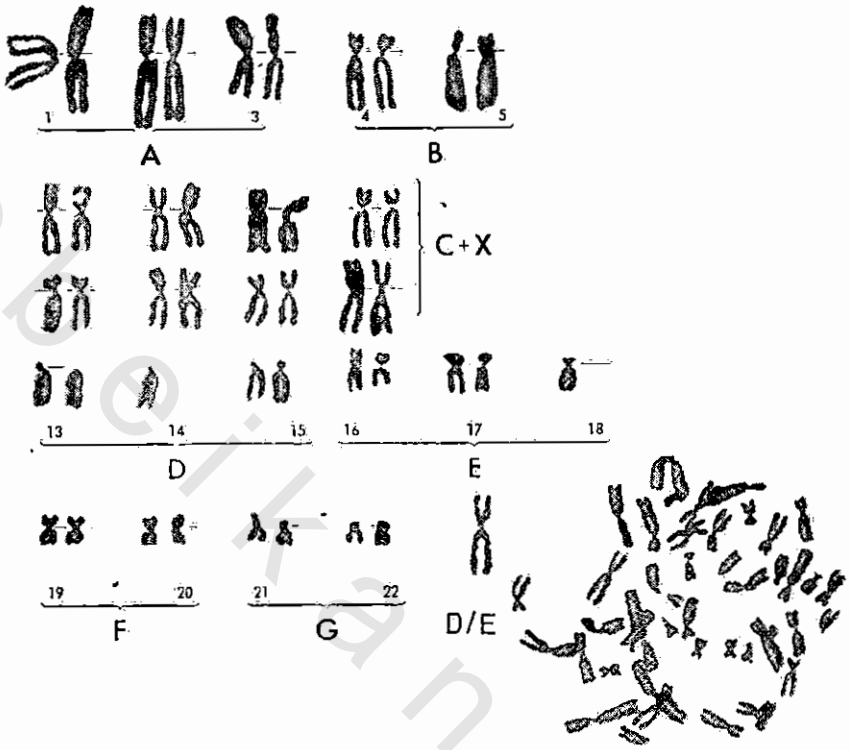

Fig. 15 — Karyotype with 45 chromosomes showing D/E translocation (Kabarity 1972)

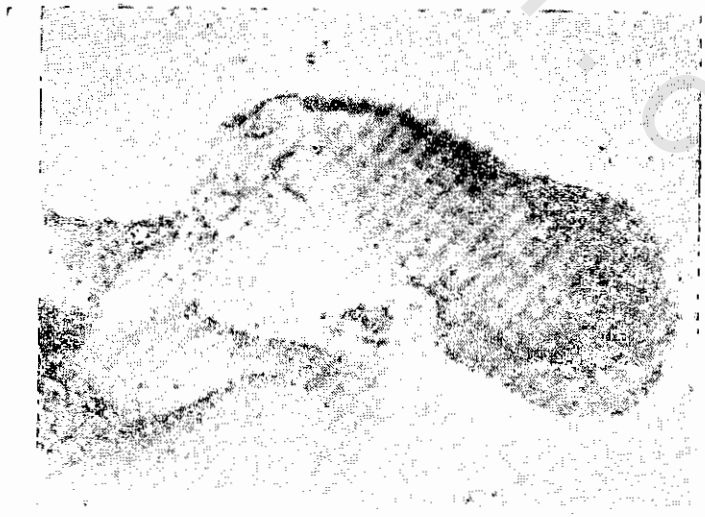

Fig. 16 — A child with D/E translocation (Kabarity et al. 1972)

21-22 reduced by about half as compared with the size of a normal chromosome. This reduction appears to be due to loss of the distal segment of the long arm although a more complete rearriangement cannot be ruled out. The presence of satellites has been frequently noted on this chromosome and although identification is difficult, the majority of workers agree that this is a chromosome 21 showing partial deletion of the long arms.

The most important question then becomes that of defining the relation between the presence of a Ph chromosome and the leukaemic nature of the cells which bear it.

Firstly, this chromosome is encountered only in the bone marrow or the blood of the patient - all other somatic cells are normal.

It must be pointed out that chromosome Ph is only observed in cases of chronic myeloid leukaemia. In allied but clincially different proliferative myeloid conditions we were not able to demonstrate a Ph chromosome working in institue of human genetic in Düsseldorf. It may be considered that this abnormal chromosome is quite specific to chronic myeloid leukaemia and that it is present in the vast majority of the marrow cells of patients.

SEX CHROMOSOMES ABNORMALITIES IN MAN

A new classification of gonadal abormalities and the state of intersex based on direct study of sex chromosomes is now in hand. The new classification is cytogenetic. It distinguishes numerical & structural amomalies with or without mosaicism.

A clear diagnose is not easy task for numerical anomalies chiefly because a mosaicism, a phenomenon which needs complete investigation and for structural anomalies mainly because of the present technique which permit detection of only the most apparent of these aberrations. This is true of both because of our imperfect knowledge of the maturation of the sex phenotype despite recent valuable experimental evidence.

Turner's Syndrome :

The history of this gonado-somatic abormality known as Turner's Syndrome (H.H. Turner 1938) started with the initial discovery of ovarien abnormality coming up to date with anomaly of the chromosome sex determinants. The first anatomical description was made by J.B. Morgagni 1749, which mentioned the essential anatomical signs : female of short nature genital infantilism and ovarien abnormalities and even including a renal malformation. Shortness and narrowness of the vagina and uterus, the presence of very thin round ligaments wide Fallopian tubes and ligaments longer than usual. Morgagni added Further "I quite clearly recognize that this woman had absolutely no ovary or even the slightest rudiments of these organs". For nearly two centuries nothing essential was added to Morgangni's description. In 1938 H.H. Turner collecting seven purely clinical observations showed that women with small stature infantile and with primary amenorrhoea may be distinguished by morphological features, the most suggestive of which are webbed neck. (Fig. 17)

The subject who was instrumental in allowing Ford et al to make this discovery on 4 April 1959 was a typical example of this disease. The bone marrow technique enabled them to count 45 instead of 46 chromosomes. (Fig. 18) After some discussion arising from the difficulty of distinguishing the X chromosomes from the 6, the authors considering that such autosomal haploidy would very probably be lethal concluded that a formula XO was likely. This was rapidly confirmed by many other authors using bone marrow technique and on skin, facia and blood. The

XO cell is distinguished both by absence of a chromosome assigned by morphology of group 6-12 X and by the absence of the chromatin body known as Barr-body. (which will be discussed later).

The frequency of XO types estimated from investigation of chromatin bodies in 10,000 newborn females among births was 0.4 per 1000. In fact, neither the age of the mother nor the father, nor the birth rank seem to influence the XO frequency.

Sex chromosomes and Barr bodies :

The sex chromatin of female mammals was discovered by Barr and Bertram 1949 in the form of a small nuclear body which was present in cells of female cats. This discovery was soon followed by others which showed that sex chromatin could be demonstrated in many other type of cells and that it was present in other mammalian species including man. The sex chromatin is now frequently called as **"Bar body"** after its discoverer.

A Barr body is a small well defined body, which stains intensely with nuclear dyes. In size it is about one micron in diameter.. Such bodies are present in a large proportion of nuclei of females but are absent in nulcei of males.

A typical shape of a Barr body is convex or wedge shaped with the rounded or pointed end pointing towards the cytoplasm. The majority of Barr bodies lies against the nuclear membrane and are seen at the periphery of the nucleus in profite, but a small proportion of Barr bodies are found in other situations within the nucleus.

One of the most interesting properties of Barr bodies is their numerical relationship with regard to the no. of X-chromosomes present. In the Normally; the maximum number of Barr bodies per nucleus in any organism or tissue is either nought (O) or one. However cells with multiple Barr bodies may be found and their existence means that more than two X chromosomes are present. Thus individuals with 3 X-chromosomes always have two Barr bodies in their cells. People with 4 X-chromosomes have 3 Barr bodies in some cells. A female was found with 5 X-chromosomes who had 4 Barr bodies in some of her cells. These findings make it clear that the maximum number of Barr bodies present in a nucleus is one less than the number of X-chromosomes. The presence of an additional Y-Chromosome does not influence this relationship.

The fact that in diploid cells the maximum number of Barr bodies is always one less than the number of X-chromosomes has given storng sup-

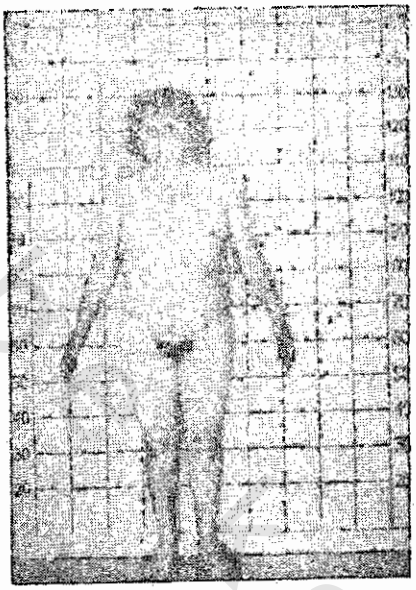

Fig. 17 — The Turner Syndrome (Mosby 1961)

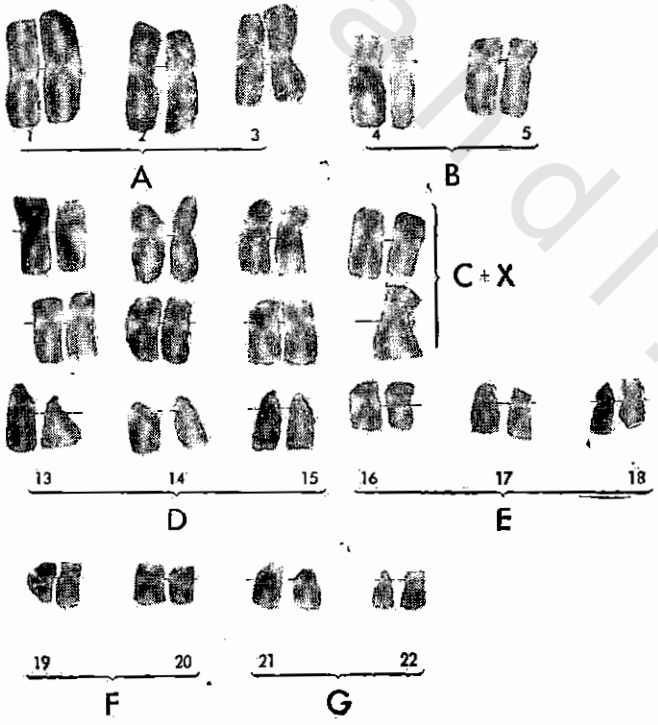

Fig. 18 — Karyotype of Turner Syndrome with XO (original)

port to the suggestion, which was first made by Ohno et al (1959) that a Barr body is formed from a single X-chromosome. This means that in the interphase nuclei of normal females one X-chromosome remains extended, while the other one becomes obviously and forms a Barr body. If more than 2 X-chromosomes are present, one again remains extended and the others form Barr bodies.

What is the function of the Barr body ? The hypothesis proposed by Mary Lyon (1961 - 1963) associates this with the concept that heterochromatin lacks major genes. It is assumed that, once sex is determined, a female like a male requires only a single X-chromosome and the sex chromation might be a means of putting the additional X-chromosome out of action. This theory has received a great deal of attention. Morphologically distinct sex chromosomes occur in a wide variety of organisms including insects, birds, amphibians and some flowering plants. It would be most desirable to have more information about the behaviour of these sex chromosomes during interphase, arrived at by techniques comparable to those in use of man and other mammals.

Type XXX

The first examples of chromosomal aberrations let to the belief that man does not escape the risk of non-disjunction any more than many experimental species. Acceptance of this possibility implies the idea of other varieties of numerical anomalies.

Study of the chromatin body also justified this work. In fact it brought to light mentally defective distinguished by the presence of two chromatin bodies in a large number of their nuclei.

In fact, subsequent research was to modify this first clinical impression. Karyotypic analyses of females with double chromatin body (Fig. 19) detected in an institution for mental deficients led to the discovery of a case of a 21 years old female with normal sex chareters. The proportions of cells with one and two chromatin bodies were almost equivalent. The XXX Karyotype was confirmed by skin and blood examination. The frequency of the XXX type was estimated by Maclean (1964) at 1.2 per 1000 live births (12 per 10,000). The risk of a birth of a triplo X girl appears to increase with maternal age but more evidence is necessary to establish this hazard. Further, following a law which appears almost general the triplo X may appear in the same family with other chromosomal aberrations.

The fertility of these women in spite of the extra X is now proven by several observations. The XXX female may in principle produce XX

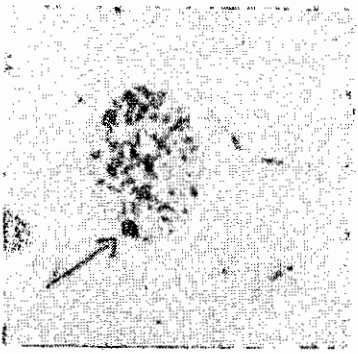

Fig. 19 — double Barr bodies in a nucleus from a patient with the Karyotype 44+ × × ×

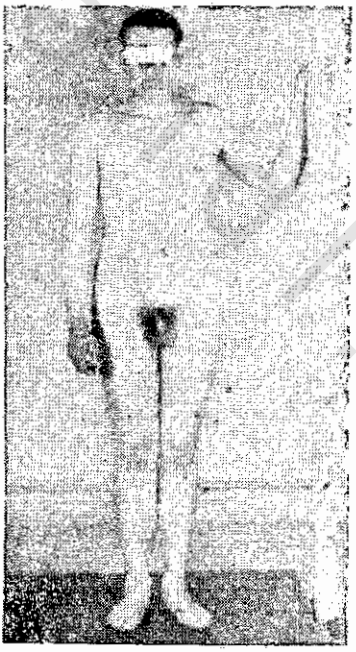

Fig. 20 — Subject with 44 +XXY Karyotype (Klinefelter Syndrome)

and X ovules in equivalent numbers. The fertility of the XX ovule and the viability of the XXX zygote are demonstrated by the very existence of XXX females. Many authors had examined many cases of XXX females, they had all normal children after Marriage.

Mental deficiency actually appears to be the most constant abnormal conseqnence of the extra X-chromosome.

Some have rightly asked whether the way of inquires did not lead to selection of institutionalized mental defectives missing females whose intelligence is compatible with normal life. Combining the results of the various inquires based on the chromatin criterion of the XXX type is 4.51 per 1000. Comparison of this result with that in the inquiry among new born girls at 1.2 per 1000, testifies to a significantly higher frequency of females of XXX constitution in the group of mentally backward, assuming that the mortality within each of these two groups is the same. However, the 10 of XXX subjects is said to be over 20.

XXY Klinefelter Syndrome :

Non-disjunction of the X chromosomes at anaphase II in oogenesis results in an XX daughter cell and the other devoid of an X-chromosome. Fertilization of the latter by Y spermatozoid should give an OY zygote which has never been identified. It is probable that this zygote is not viable. In fact, an ovule, without an X chromosome is fertile since a subject with Turner's Syndrome may draw the X from his father.

In 1942 Klinefeller described in man a syndrome involving a gynaecomastia, increase in urinary gonado trophins, hypotrophic testicles with aspermatogemesis tubular fibrosis. This description based on nine cases, brought together the essential anatomo-clinical and hormonal features of this sydrome now generally known as Klinefeller's Syndrome.

A notable aspect of the nine subjects studied aged from 17 to 38 years was the contrast between development of secondary sex characters and the consequences of testicular dysgenesis.

On the one hand, the penis, the prostate and hair cover were normal, on the other hand the testicles were very small without sperma.

The histological examination which was made in seven of these patients recorded the same essentially tubular lesions, atrophy and hylinization of the seminiferous tubules, absence of spermatogenes and inflamatory signs.

Klinefeller's Syndrome is not an example of intersexuality. The small and hard testicles migrated normally into the sacs development of the

breasts gives gynaecomastia of variable size, a common consequence of testicular hypoplasia and not a breast of feminina morphology as in the hermaphrodite, Tallness is common with macroskelia. Hair on limbs, truck and face is scanty. (Fig. 20)

The chromatin body criterion was applied to study of Klinefelter syndrome as far back as 1956. A large number of these patients 50 - 80 per cent, then appeared to be chromatin positive.

The cell type is characterized by the presence of an extra chromosome linked by morphology to group 6-12 X and by the presence of a chromoatin body at interphase.

The results of inquiries in various countries on the proportion of chromatin positive live newborn boys are more or less the same. 5 of 1191 (Moore 1959), 4 of 1890 (Bergmann 1961) 27 of 10725 (Court Brown 1964).

Presence of XXY seems to be greatly influenced by certain familial conditions. The frequency of this aberration appears to increase with maternal age.

The discovery of the karyotypic criterion was followed by inquiries into the psychic features of this XXY variety of Klienfeller syndrome. These patients are often distinguished by their passive aggressive personality with shizoid tendencies ± marked mental defeciency.

These chromatin positive subjects have usually one, two or three sex chromatin masses and therefore probaly represent XXY, XXYY, XXXY and XXXXY types with or without mosaicism. We find here the diversity observed in the newborn even through a complete karyotypic comparison is not yet possible. Mac Lean et al. however found a frequency of XX/XXY mosaics in one of the inquiries among newborn in 4 of 3000 quite close to that which they obtained for backward subjects at 2 out of 2607 (1962).

If we consider the overall results of the chromatic criterion 2.06 per 1000 chromatin positive male newborn infants and 9.71 per 1000 in mentally defective subjects, the comparison brings out an association between gonosomal aneuploidies in subjects of male phenotype and mental deficiencies which it is difficult to attribute to chance alone. This comparison pre-supposes that the risk of prepubertal mortality is equal in both groups. If it is greater for the group of mental defectives usually after 15 years, the ratio 9.71/2.06 under estimates the real position.

XYY type

The case providing the occasion for the description of the XYY type attracted attention less by his physical and psychic features proper than by the anoamalies of his progeny.

He was a male aged 44 years of average intellingence and neither his appearance nor his past indicated an abnormal chromosomal constitution. His first wife, apparently psychotic had in succession an XX primary amenorrhoeic, chromatin positive daughter with ovarian agenesis a spontaneous miscarriage; three normal boys in succession and two male twins one who died at the age of 3 days from blue disease, the other in good health.

The second wife after a spontaneous miscarriage, had a healthy dauhter then a G-trisomic daughter.

Because of these anomalies, the karyotype of this chromatin negative male was studied in two blood samples. the examination revealed the existence of 47 chromosomes with the constitution XXY. The possible origin of this XYY was discussed : meiotic non-disjunction of the Y at anaphase II in the father of the subject considered or nondisjunction in the first clearage of an XY zygote. This latter hypophysis seems to be less probable since there is an example of XO/XYY mosaic showing that differential selection does not lead to elimination of XO in favour of XYY. It should be noted that this XYY subject did not appear to suffer ill-effects from the presence of an extra Y chromosome since he was free of signs of gonado-somatic abnormalities. Other cases were described which distinguished from the proceeding case by their mental defeciency and absence of descent of the testicles. Other examples are accompanied by genital anomalies. One was complicated by a hypospodias another by hypogonadism was the most prominent aspect of another detected in a 25 year-old male with penis and scrotum of the prepubertal type and with no normal development of testicles.

CELL DIVISION

Cell division is the process through which cells are reproduced and the multicellular organisms grows. It differ from the ordinary division of physical objects. When, for example an apple is divided equally between two people, each person receives only one half of the original, but when cells divide each part is a complete cell. The daughter cell may be smaller than the parent cell immediately followings division, but they grow rapidly and soon reach the size of the original cells. Cell division is really a process of duplication or multiplication rather than division in the usual sense.

Appropriate measurements would show that the average cells size in a given tissue of a baby is about the same as that of an adult. Therefore the gross size difference is mainly dependent on the number of the cells rather than the size of cells.

Mitosis

The term mitosis is sometimes applied to cell division as a whole but when correctly employed is restricted to the division of the nucleus. Mitosis is sometimes referred to as indirect nuclear division. Another method of noclear division is known as direct division, or **amitosis,** was formely believed to be common. The series of changes covering the period from the disappearance of the interphase nucleus to the reconstitution of the daughter nuclei is divided into 4 stages. The process is identical in all organisms. The main differences being the absence of centrosome in higher plants & minor details of the structure of the spindle.

Prophase :

The prophase is first characterised by an increased in visibility of the chromosomes. The chroms decrease in length between this stage & the next. During this stage the chromosome. becomes double longitudinally but the parts do not separate until the next stage.

Metaphase :

The nuclear membrane breaks down & disappears. A spindle forms in the cytoplasm and the chromosomes "still double are arranged at the equatorial region. This arrangement is called the equational plate. The chromosomes are now at its maximum contraction & spiralization (Fig. 21).

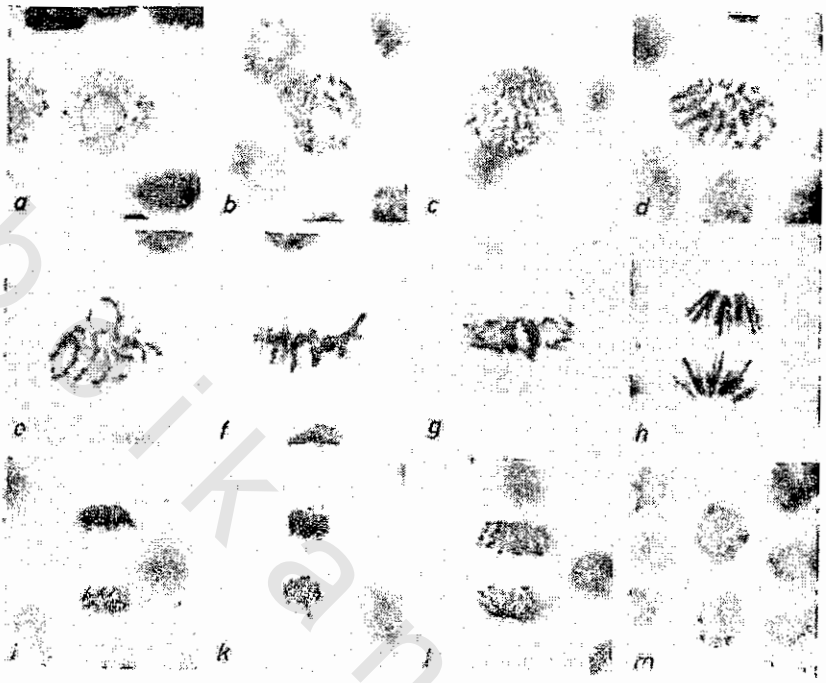

Fig 21 — Mitosis in Allium cepa root tips a-Interphase nucleus (resting stage) with large nucleolus b, c, d-different stages of prophase e-prometaphase disappearance of nucleus membrane f-metaphase g, h-Anaphase, i, k-Telophase l-transitional stage to interphase m-daughter nuclei in interphase, Original

Anaphase : Following the arrangement of the chromosomes on the equational plate, the sister chromatids separate & pass to the opposite poles of the spindle. The separation of the genes that will pass to the new cells occurs at this moment. The movement of the chromosome is centered in its centromere.

Telophase : When the movement of the chromosomes has been completed and one chromatid of each original chromosome is at one pole and one chromatid is at the other, the cell divides in two at the equatorial region & a new nuclear membrane forms around each chromosome complement. The stages of nuclear organization are now the reverse of these in prophase.

Duration of the mitotic cycle :

The duration of the mitotic cycle depends upon many physiological factors to which growth processes in general are susceptible. For the

comparison of the duration in the 4 stages of mitosis, some data are given in the next table. Interphase is the longest stage in duration but is also the most variable. Metaphase is the shortest.

	Drosophila in minutes	Chicken	Plant
Interphase	2.9	30-120	....
Prophase	3.6	30- 16	36-45
Metaphase	0.5	2- 10	7-10
Anaphase	1.2	2- 3	15-20
Telophase	0.9	3- 13	20-35
Complete cycle	9.1	67-205	78-110

Meiosis

Meiosis is a kind of cell division by which the number of chromosome is reduced to one half of their original number. The chromosome number of the cell is doubled by fertilization of the egg; should no reduction in chromosome number take place between fertilisation cycles, enormous numbers of chrom. in each cell would be the result in a few generation. A reduction division is a necessary phenomenon to zygote formation.

The formation of gametes is called **gametogenesis.** In the male this is called **spermatogenesis** and in the female **oogenesis.** For plants the equivalent terms are **microsoporogenesis** & **megasporogenesis** respectively. The processes of chromosome dividsion are identical for each. Division of the cytoplasm may be quite different.

Normally meiosis consists of two divisions. The first is usually the reduction. Thesecond is a mitotic division of the resulting haploid nucleus.

On account of the specialized arrangement of the chromosomes in the Prophase I, names have been applied to the successive chromosome configurations.

The entire process is continuous and all intermediate states may be found.

The **Leptotene** stageis the first stage periodin which the chromosomes become visible. They appear as one filament undivided into two chromatids as in the prophase of mitosis. The chromomeres are visible at this time.

During the **Zygotene** stage the homologous chromosomes begin to unite side by side. They are paired throughout their entire length. The

union of one chromosome with the other is very exact for each chromomere (gene locus) appears attracted to a corresponding chromomere on the other chromosome. Visibily the pairing is so exact that it has been described as "Chromomere by chromomeres, but actually it must be even more exact than that.

If the homologs are not so alike that all loci are duplicated on each, no pairing will result in the dissimilar areas. The zygotene union of homologous chromosome is often called. **Synapsis.** Each chromosome during the leptotene & this stage has consisted of two identical chromonemata.

Pachytnene Each of the four units which arises from the chromonemata is then called a chromatid. As these chromosomes are formed of two homologous, each is the equivalent of two of the chromosomes of the mitotic prophase. As a result the number of chromosomes is now haploid. An organism having four chromosomes in the somatic nucleus will have 2 in the pachytene, but each of these will be quadripartite. They are known as **bivalents.**

Diplotene : The appearance of the four strands is followed by an opening out of the chromatids of the homologous chromosomes. Instead of separating clearly, the chromosomes are ± held together by the so called **chiasmata.** Then as the homologs get further & further away from one another the chiasmata move toward the terminal positions of the chromosome a process known as terminalization. (Fig. 22)

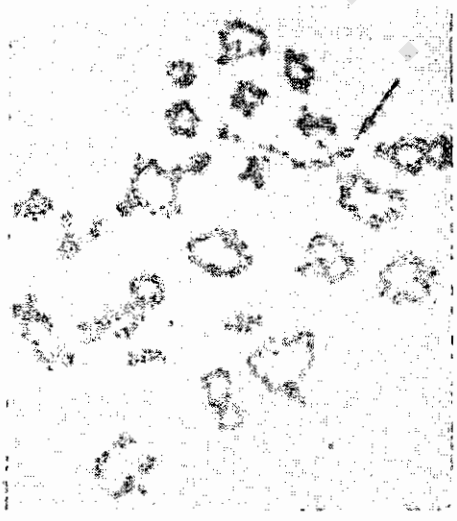

Fig. 22 — meiotic chromosome set from human testic —— XY bivalent (after Pfeiffer).

Diakinesis is produced by a maximum of contraction of the chromosomes. The process of opening out of the homologs started during the diplotene is continued during this stage.

Metaphase I the chromosomes during Metaphase I are centered on the equatorial plate where a spidle apparatus is formed first as in a mitotic division except for the fact that they are associated in homologous pairs. Then during Anaphase I the homologs separate completely one form the other. One homolog goes to one pole of the spindle. Its homologous goes to the other. At **Telophase** I, two haplcid nuclei are formed & a cell wall may separate them. The next division is not different from a normal mitosis since the two chromatids remaining of each chromosome then separate.

Interphase, Prophase II, Metaphase II, Anaphase II & Telophase II are not at all different from mitosis as previously described except for the chromosome number

In most organisms, the haploid mitosis (Meiosis II) results in the formation of four gametes called **tetrads,** each having one half of the somatic chromosome number. In some organisms, haploid cell divisions may continue and entire haploid organisms can result. This is the case in ferns, mosses & molds (gametophyte generation).

Genetic consequence of Meiosis :

There are two reasons why Meisosis is performed :

(*a*) The reduction of the chromosome number in the gametes.

(*b*) An exchange of portions of paired homolgous chromatids i.e. exchange of genes between the maternal & parental chromosomes (mother & father chromosomes).

In the simplest case, homologous chromosomes separate as wholes, resulting in the complete linkage of all genes on a single chromosome However, the phenomenon of crossing over modified this result.

Crossing over involves an exchange of portions of paired homologous chromatids. These exchanges may take place before separation of the homologs, or this exchanges may be caused by the separation.

The result of such crossing over is that, instead of the meitotic production of the original homolgous chromosome at each pole, the separated chromatids may include position of different homolgous redistribution of genes as between the members of homologous chromosome pair is thus effected.

The number of chiasmata is generally proportional to the length of the chromosome

TECHNIQUES OF STUDYING HUMAN CHROMOSOMES

(*a*) **Culture Techniques** :

There are now many techniques for studying human chromosomes, chromosome examinations have to be made by many investigators and it can be said that within a few years no large hospital or medical school will be able to manage without at least a cytogenetics laboratory.

Since the aim of cytological investigations is to establish the karyotype of the cells examined all techniques whatever their working principles and what ever the origin of the tissue must satisfy three demands :

1. dividing cells must be obtained showing enough spreading of the chromosomes.

2. Treatment must entail minimal deformation.

3. The preparations must be flattened in order to obtain photographs in which all the chromosomes are at once in focus.

Study of peripheral blood :

The cells capable of dividing in vitro are the type of small lymphocytes. The following protocol makes use of this method with some variation and can be applied a few hours after samplying. A method for preserving total blood at 80°C with addition of 15% glycerol before freezing (Atkins 1960) makes it possible according to this author, to postpone the culture for several months.

(*a*) **Culturing** :

1. 10 ml blood is withdrawn in a heparinized syringe (0.5 ml of solution of 5000 I.U. heparin per ml is drown into the syringe before blood is withdrawn).

2. The blood is decanted into a test tube inclined at 45° & the red cells allowed to sediment spontaneously. It can also be kept in the syringe the needle held upwards during sedimentation. After a period varying from 30 to 120 Min. the sedementation is sufficient to permit the withdrawal of the supernatant which contains at this moment white cells 5-10 per ml.

3. 10 ml Medium 199 is added to this patient's serum containing

white cells to obtain a final concentration of about 30-35% of the patient's serum and 65-70% of medium 199.

4. 0.2 ml. of Bacto phytohaemaglutinin is added per 10 ml of mixture which is distributed in ordinary test tubes (Pyrex) in such a way that the tubes are filled to one third their volume.

5. The tubes, carefully sealed and then incubated at 37°C for 3 days. The above mentioned procedure must be done under aseptic conditions.

(*b*) **Accumiclation of mitosis** :

The number of mitoses observed after the incubation period differs from one culture to the next. It is advisable to add 2 hours before making preparations 2 drops of isotonic solution of colchicine or a synthetic derivative at a concentration of 0.04% per ml. of medium in order to obtain the maximum number of analysable metaphases.

(*c*) **Making the preparations** :

1. After decanting the contents of one test tube into a conical centrifuge tube they are centrifuged at 800 r.p.m. for 5 min. in order to sediment the white cells.

2. The supernatant is discarded and replaced by hypotonic solution AD. 93 per cent solution of sodium citrate is added for 10 min. at 37°C.

3. Further centrifugation at 800 r.p.m. helps to remove the hypotonic solution which is replaced by Carnoy's mixture (3 : 1. absolute alcohol : acetic acid). The fixative must be added by allowing it to run down the side of the tube and by bringing the cells into suspension by tapping the tube. It is left in contact for 35 min.

4. Further centrifugation at 800 r.p.m. then removes the carnoy's fluid which is replaced by 2-3 drops of fixative in which the cells are resuspended.

5. A drop of the suspension is then placed on cold slide which immediately spread over the whole slide. It is then held above a flame to obtain rapid evaporation of the fixative necessary for flattening the preparation.

6. The preparations are allowed to dry completely in air & can be stored almost indefinitely in this form. The preparations can be stained in the usual way using 1% aceto-orcein for 5-10 minuted & then passed though a series of alcohols for dehydration and can be mounted in Canada balsam.

Study of tissues taken at biopsy :

In general, any tissue aseptically removed incapable of giving usable cultures. Whatever the method of sampling, culturing and the various manipulations are carried out according to an identical protocol.

The fragment (may be a skin biopsy) is washed in physiological saline and divided with new scalpels into small explants of about 1 mm to 2 mm. on each side. The culture is carried out in Pyrex tubes with a distal depression in which a coverslip rests. All the glassware must be of Pyrex and washed very carefully, rinsed several times in distilled water and autoclaved. Culturing consists of first spreading a drop of chick plasma over the coverslip found in the tube. Then the explants (Fragment of the material) are placed on this plasma film. The chick plasma is obtained by puncturing the marginal vein of the wing and aspiration of the blood in a syringe containing 1 ml heparin. The blood is immediately centrifuged at 3000 r.p.m. for 10 min and the plasma stored in ice in a sterile sealed tube. At this moment the addition of a drop of embryonic extract coagulates the plasma and fixes the explants to the glass (The embryonic extract from eggs incubated for 9-10 days is, in fact, the supernatant of the crushed material diluted 1 : 1 with Hank's solution.

Then the tubes closed with rubber stoppers are left for several hours or if need be over night in an incubator at 37°C. Then the culture medium is added consisting of the following ingredients per tube 5 drops of human serum (preferably of group AB,) 5 drops of Hank's solution containing penicillin 200 U/ml, streptomycin 50 Ug/ml, chloramphenicol 5 Ug/ml and 2 drops of embryonic extract.

Transfer : after a period varying from 4 to 6 days, growth of a clown of fibroblasts around the explants is observed. The explants are then removed and transferred to other tubes according to the above technique if one wishes to continue the culture. The medium contained in the tubes with coverslips is then replaced by fresh medium of identical composition.

Accumulation of mitoses :

After a further stay of 36 h in the incubator, 3 drops of embryonic extract are added to each tube without changing the medium. The tubes are again placed in the incubator for an additional period of 16 hr. At this moment a very large number of mitosis should be observed.

Fixation :

Once withdrawn from the hypotonic solution the coverslips are placed for 45 min at laboratoty temperature in Carnay's solution. 3 parts chloroform, 1 part acetic acid and 6 parts absolute thyl alcohol.

Flattening of the preparations : The preparations taken out of carnoy's are left to dry in the open air.

Staining with Unna's blue : Immerse the coverslips (always with cells on top) for 10 min. in a solution of 1 part of Unna's blue solution and 4 parts of pure neutral water. Withdrawn and lightly rinse with neutral water to remove excess stain. Leave to dry in open air to complete desiccation (5-10 min) By this means is possible to avoid the standard ethyl alcohol passage which may remove part of the dye fixed on the chromosomes.

REFERENCES

Barr, M.L and *Bertram*, E G 1949 A morphological distinction between neurones of the male and female and the behaviour of the nucleolar satellites during accelerated nucleo protein sythesis Nature Lond 163,676

Belar, K, 1926 Der Formwechsel der Protistenkerne Erg Forstw Zool 6 : 235 - 652

Bisset, K A, 1951 : Genetical implications of bacterial cytology Cold Spring Harbor Symp Quant. Biol. 16 : 376 - 380.

Bisset, K.A., 1955 : The cytology and life history of bacteria, 2nd ed.. williams & Wilkins, Baltimre : 164 pp.

Bodmer, W.F. 1961 : Effects of maternal age on the incidence of conegnital abnormalities in mouse and man, Nature Lond. 190, 1134 - 5.

Carter, C.O., Evans, K.A. and *Stewart, A.M.* 1961 : Maternal radiation and Down's Syndrome (mongolism), Lancet ii, 1042.

Chicago Conference 1966 : Standardization in human cytogenetics. Birth Defects : Orig. Article Series 2 (1966) 2. National foundation, New York.

De Lamater, E.D. 1951 : A new cytological basis for bacterial genetics. Cold Spring Harbor Symp. Quant. Biol : 9 : 122 - 126.

Denever classification 1960 : Proposition d'un systems standard de nomenclature des chromosomes mitotiques humains. (Proposal For a new standard system of nomenclature of human mitotic chromosomes. In : Ann. Genet. 1, 35 - 38

Edwards, J.H., Harden, D.G., Cameron, A.H., Grosse, V.M. and *Wolff, O.H.* 1960 : A new trisomic syndrome, Lancet 1, 787 - 9.

Esquirol, J.E.D. 1938 : Des maladies mentales considérées sous les rapports medical, hygienique et medico-legal (Mental disorders considered from the medical, hyglenic and medico legal points of view) Paris, 1838.

Ferguson-Smith, M.A. 1962 : Abnormal sex ratio in the autosomal trisomy Syndromes. Lancet ii, 337 - 8.

Ford, C.E., Imes, K.W. Miller, D.J., Mittwoch, U., Penrose L.S., Ridler, M. and *Shapiro, A.* 1959 : The chromosomes in a patient showing both mongolism and the Klinefelter's Syndrome. Lancet i, 709, 10

Ford, E.C., 1960 : The chromosomes of normal human somatic and leukaemic cells. Proc. Roy. Soc. Med. 53, 491 - 93

Gangon, J., Ducharme, J.R., Archambault, L. and *Katyk-Longtin, N.* 1962 : Mosaic with 3 lines XY / XX / XXY in an intersex with no sex chromatin. Union Med. Canada 91, 1403 - 13

Grouchy, J., De Lamy, M., Thieffry, S., Arthuis, M. and Salmon, Ch. 1963 : Deletion of the short arms of a chromosome 17 - 18 C. R. Acad. Sci. Paris 256, 1028 - 9.

Guilliermond, A., 1941 : The cytoplasm of the plant cell. Chronica Botanica, Waltham. 247 pp

Hammerling, J., 1953 : Nucleo-cytoplasmic relationships in the development of Acetabularia. Intern. Rev. Cytology 2. 475 - 498.

Hall, B. 1962 : Down's Syndrome (mongolism) wit normal chromosomes Lancet ii, 1026 - 7

Hamerton, J.L. and *Steinberg, A.G.* 1962 : Progeny of D/G translocation heterozygote in familial Down's Syndrome Lancet i, 1407.

Jacobs, P.A., Baikie, A.G., Court Brown., W.M., Strong, J.A. 1959 : The somatic chromosomes in mongolism, Lancet i, 710

Kabarity, A., H. Schade (1969) : Inheritance of a chromosome with lateral Translocation (AS). German medical monthly Vol. IVX No. 3 S. 120

Kabarity, A., Lejeune, J., Püschel, E. and *H.H. Schade* 1972 : D/E Translokation bei Meningoonzophalozele, Mikrozephalie und Pseudoanenzephalie. Dtsch. med. Wschr. 97 (1972), 2009 - 2011

Klinefiter, H.F., Jr., Reifenstein, E.G., Jr. and *Albright, E.* 1942 : Syndrome characterized by gynocomastia, aspermatogenesis, without aleydigism, and increased excretion of follicle stimulating hormone. J. Clin. Endocr. 2, 615

Langdon-Down, J.H. (1866) : Observations on an ethnic classification of idiots, Clin. Lect. and Reps. London Hospital 3, 259

Lejeune, J, Turpin, R. and *Gautier, M.* 1959 : Le mongolisme, premier exemple diaberration autosomique humaine (Monoglism a first example of human autosomal aberration. Ann. Genet. Paris 1, 41 - 9

Lejeune, J. Lafourcade, J, Berger, R. Vialatte, J. Boeswillwald, M. Seringe, P. and *Turpin, R.* 1963 : Three cases of partial deletion of the short arm of a chromosome. 5 C.R. Acad. Sci. 257, 3098 - 3102

Levan and *Hsu, T.C.* 1959 : The human idiogram. Hereditas 45 : 665 - 674

Lewis, F.J.W., Poulding, R.H. and *Woods, G.*, 1963 : Possible deletion of the short arm of chromosome 18, Hum. Chr. Newsl. 10, 10

London Conference 1964 : The London Conference on the normal human karyotype 28 - 30 August. Ann. hum. Genet. 27 : 295 - 298

Mc Clintock, B, 1929 : Neurospora. I. Preliminary observations of the chromosomes of Neurospora Crassa. Amer. Journ. Bot. 32. 671 - 678

Mittwoch, U, 1952 : The chromosome complement in a mongolian imbecile, Ann. Eugen. 17, 37

Morgangni, J.B. 1749 : Anatomical Studies of the site and causes of disease Victor Rozier

Nowell, P,C. and *Hungerford, D.A.* 1961 : A minute chromosome in human chronic granulocytic leukaemia, Science 132, 1497

Ohno, S. Kaplan, W.E. and *Kinosita, R.* 1959 : Formation of the sex chromatin by a single X-chromosome in Liver cells of Rattus norvegicus. Exp. cell res. 18, 415

Patau, K., Smith, D.W., Thermann, E., Inhorn, S.L. and *Wagner, H.P.* 1960 : Multiple congenital anomaly caused by an extra chromosome, Lancet, i, 790 - 3

Penrose, L.S. 1933 : Mental defect, 1 vol. London

Penrose, L.S. 1961 : Parental age and non-disjunction in Human chromosomal Abnormalities, 116 - 122, Staples Press, London

Sager, R.A., Palade, E.G. 1954 : Chloroplast structure in green and yellow strains of chlamydomonas. Exp. Cell. res. 7, 584

Schull, W.J and *Neel, J.V.* : 1962 : Maternal radiation and mongolism, Lancet i, 537 - 8

Seguin, E. 1846 : The moral treatment, hygiene and education of idiots. 1 vol., Paris, J.B. Bailliere

Shuttelworth, G.E. 1885 : Mentally defencient children. H.K. Lewis, London Shuttelworth, spora crassa. Amer. Jour. Bot. 40 : 124 - 144

Shuttelworth, G.E. 1909 : Mongolian imbecility, Brit. Med. J. ii, 661 Singleton, J.R. 1953 : Chromosome morphology and the chromosome cycle in the ascus of Neurospora crassa. Amer. Jour. Bot. 40 : 124 - 144

Sjostrand, F.S. and *V. Hanzon* : Ultrastructure of gogli apparatus of exocrine cells of mouse pancreas. Exp. Cell. res. 7 (1954)

Smith, D.W., Patau, K., Therman, E. and *Inhorn, S.L.* 1960 : A new autosomal trisomy Syndrome : multiple congenital anomalies caused by an extra chromosome, I. Pediat. 17, 338, 45

Spalding, J.M.K. 1961 : Down's Syndrome (mongolism) Lancet ii, 1093

Tijio, J.H. and *Levan, A.* 1956 : The chromosome number of man. Hereditas 42 : 1 - 6

Turner, H.H. 1938 : A Syndrome of infantilism, congenital webbed neck and cubitus valgus, *Endocrinology*, 23, 566

Uchida, I.A., Patau, K. and *Smith, D.W.* 1961 : The dermal pattern of the new autosomal trisomy Syndromes. Amer. J. Dis. Child. 102, 588

Wagner, H.R. 1962 : Mongolism in Orientals, Amer. J. Dis. Child (Chicago) 103, 706 - 14

Zellweger, H. Makino, K. and *Abbo, G.* 1962 : Two cases of non-mongoloid trisomy G. Ann. Paediat. 199, 613 - 24

Consulted Books :

BarthelmeB, A. : Grundlagen der Vererbung. Akademische Verlagsgesellschaft.

Brogger, A. : Translocation in Human Chromosomes. Universitetsforlaget.

Dobzhansky, Th. : Evolution, genetics and man. Science editions.

Kabarity, A. : Principles of Cytology, Said Rafat Bookshop.

Kihlman,B. : Actions of chemicals on dividing cells. Prentice-Hall, Inc.

Lenz, W. : Medizinsiche Genetik. Georg Thieme Verlag Stuttgart.

Mc Kusick, V. : Human Genetics. Prentice-Hall of India private Ltd.

Schade, H. : Korrelationen zwischen Chromosomen-Aberrationen und klinischem Status. Ferdinand Enke Verlag, Stuttgart.

Swanson, C.P. : Cytology and cytogenetics : Prentice-Hall

Swanson, C.P. : The Cell. Prentice Hall. Inc.

Turpin, R. and Lejeune, J. : Human afflictions anc chromosomal aberrations. Pergamon Press.

Wilson, G.B. : The elements of Cytogenetics. Reinhold Book Corporation.

National Library Deposit
No 2476 / 1974