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MILITARY BLOOD BANKING

GENETICS FOR THE REFERENCE AND FORENSIC TESTING LABORATORY

A MONOGRAPH

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15 September 1971

US ARMY MEDICAL RESEARCH LABORATORY
Fort Knox, Kentucky 40121

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PREFACE

Blood group serology has been a mainstay in the reference and forensic testing laboratory engaged in problems of antigen-antibody identification, crossmatch problems, transfusion reactions, paternity testing, and the performance of various tests on blood crusts, stains, saliva, semen, hair, and bone.

This monograph has been prepared for supervisors, instructors, and workers engaged in routine, specialized, and forensic testing procedures, including research. The goal has been to offer a synoptic but careful review of the fundamental aspects of genetics, cytology, and cytogenetics. The monograph is intended for individual reading and as a text in an educational program to show the basic interrelationships of these disciplines and, equally important, how they apply to problems of blood group serology encountered daily in the laboratory.

Recent advances conerning polymorphism in the blood of man are presented. These findings will result in new tools and tests for the reference and forensic testing laboratory. Thus, the complete serologic profile of man as predicated by Dr. Karl Landsteiner is rapidly becoming a reality.

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F. CRICK

M. WILKINS

A. WIENER

P. LEVINE

The scientists selected for this frontispiece contributed significantly to the science of genetics as we know it today. The works of other notable scientists, not portrayed in this composite, are described in the monograph.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Alexander S. Wiener for reviewing this monograph and especially for his most helpful suggestions.

We are indebted to the Literary Executor of the late Sir Ronald A. Fisher, F.R.S., to Dr. Frank Yates, F.R.S., and to Oliver and Boyd, Edinburgh, for permission to reprint the table on page 68 from their book "Statistical Tables for Biological, Agricultural and Medical Research."

The photographs of meiosis (Prophase I) were kindly made available by Dr. S. Ohno, Director, and Dr. John H. Melnyk, Principal Investigator, Department of Biology, City of Hope Medical Center, Durante, California.

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GENETICS

Historical Review

In the application of genetic principles to the blood groups in man, we must look to the past to understand the fundamentals of several related disciplines. It was not until the twentieth century that the importance of chromosomes was established, although Waldeyer had observed them in 1880. Basic to this discovery was the formation of the cell theory of Matthias Jacob Schleiden and Theodor Schwann in 1838 and 1839. Through the studies of Max Schültze (1825-1874) protoplasm was shown to be the fundamental substance of plant and animal cells. These early observations in cytology were extended by Gregor Johann Mendel (1822-1884), "The Father of Genetics." He used mathematics (statistical analysis) to show that there is no blending of characteristics in the germ cells of hybrid peas, but rather a reappearance in a future generation of these characteristics in specific numbers (proportions). Mendel's experiments with the garden pea (*Pisum sativum*) formed the basis of the Mendelian Laws. Another important contribution was made by August Weismann (1834-1914) when he made the important distinction between somatic (body cells) and germ cells (gametes) in the role of inheritance.

In 1841, Remak described the process of direct cellular division, and it remained until 1879 for Strasburger and Schneider to report their observations of indirect division, which was also reported by Schleicher as karyokinesis and by Flemming as mitosis. In 1890, Waldeyer made the important observation that the formation of chromosomes (nuclear filaments) was a significant occurrence in mitosis. Flemming, Strasburger, van Beneden, and Rable at the same time showed that the chromosomes divide equally between daughter cells. In 1875, Oscar Hertwig reported his discovery of fertilization (penetration of a sea urchin egg by a sperm) and union (fusion) of the sperm and egg nuclei (pronuclei) after fertilization (penetration).

Cytoplasmic discoveries include the cell center (the centriole) by Boveri and van Beneden, the reticular or Golgi apparatus by Golgi in 1898, and the chondriome (granular and filamentous forms in the cytoplasm) described by Altmann (1890) and Benda (1897).

In 1871, F. Miescher isolated animal cell nuclei from which he extracted a substance that he named nuclein, now known as nucleic acid.

Whereas Mendel's principles of inheritance were formulated in 1865, it was not until 1900 that the scientific world recognized the importance of Mendel's work. Indeed, two scientists contemporary to Mendel may not have even known of Mendel's outstanding publication. They were Charles Darwin, author of "The Origin of Species by Means of Natural Selection" in 1859, and Sir Frances Galton who used mathematics in studies of human heredity (medical genetics).

However, at the beginning of the twentieth century, the pace of experimental work quickened and Mendel's Laws were rediscovered independently by three scientists at the same time (1900). Carl Correns in Germany, Hugo De Vries in Holland, and E. von Tschermak in Austria made known their findings confirming Mendel's original observations and it was at this time (1900) that genetics was recognized as a science.

Cytology and cytogenetics developed rapidly in the first half of the twentieth century. In 1902, chromosomal behavior was noted by Correns, Sutton, and Boveri. At Columbia University, the teachings of Dr. Edmund B. Wilson (1848-1935) on chromosomes and heredity played an important role in this expansion of cytology and cytogenetics. Among this group at Columbia was the Nobel Prize recipient, Dr. Thomas Hunt Morgan (1866-1945), who used the fruit fly, *Drosophila melanogaster*, in genetic studies. Through their contributions in genetics, cytology, and cytogenetics, the students from the Columbia University era of Wilson and Morgan became scientists of renown.

The blending of genetics and cytology led to the recognition of meiosis (reduction in chromosome number) in early 1900. Nevertheless many problems remained to be defined before chromosomes were accepted to be the carriers of inheritable characters or units. In 1911, genetic nomenclature was defined much as we know it today by Johannsen when he introduced the terms genotype and phenotype. The genotype referred to inherited genes and this in turn determined the phenotype, expression or characteristic of an organism. The term gene was created by Johannsen who defined it as the following:

> The gene is nothing but a very applicable little word, easily combined with others, and hence may be useful as an expression for the "unit factors," "elements," or "allelomorphs" in the gametes, demonstrated by modern Mendelian researches.

Returning to our Columbia University group under T. H. Morgan, in 1910, A. H. Sturtevant, H. J. Muller, and C. B. Bridges started the famous studies on the fruit fly, *Drosophila melanogaster*. During the next two decades they showed that chromosomes do carry the genes and these genes are arranged linearly along the length of the chromosomes in bead-like fashion.

By 1940 cytogenetic methods had provided a better understanding of the mechanism of heredity. The time was ripe for the biochemical geneticists to begin serious study of gene function. We recall that Mieschер had discovered nucleic acid in the nucleus many years prior to this era. It was now appreciated that two kinds of nucleic acid existed: deoxyribonucleic acid (DNA) found mainly in the nucleus and ribonucleic acid (RNA) found in both the nucleus and the cytoplasm. Investigators now wanted to know two things: (1) How do genes act to control metabolism? (2) What is the chemical nature of genes (genetic material)?

In 1941, Beadle and Tatum reported that they had isolated mutants of the mold, *Neurospora*, which were unable to carry out certain metabolic steps. From this finding came the one gene - one enzyme hypothesis.

In 1924, Feulgen reported that his stain was specific for DNA and in 1936, Caspersson, using spectrophotometric technics, proved that chromosomes contain a high concentration of nucleic acid. Application of the staining technic of Feulgen showed further that this nucleic acid is specifically DNA.

The rapid pace of research attracted scientists other than biologists and chemists. This includes the work of Avery, MacLeod, and McCarty and the role of DNA in pneumococcus. That DNA was, indeed, the important genetic material was further supported by Delbrück's finding in 1952 that the hereditary substance of T series of bacteriophages infecting *E. coli* was the DNA. Finally, in 1955 Allfrey, Mirsky, and Stern showed that the DNA content of sperm is one-half that found in diploid somatic cells.

With this background the structure of DNA was ready for serious investigation by numerous investigators. The list includes Maurice Wilkins, Linus Pauling, Joshua Lederberg, John Kendrew, Max Perutz, Rosalind Franklin, Erwin Chargaff, and, of course, James D. Watson and Frances Crick, using X-ray diffraction and other technics. It was Crick and Watson, in 1953, who proposed a structure for DNA. They, with Maurice Wilkins, were awarded the Nobel Prize for Medicine and Physiology in 1962.

GENETICS

The blood groups, factors, and other polymorphisms of blood have contributed much to the study of human biology, and in particular to advances in the field of genetics. The exciting story begins when Gregor Mendel's original genetic findings (1865) were rediscovered independently in 1900 by DeVries, Correns, and Tschermak. It was then that the scientific basis for new disciplines was created. These include the rapid expansion of genetics, cytology, blood group serology, and immunology into the sciences we know today: cytogenetics, immunohematology, immunochemistry, immunogenetics, and immunoanthropology.

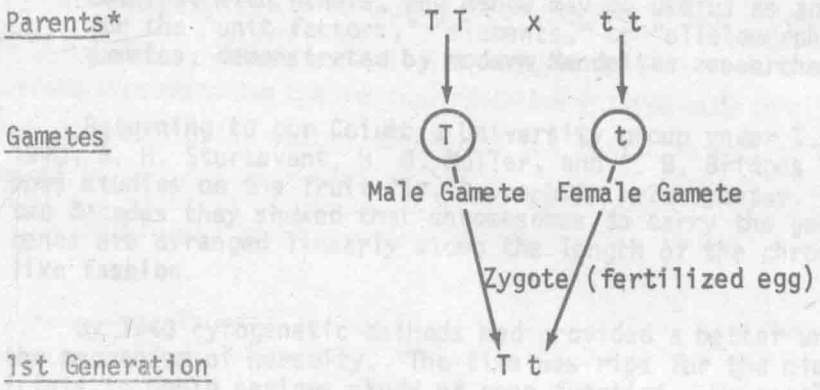
Mendel's Laws

Mendel crossed pure lines of garden peas (*Pisum sativum*) in a series of experiments and by following various characteristics in the progeny of the crosses for several generations, he formulated several hypotheses based upon his observations. These have since become known as Mendel's Laws:

Law of Segregation. Each member of a single pair of genes passes to separate gametes.

Law of Independent Assortment. Members of different pairs of genes segregate to gametes independently of other pairs.

The classic technic for illustrating crosses is the assignment of letters for factors such as T for Tall and t for dwarf:



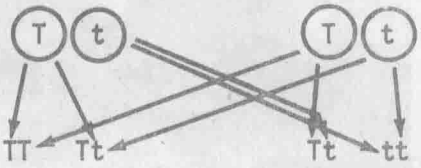
T = Tall (Dominant Gene).
t = Dwarf (Recessive Gene).
* = Homozygous for each trait.

Gene Expression (senses) = Tall (phenotype)

Genetic Symbols Tt (genotype)

Continuing with a Heterozygous Cross Tt x Tt

Gametes



2nd Generation

F1

3 Tall

1 Dwarf

	T	t
T	TT#	Tt#
t	Tt#	tt°

#3 Tall

°1 Dwarf

Mitosis and Meiosis

Cells divide and during this process the cell nucleus goes through a series of transformations which have significance in somatic or body cells for growth and repair of tissue. This is known as mitosis. Somewhat similar, but far more complicated, is the series of mitotic and meiotic processes that occur in the germ cells (gametes).

There are four unique structures in the mitotic figure:

1. Centrosome.
2. Chromosomes.
3. Aster.
4. Spindle fibers.

The mitotic process has the following stages:

1. Interphase.
2. Prophase.
3. Metaphase.
4. Anaphase.
5. Telophase.

Figures 1-4

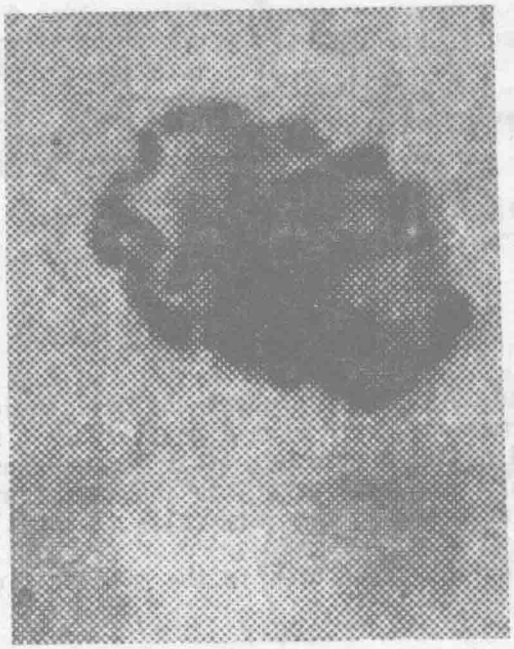


Fig. 1. Prophase.

Fig. 2. Metaphase.

Fig. 3. Anaphase.

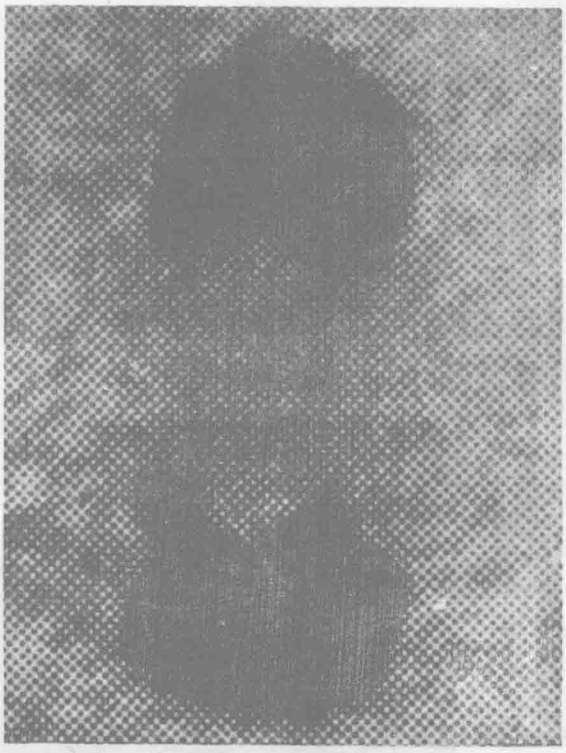


Fig. 4. Telophase.

Meiosis

Meiosis is divided into two phases: meiosis I with a significant first prophase consisting of five stages, and meiosis II.

1st Prophase

a. Diagrammatic (Figures 5-9).

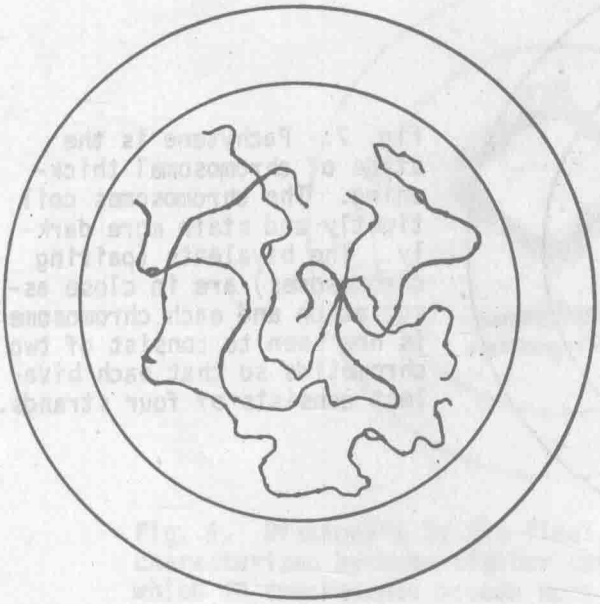


Fig. 5. Leptotene is characterized by the first appearance of the chromosomes as thin threads. Although the DNA has duplicated prior to this stage, the threads still appear single.

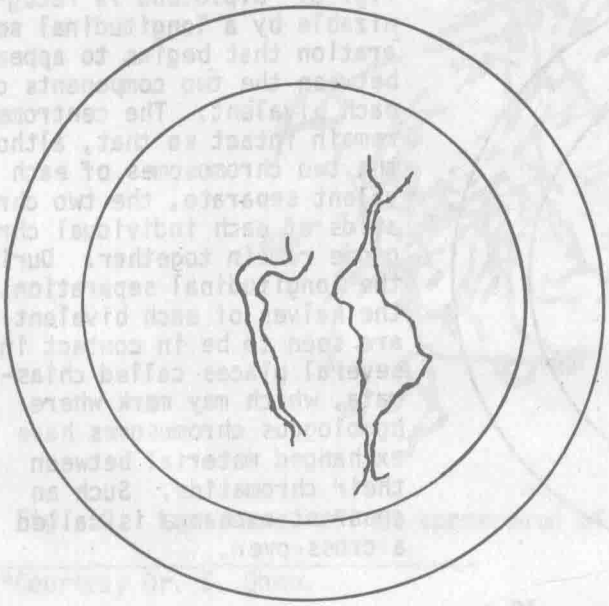


Fig. 6. Zygotene is characterized by pairing (synapsing) of the homologous chromosomes. The two members of a pair lie parallel to each other in point-for-point association to form bivalents. This pairing does not occur in mitosis.