The Chromosome Complements of Human Somatic Cells[1]

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HISTORICAL

THE CHROMOSOME NUMBER OF MAN has been a question of unusual interest to biologists ever since it became known that these small deeply staining bodies are constant in number for a given species of animal or plant (cf. Stern, 1959). Arnold in 1879 published drawings of human tumor cells in division. Flemming in 1881–82 demonstrated mitosis from a corneal cell in which some twenty large and small, V, J and rod-shaped chromosomes were recognizable. The first attempt to determine the number of chromosomes in human cells was that by Hansemann, who, in 1891, reported three cells from "normal human tissues" with 18, 24 and more than 40 chromosomes, respectively. Since this early date, a number of other investigators, using both germinal and somatic tissues, have attempted to determine the exact number of chromosomes in man. Actual counts have ranged from 16 to 32, and there was no unanimity of opinion as to the true number. De Winiwarter in 1912 claimed that there were 47 chromosomes at metaphase in spermatogonia and 23 autosomal bivalents plus an unpaired X in primary spermatocytes. Wieman (1917) was the first to report the presence of XY chromosomes; Evans (1918) was the first to find a diploid number of 48 chromosomes in spermatogonia. In 1921 Painter reported in *Science* the presence of a small Y chromosome in males. In this same paper he stated that in spermatogonia "the counts range from 45 to 48 apparent chromosomes, although in the clearest equatorial plates so far studied only 46 chromosomes have been found." He went on to conclude that the diploid chromosome number in man is either 46 or 48. Two years later, Painter came to the conclusion that the correct diploid number was 48 in both sexes. It is interesting to note, however, that the materials used in his studies were testicular biopsies from three (two Negroes and one White) insane individuals. Whether or not chromosomal variations were present in these individuals was difficult to determine. In this same paper, noting

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the occurrence of giant or tetraploid spermatogonial cells, Painter suggested the possibility of an XXY hemaphrodite originating in a manner similar to the findings by Bridges (1922) in which XXY flies were derived from triploid *Drosophila*.

Painter's conclusion that the diploid chromosome number of man is 48 was supported by most authors in the following decades. However, de Winiwarter and Oguma (1926) adhered to the view that there was only a single sex-chromosome in spermatogonia. Koller's (1937) account of the behavior of the sex chromosomes during spermatogenesis conclusively proved the existence of the XY sex chromosome condition. From then on, the value of $2n = 48$ was generally accepted.

Early in 1956, Tjio and Levan made the surprising announcement that consistent counts of 46 chromosomes were obtained in lung fibroblast-like cell cultures established from four aborted Swedish embryos. In the same year, their counts were confirmed by Ford and Hamerton in testicular preparations from three British individuals.

An earlier solution to the question was probably prevented by problems of technical nature. Cytologists had been greatly handicapped in the study of mammalian and avian chromosomes by the fact that most species possess a large number of small chromosomes which usually crowd the metaphase plate and make counting and observation of individual chromosomes very difficult. With the exception of de Winiwarter, practically all early investigators working upon the spermatogenesis of man have used stale tissue—such as that obtained from executed criminals—in which the testis had remained in the body for some time after death. Painter (1923) stressed the cytological advantages of using freshly biopsied materials which were immediately fixed. Evans and Swezy (1929) expressed a similar view by pointing out that postmortem changes could alter the chromosome picture, giving rise to clumping, within ten or fifteen minutes between cessation of heart beat and autopsy. A determined effort was made by them to reduce the time intervals between death and fixation of cells. Their finest results came from cases in which testicular fragments were removed at the foot of the gallows and fixed in less than one minute. Nevertheless, the drawings of the best metaphase plates in these studies still show a considerable chromosome crowding and overlapping. A similar situation existed in several earlier studies in which adult somatic and embryonic tissues were used. However, the remarkable results obtained by these earlier investigators under the circumstances existing at the time command admiration and respect.

Before describing the number and morphology of human metaphase chromosomes in the light of modern studies, which are based primarily on somatic cells, the importance of studying chromosome morphology and behavior in spermatogenesis should be emphasized. The classical studies of de Winiwarter, Painter, Evans and Swezy, Koller, and others in establishing metaphase morphology of various chromosomes, in analyzing the sex chromosomes, and in following meiotic chromosome behavior certainly represent major triumphs of human cytology. The chromosomes in spermatogonia and spermatocytes are im-

portant as sources for comparison and verification. In addition, in view of the fact that the structure of metaphase chromosomes has only limited use for the detail required for a cytogenetic map, studies on pachytene chromosome structure such as those initiated by Schultz and St. Lawrence (1949) and followed by Yerganian (1957) and Kodani (1957a) should be actively pursued.

RECENT TECHNICAL ADVANCES IN THE STUDY OF HUMAN CHROMOSOMES

The rapid development of tissue and cell culture techniques in the past two decades has stimulated an experimental attack on many fundamental and applied problems of cell biology. The utilization of cell culture techniques also provides a number of advantages for cytological studies. For example, cell cultures present extremely favorable conditions for direct observation and photography of cells in the living state. The fact that the growth zone often consists of only a single layer of cells facilitates experimental treatments as well as cytological fixation and staining. Mitotic activity is usually more enhanced *in vitro* than *in vivo*, and it can be subjected to experimental control. Cell cultures also have a great advantage over sectioned histological preparations, which were used exclusively in earlier studies of human chromosomes, in that the cells in culture are more flattened and stretched on the substrate and no cellular material is lost or added by sectioning. Furthermore, cell culture techniques make it possible to compare chromosome constitutions of various tissues of the same individual, particularly useful in connection with the problem of somatic mosaicism. One possible criticism of the use of cultured cells for chromosome studies may be the known phenomenon of karyotypic changes which occur during growth *in vitro* (cf. Hsu, 1959). However, it is now possible to maintain a euploid condition of human cell lines for a considerable length of time without obvious chromosomal alterations (Tjio and Puck, 1958a; Chu and Giles, 1959a). Moreover, in the case of short-term cultures, such as those of bone marrow cells, this question does not arise.

As early as 1929, Kemp, among others, had used tissue cultures of human embryonic heart, liver and spleen to study chromosomes. Unfortunately, this approach was for many years almost completely neglected by cytologists. Similarly, the effect of a hypotonic medium in spreading chromosomes was not realized until 1952 when Hsu and Hughes accidentally and independently rediscovered this simple and very useful technique, which had been noted earlier by Eleanor Slifer (1934) and Margaret Lewis (1934). The favorable results thus obtained have since stimulated a great number of investigations on the chromosome cytology of mammalian and avian species. This technical advance also led Tjio and Levan to the finding of the new chromosome number in man, thus opening the modern reinvestigation of the subject.

The prime difficulty encountered in human cytology in the past has been the scarcity of controlled material and its capricious availability. This is particularly the case when surgical procedures are involved. While the source of material for study of human spermatogenesis is still limited, somatic cells offer a ready and favorable source of materials for chromosome and other studies. Tech-

niques are now available for short or long term cultivation of human cells of different origin derived by biopsy or autopsy from both adult and young. The development of culture techniques for chromosome analysis by Ford and his associates (1958), who have used human bone marrow cells; by Puck, Cieciura and Robinson (1958), Lejeune, Gautier and Turpin (1959a,c), Fraccaro, Kaijser and Lindsten, Harnden (in press) and others, who have used tiny skin biopsies; and by Hungerford and co-workers (1959), who have used leukocytes from peripheral blood, are particularly noteworthy. It appears that not only is an extensive cytological survey of human populations possible, but concentrated investigations on members of families with certain particular genetic constitutions are also feasible.

THE KARYOTYPE OF MAN

Since 1956, the chromosome number of 46 has been found in testicular preparations and in cultures of normal tissues of different origins by a number of workers (Ford, Jacobs and Lajtha, 1958; Tjio and Puck, 1958a,b; Chu and Giles, 1959a). Chu and Giles (1959a) concluded that every pair of homologous chromosomes of the human complement can be individually recognized. Furthermore, statistical analyses indicate that homologous autosomes from cells of the same or different individuals do not differ significantly either in relative length or in centromere position. The only difference between chromosome complements of the two sexes resides in the sex chromosomes. There are no significant differences among the X chromosomes or among the Y chromosomes from different individuals.

An idiogram of the human karyotype based on the results of chromosome measurement has been presented (Fig. 1). The autosomes, designated in Arabic numerals, of the human haploid chromosome complement are arranged in order of decreasing total length and of relative centromere positions. If two chromosomes are of equal length, the one having the more nearly median centromere is placed first. The sex chromosome pair, X and Y, is placed at the end. This

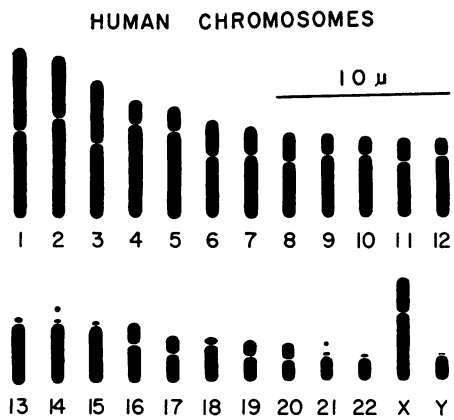

FIG. 1. Idiogram of the human haploid chromosome complement, including the sex pair.

system is adopted because it is the simplest and the least arbitrary. This idiogram is in almost complete agreement in cytological detail with those independently proposed by Tjio and Puck (1958b), by Ford, Jacobs, and Lajtha (1958), by Lejeune, Turpin, and Gautier (1959b), by Böök, Fraccaro, and Lindsten (1959), and others. The only major difference is in the systems employed in numbering the chromosomes. The need for a unified system to avoid confusion and to serve as a working basis is apparent. Hope was generally expressed, during recent discussions with a number of these workers, that a uniform nomenclature will soon be adopted.

The total number of cases in which the diploid number 46 has been recorded is now well over 200. There remain, however, the reports by Kodani (1957a, b; 1958a, b) of supernumerary chromosomes in man resulting in chromosome counts of 46, 47 and 48. Four possible explanations of these results, which are in disagreement with all other recent findings, may be considered. Firstly, the possibility of technical error cannot be dismissed. Secondly, on the basis of Kodani's observations, the basic 46 chromosomes, recognizable by their size and shapes, are common to all individuals. In those with one or two supernumeraries, no multiple chromosome association has been found. These facts seem to rule out the explanation of the origin of chromosome polymorphism by a Robertsonian type of chromosome evolution. By the same token, the lack of homology between these supernumeraries and any chromosomes of the basic set, and the absence of multivalent association in meiosis, make the explanation of the extra chromosomes as trisomics or tetrasomics unlikely.

Thirdly, despite the absence of any known cases in mammals, there exists a remote possibility of somatic elimination of supernumeraries, which would prevent their detection in individuals from whom somatic cells alone have been studied. However, the diploid number of 46 has been repeatedly found in primary spermatocytes and spermatogonial cells by Ford and associates (1958). In cases where both somatic and germinal tissues of the same individuals have been examined, there is no evidence of chromosome elimination (cf. Chu and Giles, 1959a). Examination of a number of embryonic tissues has also failed to show any evidence of chromosome elimination, even at the early stages of development (Chu and Giles, 1959a and unpublished).

The fourth possible explanation of Kodani's results may be that differences exist in various human populations. According to his observations, supernumeraries seem to occur with a much higher frequency in certain Oriental populations, and, in one instance, supernumeraries were found in a Caucasian individual (Kodani, 1958b). It would be interesting to examine the somatic chromosomes of those reported to have supernumeraries. Additional independent examinations of individuals from these populations is highly desirable in order to clarify this point. Recently, Makino and Sasaki (1959) reported six Japanese cases in which cultured embryonic cells were used, all showing 46 somatic chromosomes.

On the basis of present overall evidence, it is reasonable to conclude that 46 is the correct basic diploid chromosome number in man. The author wishes to stress the point that there is a great degree of constancy, in both chromosome

number and morphology, of this basic set in normal individuals from the different human populations which have been studied so far. This information is essential for the analysis of radiation-induced human chromosome aberrations (Chu and Giles, 1959b), as well as for the study of naturally occurring human chromosome variations. On the other hand, it should also be kept in mind that chromosome studies in man are just beginning, and variations in number as well as in morphology and structure are to be expected here as in other more thoroughly studied species. Indeed, in this year, there have been significant discoveries of some most interesting chromosomal variations found in individuals with various hereditary conditions. The genetical and clinical implications of these will be explored by the next speaker, who is among those responsible for these discoveries.

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