Human Chromosome Complements in Normal Somatic Cells in Culture

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THE STUDY OF HUMAN CHROMOSOMES has received considerable attention and interest in recent years especially since the discovery of a new diploid chromosome number—46 (Tjio and Levan, 1956; Ford and Hamerton, 1956) and subsequent reports of the possible existence of chromosome number variation in man (Kodani, 1957a, b; 1958a, b). In view of the significance of the problem, the need for independent observations on various human populations is apparent. Furthermore, detailed information on human chromosome cytology appears essential in connection with experimental cytogenetical studies of human cells *in vitro* involving such problems as mutation, genetic recombination, chromosome structural changes induced by physical and chemical agents, and carcinogenesis.

In the course of studies in this laboratory using tissue culture techniques for an analysis of radiation-induced aberrations in human chromosomes, biopsy materials from a number of human individuals have been established in culture. The present paper reports the results of chromosome number determinations in these materials. In addition, a detailed analysis is presented of the human karyotype based on studies of the chromosome complements of these normal human somatic cells.

MATERIALS AND METHODS

Source of materials.—Surgical biopsies and fetal tissues from a number of human subjects have been obtained through the kind cooperation of members of the Departments of Surgery, Obstetrics and Gynecology, and Internal Medicine at the Yale University School of Medicine. Table 1 lists these materials in terms of the specimen number, tissue of origin, and sex, age, and race of the individuals involved. Most individuals, especially the new-born babies and young children, were normal and healthy. Specimen Y7 was from a woman with idiopathic thrombocytopenic purpura. Histological study of this splenic biopsy showed normal morphology. The bone marrow specimen Y9 was from a rib removed during thoracic surgery. Specimens Y15 and Y24 were from kidneys removed because of hydronephrosis; specimen Y16 came from a testicular biopsy involving a patient diagnosed with retroperitoneal lymphosarcoma. In this case, aspermatogenesis persisted for a brief period following radiation therapy. The biopsy was obtained after apparent recovery and after the man had fathered a child. Parallel histological and cytological studies indicated normal

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TABLE 1. A SURVEY OF HUMAN SOMATIC CHROMOSOME NUMBERS BASED ON CELLS IN TISSUE CULTURES FROM 34 DIFFERENT INDIVIDUALS

Specimen No.	Source	Sex and age of individual	Race[a]	Chromosome number of apparently intact diploid cells		
				45	46	47
Y 7	spleen	F, 58	W		2	
Y 9	bone marrow	F, 63	W		8	
Y 13	tonsil	M, 6	W		6	
Y 15	kidney	F, 21	W		97[b]	
Y 16	testicular fibroblast	M, 34	W		20	
	spermatocyte	"			4	
—	muscle (Sloan-Kettering)	sex unknown, embryonic	unknown		3	
—	thyroid (Tulane)	male pseudo-hermaphrodite, 28	W		8	1
Y 24	kidney	M, 75	W		12[b]	
Y 25	skin-muscle	M, 2 month embryo	W	1	76[b]	
	kidney	"			2	
	lung	"			12	
Y 32	foreskin	M, new born	W		1	
Y 33	foreskin	M, new born	N		3	
Y 34	foreskin	M, new born	W		37	4[c]
Y 40	foreskin	M, new born	W	2	10	
Y 41	foreskin	M, new born	N		22	
Y 45	foreskin	M, new born	W		13	
Y 46	foreskin	M, new born	W		15	
Y 47	foreskin	M, new born	W		10	
Y 49	foreskin	M, new born	N		17	
Y 58	foreskin	M, new born	W		5	
Y 60	foreskin	M, new born	W		18	
Y 66	bone marrow	M, 82	W		8	
Y 86	foreskin	M, new born	W		6	
Y 89	foreskin	M, new born	W		28[b]	1
Y 90	foreskin	M, new born	W		9	
Y 91	foreskin	M, new born	W		2	
Y 93	foreskin	M, new born	W		7	
Y 115	foreskin	M, new born	W		24	
Y 117	foreskin	M, new born	W		31	
Y 121	foreskin	M, new born	W		17	
Y 128	foreskin	M, new born	W		20	
Y 140	foreskin	M, new born	N		9[b]	
Y 141	foreskin	M, new born	W		12	
Y 144	foreskin	M, new born	W		4	
Y 148	foreskin	M, new born	W		33	

a) W (American Whites); N (American Negroes).
b) includes 1 to 3 cells having 46 diplochromosomes.
c) see text for discussion of these counts.

morphology and spermatogenesis. The bone marrow specimen Y66 was taken by ilium puncture from a man with pernicious anemia.

The embryonic muscle cell culture was obtained from the laboratory of Dr. Alice E. Moore, Sloan-Kettering Institute for Cancer Research, New York City. The thy-

roid culture was initiated in the laboratory of Dr. William Sternberg at the School of Medicine, Tulane University, New Orleans, Louisiana. It came from a thyroid biopsy involving a 28 year old male pseudohermaphrodite in a family studied genetically by Dr. Sternberg and Dr. H. W. Kloepfer of the same University. The generous cooperation of the Yale Medical staff and of others who supplied materials is gratefully acknowledged.

Tissue-culture techniques.—Immediately after removal, tissue specimens were placed in sterile moist containers including a gauze soaked with physiological saline (Ringer's solution). The bone marrow specimens were injected first into a vial containing Hanks' balanced saline with heparin (1:20,000). The specimens were either cultured immediately or stored at 4° C for a few hours to overnight before culturing.

In establishing cultures, the specimens were first rinsed with Hanks' balanced saline at room temperature, cut into pieces of 1–2 mm^3 in size, and trypsinized according to the method of Rappaport (1956). For cell dissociation, 0.2 per cent trypsin (Difco 1:250) in phosphate-buffered (Na_2HPO_4—KH_2PO_4) saline was generally used. Trypsinization was continued for 5–10 minutes with embryonic materials and for 30–60 minutes with other materials after which a sample of cell suspension was taken for microscopic examination and cell counts in a hemacytometer. The cells were then centrifuged and resuspended in growth medium. For bone marrow, the cells were centrifuged directly and resuspended in growth medium.

The growth medium used contained 75 per cent Eagle's synthetic supplement (Eagle, 1955; Eagle *et al*, 1956), 20% non-pooled human serum (Obtained from the Philadelphia Serum Exchange, 1740 Bainbridge Street, Philadelphia, Pennsylvania.) with blood type predetermined and 5 per cent beef embryo extract ultrafiltrate (Obtained from Microbiological Associates, Inc., Bethesda, Maryland.).

A cell suspension of 1–3 $\times$ 10^5 cells per ml. was inoculated into either 3 oz. prescription bottles or depression test tubes (Leighton tubes) containing removable clean sterile 30 x 11 mm cover slips, and incubated at 37° C. Viable cells settled within 12 hours and began to proliferate on glass. The medium was changed after two to three days. In about a week when the cell population in culture bottles had increased approximately 5 to 10 fold, the cells were dissociated by means of trypsinization and subcultured in the same medium.

It is often desirable to test each individual batch of serum before use for a particular cell line, since serum toxicity of unknown nature may affect cell growth or cause undesirable cellular and chromosomal alterations. Although most tissues are potentially capable of initiating cell proliferation *in vitro*, not every biopsy specimen was established as a cell line with active growth. Growth rates also vary in different cases. Cytological studies were made as soon as feasible, usually at the second transfer—about one week after the initiation of a culture. However, many cell lines were reasonably stable and exhibited no obvious cellular or chromosomal alterations after three to six months in culture. Morphologically, most cells were fibroblast-like, but the cells from kidney cultures were epithelium-like.

Cytological techniques.—The general procedure in preparing slides for chromosome studies was similar, with a few modifications, to that previously described (Chu and Giles, 1957, 1958a). In order to accumulate metaphases, colchicine pretreatment was employed. A final colchicine concentration of 10^{-6} gm. per l. was incorporated into

the culture medium 2–5 hours before fixation. Chromosome spreading was achieved by incubation of cells on cover slips in hypotonic saline (5 per cent full formula Hanks' with 95 per cent Hanks' minus NaCl) at 37° C for 15 to 20 minutes. The cells were then fix-stained by inverting the cover slip over a drop of aceto-orcein (1 per cent of natural orcein (Obtained from G. T. Gurr, Ltd., London, England.) in 45 per cent acetic acid containing 2 per cent by volume of 1N HCl) on a clean slide. The preparation was placed between layers of bibulous paper and pressed very lightly. It was then either sealed with Krönig cement (Obtained from Riedel-de Haën AG. Seelze bei Hannover, Germany.) as a temporary preparation or rendered permanent by the following procedure: the preparation was dipped in liquid nitrogen for 3–5 seconds; the cover slip was carefully separated from the slide by prying off with a razor blade; dehydration and clearing followed by passing both the slide and cover slip through a series of baths—(1) pre-chilled 1:1 acetone-tertiary butyl alcohol (TBA) for 30 min. at −10° C (in freezer compartment of a refrigerator), (2) TBA containing 1 per cent 1N HCl for 15 sec. at room temperature, (3) 1:1 xylene-TBA for 1–2 min., and (4) two successive xylene baths for 2 min. each; the preparation was then mounted in Canada balsam.

Chromosome studies.—Chromosome counts, idiogram analyses, and photomicrographs were made in the same manner as previously described (Chu and Giles, 1958a). All chromosome counts were exact counts from single intact cells. Chromosome measurements were made either from camera lucida drawings of individually centered chromosomes or from enlarged prints (ca 3000×). Measurements were estimated to the nearest 0.5 mm of a metric ruler and recorded as "units". Standard errors were calculated and the significance of differences tested at the 95 per cent level of fiducial probability.

RESULTS

Chromosome number.—Somatic chromosome counts have been made of cells in culture derived from 34 different human subjects (Table 1). Regardless of race, sex, age, or tissue, in all cases the diploid chromosome number was 46. In one individual (Y16), 4 primary spermatocytes were analysed in which 23 pairs of chromosomes were clearly seen at metaphase I. This is in agreement with the diploid chromosome number determined in testicular fibroblasts *in vitro* derived from the same individual. In one of the fetuses (Y25) chromosome counts were the same in cells derived from skin-muscle of a fore limb, kidney, and lung. Of particular interest is the case of a male pseudohermaphrodite. Buccal cell smear preparations were sex chromatin-negative indicating the presumptive genetic sex as male. In agreement with this observation, chromosome determinations using thyroid biopsy material grown *in vitro* showed that this individual has 46 chromosome including an X and a Y chromosome.

In a few exceptional cells, deviations of one chromosome from normal were observed. These counts may represent either instances of somatic aneuploidy, originally present or induced during cultivation, or artifacts resulting from errors in technique. In one instance, Y34, an actual chromosome number alteration was observed during cultivation. Among 29 cells analyzed at the fourth transfer—two weeks

after the initiation of the culture—only one cell was suspected of having an extra chromosome. Later at the ninth transfer (after nearly three months in culture) three such cells were found among an additional 12 cells examined. The extra chromosome had a submedian centromere, and was approximately the size of chromosome number 8 (see below). The chromosomes in the normal complement showed no visible change.

In some cultures tetraploid cells and cells with diplochromosomes were present in which exact chromosome counts were possible. The overall frequencies of these cells was well below 5 per cent. The results of chromosome counts in such cells confirmed the basic diploid number, i.e., 46.

Chromosome morphology.—Representative photomicrographs of human chromosomes from individual somatic cells in culture are presented in Figures 1 and 2. Camera lucida drawings of five additional cells derived from different individuals of both sexes are shown in Figures 3 and 4. Homologous chromosomes and the sex pair were identified initially by chromosome matching and then more accurately by measurements. The method of Rothfels and Siminovitch (1958) for identification of homologous chromosomes was employed. The total length and arm lengths of each chromosome were measured and the arm index calculated by dividing the length of the short arm into that of the long arm. Utilizing these data, homologues were convincingly paired in each individual cell. The X and Y chromosomes were first recognized in male cells and the pair of X chromosomes then identified in female cells. There was no evidence of chromosomal heteromorphism among the autosomes.

At metaphase, the longest chromosome measures from 8 to 10 microns, and the smallest, the Y, from 1.2 to 1.5 microns. Since the length of a chromosome depends on the state of condensation and is also affected by the colchicine treatment employed in these studies, it is desirable to express relative lengths in terms of percentage of the haploid complement. Furthermore, in order to avoid the differences between sexes, these percentages were calculated on the basis of total autosome length only. In Table 2, the relative lengths of individual human chromosomes are expressed in terms of their mean percentage of the total length of the haploid autosomal complement calculated from measurements of one female cell and three male cells from different individuals, i.e., the average of eight homologous chromosomes. The mean arm index of all chromosomes, based on measurements from these and three additional male cells each from a different individual, is also included. It is evident that every pair of homologous chromosomes of the human complement can be individually recognized. Furthermore, appropriate statistical tests show that homologous autosomes from cells of the same or of 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. Similarly, there are no significant differences among the X chromosomes or among the Y chromosomes from different individuals.

An idiogram of the human karyotype (Fig. 5) has been constructed on the basis of the above results. The chromosomes of the haploid set are arranged in order of decreasing lengths, following the classical procedure. If two chromosomes are of equal length, the one with the more nearly median centromere is placed first. Arabic

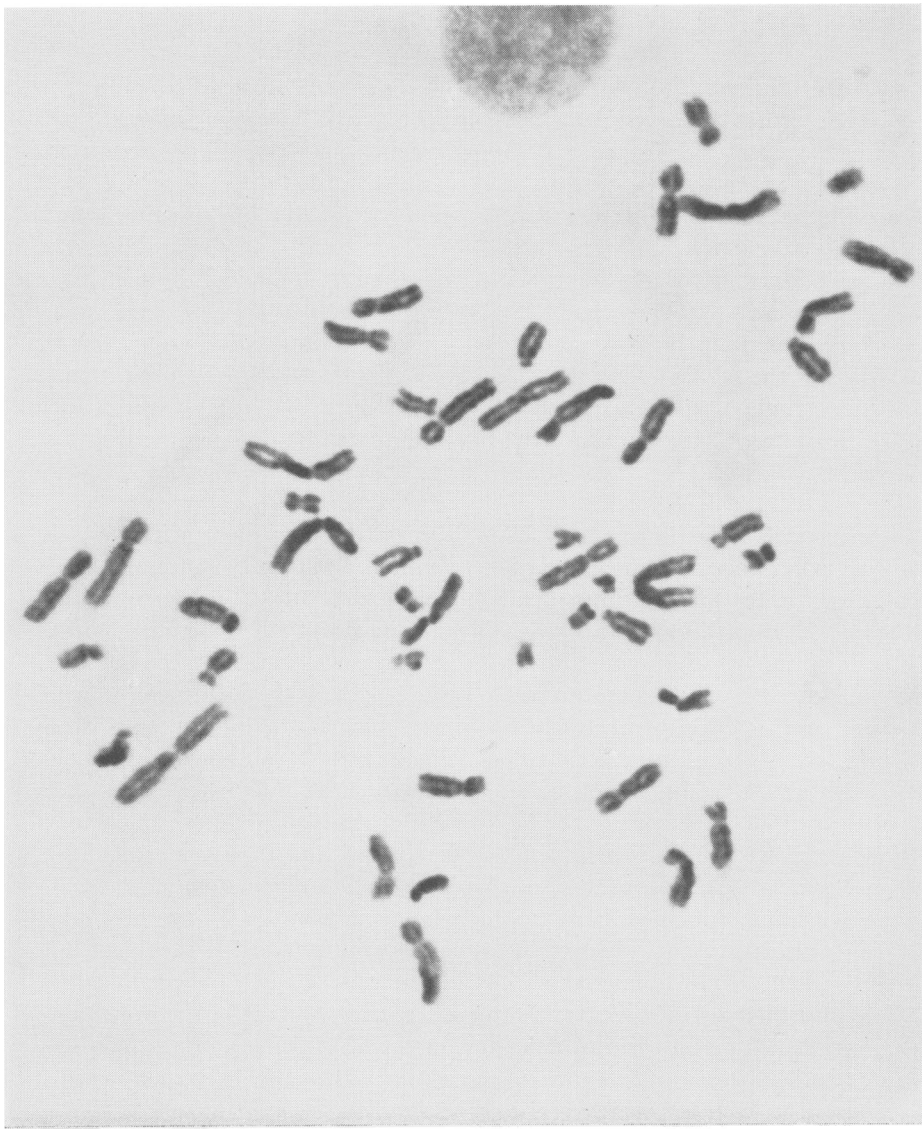

FIG. 1. Normal diploid complement (2n = 46) of a cell in a culture derived by foreskin biopsy (specimen Y 89) from a new born White male. Acetoorcein stain. 2100×.

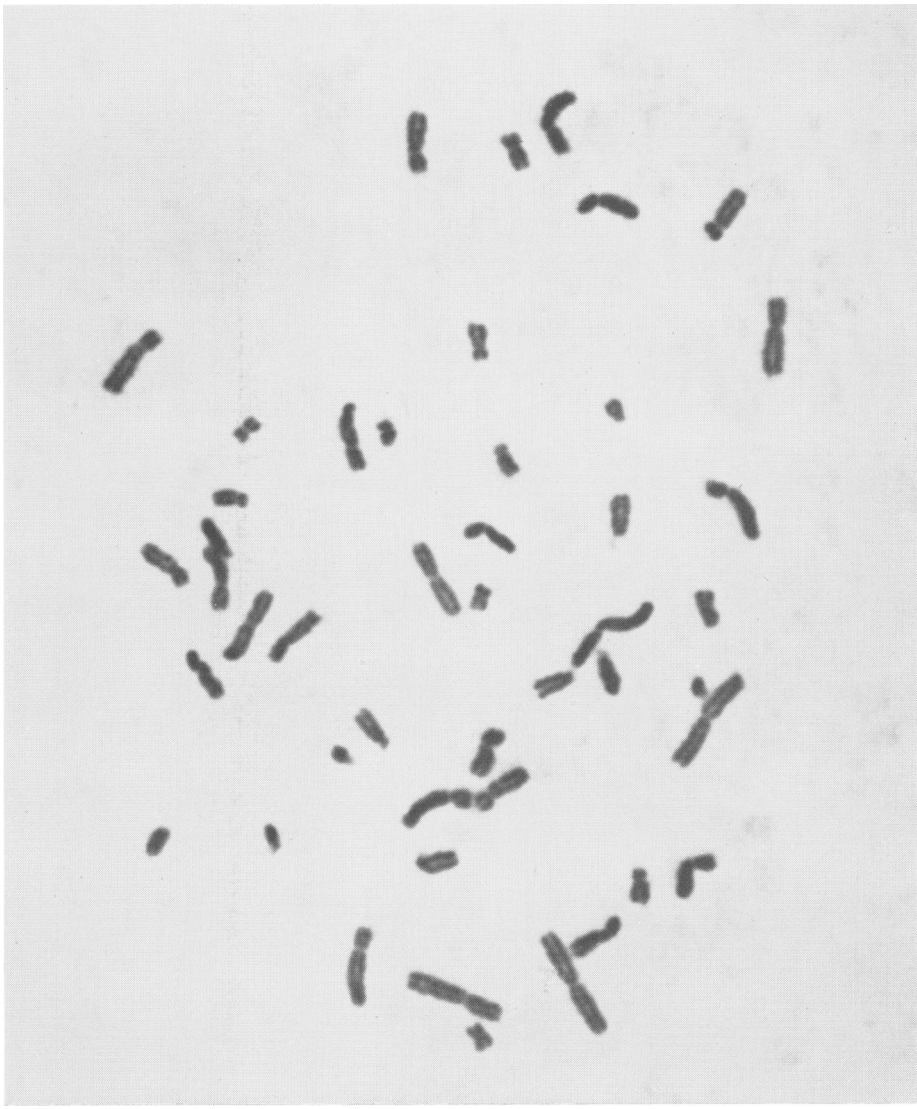

FIG. 2. Normal diploid complement (2n = 46) of a cell in a culture derived by bone marrow biopsy (specimen Y 66) from an 82 year old White male. Acetoorcein stain. 1800×.

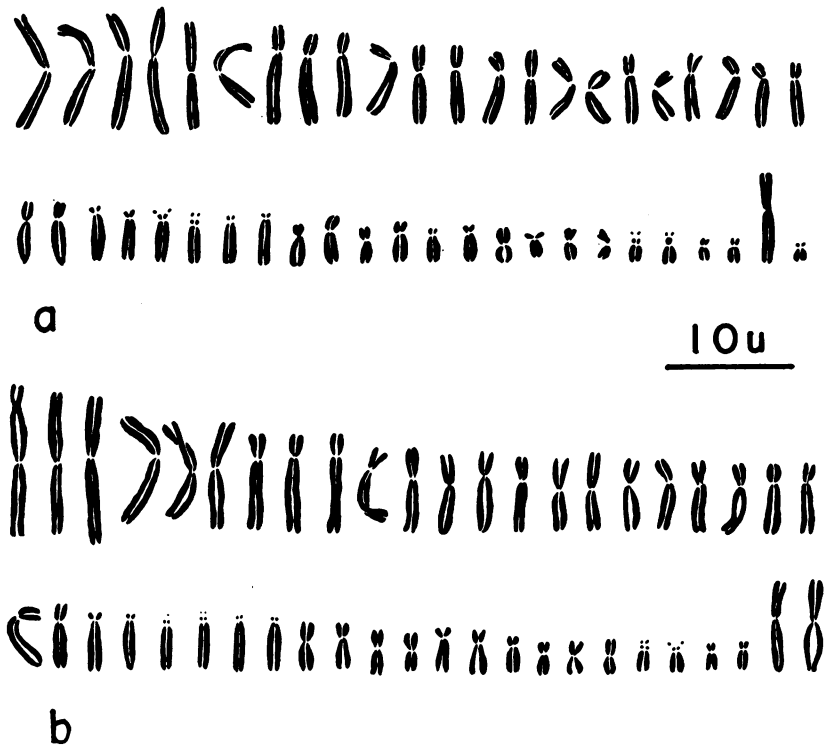

FIG. 3

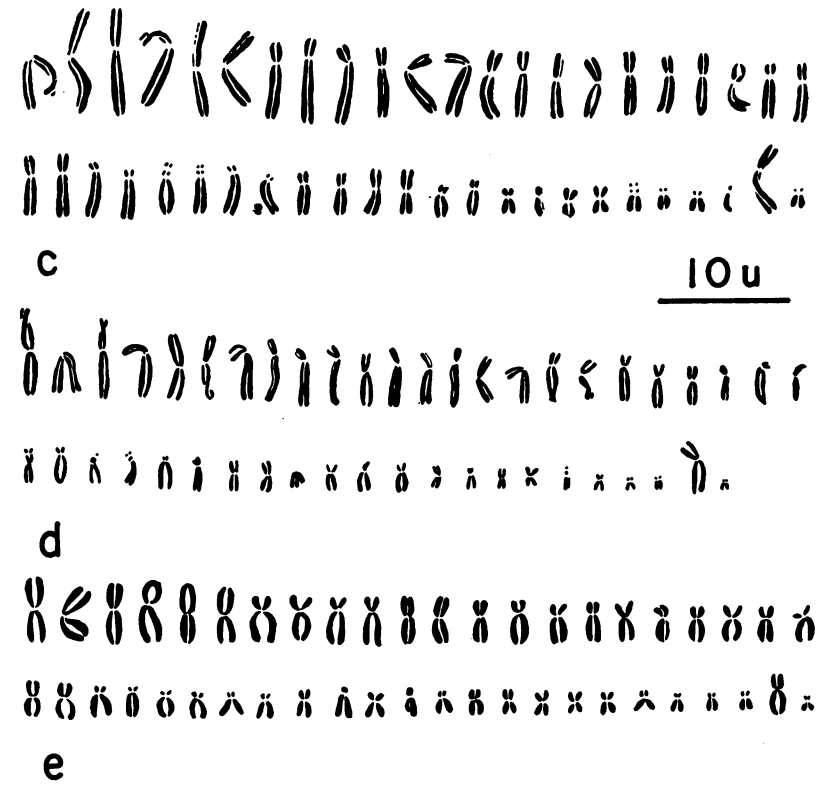

FIG. 4

FIGS. 3 and 4. Idiograms of human diploid cells growing *in vitro* derived from five separate individuals as follows: a. skin-muscle (specimen Y 25), male; b. kidney (specimen Y 15), female; c. foreskin (specimen Y 89), male; d. foreskin (specimen Y 34), male; and e. foreskin (specimen Y 45), male.

numerals have been used to designate individual autosomes. The sex chromosomes, X and Y, are placed last.

Using the system of Tjio and Levan (1956) the chromosomes have been classified into 3 groups: M chromosomes (median-submedian centromeres), S chromosomes (subterminal centromeres) and A chromosomes (acrocentric chromosomes—those chromosomes having nearly terminal centromeres) (Table 2). On this basis, the human diploid chromosome complement consists of 9 pairs of M, 8 pairs of S, and 5 pairs of A autosomes in addition to the sex pair. The Y chromosome is acrocentric. In addition to the method just described, certain members of the complement have characteristic morphological features which aid in individual chromosome identification.

To facilitate description and identification, human somatic chromosomes can be arranged in 7 "natural" sub-groups (Table 3) as follows:

I. The five longest chromosomes can be easily and unequivocally recognized. Chromosomes 1, 2, and 3 are M chromosomes while 4 and 5 are S chromosomes.

TABLE 2. CHARACTERIZATION OF HUMAN CHROMOSOMES IN TERMS OF LENGTH AND ARM INDEX. THE RELATIVE LENGTHS OF INDIVIDUAL CHROMOSOMES ARE EXPRESSED IN TERMS OF THEIR MEAN PERCENTAGE OF THE HAPLOID AUTOSOMAL COMPLEMENT. ARM INDICES WERE DETERMINED FOR EACH CHROMOSOME BY DIVIDING THE LENGTH OF THE SHORT ARM INTO THAT OF THE LONG ARM. FOR FURTHER DETAILS, SEE TEXT.

Chromosome designation	Mean percent haploid autosomal complement	Mean arm index	Position of centromere†
1	9.53 ± 0.02*	1.07 ± 0.00*	M
2	9.15 ± 0.05	1.48 ± 0.01	M
3	7.60 ± 0.14	1.16 ± 0.01	M
4	6.57 ± 0.30	2.89 ± 0.03	S
5	6.10 ± 0.05	3.17 ± 0.22	S
6	5.88 ± 0.10	1.77 ± 0 07	M
7	5.45 ± 0.03	1.89 ± 0.10	M
8	4.90 ± 0.00	1.65 ± 0.07	M
9	4.90 ± 0.00	2.40 ± 0.23	S
10	4.72 ± 0.00	2.31 ± 0.12	S
11	4.55 ± 0.03	2.12 ± 0.10	S
12	4.46 ± 0.05	3.13 ± 0.31	S
13	3.60 ± 0.03	9.53 ± 0.57	A
14	3.43 ± 0.04	9.67 ± 0.27	A
15	3.34 ± 0.06	11.94 ± 1.80	A
16	3.17 ± 0.04	2.07 ± 0.04	S
17	2.79 ± 0.06	1.60 ± 0.06	M
18	2.58 ± 0.04	3.75 ± 0.43	S
19	2.32 ± 0.09	1.95 ± 0.18	M (S)
20	2.02 ± 0.06	1.28 ± 0.03	M
21	1.59 ± 0.08	6.83 ± 0.17	A
22	1.25 ± 0.06	6.00 ± 0.00	A
X		2.05 ± 0.14	S (M)
Y		5.00 ± 0.00	A

*95% level of fiducial probability.

† M (median and submedian); S (subterminal); A (acrocentric).

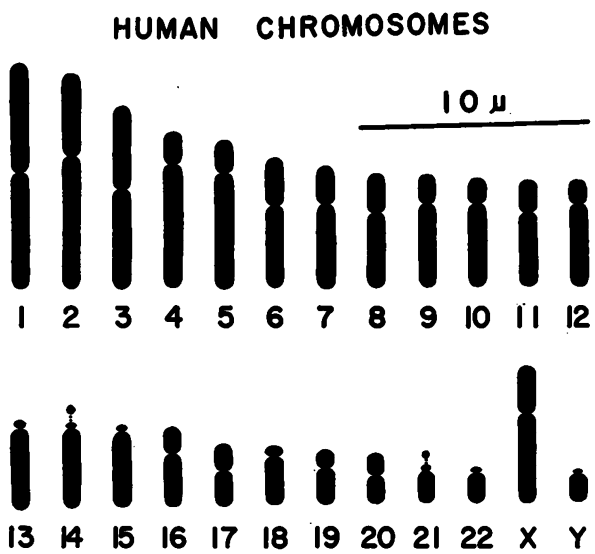

FIG. 5. Idiogram of the human haploid chromosome complement, including the sex pair. The autosomes are arranged in order of decreasing total length and of relative centromere positions. If two chromosomes are of equal length, the one having the more nearly median centromere is placed first.

TABLE 3. HUMAN SOMATIC CHROMOSOMES GROUPED TO FACILITATE IDENTIFICATION. FOR FURTHER DETAILS, SEE TEXT.

Group Designation	Chromosome classification on basis of centromere position*		
	M	S	A
I	1, 2, 3	4, 5	
II	6, 7, 8	X	
III		9, 10, 11, 12	
IV			13, 14, 15
V	17	16, 18	
VI	19, 20		
VII			21, 22, Y

* M (median and submedian); S (subterminal); A (acrocentric)

II. The X chromosome is about the same length as chromosome 5 but with a more median centromere. Chromosomes 6, 7, and 8 are M chromosomes, shorter than the X, but not easily distinguishable from each other without actual measurements.

III. Chromosomes 9 to 12 represent the most difficult group of all for individual chromosome identification, although all are clearly S types.

IV. The three pairs 13 to 15 are acrocentric chromosomes, and are clearly shorter than the chromosomes of group III. The shorter arm of chromosome 13 appears to be longer than the shorter arms of the other two chromosomes in the group. Chromosome 14 has a small satellite on the shorter arm. Tjio and Puck (1958) have recently

reported heterozygosity with respect to the size of the satellites of this chromosome pair in cells of two female individuals.

V. This group includes 3 chromosomes (16, 17, and 18) which are easily distinguishable from each other. In length, chromosome 16 approaches the acrocentric chromosome 15. Chromosome 17 is the only M chromosome in this group; chromosome 18 has a subterminal centromere.

VI. This group contains chromosomes 19 and 20, the latter being particularly easy to identify in the complement because of its size and its nearly median centromere.

VII. This final group includes two pairs of small acrocentric chromosomes, and the Y chromosome in the case of male cells. Chromosome 21 has a small satellite, com-

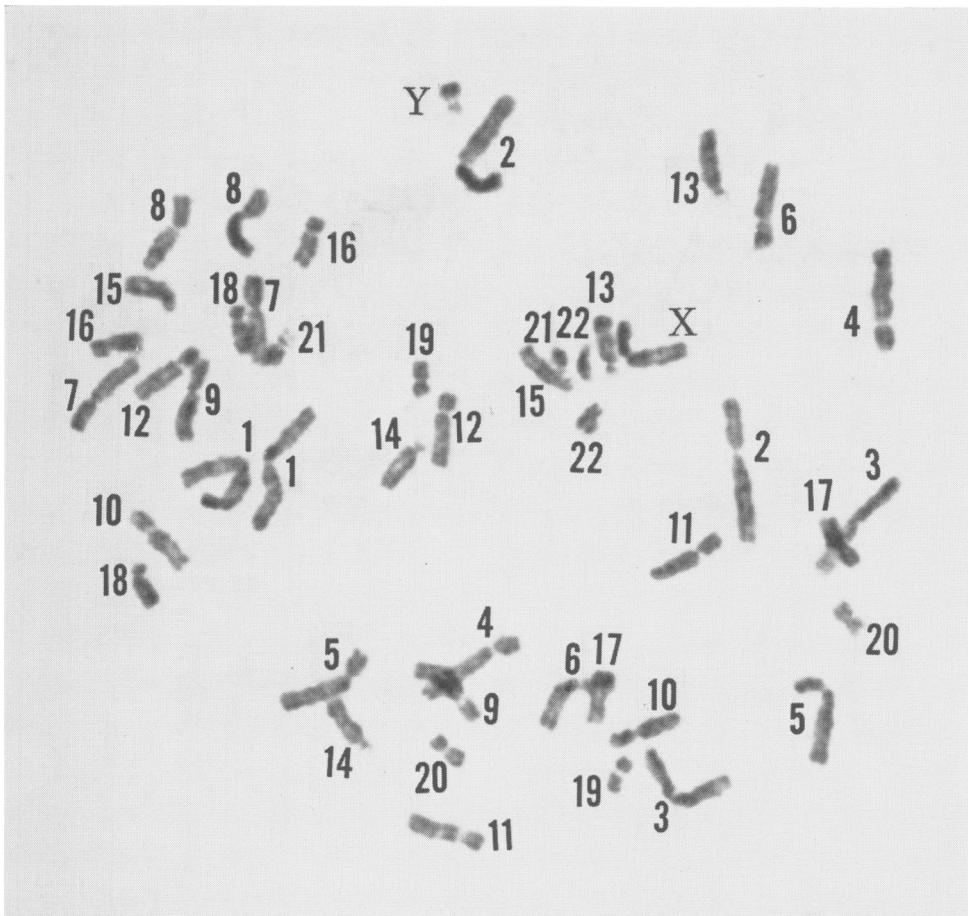

FIG. 6. Normal diploid complement (2n = 46) of a cell at metaphase in a culture derived by foreskin biopsy (specimen Y 89) from a new born White male. All chromosomes have been identified by number (cf. figure 5). Acetoorcein stain. 2150×.

parable in morphology to that of chromosome 14, on its shorter arm. Chromosome 22 is acrocentric, smaller than 21, and without a satellite.

The Y chromosome is apparently the smallest in the whole complement, but differs very little in length from chromosome 22. In most figures it also appears to be acrocentric, not telocentric. The presence or absence in the somatic complement of this fifth small acrocentric chromosome has been successfully used to diagnose the sex of the individual from whom cells have been derived (Ford, Jacobs and Lajtha, 1958).

Figures 6 and 7 present two photomicrographs—one of a male and the other of a female cell—in which all chromosomes have been identified on the basis of the criteria just described and are individually labelled. Figure 8 shows another female cell in which the four satellited chromosomes are indicated.

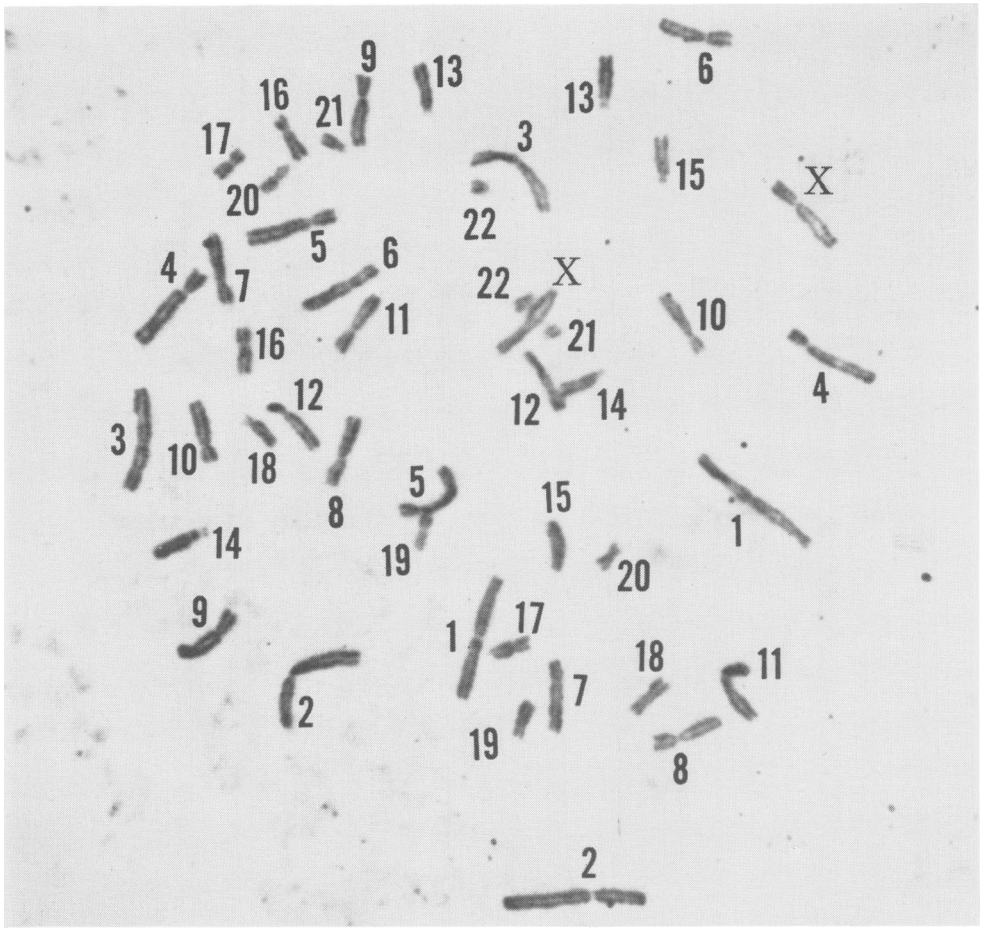

FIG. 7. Normal diploid complement (2n = 46) of a cell at early metaphase in a culture derived by kidney biopsy (specimen Y 15) from a 21 year old White female. All chromosomes have been identified by number (cf. figure 5). Acetoorcein stain. 1650×.

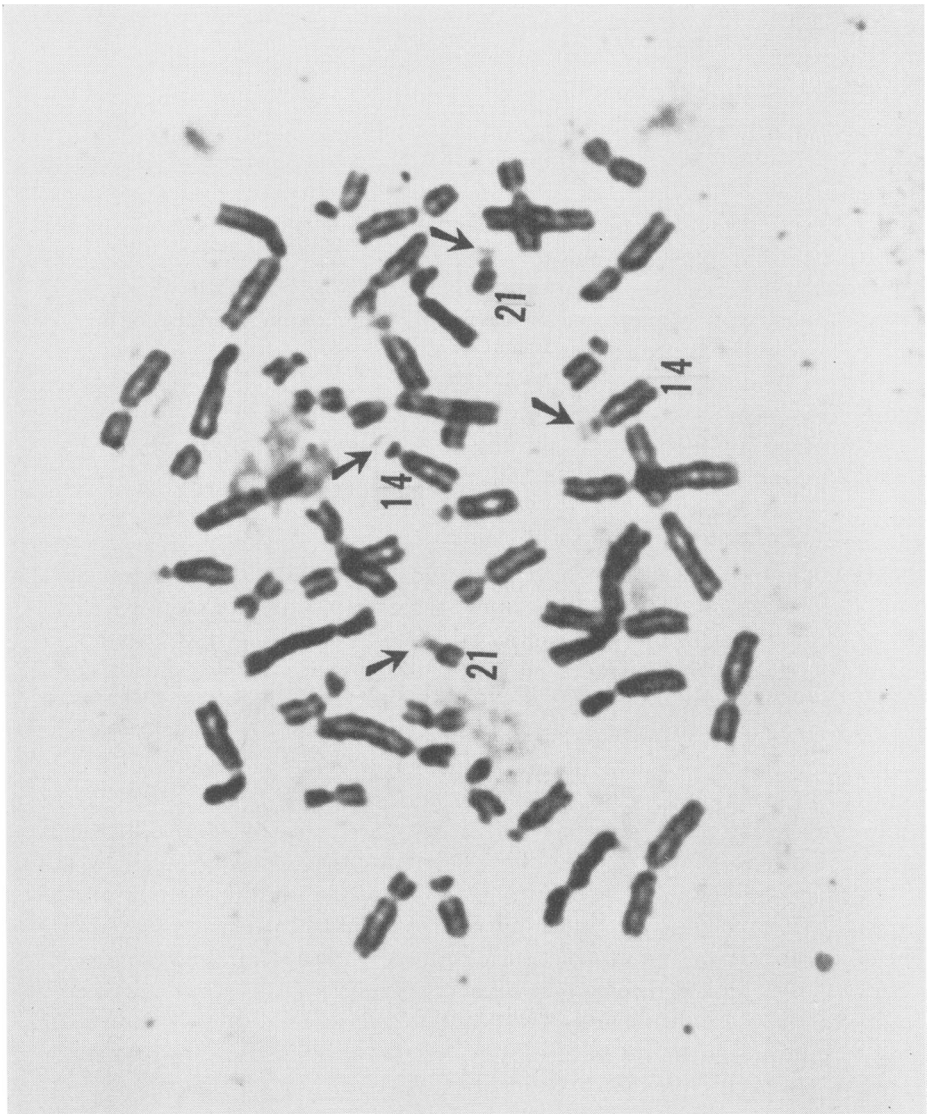

FIG. 8. Another cell from the same individual as in figure 7. This preparation shows the four chromosomes (labelled as two 14's and two 21's) having satellites (indicated by arrows) on their shorter arms. Acetoorcein stain. 2950×.

DISCUSSION

With the application of tissue culture and suitable cytological techniques, information has been obtained on chromosome number and morphology in somatic cells from 34 normal human individuals. The results indicate that regardless of race (Negro or White), sex, age, or tissue used, in all cases the diploid chromosome number was 46. These observations provide additional evidence in support of similar findings by other workers based on different human populations, both European and American (Tjio and Levan, 1956; Ford and Hamerton, 1956; Hsu, Pomerat and Moorhead, 1957; Syverton, 1957; Bender, 1957; Ford, Jacobs and Lajtha, 1958; Tjio and Puck, 1958; Tjio, 1958; Puck, 1958.) The total number of cases in which the diploid number 46 has been recorded is now well in excess of 100.

There remain, however, the reports by Kodani (1957a, b; 1958a, b) of supernumerary chromosomes in man resulting in diploid chromosome numbers of 46, 47, and 48. Two possible explanations of these results, which are in disagreement with all other recent findings, may be considered. On the basis of Kodani's observations, supernumeraries seem to occur with a much higher frequency in certain populations, such as Orientals. Additional independent examinations of individuals from these populations appear highly desirable in order to clarify this point. Secondly, despite the absence of any known case in mammals, there exists the remote possibility of somatic elimination of supernumeraries, thus preventing their detection in individuals from whom somatic cells alone have been studied. However, the diploid number of 46 has also been repeatedly found in primary spermatocytes and spermatogonial cells by Ford and Hamerton (1956) and by others (cf. Ford, *et al.*, 1958). Although the number of instances in which chromosome determinations have been made from both germ line and somatic cells of the same individual is not large, available data (Y16 in this report; Hamerton, personal communication) do not suggest such chromosome number variation. Examination of a number of embryonic tissues, both by Tjio and Levan (1956) and by the present writers as reported in this paper, fail to show any evidence of chromosome elimination even at the early stages of development. It is, of course, difficult to rule out the possibility that such elimination occurs at the initial cell divisions of a fertilized egg. Studies of the somatic chromosomes of those individuals reported to have supernumeraries would be particularly interesting. In view of the present overall evidence, it is perhaps reasonable to conclude that 46 is the correct basic diploid chromosome number in man.

The present karyological analysis also leads to the identification of individual chromosomes, including the sex pair. It is evident that metaphase chromosomes in somatic cells assume a more clearly definable morphology than do chromosomes in either meiotic or spermatogonial metaphases. Somatic cells in tissue culture provide additional advantages in having increased mitotic activities and in permitting more efficient cytological treatments. On the basis of detailed microscopical comparisons and actual measurements, an idiogram of the human karyotype has been constructed. This idiogram is in general agreement with the idiograms presented by Hsu (1952) and more recently by Ford *et al.* (1958) and by Tjio (1958). The present idiogram is also roughly comparable to the one proposed by Kodani (1957a) based on measurements of meiotic first metaphase chromosomes and to another by the same author

(1958b) in which relative chromosome arm lengths of certain chromosomes can be inferred on the basis of the pairing configurations of bivalents at the meiotic metaphase.

Statistical analysis has established that there are no significant differences in the morphology of individual chromosomes (based on determinations of relative lengths and arm indices) among several individuals examined in this study. The human sex chromosomes are heteromorphic, but there are no morphological differences among X chromosomes or among Y chromosomes derived from different individuals. Since this idiogram is in general agreement with those prepared by others, this conclusion on chromosome morphological constancy is probably generally true. The heterozygosity of satellites on chromosome 14 (Tjio and Puck, 1958) has not been observed in the present group of samples.

The occurrence of pronounced heteromorphy in the human sex chromosomes has important implications for such phenomena as the mechanism of sex determination, sex linkage, and the cytological diagnosis of sex. On the basis of somatic length the X chromosome is 4 to 5 times longer than the Y. Heterochromaticity has been reported to occur in the entire shorter arm of the X (Kodani, 1957a) and in a fairly large region close to the centric end of the Y (Tjio and Puck, 1958). During meiosis, Sachs (1954) has noted the absence of normal pairing between the X and Y. In addition, Kodani (1957a) has found that the X and Y associate at meiosis in only 60% of the cells. When they do pair, the region of association is limited to the tips of shorter arms of the two chromosomes. The great difference in total length, the presence of long heterochromatic regions, the limitation of pairing regions, and the high frequency of nonconjugation all lead to the conclusion that there is little if any homology between the human X and Y chromosomes.

The presence or absence of a Y chromosome can be used to diagnose the genetic sex of cells (Hsu *et al.* 1953). This procedure is particularly useful as a direct test of the validity of the diagnosis of the genetic sex of intersexual individuals on the basis of the presence or absence of "sex chromatin" (Barr *et al*, 1950; Moore *et al*, 1953). The study by Ford *et al.* (1958) on a case of Klinefelter's syndrome and the present report on a male pseudohermaphrodite are cases in point. In both instances, the diagnosis of genetic sex by means of the sex chromatin method was verified by direct chromosome examination.

The pronounced heteromorphy of the human XY chromosome pair would also appear to provide a likely cytological explanation for the distinction between female and male cells based on sex chromatin. It is evident that the major cytological difference between cells of the two sexes resides in this heteromorphic pair of chromosomes. Since the X is much larger than the Y and its entire shorter arm is reported to be heterochromatic (Kodani, 1957a), a reasonable hypothesis appears to be that sex chromatin represents the heterochromatic region of the X chromosome and regularly appears only when both X chromosomes are present in a female cell. Evidence in support of this hypothesis has been reported by Reitalu (1957).

Finally, the two pairs of autosomes with satellites (chromosomes 14 and 21) are probably nucleolus-organizing chromosomes. The finding (Chu and Giles, 1958b) that the human somatic interphase nucleus contains basically four spherical nucleoli—

one large and one small pair—supports this view. Detailed cytological studies of the nucleolar constitution of human somatic cells will be published later.

SUMMARY

Studies have been made on chromosome number and morphology in somatic cells in tissue cultures derived from 34 normal human subjects including 29 American Whites, 4 American Negroes and one of unknown race. The results indicate that regardless of race, sex, age, or tissue, in all cases the diploid chromosome number was 46.

Karyotype analyses have resulted in the identification of each individual human chromosome pair, including the sex pair. On the basis of relative chromosome lengths and arm indices, an idiogram of the human karyotype has been constructed in which each individual pair of chromosomes is designated by number. Statistical analysis has further established that homologous chromosomes from cells of the same or of different individuals do not differ significantly either in relative length or in centromere position. Similarly, there are no significant differences among X chromosomes or among Y chromosomes derived from different individuals.

To facilitate description and identification, human chromosomes have been grouped into 7 sub-groups. The morphological characteristics of chromosomes in each subgroup are described.

Two pairs of autosomes have satellites on their shorter arms. The presence of two pairs of nucleoli in somatic nuclei of both sexes supports the view that these pairs of satellited autosomes are nucleolus-organizing chromosomes.

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ADDENDUM

Since the present manuscript was submitted for publication, a paper by Tjio and Puck describing their observations on the human karyotype has appeared (Proc. Nat. Acad. Sci. U. S. 44: 1229–1237, 1958). The results of the two studies are in substantial agreement as to cytological details, the principal difference being in the systems employed in numbering chromosomes.

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