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Chromosomes of man and their relationship to cancer and diseases of the reproductive system

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THE CHROMOSOMES OF MAN AND THEIR RELATIONSHIP TO CANCER AND DISEASES OF THE REPRODUCTIVE SYSTEM

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INTRODUCTION AND HISTORY

In 1891, Hansemann (19) opened the question of the number of chromosomes in human cells by reporting counts of 18, 24, and more than 40 chromosomes respectively in three cells from "normal human tissue." At that time he considered the diploid (2n) chromosome number of man to be 24. Between this time and 1912, counts ranging from 16 to 36 were reported, with 24 being the favorite number (3,39). In 1912, Winiwarter (40) asserted that human cells contain 47 chromosomes at metaphase in spermatogonia; and 23 bivalents plus an unpaired X in primary spermatocytes. By thus reporting, he placed man in the X-0 category of sex determination. In 1921, however, Painter (32) reported the presence of a small Y-chromosome in the testicular cells of three males. He also stated that the diploid number was either 46 or 48, and further investigation was necessary before the exact number could be established. Painter (33), after some further investigation, declared in a long report in 1923, that the chromosome number of man is 48 and that he has an X-Y type of sex determination. He submitted a great deal of evidence, in the form of drawings of chromosomes observed at metaphase, to support his conclusions.

This apparently satisfied all workers in the field of cytogenetics, because all subsequent reports agreed that the chromosome number was 48 and counts other than this were discredited and assumed to result from technical errors. In fact, counts other than

48 were not published, and some work which showed 46 chromosomes was postponed because the workers believed 48 should be present (37).

In 1956, however, Tjio and Levan (37) re-opened the question of chromosome number in man by suggesting that 46 was indeed the correct number. They utilized some new-found techniques (mainly pre-treatment of the cells in hypotonic solutions to cause spreading of the chromosomes, incubation of cells in culture with colchicine so that more cells appeared in metaphase, and the squashing of cells onto slides rather than sectioning as previously had been done), and produced some rather convincing photomicrographs to support their claims. (See Fig. 1.)

Since 1956, this field of investigation has blossomed. Ford, Jacobs, and Lajtha (13) devised a method by which chromosomes could be counted in bone marrow cells after a short-term tissue culture. Thus, a source of cells for chromosome counts and analyses became easily available from any patient.

Since this time, chromosome counts and analyses have been done on many patients; much light has been shed on the etiology of previously baffling conditions, such as mongolism. The investigations now going on promise to give important and exciting insights into the etiology of such puzzling conditions as leukemia.

Thus, the re-opening of the question of chromosome number in man has unfolded new horizons for medical research.

MATERIAL AND METHODS

Portions of the bone marrow aspirates taken from four patients for diagnostic purposes were used. These were handled according to the method of Ford and Lajtha (10). which is as follows:

1. 1 ml. of bone marrow aspirate is placed in 16-18 ml. of Ringer's solution with heparin, 1:20,000 at room temperature.
2. Fifteen to thirty minutes later, spin down for 10-15 minutes at 1500 r.p.m. Remove supernatant and add 2-3 ml. of glucose-saline solution (0.6 gm. glucose and 0.7 gm. NaCl in 100 ml. H_2O) at room temperature.
3. Mix with AB serum at room temperature to give a final concentration of between 70 and 90%.
4. Withdraw into syringe and rock in horizontal position for 1-2 minutes to insure oxygenation of the RBC's and return to the bottle.
5. Incubate immediately at 37° C. in a H_2O bath.
6. Six hours later inject a volume equal to one-tenth the culture volume of 0.04% colchicine in glucose-saline solution.
7. One hour later remove from H_2O bath and spin down gently. Remove nearly all the supernatant fluid (SNF), and add 2-4 ml. of 1.1% sodium citrate solution. Replace in water bath.
8. Fifteen minutes later transfer to centrifuge tube and spin down gently. Remove nearly all the SNF and re-suspend sedimented cells in the remainder. Fix in acetic-alcohol, 1:3, freshly made up.

9. Thirty minutes later spin down. Re-suspend cells in a small amount of SNF as in Step 8. Add 80% alcohol and leave 15 minutes.
10. Spin down and treat as in Step 9. Add 50% alcohol. Leave 15 minutes.
11. As in Step 10. Change to water and leave 15 minutes.
12. Spin down and re-suspend cells in a small amount of SNF. Add 1N HCl kept at room temperature, and hydrolyze in a water bath at 60° C. After 4 minutes stop hydrolysis by chilling tube in ice water.
13. Spin down. Add Feulgen (Schiff's) reagent for 1 hour.
14. Spin down and re-suspend cells. Add chilled 45% acetic acid. Repeat this to wash out the Feulgen reagent. The cells should now sediment quickly.
15. Make squash preparations by flicking tube to bring cells into suspension; place a drop on a clean glass slide, cover with a cover glass immediately. Warm gently over an alcohol lamp, and then squash between layers of filter paper on a flat surface.
16. Make permanent by freezing on dry ice for 3-4 minutes, prying off the cover glass with a razor blade, dehydrating in 95% alcohol, and mounting.

RESULTS

Personal Results:

In none of the four preparations made could cells be identified which contained chromosomes. This undoubtedly was due to technical difficulties, as the success of this and similar methods is evidenced by many reports which are substantiated by photographs. Several reasons can be postulated for the failure seen in these preparations. First, in two instances probably no marrow fragments were obtained (in one case due to the acellularity of the patient's marrow, and in another due to an error in dividing the aspirate). Second, in one case the preparation was contaminated by bacterial growth. In the final case, the bone marrow cells probably died prior to incubation by allowing them to stand overnight (as was described by Ford, et al.) (13). Because of time limitations, further attempts to reproduce this method were not made.

Previously Published Chromosome Counts:

As stated previously, Tjio and Levan (37) re-opened the question of the chromosome number in man by reporting consistent counts of 46 in cells in tissue culture. These cells had been obtained from legally aborted human embryos and were probably fibroblasts. The cells were pretreated with hypotonic solutions and incubated 12-20 hours in a dilute colchicine solution prior to fixation. These authors presented rather convincing proof for their claims. (See Fig. 1.)

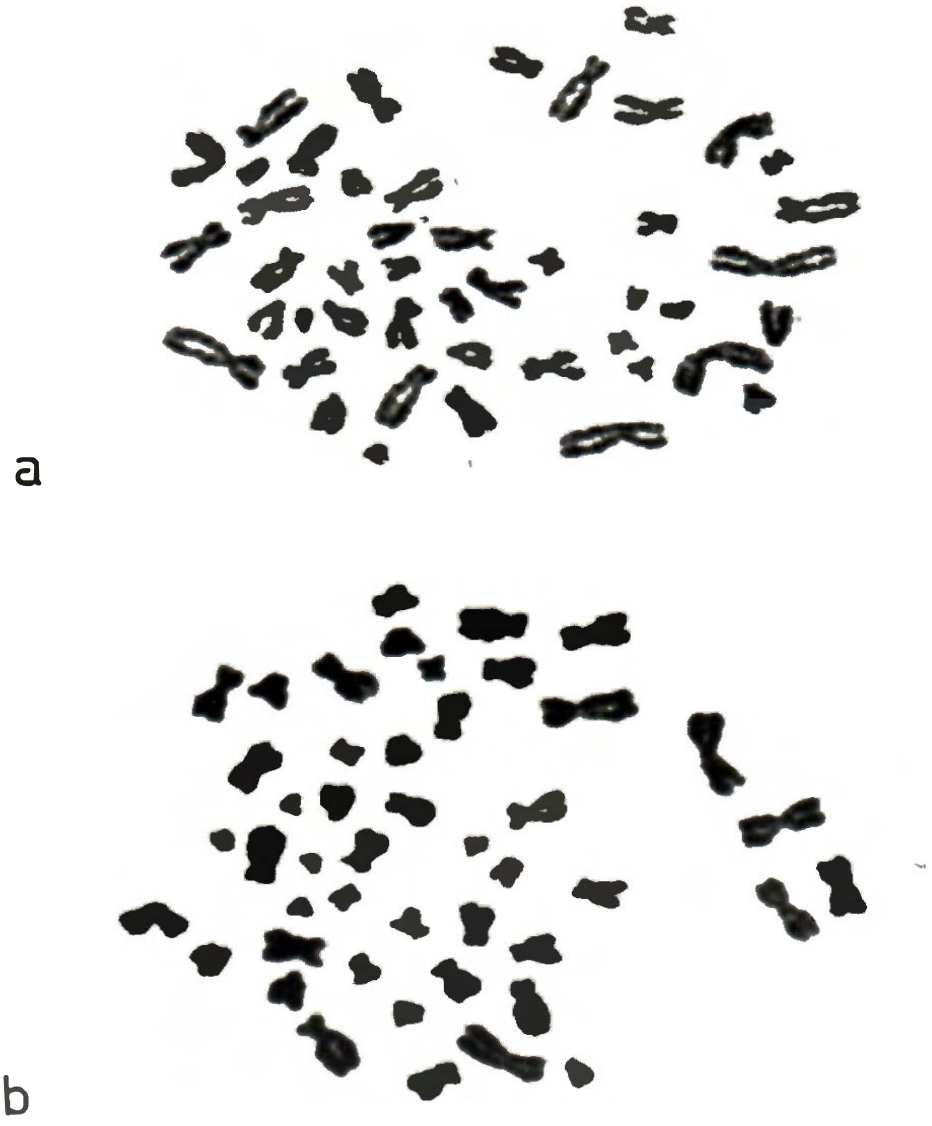

Figure 1. Chromosomes in tissue culture of lung from aborted embryo. (a) early metaphase, (b) late metaphase. (From Tjio & Levan, Hereditas 42:1-6, 1956.)

In total, they counted 261 cells. Only 4 cells showed counts of 47 or 48 chromosomes. Many cells with counts less than 46 were found, but all were obviously damaged.

Other reports soon confirmed the findings of Tjio and Levan. Ford and Hamerton (11) reported finding 46 chromosomes in preparations of fresh testis obtained from three patients. These authors

also report counts of fewer than 46 chromosomes in apparently undamaged cells, but assume that they were damaged, since the majority of cells contain 46 chromosomes.

In 1958, the first large series (22 patients) in which chromosome counts had been done, appeared (13). In this undertaking, Ford, Jacobs, and Lajtha had attempted to determine the diploid (2n) number of chromosomes in man, and, if possible, to identify the sex chromosomes. Table 1 summarizes their work.

Case	Cells Counted	Counts									Sex		Clinical Condition
		42	43	44	45	46	47	48	49	50	True	Dx	
10	34	1	1	4	4	22	2				F.	F.	Pregnancy Anemia
12	50	2	4	6	3	27	3	2	1	2	M.	M.	Iron Deficiency Anemia
14	70	6	1	9	9	43	1			1	F.	F.	Pernicious Anemia
15	15			1	1	13					F.	F.	Iron D. A.
19	17			1	1	5	3	2	1	4	M.	M.?	Iron D. A.
24	94	3		4	7	73	3	2	1	1	F.	F.	Undiag. fever; Very active normal bone marrow
26	27	1		2	2	17	3	1	1		F.	F.?	Preg. Anemia
30	22			1	3	15	2			1	M.	F.?	Polycythemia Vera
34	24				4	17	2			1	F.	F.	Lymph. Leukemia
35	35	1			4	22	3	2	1	3	M.	M.	Hemolytic Anem.
36	47	4		1	8	34					F.	F.	Iron D. A.
38	22	2	1	1	3	13		2			F.	F.	Preg. Anemia
43	28	1	1	2		19	4	1			F.	F.	Pern. Anemia
44	26	1		1	1	22		1			F.	F.	Blast Cell Leukemia
8 Others	44	3	1	3	4	25	5	2	1				
Total	555	25	9	36	54	367	31	15	6	12			

Table 1. Summary of chromosome counts on 22 patients. (Ford, et al., 1958)

The authors explained the variation from 46 seen in the table by pointing out three ways in which they could have arisen: (a) through counting chromosomes in cells where fixation and spreading of chromosomes was inadequate, (b) early division of centromeres (giving rise to colchicine anaphases) which produced structures counted as two bodies which in reality were the two chromatids (arms) of one chromosome, and (c) cells are likely to be ruptured in squash preparations and consequently to lose fragments of cytoplasm containing one or more chromosomes.

In the same report (13), Ford et al. undertake an analysis of the chromosomes in order to identify the sex chromosomes (see discussion under Karyotype below).

In another large report of 34 patients, Chu and Giles (7) have reported chromosome counts of 46, with only a small minority of the cells showing counts other than this. (See Fig. 2 and 3.) Tjio and Puck (38) have also reported 13 patients with chromosome counts of 46. All in all, the literature to date reveals that cells from over 100 patients have been found to contain 46 chromosomes.

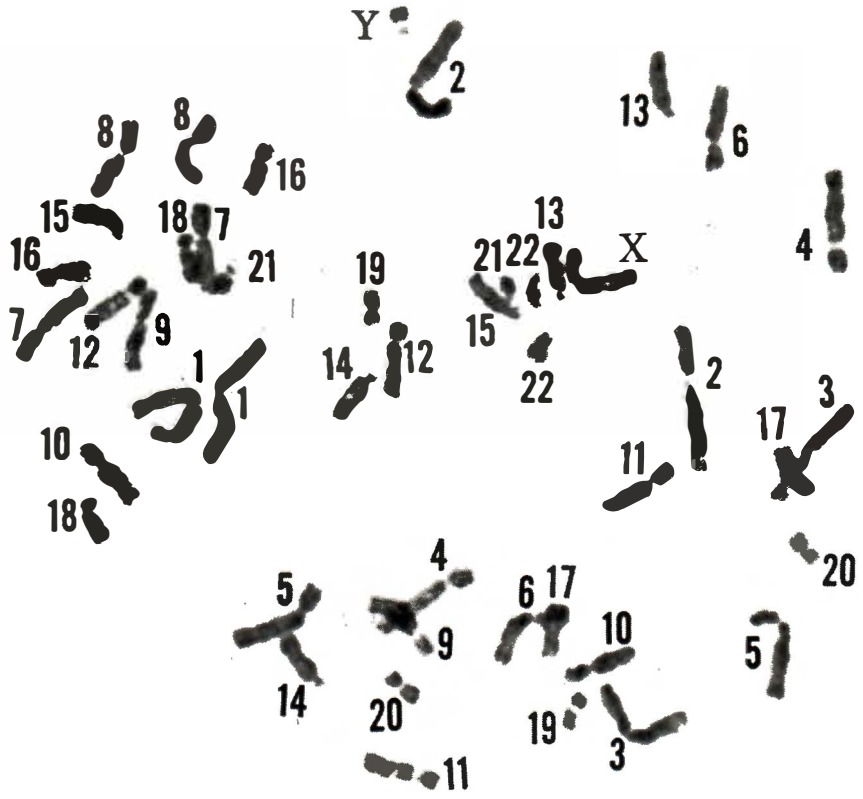

Figure 2. The chromosomes from a cell (fibroblast) from tissue culture. Chromosomes are labeled with numbers according to length. Chromosome #21 has a satellite. The patient was a male. (From Chu & Giles, Am. J. Human Genet. 11:63, 1959)

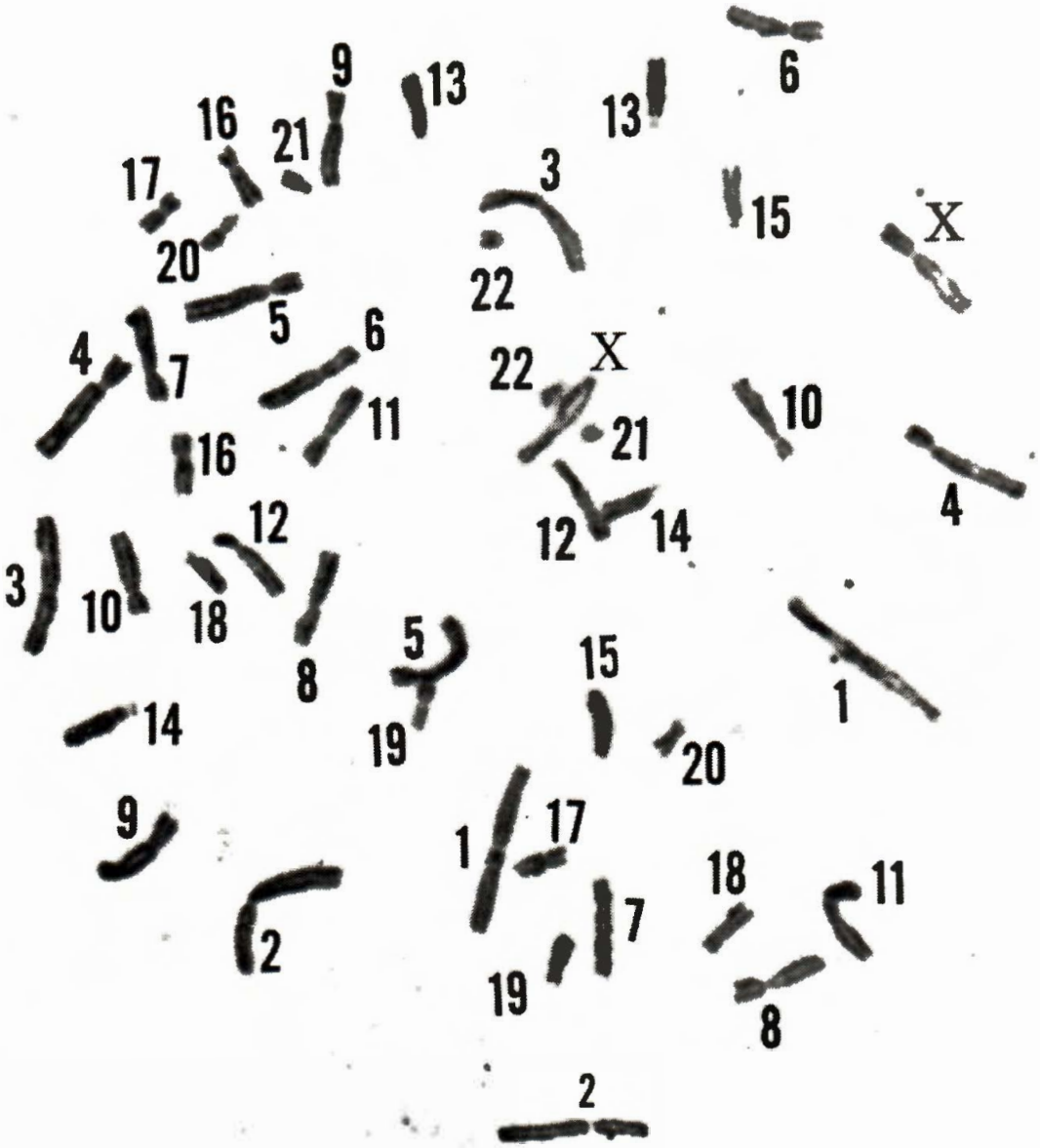

Figure 3. Chromosomes from a cell (fibroblast) in tissue culture, labeled with numbers according to length. The patient was a female. (From Chu & Giles, Am. J. Human Genet. 11:63, 1959)

Since the report of Tjio and Levan in 1956, only Kodani (27, 28, 29) has reported counts other than 46. His work has included counts on 36 Japanese and 8 Caucasians. All of his specimens have been testicular tissue and counts have been made on spermatogonia and the chromosomes have been bivalents. His counts on Japanese have included 21 specimens with 48 chromosomes, 2 with 47, and 13 with 46. In Caucasians, 1 patient has had 48, and 7 have had 46 chromosomes. Kodani presents some photographs showing quite small chromatin particles which the author claims are supernumerary chromosomes.

If man does indeed have one or two supernumerary chromosomes of the size which Kodani describes, they must exert little or no genetic influence. These findings, if substantiated, will cast doubt on the validity of a cause and effect relationship between supernumerary chromosomes and clinical conditions such as mongolism. Since, however, the sex chromosomes are involved in the other clinical conditions with which abnormal counts have been described (e.g. Klinefelter's syndrome), this finding will have little effect on the theories of the etiology of these diseases. Kodani's findings await substantiation by other workers.

IMPORTANT FACTORS OTHER THAN NUMBER CONCERNING CHROMOSOMES

The Karyotype:

As pointed out by Stern (36), the number of chromosomes is perhaps the least important characteristic of human chromosomes. As one can readily imagine, chromosomes could easily split or join together within a species, and form more or fewer chromosomes, respectively, and the amount of genetic material would not have changed. With this in mind, further description of the chromosomes has been devised in the form of the karyotype. Stated simply, this is a description of the morphology of the chromosomes, including the size, shape, and other individual characteristics of the chromosomes, e.g. nucleoli or satellites. The position of the kinetochore (the non-staining area in a chromosome separating the two arms) is also quite valuable in the classification of the individual chromosomes (36).

Recently, two workers (7, 38) have worked extensively on the karyotype of man. Chu (7) presents his evidence on an analysis of 34 patients. He found a very small standard deviation in the mean lengths (expressed as percentage of the total somatic chromosomal length) and arm ratios (obtained by dividing the small arm length into the long arm length). (See Table 2.) Tjio and Puck (38), working with four male and four female metaphase cells, found essentially the same figures. (See Table 2.) Tjio, unlike Chu, included the X and Y lengths in the total chromosome length. This

Table 2. Analysis of Human Chromosomal Karyotype. Length is expressed as a percentage of total haploid autosomal complement. Arm index is long arm/short arm. (After Chu & Giles, 1959 and Tjio & Puck, 1958)

Chromosome Number		Mean % of Haploid Autosomal Complmt.		Mean Arm Index		Position of Centromere	
Chu	Tjio	Chu	Tjio	Chu	Tjio	Chu	Tjio
1	1	9.53±0.02	9.6	1.07±0.01	1.08	M *	M *
2	2	9.15±0.05	8.7	1.48±0.01	1.56	M	M
3	3	7.60±0.14	7.4	1.16±0.01	1.20	M	M
4	4	6.57±0.30	6.8	2.89±0.03	2.85	S*	S*
5	5	6.10±0.05	6.2	3.17±0.22	3.18	S	S
6	6	5.88±0.10	5.8	1.77±0.07	1.69	M	M
7	7	5.45±0.03	5.0	1.89±0.10	1.31	M	M
8	8	4.90±0.00	4.7	1.65±0.07	1.50	M	M
9	9	4.90±0.00	4.7	2.40±0.23	1.92	S	M
10	10	4.72±0.00	4.6	2.31±0.12	2.40	S	S
11	11	4.55±0.03	4.6	2.12±0.10	2.78	S	S
12	12	4.46±0.05	4.5	3.13±0.31	3.13	S	S
13	18	3.60±0.03	3.7	9.53±0.57	8.00	A*	A*
14	19	3.43±0.04	3.4	9.67±0.27	7.33	A	A
15	20	3.34±0.06	3.1	11.94±1.80	10.50	A	A
16	13	3.17±0.04	3.4	2.07±0.04	1.78	S	M
17	14	2.79±0.06	3.1	1.60±0.06	2.83	M	S
18	15	2.58±0.04	2.6	3.75±0.43	3.75	S	S
19	16	2.32±0.09	2.3	1.95±0.18	1.43	M(S)	M
20	17	2.02±0.06	2.2	1.28±0.03	1.29	M	M
21	21	1.59±0.08	1.9	6.83±0.17	3.67	A	S
22	22	1.25±0.06	1.8	6.00±0.00	3.33	A	S
X			6.3	2.05±0.14	1.94	S(M)	M
Y			2.0	5.00±0.00	-	A	-

* M is median-submedian,
S is subterminal.
A is acrocentric,
nearly terminal.

undoubtedly accounts for some of the differences between the findings as presented in Table 2.

The chromosomes are thus divided into three groups. The "M" group has a median-submedian kinetochore and an arm ratio of 1-1.9. The "S" group has a subterminal kinetochore and an arm ratio of 2-4.9, while the "A" group has a nearly terminal kinetochore and an arm ratio of 5 or more. Using this grouping, Chu and Giles (7) found nine M-chromosomes, nine S-chromosomes, (including the X-chromosome) and six A-chromosomes (including the Y-chromosome), making 22 autosomes and the X and Y-chromosomes. Tjio and Puck (38) found eleven M-chromosomes, nine S-chromosomes, and three A-chromosomes. They could not classify the Y-chromosome. Tjio and Levan (37) had previously reported 10 M-, 10 S-, and 3 A-chromosomes. The differences in classification come because some chromosomes have borderline values for arm ratio.

Another means of placing chromosomes into groups has been put forth by Ford, et al. (13). These groupings are on the basis of length, and have been helpful in identifying the sex of patients. In a complete complement, i.e. 46 chromosomes, these authors found five very short acrocentric chromosomes in the male, and only four in the female. Both sexes had 16 other short chromosomes. In females, there were 16 medium length chromosomes but only 15 in the male. Both sexes had 10 quite long and easily identifiable chromosomes. From these findings it is seen that the Y must be one of

the very shortest chromosomes and the X one of the medium length chromosomes. (See Fig. 4.)

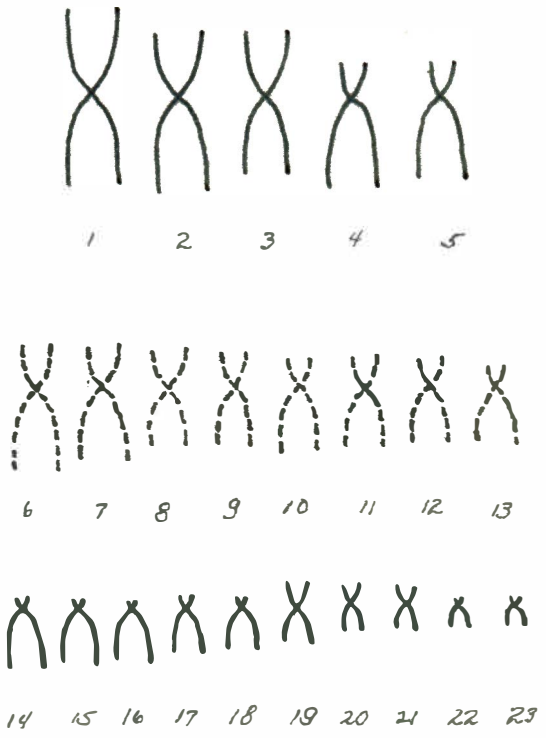

Figure 4. Diagram of the human (European female) haploid chromosome-complement based on measurements of the individual chromosome arms. The male chromosome-complement has one less chromosome in the group 6-13, and one more of the type similar to 22 and 23. (From Ford, et al., Nature 181:1567, 1958)

Ford, et al. were able to diagnose sex correctly thirteen times in fourteen attempts using this method, as seen in Table 1. (13). Other authors have also had success using this method (14, 15, 16, 23, 24, 26, 31, 38).

Other details of chromosome morphology are not so helpful in overall classification. For instance, Tjio and Puck (38) state that the number 18 and number 21 chromosome have satellites, i.e. a small chromatic area near the chromosome with an achromatic "thread" connecting to the chromosome. (See Fig. 2.) Kodani (28) states that the L- (number 12) chromosome has a nucleolus and that another smaller chromosome (which he does not identify) also has a nucleolus in prophase which later disappears in metaphase.

Ford and Hamerton (11) express the length of the total chromosome complement in morgans. A morgan is a function of the number of chiasmata, 50 centimorgans equalling 1 chiasma. In 23 cells they analyzed, they found a mean of 55.9 chiasmata per cell. On this basis, the chromosome length was stated to be 27.9 morgans. This technique is definitely less useful than the others described above. An interesting hypothesis arises from this, however; this states that each chiasma represents a single genetic crossover. A high number of chiasmata implies a genetic system in which there is rapid reassortment of the genotypic variability. This is the mark of a plastic species able to adapt itself readily to adverse environmental circumstances.

X - Y Configuration:

The X-Y chromosome configuration is known to control sex. A number of genes, such as the ones controlling color-blindness, hemophilia, and testicular feminization have been definitely located

on the X-chromosome, because of the sex-linked character of the phenomena which these genes produce. This, plus the fact that disturbances in sexing, e.g. hermaphrodism, are probably in some way related to the sex chromosomes, makes their identification extremely desirable.

As stated above, the X- and Y-chromosomes have been definitely placed in two groups of chromosomes by Ford, et al. (13), but their positive identification has been more difficult. Ford and his group and Jacobs and her group of workers have been unable to definitely identify the X- and Y-chromosomes (13, 14, 15, 16, 23, 24, 25, 26). This difficulty arises because the X- and Y-chromosomes are very similar in size to other chromosomes in their groups.

The problem of identifying the X- and Y-chromosomes is much easier when cells in prophase or early metaphase are examined. In this stage of mitosis the chromosomes form pairs, and because of the unequal size of the X and Y, this bivalent is asymmetric. Kodani (28) has presented a detailed diagram of the structure of the X-Y bivalent in prophase. (See Fig. 5.)

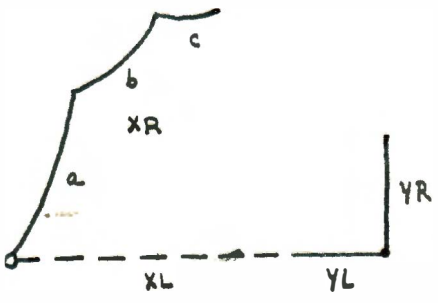

Fig. 5. The X-Y structural characteristics are shown diagrammatically. The right arm (XR) of the X consists of 3 segments, a, b, c. The broken line indicates the heterochromatic nature of the left arm of the X (XL). YR and YL denote respectively the right and left arms of the Y. The circle indicates the kinetochore. (After Kodani, Proc. Int. Genet. Symp. Cytol. Suppl. 1957, 103-7)

In this, the XY consists of a large two-armed chromosome, and a smaller, two-armed chromosome. The X (larger) has a well condensed and highly chromatic arm and another uncondensed and poorly staining arm. The euchromatic arm (XR) is often bent near the middle, giving rise to two segments, the distal of which is sometimes found to be bent near its free end.

The Y consists of two arms with a ratio of about 2:1. The short arm is connected to the heterochromatic arm of the X at first metaphase.

On the basis of measurements of chromosomes, and the subsequent pairing of these, X has been tentatively identified in male cells. On the basis of these measurements, chromosomes with similar measurements have been paired and labeled as the X-chromosomes in female cells. Tjio and Puck (38), using this method, place X in the fifth position with regard to length, and the Y in the twenty-first. Chu and Giles (7) do not attempt to label the X and Y in their analysis of karyotype, although they have apparently identified these two chromosomes to their satisfaction (see Table 2), and state that X is sixth in the order. Ford et al. (14, 15) place the X seventh in the order, while Nilsson et al. (31) place X sixth in order, thereby agreeing closely with Chu and Giles. (They are in close agreement with respect to the arm ratio of X, also.)

It is obvious from the above statements that the similarity

in size between the chromosomes in the group where X is found makes identification difficult. Further work must be done before a positive identification of X and Y can be made with certainty.

Tjio and Puck (38) present an interesting sidelight to the problem of the X-Y identification. Assuming that their measurements of the chromosomes are correct (see Table 2), then the presence of an XX in females gives their cells about 4% greater chromosomal volume. Potentially, at least, the female has a richer genetic capacity. This factor could underlie some of the sex differences, e.g. difference in longevity and tolerance to hypertension, which were previously thought to be functional.

DNA Content:

Of less importance to the cytogeneticist, but important to the overall understanding of chromosomes, is the DNA (desoxyribose nucleic acid) content of a cell. Within a given species, the DNA content has been found to be constant, and since DNA is probably responsible for the transmission of genetic specificity, its quantity would seem to be a fundamental property of a cell (36).

It is of interest then, that the sperm of 21 sterile men was found to contain one-half the amount of DNA found in sperm of fertile men. Furthermore, if X is taken as the amount of DNA in the sperm of these sterile men, then 2X must be the amount in normal sperm. In the 21 sterile men, the amount of DNA in primary spermatocytes was found to be 4X (8X was found in normal men) and

in secondary spermatocytes it was found to be 2X (4X in normal men). This indicates that meiosis in these 21 men had taken place in a manner similar to that in normal men, with a reduction in the DNA content as the number of chromosomes was reduced.

SOMATIC AND TISSUE CULTURE CHROMOSOMES

It must be noted that chromosomes have never been counted in somatic cells without first subjecting them to tissue culture. Recent attempts to do so in bone marrow cells without prior culture have failed to give satisfactory results (9). While the large number of counts of 46 chromosomes in tissue culture cells and the consistency of the morphology of these chromosomes strongly suggests that 46 is indeed the correct number, it must be realized that cells are known to do strange and unpredictable things in tissue culture.

As a consequence of nondisjunction and a variety of other aberrant division processes, cells originate with heteroploid, tetraploid and higher multiple chromosome numbers (12, 22, 36). At the same time, multipolar mitoses followed by multiple cell divisions will lead to cells with subdiploid constitutions. (36)

Superimposed on these processes spontaneous chromosome breakages occur, resulting in chromosomal reorganizations of various types. These include translocations, formation of ring chromosomes, chromosomes with two kinetochores and acentric chromosome fragments (2, 36). (See Fig. 6.)

The cells of most, if not all, tumor strains have highly aberrant karyotypes. The majority of strains are characterized by hyperdiploid chromosome numbers. Even cells with identical or similar counts may have very different karyotypes (36). Recently,"normal" human cells from the synovial lining of the knee were placed in

tissue culture. Between the seventh and eighth passage, heteroploid transformation took place with chromosome counts of 130 ±. The cells had been found to contain 46 chromosomes at the fifth passage (22).

The above findings carry the implication that counts on chromosomes in tissue culture cells may not be accurate. It must be noted, however, that the chromosome aberrations found in the tissue culture of synovial cells did not take place until after six and two-thirds months of culture. This is certainly far out of the range of time in which cells are cultured which are then subjected to chromosome counts (see "Method").

RELATIONSHIP TO CANCER

Cells in tissue culture are known to undergo spontaneous chromosome aberrations, due either to aberrant division processes or to chromosome breakages (12, 22, 36). X-ray is known to increase the incidence of the aberrations in short-term tissue cultures, and there is a relationship between the dose of X-ray and the number of aberrations seen (4). Cells with these abnormal karyotypes have a selective advantage over their normal counterparts in tissue culture. The changes must be viewed as giving the cells properties which better adapt them to life in tissue culture.

After these changes have taken place, these cells resemble malignant cells very closely, especially those malignant cells which have been maintained in tissue culture. Heteroploid chromosome numbers are common in malignant cells maintained in tissue culture (12, 36). Recently, Hsu, et al. (22) cultured cells from normal synovial lining obtained by biopsy. After six and two-thirds months of tissue culture, heteroploid transformation took place, with 130 + chromosomes being present in these cells. The cells, which now had an appearance of malignancy, were then transplanted into human volunteers. In the volunteers, the cells taken from normal tissue exhibited malignant behavior.

Cancer is a disease whose basic characteristic is the hereditarily transmissible abnormality in cells that is manifested by reduced control over growth and function. This leads to serious

adverse effects on the host through invasive growth and metastasis (35).

Cancer can be caused by various agents; there are hundreds of chemicals which are known to be carcinogenic, and several types of physical energy have been correlated with neoplastic change. In animals, certain viruses have been shown to cause various types of neoplasm. And, as shown above, normal cells may exhibit malignant behavior after a period of tissue culture.

In all these instances, however, the exact change which takes place in the cell which confers the neoplastic properties on the cell is not known. The change is obviously a genetic one, because the neoplastic property is transmitted to all the descendents of the changed cell. The above facts, i.e. the abnormal chromosome complements in malignant cells in tissue culture, the change in normal cells to an appearance of malignancy, and the frank malignant behavior of "normal' cells after the change in chromosomal appearance and number has taken place, all point to some relationship between malignancy and chromosome changes. Since the elucidation of the basic mechanism of carcinogenesis would be such a giant step forward, more work in this direction is certainly warranted.

DISEASES MANIFESTING ABNORMAL CHROMOSOME NUMBER OR MORPHOLOGY

Acute Leukemia:

In 1958, Ford, Jacobs, and Lajtha (13) reported a case of "active" leukemia, presumably an acute leukemia of some type, in which the marrow cells when prepared according to the method described above, persistently showed counts of 44 chromosomes, plus an additional minute fragment. In a few cells, 88 chromosomes plus two identical fragments were found. The authors noted the unusual aspect of this case, and further stated that abnormal chromosome counts had been found in radiation-induced leukemias in mice, but the numbers were always hyperdiploid, e.g. 41, 42, 43, or 44, and had never been less than the diploid number of 40 (12).

Shortly thereafter, in 1959, Baike, Court Brown, Jacobs, and Milne (2) reported detailed studies on one patient with acute myelomonocytic leukemia, and chromosome counts on four other cases of acute leukemia, six cases of chronic leukemia, and two cases of myelomatosis.

In the one case of acute myelomonocytic leukemia, persistent chromosome counts of 46-50 were found, and these persisted over a seven-month period of time, as shown in Table 3.

Sample	Date	Site	Chromosome Number <43	43	44	45	46	47	48	49	50	>50	4n	8n	>8n	Cell Counts
A	9/3/58,	Sternum	2	-	3	2	24(8)*	45	75	27	6	-				184
B	10/15/58	"	-	1	1	1	12(5)	25	28	9	1	-	1		1	80
C	1/5/59	"	-	1	-	2	9(2)	25	46	15	4	1	1			104
D	4/2/59	"	-	-	1	2	9(7)	34	22	12	1		2	1		84
E	4/20/59	"	-	1	-	2	7(4)	36	25	15	8	1	5			100
F	" "	Lumbar Spine	-	-	1	1	4(1)	7	9	13	3	1	-			39
G	" "	Iliac Crest	-	-	-	3	5(2)	18	20	6	1	7	2	1		63

* Number of cells in which no structural abnormalities found

Table 3. Chromosome counts on bone marrow cells from a patient with acute myelomonocytic leukemia. (After Baike, et al., 1959)

It is interesting to note that on the first three occasions, the counts ranged from 46-50, with a modal number of 48. There were ring chromosomes and chromosome fragments present at these times; eighteen days before death, the modal number had changed to 47, and ring chromosomes and fragments were still present. In addition, however, an abnormally large submetacentric chromosome was present in three cells. (See Fig. 6.)

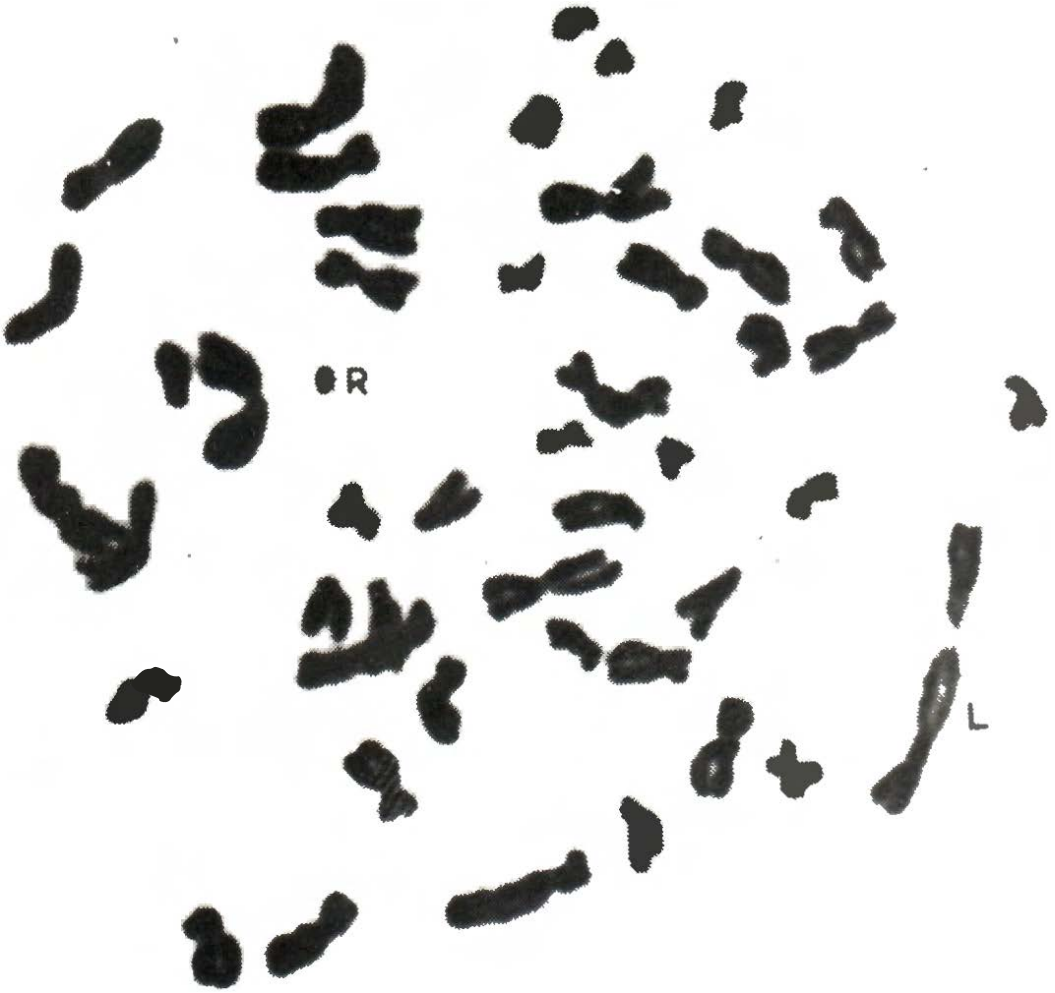

Figure 6. Photomicrograph of a sternal marrow cell from a patient with acute myelomonocytic leukemia. Fifty chromosomes are present, including ring chromosomes (R) and a large atypical chromosome (L). (From Baike, et al., Lancet 11:425-28, 1959)

To confirm that the abnormal counts were present only in bone marrow, a skin biopsy was taken thirteen days prior to death and placed in tissue culture. Chromosome counts on this material revealed a count of 46 in 36 of 37 cells counted, indicating that this patient did not have an inherent chromosome abnormality.

Four other cases of acute leukemia were examined by Baike, et al. (2). One of these had an abnormality of chromosome number, the counts ranging from 46-50 as in the first case described. Two other cases had counts of 46, but morphologic abnormalities were present. These were probably translocations of chromosome arms. In only one case of acute leukemia could no abnormalities, either in number or morphology, be found. Likewise, the six cases of chronic leukemia and two cases of myelomatosis had no chromosomal abnormalities.

Mongolism:

Mongolism was one of the first serious congenital conditions to be investigated with regard to the chromosome number. This is due in part to the relationship of mongolism with an increased incidence of acute leukemia. Jacobs, et al. (25), early in 1959, published findings on six mongoloid idiots. Bone marrow aspiration from all of these had been prepared to allow counts of chromosomes. In all of the patients, the predominant number of cells contained 47 chromosomes; 46 of these appeared very similar to those seen in normal individuals. In addition, there was a very small

acrocentric (i.e. with a terminal centromere) chromosome.

Because mongolism is associated with increasing maternal age and the mongol resembles antigenically its mother more closely than its father, it is suggestive of a disorder in oögenesis. This could be explained by nondisjunction, where one chromosome of a pair moves with its mate to the same pole during some stage in meiosis, producing an ovum with a chromosome number of n plus 1 (i.e. 23 plus 1). The alternate explanation is the occurrence of nondisjunction <u>early</u> in embryonic life, producing two cells, one with 2 n plus 1 and the other with 2 n minus 1. It is assumed that the 2 n plus 1 cell then forms the stem line.

<u>Problems Related to the Reproductive System:</u>

1. <u>Klinefelter's syndrome.</u> This syndrome is associated with gonadal dysgenesis. The patients show a male phenotype, but fail to develop secondary sex characteristics at puberty. The testes are small and atrophic, and oligo- or azoospermia is present. Pubic, axillary, and facial hair is scanty or absent. The general body habitus is female in character and characteristically the extremities are long. A high-pitched voice may be associated with the clinical condition (20).

Jacobs and Strong (26) described such a case in 1959. The patient was chromatin-positive on buccal smear and peripheral blood smear. A bone marrow aspiration prepared for counting the chromosomes gave the results in Table 4.

Chromosome number	45	46	47	48	49	Total
Number of cells	2	7	29	5	1	44

Table 4. Chromosome numbers in bone marrow cells from a case of Klinefelter's syndrome (Jacobs, P. A. & Strong, J. A., 1959)

It is obvious that the majority of cells contained 47 chromosomes. The authors considered counts other than 47 to be due to technical errors (see Ford, et al. (12)). A detailed analysis of eight cells containing 47 chromosomes was done. The following complement was found:

5 small acrocentric chromosomes (as in a male)

16 small chromosomes (same in both sexes)

16 medium sized chromosomes (as in a female)

10 large chromosomes (same in both sexes).

The above complement leads one to the conclusion that the patient has an XXY type of sex determination. This type of sex chromosome complement is known in Drosophila melanogaster, but is associated with a female phenotype. Many such female flies are fertile. It becomes obvious that the presence of Y in humans is the strong determining factor for a male phenotype.

Two other cases of Klinefelter's syndrome associated with abnormal chromosome counts have been described. One, described by Ford et al. (14) was in a patient showing the characteristics of both Klinefelter's syndrome and mongolism. A chromosome count re-

vealed most of the cells to contain 48 chromosomes. One of the extra chromosomes was the small acrocentric chromosome associated previously with mongolism, and the other was a medium-sized chromosome, similar to those in the group where X is to be found. (See Figs. 7 & 8.)

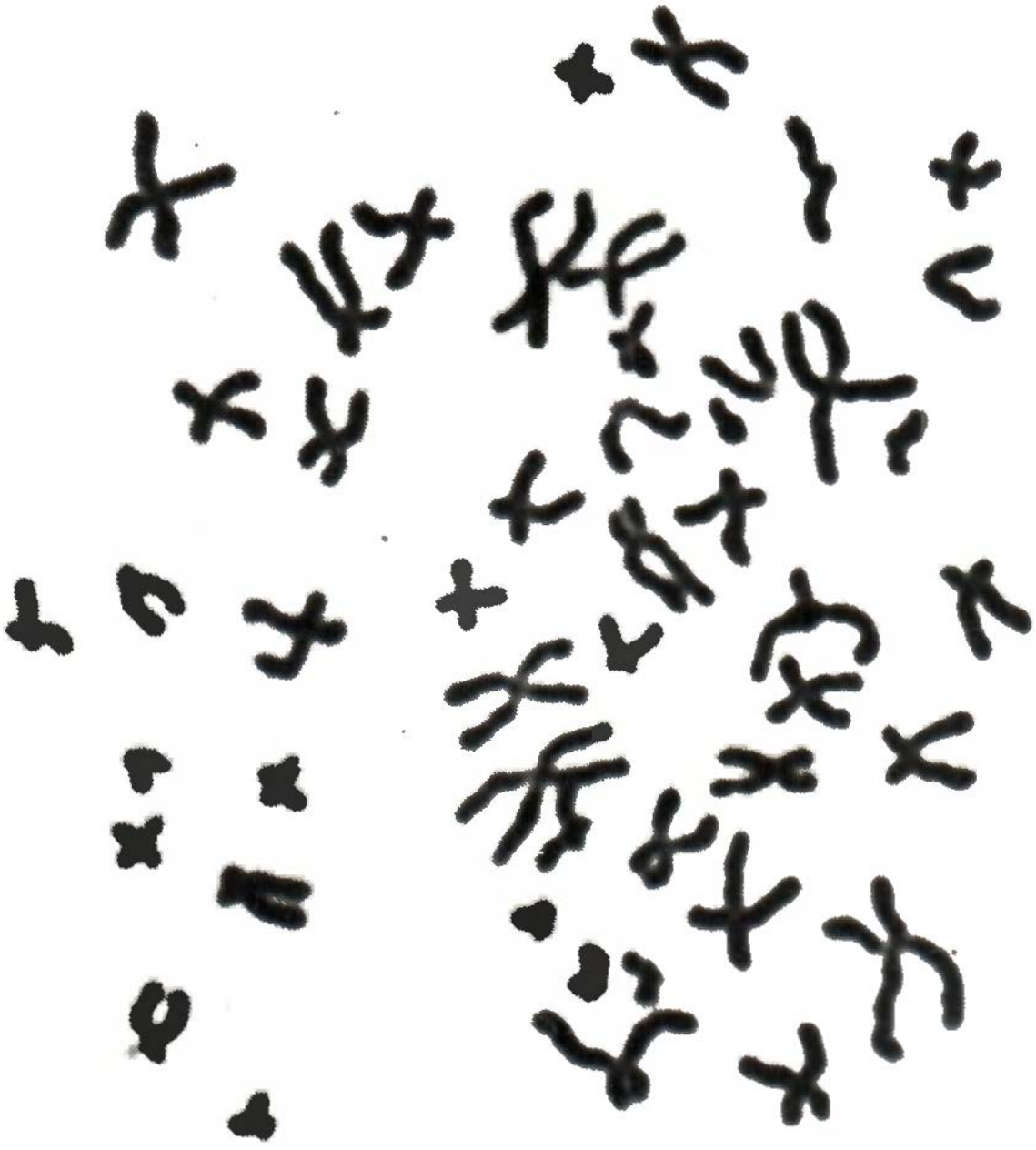

Figure 7. Mitotic metaphase in a bone marrow cell from a patient with both Klinefelter's syndrome and mongolism. Forty-eight chromosomes are present. (From Ford, et al., Lancet 1:708-10, 1959)

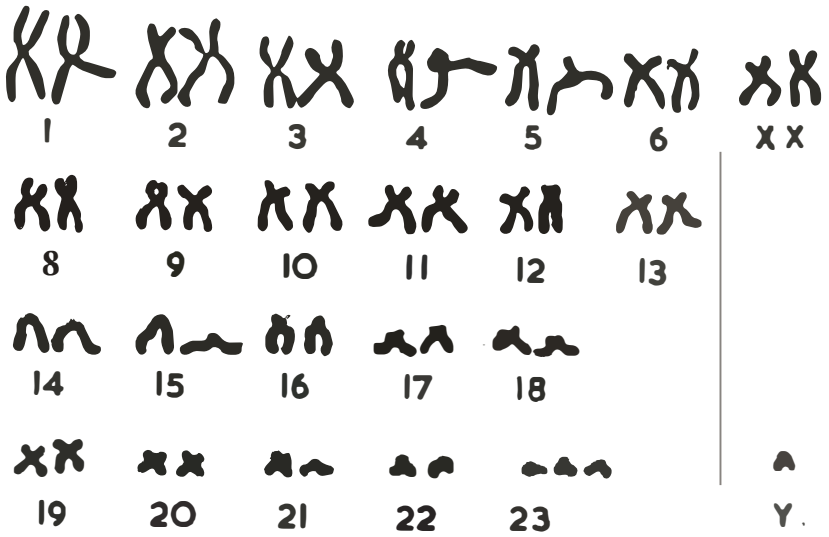

Figure 8. Chromosomes shown in Fig. 7, arranged to size; the X-chromosomes are seventh in this order. (From Ford, et al., Lancet 1:708-10, 1959)

The conclusion was that this patient was also XXY with respect to the sex-chromosomes.

The final case of Klinefelter's syndrome associated with abnormal chromosome counts was also described by Ford, Polani, Briggs and Bishop (16). This case demonstrated all of the clinical aspects of Klinefelter's syndrome. Chromosome counts were undertaken and showed the results in Table 5.

Chromosome number	41	42	43	44	45	46	47	48	49	Total
Number of cells	1	0	0	2	3	13	44	1	1	65

Table 5. Chromosome counts in 65 cells from a patient showing Klinefelter's syndrome. (After Ford, et al., Nature 183: 1030, 1959)

It can be quickly seen that the predominating number is 47, as would be expected from previous reports. However, a high proportion of the cells (20% of the total) show 46 chromosomes. This is indeed higher than had been obtained in other preparations, and higher than would be expected from damage alone with loss of only one chromosome. In addition, the loss of the same chromosome in five cells and probably eight cells (as observed by the authors) seems extremely unlikely. Therefore, the authors conclude that this patient has the added feature of probably being a true mosaic (an individual with cells having two or more different chromosome numbers.)

Twelve cells containing 47 chromosomes were selected for analysis. In these there were five short acrocentric and sixteen other short chromosomes, as in a male, and sixteen medium-length chromosomes, as in a female. This, as noted above, has been observed in other cases of Klinefelter's syndrome and is presumed to be due to the presence of an XXY sex-chromosome complement coupled with twenty-two pairs of normal autosomes.

Similar analysis of eleven cells containing 46 chromosomes was undertaken. Findings in these cells were four short acrocentric chromosomes and sixteen other short chromosomes, as in a female, and sixteen medium-length chromosomes also as in a female. The conclusion is that these cells were XX with twenty-two pairs of normal autosomes.

While loss of a chromosome is known to result in a cell's rapid elimination, the loss of the Y in an XXY individual would be a step toward normality, and such a cell might, therefore, have a small selective advantage over its progenitors. At the same time, the other cell resulting from such a non-disjunctive cell mitosis could conceivably have an XXYY sex-chromosome complement. This would be more abnormal, and this cell would be expected to perish.

2. Turner's Syndrome. Patients exhibiting this syndrome are phenotypical females whose ovaries fail to develop and remain as small fibrotic streaks or chords in each broad ligament. The clinical manifestations can be directly related to estrogen deficiency: these include infantile sex organs, lack of breast development, primary amenorrhea, diminished public and axillary hair, delayed epiphyseal closure, and osteoporosis. Association with short stature, webbing of the neck, wide chest, cubitus valgus and coarctation of the aorta are rather frequent. The patients are sterile, of course (20). A rather high percentage (approximately 80%) of patients have chromatin-negative patterns on buccal smear and leukocyte examination (34).

As long ago as 1956 attempts were made to determine the chromosomal sex of patients with this syndrome by utilizing results of studies on red-green colorblindness (34). (Red-green colorblindness is caused by a sex-linked recessive gene carried on the X-chromosome. Its presence on the X in a male leads to the clinical

manifestations of red-green colorblindness. Both X-chromosomes in the female must carry the gene for the clinical condition to be manifest, however. The condition, therefore, is much more common in males, occurring in 7-8% of the general population. Only about 0.6% of the females are so affected.)

In a group of 25 females with Turner's syndrome, 16% were found to have red-green colorblindness. This was much greater than expected, and suggested that the patients were of X-Y genotype, or perhaps X-0.

Direct studies on the chromosomes were the only answer to the question. These came in 1959, when Ford, Jones, et al. (15) reported a case of Turner's syndrome having only 45 chromosomes. The authors suggested that the missing chromosome was one of the X's. Three chromosomes of almost equal length were present, making identification of X impossible, but the length of all three was similar to that of the X measured in X-Y bivalents seen at metaphase in primary spermatocytes. The authors thought, therefore, that the patient was X-0 in type, and was the result of nondisjunction (both X-chromosomes moving to one pole) during meiosis. (See Figs. 9 & 10.)

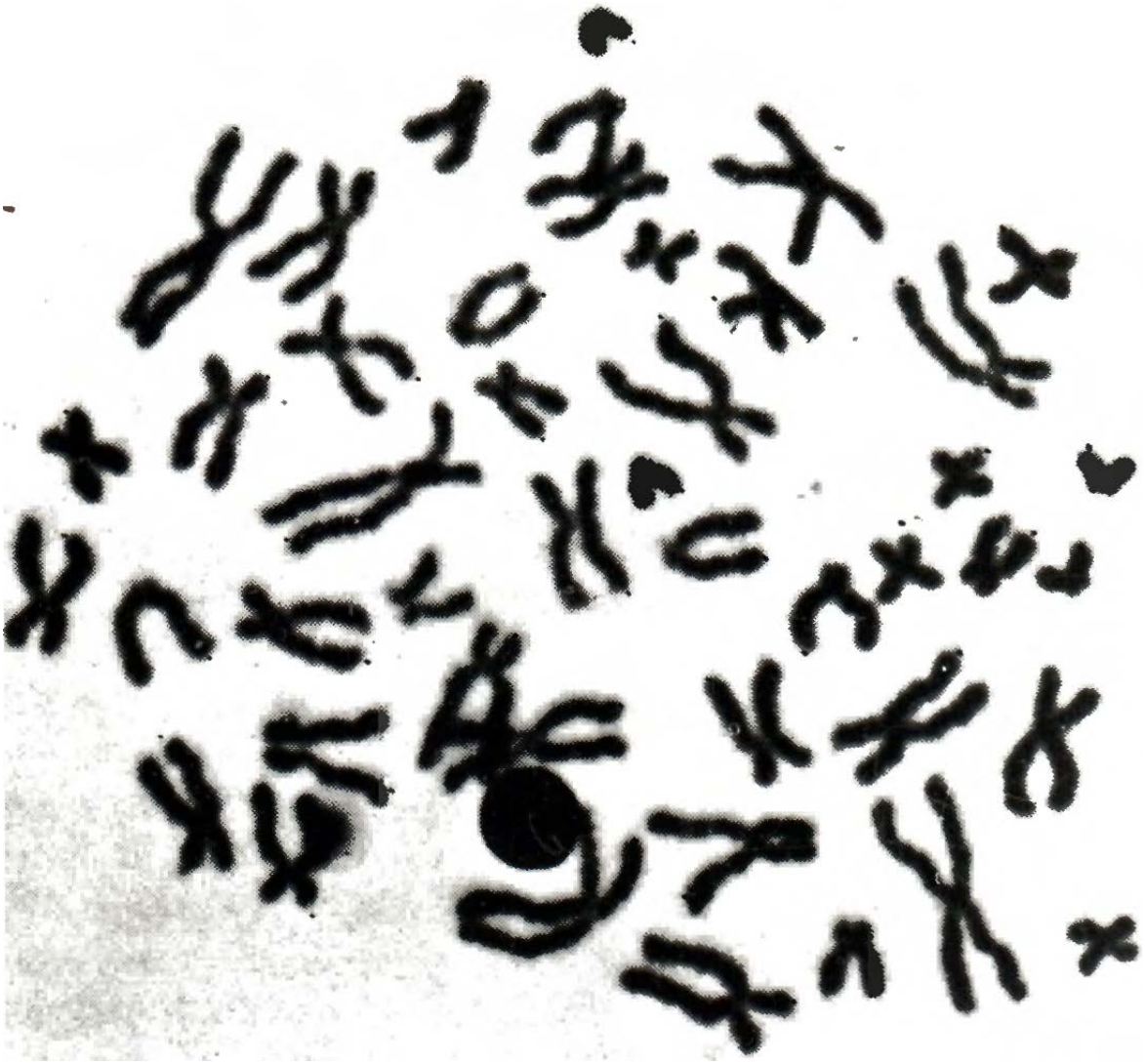

Figure 9. Mitotic metaphase in a bone marrow cell from a patient with Turner's syndrome. Forty-five chromosomes are present. The large black spot is probably an oil droplet. (From Ford, et al., Lancet 1:711-13, 1959)

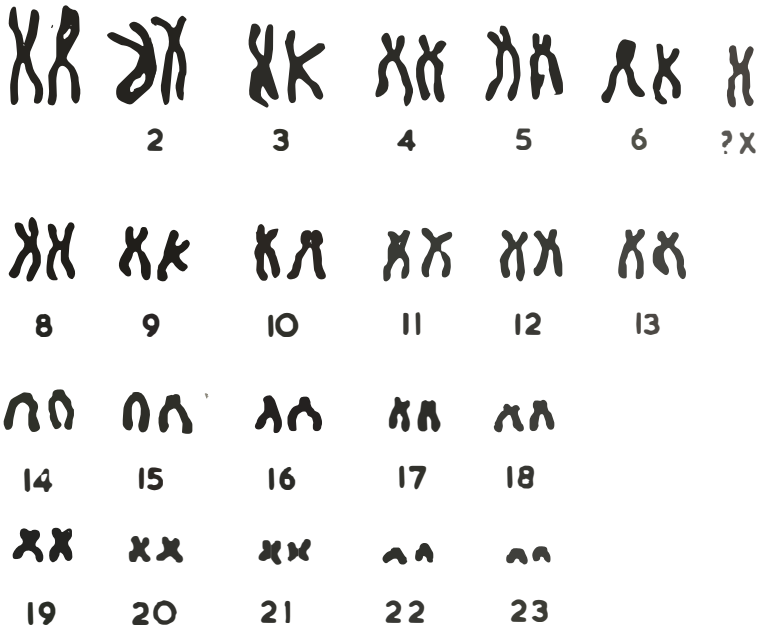

Figure 10. The chromosomes shown in Fig. 9 arranged to size. (From Ford, et al., Lancet 1:711-13, 1959)

Two further cases of Turner's syndrome have been examined (16), and both show a chromosome number of 45. Analysis of the cells showed complements consistent with an X-O diagnosis.

3. Superfemale. It is obvious that nondisjunction during oögenesis or spermatogenesis can produce ova containing XX, X, or O sex-chromosomes and sperm with XY, X, Y, or O sex-chromosomes. In Drosophila melanogaster combinations of such ova and sperm are known to produce YO (non-viable), XO (sterile male), XXY (fertile female) and XXX ("super" female) combinations in addition to normal XX and XY combinations. Since XO and XXY combinations have already been found in humans, other combinations could be expected. Such is the case.

Jacobs, Baike, Court Brown, MacGregor, Maclean and Harnden (24) have recently reported such a case. The patient is a 35-year-old female. Her mother was 41 and her father 40 at the time of her birth. She is third in a sibship of three girls. Her chief complaint when first seen was primary amenorrhea at age 22. Menstruation had ceased three years prior to that, after five years of extremely irregular menstrual bleeding. This patient's breasts were underdeveloped and her external genitalia infantile in character. Urinary gonadotropin excretion was persistently high. Seven years ago, at laparotomy, the ovaries appeared post-menopausal.

Chromosome studies were done on bone marrow cells. The majority of the cells examined contained 47 chromosomes. A detailed

study on thirteen cells showed an extra chromosome in the medium-sized group where the X is known to be found. A tissue culture of a skin biopsy confirmed the chromosome count of 47. Photographs of the chromosomes show convincing evidence to support the conclusion that this patient is an XXX human "super" female.

4. Testicular Feminization. Other syndromes involving the reproductive system and leading to signs and symptoms usually due to estrogen or androgen deficiency are known. Such a syndrome is that of testicular feminization (23). These patients are female in phenotype. Pubic and axillary hair is absent or scanty, the habitus is female, and there is primary amenorrhea. The vagina is usually incompletely formed. Many have inguinal hernias, and testes can be found in the canals; less frequently testes are found in the labia majora or in the abdomen. There may be a rudimentary uterus and fallopian tubes. This condition is familial and is transmitted through the maternal line.

Chromosome counts on these patients show 46 chromosomes to be present (23). Analyses show the complement to be identical with that found in normal males (i.e. five short acrocentric chromosomes, sixteen other short chromosomes, fifteen medium-sized chromosomes, and ten long chromosomes). These findings indicate that the condition is probably due to a sex-linked recessive gene or a sex-limited dominant autosomal gene. This is consistent with family analyses which show that the ratio of females to normal males plus affected males is approximately 1:1.

DISCUSSION

Biological Constancy of the Chromosome Complement:

Since 1923, when Painter stated that man has 48 chromosomes, this number has been accepted as the chromosome number for man. Studies on other animals have shown the chromosome number to be a biological constant, and the same constancy is assumed to be present in man. Indeed, the whole explanation of the perpetuation of a species depends on this constancy, claiming that a chromosome reduction takes place in meiosis, so that the 2n number of chromosomes is reestablished in the fertilized gamete. One might say that the field of genetics makes the biological constancy of the chromosome number within a species one of its basic premises.

The finding of Tjio and Levan that man's cells contain only 46 chromosomes has not changed the concept of a constant chromosome number. To date, only Kodani disagrees with this premise, and his work awaits confirmation.

If one assumes that the chromosome number in man is a constant (and most workers have agreed on this), then the finding of abnormal counts in the cells of persons with previously baffling diseases offers a logical explanation for the etiology of these diseases. Such is the case with mongolism, Klinefelter's syndrome, and Turner's syndrome, where abnormal chromosome counts are now known to be present. If, however, one accepts the work of Kodani and assumes that small supernumerary chromosomes are frequently

present in man, then one must conclude that these small chromosomes exert little or no genetic effect. This conclusion makes the finding of a small chromosome in the cells of patients with mongolism carry little, if any, significance as an etiologic factor in the disease. The finding of an extra chromosome or the absence of one, as in Klinefelter's and Turner's syndromes, respectively, can still be associated with the etiology of these diseases, as the chromosome involved is the X rather than a small, acrocentric chromosome.

Obviously, more work is needed in this field in order to prove or disprove the presence of supernumerary chromosomes in man. Until then, one can only say that the concept of constant chromosome number within a species is a time-honored one, and that adherence to it now offers an explanation of the etiology of diseases where deviations from this number have been found.

While the above argument continues, study of the morphology of the individual chromosomes gives more information about them. The extremely small standard deviation in chromosome length and arm ratio found by Chu and Giles is almost unbelievable; in fact, one might expect this much deviation merely from errors in measurement. The findings of Tjio and Puck are in such close agreement with those of Chu and Giles, however, that one immediately concludes that the morphology of the individual chromosomes is quite constant. If one accepts the premise that genes are carried on the chromosomes, that there is a constant number of genes, and that the

genes of identical chromosomes pair up, with gene pairs in apposition, and that this happens not only within a given individual but also between individuals in the process of fertilization, then one would have concluded that chromosomes must be remarkably similar from individual to individual. If this were not so, there would be imperfect pairing of chromosomes when a sperm fertilizes an ovum.

It is gratifying to find that chromosome morphology is almost constant from individual to individual, as this is direct evidence for a belief which has been held since the beginnings of genetics, dating back one hundred years to Gregor Mendel's time.

Mechanisms of Development of Abnormal Chromosome Complements:

Two mechanisms are known by which abnormal chromosome complements may arise. Knowledge of these has come from the extensive genetic studies which have been done on other species.

The first of these mechanisms involves a complete chromosome and gives rise to an abnormal chromosome number. During the process of mitosis (or meiosis), both chromosomes of one pair move to one pole, rather than the pair splitting, with one chromosome going to each pole. This gives rise to two cells with abnormal counts. If the error took place during mitosis, one cell has 2n plus 1, and the other cell 2n minus 1 chromosomes. If the end products are ova and sperm, the chromosome number could be either n plus 1 or n minus 1. Such a mechanism is called nondisjunction and probably takes place in gametogenesis of one of the parents of patients with such

disorders as Klinefelter's or Turner's syndromes. An alternative explanation is that the error takes place very early in the embryonic life of such patients; one of the cells then perishes and the other forms the stem line. If both cells survive, or if normal cells survive along with one of the cells with an abnormal chromosome number, then the individual becomes a mosaic, such as the one described by Ford *et al*.

On the other hand, changes in chromosome complement may involve only parts of chromosomes. Such changes may have nothing to do with cell divisions. Such a phenomenon is *translocation*, in which a portion of one chromosome, e.g. one arm, breaks off and becomes attached to another chromosome. This change then persists through succeeding mitoses, and two abnormal chromosomes are present. This process may, of course, involve more than one chromosome, and result in extremely bizarre chromosome complements. The more deranged the chromosomes become, the more aberrant the cell becomes. There is a point beyond which this process cannot go, as the cell will become non-viable. This process is probably quite common, but one fails to see such cells, because they either die or fail to undergo another mitosis.

There may be an exchange of genetic material between chromosomes, or a rearrangement of genetic material within a chromosome, without a visible change in chromosome morphology. Such a mechanism is thought to be at play during the phenomenon of

"crossing over." This takes place, or at least potentially occurs, wherever a chiasma occurs on a chromosome, and judging from the number of chiasmata which are visible, it is quite a frequent occurrence.

Relationship of Chromosome Changes to the Theories of Carcinogenesis:

As noted above, there seems to be some relationship between chromosome changes and malignant behavior. It is generally agreed, however, that the chromosome aberrations which can be seen are not the cause of the malignant behavior exhibited by cells showing abnormal chromosome complements. Baike, et al. (2) offer three explanations for the presence of the abnormal chromosome complements seen in their cases of acute leukemia. First, the chromosome changes may arise as epiphenomena in a grossly disordered bone marrow; second, the visible chromosome changes may include the fundamental change which has conferred the neoplastic qualities on the marrow cells; and third, the chromosome changes may be the remote consequences of a more subtle alteration in the genetic constitution of the leukemic cell. The authors feel that the last possibility is the most likely, and reject the first two.

Certainly there must be some relationship between chromosomes and cancer, because cancer has a genetic component and the chromosomes are the genetic material of a cell. The change which occurs in the genetic material is not known; however, it is generally agreed that the change must be very small, at least in the beginning.

Armitage and Doll (1) have proposed a two-stage theory of carcinogenesis. The first stage is the exposure to a carcinogenic agent; this induces a change in the cell which confers on its descendents a faster rate of multiplication than the unchanged cells. Frank malignant change is not manifest, however. The number of cells so changed would depend on the concentration of the carcinogenic agent.

If "aging" were then regarded as something that occurred as a result of prolonged asexual multiplication, and cancerous degeneration was the end result of aging, it would follow that cancer would appear in the descendents of the changed cell sooner than in the surrounding tissue, but only after a long latent period. This malignant change is the second stage.

Such a theory would explain several facts which are known about human cancer: (a) the differences between the shapes of the curves relating mortality to age, observed for different sites; (b) the long latent period observed after exposure to a carcinogen before a tumor develops; and (c) the apparent linearity of the relationship between cancer incidence and the concentration of carcinogen to which the patient is exposed.

Presently there are several other theories of carcinogenesis. The first of these, and one which has wide support, is the somatic mutation theory. A mutation is a change which takes place in a gene which confers new properties on it. There are two criteria

which this change must fulfill in order to be called a mutation; first, the change must be permanent and the mutated gene must duplicate itself in its altered form, and second, the altered gene must be independent and able to segregate from its normal, unchanged partner. This segregation can occur only during gamete formation, and, therefore, only germinal mutation satisfies both of these criteria (30). Somatic mutation is known to occur in plants, where it is more easily studied because of the linear growth of plants. There is no reason to believe that it does not occur in somatic cells of humans at least as frequently as in germinal cells, i.e. a rate of one mutation in 100,000 cells during one cell generation (5).

It is believed that a series of somatic mutations must occur in a cell before frank malignant change takes place. This stepwise theory of cancer etiology assumes that the changed cells are capable of further proliferation, and that the successive mutations confer a certain advantage on the affected cells. It is likely that the second mutation is more apt to occur in a cell where the first mutation has already occurred, and that the third is more apt to occur in a cell where two mutations have occurred. Evidence now points to a six-step process, in which six mutations would be necessary before all control of cell growth and reproduction is lost (5).

A second popular theory of carcinogenesis is the <u>viral theory.</u>

That viruses can cause a wide variety of tumors in animals is well known and documented (17, 18). One might expect these viruses to be quite complex, consisting of nucleic acid associated with protein and possibly other chemical compounds in unknown quantity (18). Very simply, by analogy with non-tumor viruses, this is probably DNA or RNA associated with a protein.

This structure would explain many phenomena associated with tumor viruses. For example, viral replication and specificity is probably associated with the DNA, while the immunologic reactions are associated with the protein.

The exact mechanism by which viruses cause tumors is, of course, not known. It is possible that these viruses invade a cell and associate themselves with the DNA present in the genes. By so doing, a genetic change is effected in the controlling mechanisms of cell growth and reproduction. Or, the virus may invade the cell and form a bit of genetic material by itself. This would be self duplicating in succeeding generations, and its presence would confer neoplastic qualities on the cell and its descendents. A third alternative is that the mere presence of the virus causes a change in the controlling mechanisms of growth and cellular reproduction, and the virus does not join with the DNA of the chromosomes to form an autogenous bit of genetic material.

Such a theory cannot explain the long latent periods of most cancers, and the increasing incidence of cancer seen with increasing

age. If one proposes a long incubation period in these viruses, then one can partially explain these phenomena. Such a latent period (6-8 months) is seen in mouse leukemias which are caused by a virus injected into newborn mice (17).

In viral-induced neoplasms in animals, there are other mechanisms which could come into play in the transmission of malignancy (17). One of these is transplantation, where neoplastic cells are transmitted from one animal to another. Such great pains are taken to produce cell-free filtrates that if transplantation does occur, it must be rare, and due to experimental error.

The second possibility is induction, which has been mentioned above. This assumes that cells take on neoplastic properties when certain viruses are present. There is no change in the genetic material, but the functioning of the cell is altered by the presence of the virus. This seems less likely than the theory of viral incorporation into the genetic material, or the formation of new genetic material by the virus.

The only other popular theory of carcinogenesis which is presently entertained is the plasmagene theory (30). A plasmagene is a bit of genetic material, like a gene, which is located in the cellular cytoplasm outside the cell nucleus. These plasmagenes reduplicate themselves from generation to generation just as genes do, and may undergo all of the phenomena characteristic of genes, including mutation.

The plasmagene theory states that tumor "agents" can arise de novo within the cell. Tumors which can be propagated only by cell transplantation would be caused by somatic mutation in a plasmagene, which would still remain an integral part of the cytoplasmic system in which it arose. Tumors which can be propagated by cell-free extracts would owe their origin to a plasmagene which by mutation or series of mutations becomes an "agent" or virus with the ability to infect other cells by injection.

Close examination of the above theories reveals that they are really not far apart in their considerations. Since each theory explains part of the known facts about carcinogenesis, reconciliation of the theories could provide a logical theory to explain most of the facts known about carcinogenesis.

Burnet (5) has proposed such a reconciled theory. He states that cancer viruses represent one potent means by which what is functionally equivalent to a somatic mutation can be produced. This could be extended to include plasmagenes. This theory, then, places certain viruses among the hundreds of chemicals and several types of physical energy which can act as evokers of the neoplastic reaction. One can go a step further, and accept the part of the plasmagene theory which states that somatic mutations in plasmagenes can give rise to tumor agents or viruses. If one does this, then the whole theory becomes interacting; viruses can cause somatic mutations, which then lead to neoplasm, or somatic mutation can give rise to

tumor viruses, which then cause neoplasm.

It is obvious that visible chromosome changes, even though often seen in malignant cells, are thought to play no role in carcinogenesis. It is possible, though, that these chromosomal aberrations may reflect the more subtle changes which are thought to take place. It takes little imagination to envision chromosome breaks occurring after somatic mutations or after union of a virus with a chromosome. Viral particles which act as autogenous new genetic material may be visible, and conceivably could be "ring" chromosomes as seen in Fig. 5.

Chromosomal aberrations are not necessary for malignant behavior, however, as evidenced by normal chromosome complements seen in many malignant cells (2, 13). The exact relationship between the theories of carcinogenesis and chromosomal aberrations obviously remains to be elucidated. Until this relationship becomes clear, one must view the deranged chromosome complements often seen in malignant cells as a new and valuable adjunct to the investigation of cancer etiology.

SUMMARY

New work on the chromosome number of man in 1956 caused a revision in thinking on this subject. The chromosome number was conclusively shown to be 46, rather than 48 as previously thought. Development of new techniques of tissue culture was combined with incubation of cells in hypotonic solutions to cause spreading of the chromosomes and in dilute solutions of colchicine to cause arrest of mitosis in metaphase where the chromosomes could be easily seen. Further refinement of the technique came when easily accessible bone marrow cells were used in short-term tissue culture.

Extensive study of the chromosome number and morphology by several groups has shown that both are remarkably constant from individual to individual. Much time and effort has been spent in attempting to identify the X- and Y-chromosomes.

Study of the chromosomes of individuals with manifestations of several diseases has further elucidated the etiology of these diseases. Mongolism has been shown to be associated with an extra, small chromosome. Klinefelter's syndrome has been shown to have a sex-chromosome pattern of XXY, and a total chromosome number of 47. Turner's syndrome has a sex-chromosome pattern of XO, with only 45 chromosomes. "Females" with testicular feminization have been shown to be XY individuals, and one individual with XXX sex-chromosomes has been found.

Many of these diseases had been thought to be associated with sex-chromosome derangements, and study of the chromosomes has borne this premise out.

Of even more interest is the possible relationship of chromosome derangements and cancer. Mongolism is known to be associated with an increased incidence of leukemia. Several patients with acute leukemia have been shown to have abnormal chromosome number and deranged morphology. Tissue cultures of malignant cells are known to have abnormal chromosome complements, and recently a tissue culture of "normal" cells underwent a transformation of chromosome number with 130 plus chromosomes appearing after six and two-thirds months of culture. These cells demonstrated malignant behavior when transplanted into human volunteers.

At present, no concrete relationship between abnormal chromosome counts and malignancy can be shown. In fact, most authorities deny any cause and effect relationship between chromosome changes and malignancy. Rather, the theories of carcinogenesis consider somatic or plasmagene mutation or viruses as the causative agents in cancer. Examination of these theories reveals that many of their premises can be reconciled, so that a single, logical explanation can be put forth for the etiology of cancer. Chromosome abnormalities which are seen in malignant cells are thought to be the result of physiologic changes in the cell which are due to the subtle changes of mutation and/or virus.

Further work in the field of chromosomes and their relation to disease will undoubtedly reveal new facts about these little bits of protoplasm which do so much to determine man's physical characteristics.

ACKNOWLEDGEMENTS

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GLOSSARY

acrocentric - (adj.) description of a chromosome having a terminal or nearly terminal centromere.

crossover - an interchange of factors or genes between homologous chromosomes of a hybrid.

diploid (2n) - having two sets of chromosomes, as normally found in the somatic cells of higher organisms.

genotype - the fundamental hereditary constitution (or assortment of genes) of an individual.

haploid (n) - having a single set of chromosomes, as normally carried by a gamete, or having one complete set of nonhomologous chromosomes.

heteroploid - possessing a chromosome total that is not an exact multiple of the haploid number.

nondisjunction - nonseparation of a chromosome pair in mitosis in consequence of which both chromosomes pass to one daughter cell.

phenotype - the outward visible expression of the hereditary constitution of an organism.

tetraploid (4n) - having four full sets of homologous chromosomes.

translocation - the joining of a part of one broken chromosome with the part of another.

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