THE CHROMOSOME NUMBER OF MAN

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ESTACION EXPERIMENTAL DE AULA DEI, ZARAGOZA, SPAIN, AND CANCER CHROMOSOME
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WHILE staying last summer at the Sloan-Kettering Institute, New York, one of us tried out some modifications of Hsu's technique (1952) on various human tissue cultures carried in serial *in vitro* cultivation at that institute. The results were promising inasmuch as some fairly satisfactory chromosome analyses were obtained in cultures both of tissues of normal origin and of tumours (LEVAN, 1956).

Later on both authors, working in cooperation at Lund, have tried still further to improve the technique. We had access to tissue cultures of human embryonic lung fibroblasts, grown in bovine amniotic fluid; these were very kindly supplied to us by Dr. RUNE GRUBB of the Virus Laboratory, Institute of Bacteriology, Lund. All cultures were primary explants taken from human embryos obtained after legal abortions. The embryos were 10—25 cm in length. The chromosomes were studied a few days after the *in vitro* explantation had been made.

In our opinion the hypotonic pre-treatment introduced by HSU, although a very significant improvement especially for spreading the chromosomes, has a tendency to make the chromosome outlines somewhat blurred and vague. We consequently tried to abbreviate the hypotonic treatment to a minimum, hoping to induce the scattering of the chromosomes without unfavourable effects on the chromosome surface. Pre-treatment with hypotonic solution for only one or two minutes gave good results. In addition, we gave a colchicine dose to the culture medium 12—20 hours before fixation, making the medium 50×10^{-9} mol/l for the drug. The colchicine effected a considerable accumulation of mitoses and a varying degree of chromosome contraction. Fixation followed in 60 % acetic acid, twice exchanged in order to wash out the salts left from the culture medium and from the hypotonic solution that would otherwise have caused precipitation with the orcein. Ordinary squash preparations were made in 1 % acetic orcein. For chromosome counts the squashing was made very mild in order to keep the chromosomes in the metaphase groups. For idiogram studies a more thorough squashing was preferable. In many cases single cells were squashed

under the microscope by a slight pressure of a needle. In such cases it was directly observed that no chromosomes escaped.

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With the technique used exact counts could be made in a great number of cells. Figs. 1 *a* and *b* represent typical samples of the appearance of the chromosomes at early metaphase (*a*) and full metaphase (*b*), showing the ease with which the counting could be made. In Table 1 the numbers of counts made from the four embryos studied are recorded.

TABLE 1. *Number of exact chromosome counts made.*

Embryo No.	Number of cultures	Number of counts
1	5	15
2	10	98
3	3	119
4	4	29
Total	22	261

We were surprised to find that the chromosome number 46 predominated in the tissue cultures from all four embryos, only single cases deviating from this number. Lower numbers were frequent, of course, but always in cells that seemed damaged. These were consequently disregarded just as the solitary chromosomes and the groups with but a few chromosomes, which were frequent. In some doubtful cases the numbers 47 and 48 were counted (in four cases not included in the table). This may be due to one or two solitary chromosomes having been pressed into a 46-chromosome plate at the squashing. It is also possible that deviating numbers may originate through non-disjunction, thus representing a real chromosome number variation in the living tissue. This kind of variation will probably increase as a consequence of the change in environment for the tissue involved in the *in vitro* explantation. HSU (1952) reports a certain degree of such variation in his primary cultures. LEVAN (1956), studying long-carried serial subcultures, found hypotriploid stemline numbers in two of them, and a near-diploid number in a third culture. In this culture one cell with 48 chromosomes was analysed. Naturally, at that time, this was thought to represent the normal diploid number.
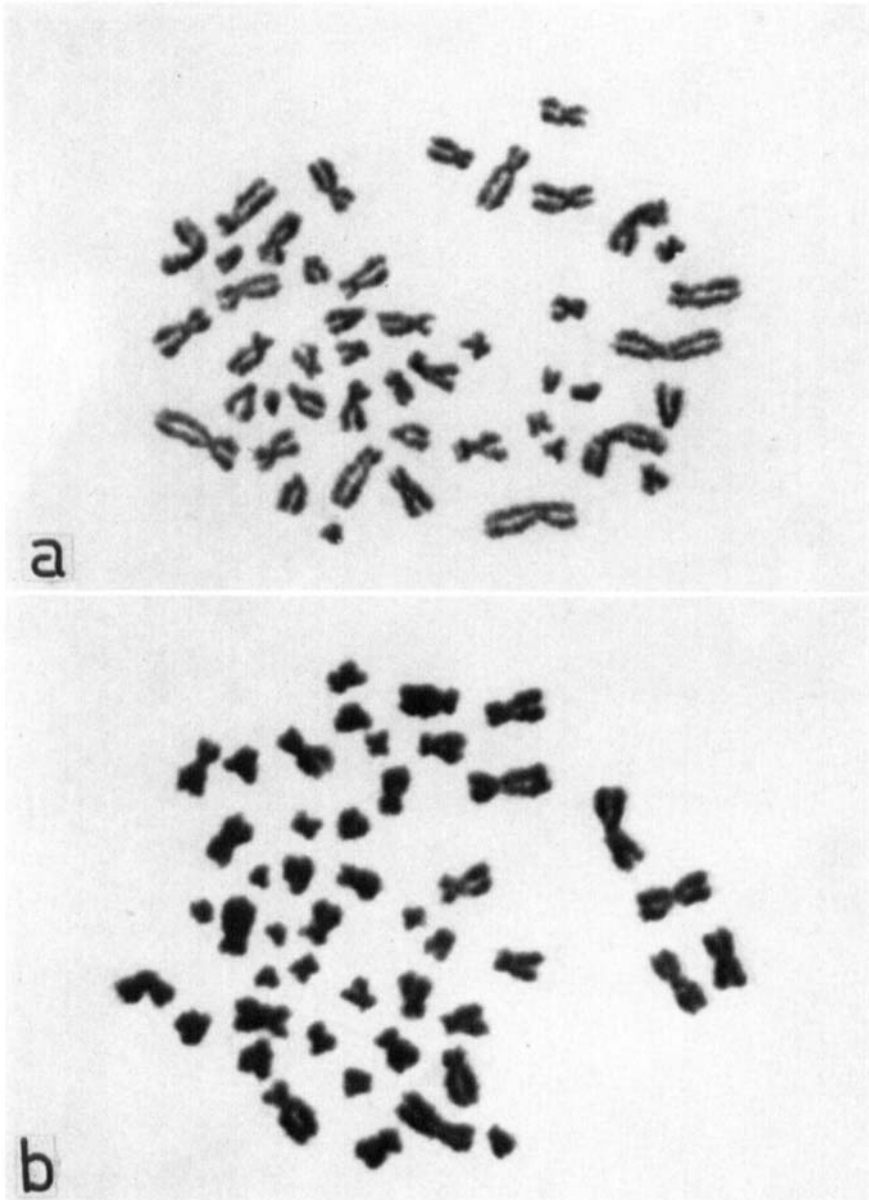
Fig. 1. Colchicine-metaphases of human embryonic lung fibroblasts grown *in vitro*. *a*: early metaphase, *b*: full metaphase. The two cells are from embryos 2 and 3 (Table 1), respectively. — ×2300.

CHROMOSOME MORPHOLOGY

Some data on the chromosome morphology of the 46 human chromosomes will be communicated here. The detailed idiogram analysis will be postponed, however, until we are able to study individuals of known sex, the sex of the present embryos being unknown. The comparative study of germline chromosomes in spermatogonial mitoses constitutes an urgent supplement to the present work.

In Fig. 2 four cells are analysed ranging from late prophase (*a*) to late c-metaphase (*d*). The chromosomes of metaphases with moderate colchicine contraction vary in length between 1 and 8 μ (Fig. 2 *b*), but the entire range of variation of Fig. 2 is from 1 to 11 μ. The chromosome morphology is roughly concordant with the observations of earlier workers, as, for instance, the idiogram of Hsu (1952). The chromosomes may be divided into three groups: M chromosomes (median-submedian centromere; index long arm : short arm 1—1,9), S chromosomes (subterminal centromere; arm index 2—4,9), and T chromosomes (nearly terminal centromere; arm index 5 or more).

The M and S chromosomes are present in about equal numbers (twenty of each), while six T chromosomes are found. The classification of the three groups is arbitrary, of course, since gradual transitions of arm indices occur between the three groups. Certain submedian M chromosomes are hard to distinguish from some of the S chromosomes, and the most asymmetric S chromosomes approach the T group.

The chromosomes are easily arranged in pairs, but only certain of these pairs are individually distinguishable. Thus, the M chromosomes include the three longest pairs, which can always be identified. The two longest pairs are different: the second having a decidedly more asymmetric location of its centromere. The two or three smallest M pairs are also recognizable. Between the three longest and the three shortest pairs there are four intermediate pairs that cannot be individually recognized.

The S chromosomes are hardly identifiable, since they form a series of gradually decreasing length. The largest pair, however, is characteristic. Certain chromosomes were seen to have a small satellite on their short arms. Secondary constrictions, too, have been observed now and then, so that it may be hoped that the detailed morphologic study will lead to the identification of more chromosome pairs. The T chromosomes are recognizable; they constitute three pairs of middle-sized chromosomes. Unlike the mouse chromosomes, the human T chromosomes evidently have a small shorter arm.
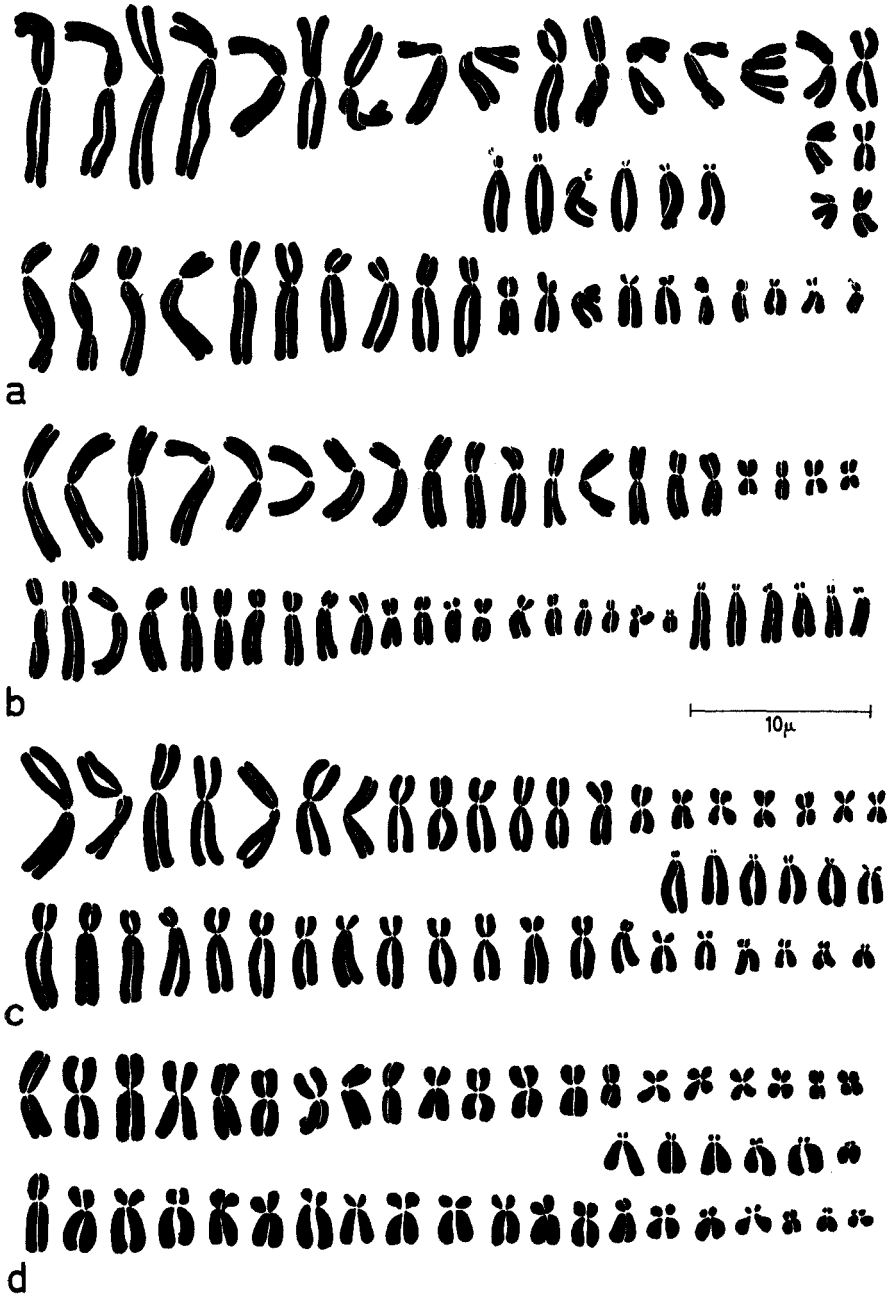
Fig. 2. Four idiogram analyses of human embryonic lung fibroblasts grown *in vitro*. The chromosomes have been grouped in three classes: M (top row), S (bottom row), and T (in between, except in *b*, where T is at the end of the S row). Within each class the chromosomes have been roughly arranged in diminishing order of size. — ×2400.

CONCLUSION

The almost exclusive occurrence of the chromosome number 46 in one somatic tissue derived from four individual human embryos is a very unexpected finding. To assume a regular mechanism for the exclusion of two chromosomes from the idiogram at the formation of a certain tissue is unlikely, even if this assumption cannot be entirely dismissed at this stage of inquiry. Our experience from one somatic tissue in mice and rats, *viz.*, regenerating liver, speaks against this assumption. The exact diploid chromosome set was always found in regenerating liver.

After the conclusion had been drawn that the tissue studied by us had 46 as chromosome number, Dr. EVA HANSEN-MELANDER kindly informed us that during last spring she had studied, in cooperation with Drs. YNGVE MELANDER and STIG KULLANDER, the chromosomes of liver mitoses in aborted human embryos. This study, however, was temporarily discontinued because the workers were unable to find all the 48 human chromosomes in their material; as a matter of fact, the number 46 was repeatedly counted in their slides. We have seen photomicrographs of liver prophases from this study, clearly showing 46 chromosomes. These findings suggest that 46 may be the correct chromosome number for human liver tissue, too.

With previously used technique it has been extremely difficult to make counts in human material. Even with the great progress involved in HSU's method exact counts seem difficult, judging from the photomicrographs published (HSU, 1952 and elsewhere). For instance, we think that the excellent photomicrograph of HSU published in DARLINGTON's book (1953, facing p. 288) is more in agreement with the chromosome number 46 than 48, and the same is true of many of the photomicrographs of human chromosomes previously published.

Before a renewed, careful control has been made of the chromosome number in spermatogonial mitoses of man we do not wish to generalize our present findings into a statement that the chromosome number of man is $2n=46$, but it is hard to avoid the conclusion that this would be the most natural explanation of our observations.

SUMMARY

The chromosomes were studied in primary tissue cultures of human lung fibroblasts explanted from four individual embryos. In all of them the chromosome number 46 was encountered, instead of the expected number 48. Since among 265 mitoses counted all except 4 showed the number 46, this number is characteristic of the tissue studied. The possible bearing of this result on the chromosome number of man is discussed.

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