A STUDY OF THE KAROTYPE OF A MONOLAYER CULTURE OF CARCINOMA OF THE HUMAN STOMACH

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As a result of improvements in the technique of explantation and prolonged cultivation of normal and malignant cells in vitro, during recent years a new trend in genetics has developed and progressed – the genetics of somatic cells [2, 4, 18]. A result of this has been the rapid development of cytogenetics, for it has become possible to obtain histological preparations not only for counting, but also for identifying the individual chromosomes in malignant and normal cells [5, 7, 10, 11, 19].

The old hypotheses regarding the connection between carcinogenesis and changes in the mitotic process and the number of chromosomes [6, 8, 20] have again become the subject of wide discussion [9, 15].

Malignant diseases of the human stomach are of particular interest in oncology. Karyological investigations of carcinoma of the stomach were first undertaken by Hauser in 1898 [10a]. In 1938, during investigation of biopsy material [1], it was shown that the number of normal mitoses was about 80%, whereas anomalies of mitosis were present in 15.4-22.4% of cases; the number of chromosomes varied from 38 to 165, and in the great majority of cells it was close to diploid. Structural changes were also noted in the chromosomes.

Recent clinical investigations [12, 13, 14, 16] of patients in late stages of cancer, using cytogenetic analysis, have shown that cells of carcinoma of the stomach possess individual specificity for each patient (modal number of chromosomes, character of heteroploidy, presence of chromosome markers, trisomia and monosomia of individual chromosomes, and the presence of chromosome fragments).

In 1959, the Cave (Carcinoma ventriculi) strain of gastric carcinoma cells was obtained from a female patient [3]. The object of the present investigation was to make a cytogenetic examination of this strain.

EXPERIMENTAL METHOD

The 65th passage of a culture of Cave cells was used for the experiments. The culture was seeded into Wassermann tubes containing cover glasses, which were extracted on the 2nd-3rd day. Division of the cells in the culture was preliminarily synchronized by Newton's method [17]. The cells on the cover glasses were treated with a hypotonic solution and prefixed in it with a few drops of freshly prepared fixer (3 ml absolute alcohol, 1 ml glacial acetic acid, and 0.5 ml of a 40% solution of formalin) for 15 min. Fixation was then carried out in the same fixing solution for 10 min. The fixed preparations were dried in air at room temperature for not less than 2 h. Before being stained, the dried preparations were hydrolyzed in a 1N solution of HCl at 60° for 7 min, rinsed with distilled water, and then stained by Unna's method, also for 7 min. The stained preparations were again rinsed with distilled water, passed quickly through alcohol and xylol, and mounted in Canada balsam. We thus had a permanent preparation for making the chromosome analysis of the culture.

The chromosomes were stained blue in these preparations. Cells in the stage of the metaphase plate were analyzed. Chromosomes were counted visually under the MBI-3 microscope with a green filter (magnification 900x). For

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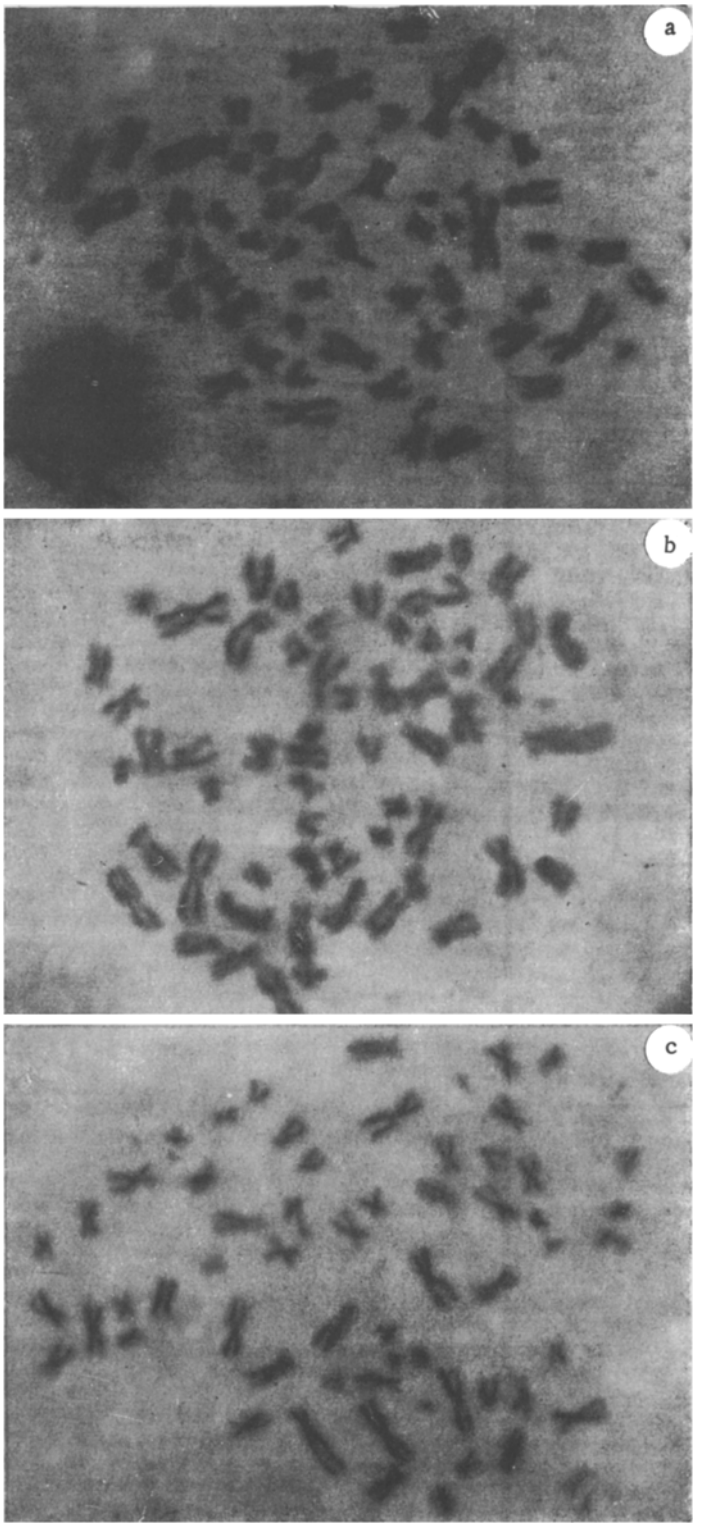

Fig. 1. Cells in the stage of metaphase plates with the following chromosome numbers: 59 (a), 60 (b), and 62 (c).

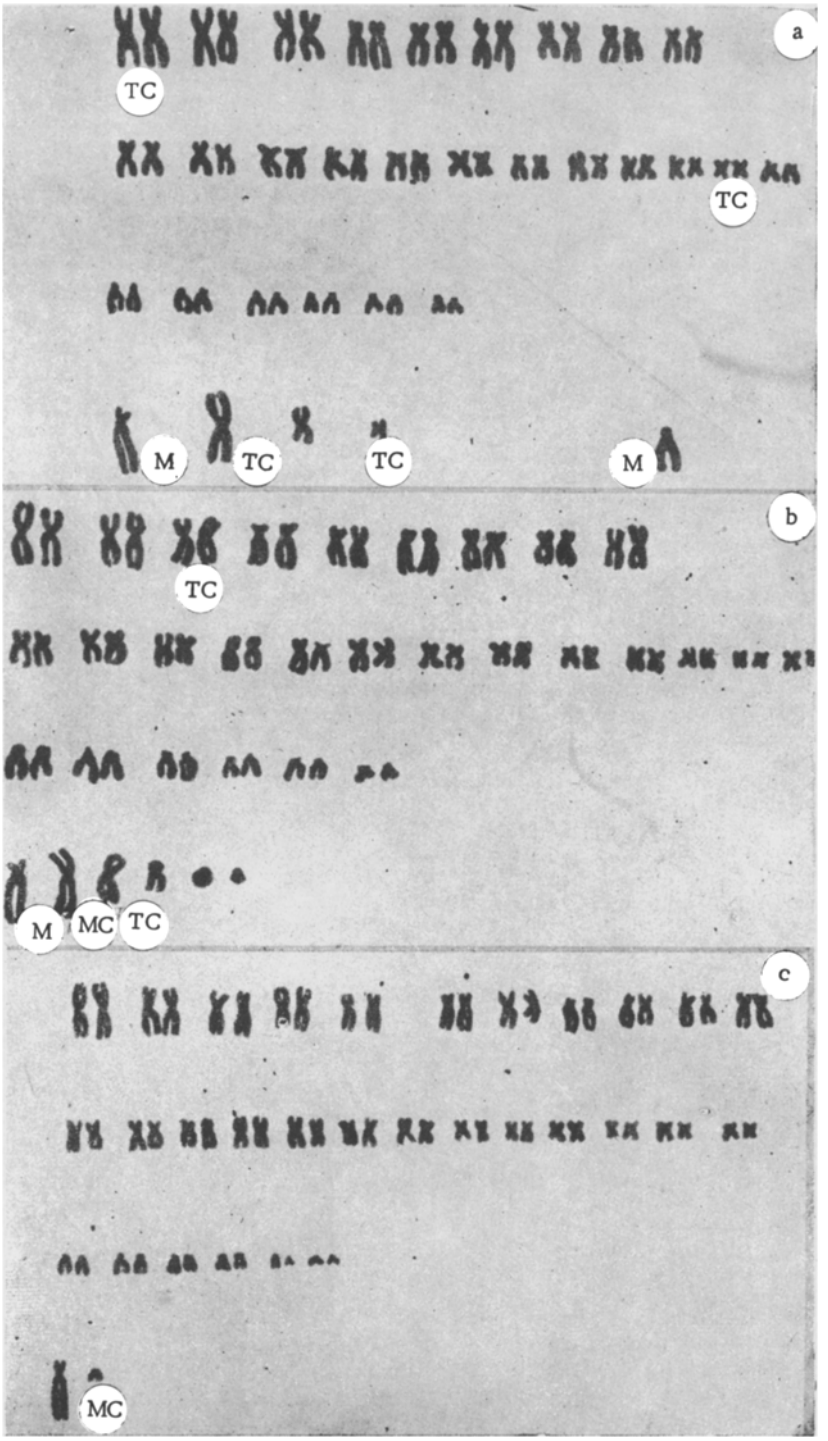

Fig. 2. Idiograms of cells with chromosome numbers of 59 (a), 60 (b), and 62 (c). M) marker chromosome; MC) monosomia; TC) trisomia.

Results of Counting the Chromosomes in the Cells of a Monolayer Culture of Carcinoma of the Human Stomach

Cave strain	No. of chromosomes																								Total No. of cells
	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	109	110	111	112	113	114	115	116	
Number of cells	1	–	–	3	2	1	–	1	5	25	8	26	9	13	3	1	1	–	–	–	–	–	–	1	100

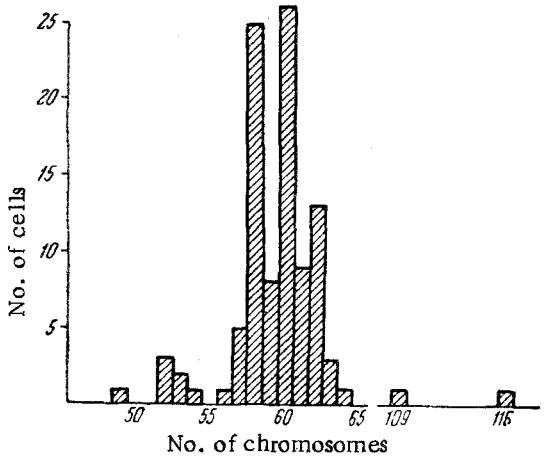

Fig. 3. Histogram of cells of the Cave strain.

photography, the MBI-6 microscope was used (objective 60×, eye-piece 15×, 20×), with a green filter and a plate measuring 9 × 12 cm. The most suitable distribution of the chromosomes was photographed in the stage of the metaphase plate for construction of idiograms.

EXPERIMENTAL RESULTS

The chromosomes were counted in 100 cells. It may be seen from the table that the number of chromosomes in the cells of the Cave strain varied from 49 to 116.

A histogram was constructed from the results of these counts (Fig. 3) showing that the Cave strain possesses 3 modal chromosome numbers – 58, 60, and 62. Idiograms of three cells of the Cave strain were constructed (Fig. 1), with chromosome numbers of 59, 60, and 62 (Fig. 2). To construct the idiograms, certain fundamental principles of the classification of human chromosomes adopted at the Denver conference were used. The chromosomes were classified, firstly by their length, and secondly by the arrangement of their centromeres. Unpaired chromosomes were placed in a separate group regardless of whether they were a sign of monosomia or trisomia.

In all three idiograms, a marker chromosome can be seen (a long, subtelocentric chromosome), which is found in most cells of this strain. In the idiogram of the cell with a chromosome number of 59 (Fig. 2, a) there is an additional marker chromosome – a large acrocentric chromosome.

Besides the marker chromosomes, other unpaired chromosomes were revealed. In these cases monosomia or trisomia may be postulated. Small chromosome fragments can be seen in the idiogram with the chromosome number of 60 (Fig. 2, b). We found none of the annular chromosomes observed by other writers [12, 14].

Hence the Cave strain is distinguished by the relative cytogenetic homogeneity of its cells: in 81% of cases the chromosome number varies from 58 to 62, with a well marked peak in the region of 58 and 60.

SUMMARY

The results of studying the karyotype of cells obtained from a monolayer culture of human cancer of the stomach (Cave strain) are presented. The relative cytogenetic homogeneity of the cells of this strain was established: The number of chromosomes in 81% of the cells varied from 58 to 62 with a pronounced peak within the range of 58-60. A characteristic chromosome (long, subtelocentric), revealed in the majority of the cells, was a marker for the given strain. Some cells had an additional marker chromosome – a large and acrocentric one. It is assumed that monosomia and trisomia in individual chromosomes are present. In some cells small chromosome fragments were revealed.

LITERATURE CITED

1. A. G. Andres, and E. O. Stepanskaya. Arkh. biol. nauk 51, 3, 26 (1938).
2. N. B. Varshaver. Tsitologiya, 6, 653 (1961).
3. Ya. V. Dobrynin, and R. P. Dirlugyan. Vopr. onkol., 5, 47 (1961).
4. O. N. Panchenko. Uspekhi sovr. biol. 53, 2, 169 (1962).
5. U. Min. Vopr. onkol., 7, 9 (1961).

6. Th. Boveri, Zur Frage der Entstehung maligner Tumoren, Jena, 1914.
7. C. E. Ford and J. L. Hamerton, Stain Technol., 1956, Vol. 31, p. 247.
8. D. Hansemann, Arch. path. Anat., 1890, Bd. 119, S. 299.
9. T. S. Hauschka, Cancer Res., 1961, Vol. 21, p. 957.
10. T. C. Hsu and C. M. Pomerat, J. Hered., 1953, Vol. 44, p. 23.
10a. G. Hauser. Cited by A. G. Andres and É. O. Stepanskaya.
11. The Human Chromosome Study Group. A. Proposed Standard System of Nomenclature of Human Mitotic Chromosomes. J. Hered., 1960, Vol. 51, p. 214.
12. T. Ishihara, G. E. Moore, and A. A. Sandberg, J. nat. Cancer Inst., 1961, Vol. 27, p. 893.
13. U. Ising and A. Levan, Acta Path. microbiol. scand., 1957, Vol. 40, p. 13.
14. A. Levan, Hereditas (Lund.), 1956, Vol. 42, p. 366.
15. A. Levan and J. J. Biesele, Ann. N. Y. Acad. Sci., 1958, Art. 6, Vol. 71, p. 1022.
16. S. Makino, T. Ishihara, and A. Z. Tonomura, Krebsforsch., 1959, Bd. 63, S. 184.
17. A. A. Newton and P. Wildy, Exp. Cell Res., 1959, Vol. 16, p. 624.
18. T. T. Puck., Revs. Mod. Phys., 1959, Vol. 31, p. 433.
19. J. H. Tjio and T. T. Puck, J. exp. Med., 1958, Vol. 108, p. 259.
20. O. Winge, Z. Zellforsch., 1930, Bd. 10, S. 683.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
