A Preliminary Note on the Chromosomes of the McCoy Synovial Cell Line Cultivated *in vitro*

By

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(With 7 Text-figures and 2 Tables)

Earle and his associates (1943a, b) established a cell line in tissue culture called the "L" strain which was isolated from subcutaneous tissues of the C3H mouse. The original stock and its derivatives have been widely distributed and maintained at many laboratories all over the world. Largely as a result of this stimulus, many established cell lines from human as well as mammalian tissues of both normal and malignant origin have been employed as a useful tool for such research fields as cytology, virology, radiation biology and biochemistry.

One of the most fascinating problems is to determine the chromosomal status in cell populations after continuous cultivation *in vitro*. Many cytologists have studied mammalian chromosomes in tissue culture extensively (c.f. Hsu 1961, Chu and Giles 1958, Chu *et al.* 1958, Levan 1959). One of the important findings reported by Hsu and Klatt (1959) was that, in primary cultures or in early transfers of normal tissues, euploid cells usually dominate in the population. Heteroploid transformation takes place in the course of long-term cultivation of such cells. The stem cells are thus heteroploid with chromosome numbers between the diploid and tetraploid limits in established cell lines. Levan and Biesele (1958) observed a gradual increase of polyploids in cultures of certain mouse embryonic tissues. They demonstrated that polyploids have some advantages in survival over the diploids in the *in vitro* environment.

Heteroploid stem elements in established cell lines are, however, not always stable as to definite chromosome patterns and numbers, but there is a tendency for gradual decreases in the number of chromosomes concomitant with morphologic alterations in the course of serial culture passages.

The present report is concerned with the chromosomal condition of the McCoy cell line after continuous cultivation *in vitro*. Special attention is directed to an inquiry into the chromosomal changes in both morphology and number found in successive subcultures.

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Materials and methods: The McCoy line under study was derived by Pomerat (unpublished), and briefly described by Hsu and Moorhead (1957), from cells of the synovial fluid in the knee joint of a patient suffering from degenerative arthritis in October 1955. The cells were typically spindle-shaped. Culture fluid employed for their maintenance was Eagle's semi-synthetic medium containing 10 per cent horse serum.

Samples used were kindly provided by Dr. L. Hayflick of the Wistar Institute, Philadelphia, Pennsylvania, and by Dr. Charles A. L. Stephens, Jr., of the University of Arizona, Tucson, Arizona. The first sample received in February 1961 was derived from Philadelphia stock and the second from the Arizona stock in December 1961. These will hereafter be referred to as P and A stocks.

For chromosome observations squash preparations in combination with colchicine and hypotonic sodium citrate solution treatment prior to fixation were made with the use of acetic orcein. For experimental purposes the cells on the 3rd day after subculture were exposed to 400r of gamma irradiation from a cobalt-60 source at a dose rate of 45.9 r per minute at the focal distance of 50 cm. Squash specimens were then prepared from such cells 24 and 48 hours of post-irradiation.

Results

1) *Chromosome number*

The result of the chromosome counts is listed in Table 1. As seen in the table, the modal chromosome number of cells in the McCoy line was found to be 66 in both the P and A stocks.

In the P stock, 16 cells out of 30 metaphases formed the stem-line of the population. Variation in the range of chromosome numbers at this period was rather narrow, showing a typical unimodal distribution. The A stock had the same modal value of 66 as in the former case, although the fluctuation around the stem-line number was fairly wide (Table 1). The cells with chromosome numbers below the modal value were encountered very frequently. Consequently, the histogram showed an eccentric distribution toward the lower numbers (Fig. 1A).

2) *Chromosome morphology*

Chromosome analyses were made from drawings with the aid of a camera lucida or from photographs enlarged to appropriate sizes.

A representative metaphase plate and its karyotype from the P stock are shown in Figures 2 and 4. Using the system of Tjio and Levan (1956), chromosome arm indices of each individual chromosome were calculated. The chromosomes were classified tentatively into 3 groups: M chromosomes (median-submedian centromeres), S chromosomes (subterminal centromeres) and T chromosomes (nearly terminal centromeres). The stem cell comprised of 20 M and S chromosomes and 46 T

elements.

The A stock was notable in that several marker chromosomes were present in every cell (Figs. 3, 5 and 6). These markers were designated as A, B, C, D, and *m* in the descending order of size (Fig. 7). The A chromosome was a large dicentric

Table 1 Distribution of chromosome numbers in the McCoy strain

	Chromosome numbers													Total
	60	61	62	63	64	65	66	67	68	69	70···76···83			
Philadelphia stock February, 1961		1		1	1	4	16	4	1		2			30
Arizona stock December, 1961														
ABC*m*	1	1	3	11	13	22	26	3		1				81
ACD*m*					5	2	2							9
AC*m*	1		1	1										3
Others		1		2	4	2	4	1	1		1	1	1*	18
Total	2	2	4	14	22	26	32	4	1	1	1	1	1	111

* = A cell with a ring element.

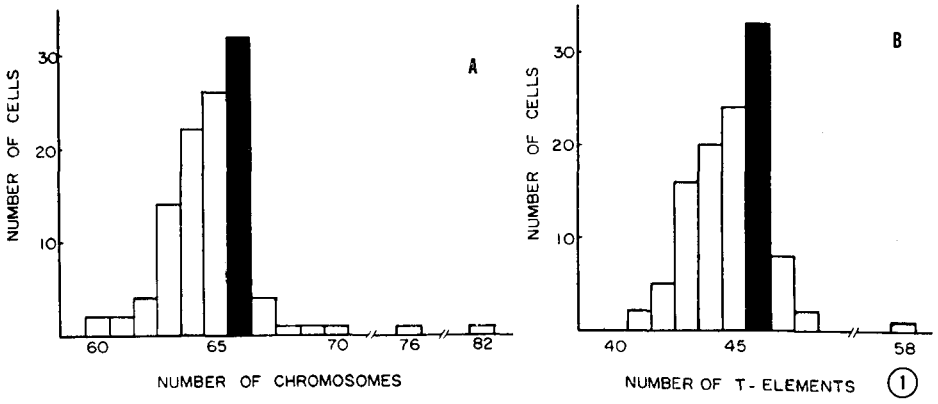

Fig 1A. Distribution of chromosome numbers in the Arizona stock cells (McCoy strain). Fig. 1B. Distribution of the number of T chromosomes in the Arizona stock cells.

element with a secondary constriction in the mid-portion of the chromatids between the centromeres. The B element was a member of small-size chromosomes with median-submedian centromeres. Since the split between the chromatids could not be clearly seen, this element always showed a figure like the letter Y. There were two secondary constrictions on one arm with fused chromatids. The C

chromosome had a nearly terminal centromere. It also had two or three secondary constrictions. The D element was rather rare and was sometimes heteropyknotic in nature. There seemed to be more than two secondary constrictions in this chromosome. The *m* chromosome was of minute size with a dot-like shape. Its length was approximately one-third that of the smallest chromosome.

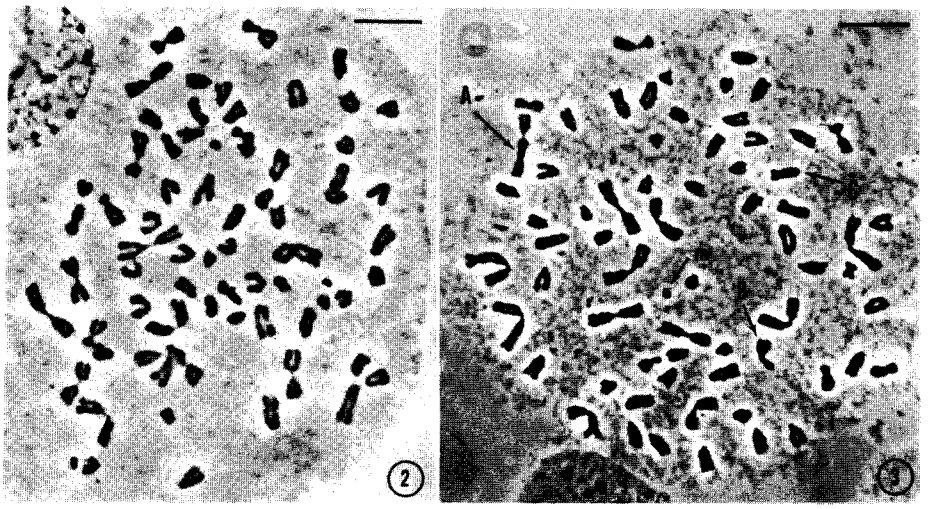

Fig. 2. A metaphase from the Philadelphia stock (66 chromosomes). The scale of magnification is given in the upper right area of this and of each succeeding figure representing 10μ. Fig. 3. A metaphase from the Arizona stock (66 chromosomes). Arrows indicate marker chromosomes (A, B, C and *m*).

The remainder of the complement consisted of ordinary M, S and T chromosomes resembling those found in the P stock. The occurrence of M and S chromosomes was almost constant in contrast to that of the T elements which were fairly labile. Therefore, numerical variation seemed to be caused by excess or loss of T chromosomes (Fig. 1B).

Cells having A, B, C and *m* marker chromosomes (ABC*m*-type) predominated in the population. Approximately 73 per cent of the cells observed belonged to this category (Table 1). There also were several cell patterns determined by different combinations of the markers, such as ACD*m*, AC*m*.

3) *Effect of gamma irradiation on the McCoy cell population*

The result of the preliminary experiment is shown in Table 2. It was observed that there were various kinds of chromosomal abnormalities induced by 400r of gamma irradiation. The cells whose chromosomes appeared morphologically unaffected by the radiation were chosen for the present observation.

As seen in the table, there was a decrease in the number of ABC*m*-type

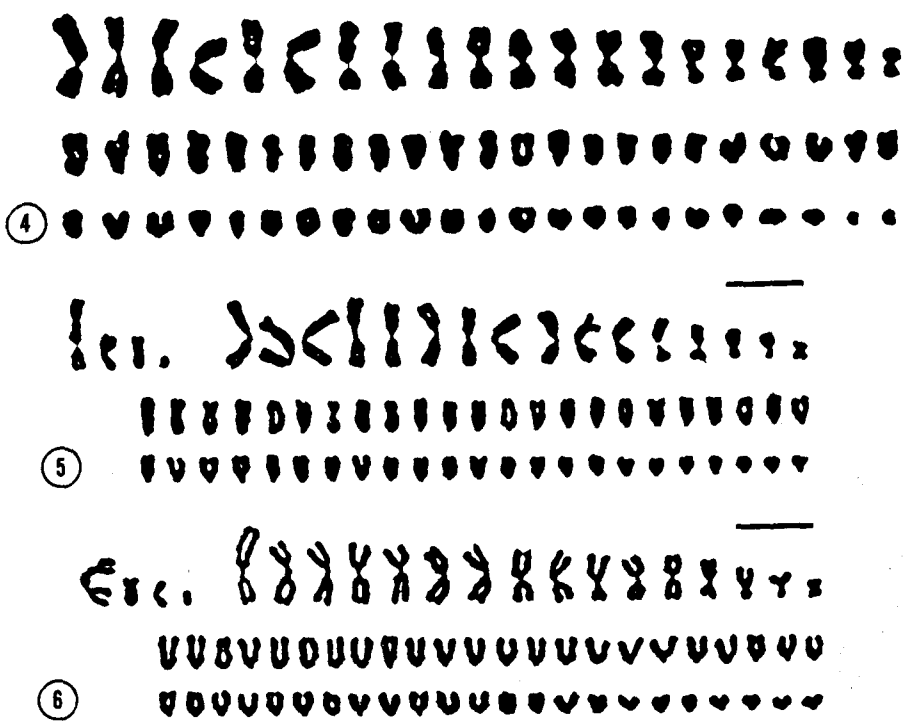

Fig 4. A karyotype of metaphase from the Philadelphia stock (66 chromosomes). Figs. 5 and 6. Karyotypes of metaphases from the Arizona stock. Fig. 5. ABC*m*-type (65 chromosomes). Fig. 6. ACD*m*-type (66 chromosomes). Figs. 4 and 5 correspond to Figures 2 and 3, respectively.

Table 2 Morphological classification of cell types in the McCoy strain

Cell type	Control	Co^{60} 400 r	
		24 hrs	48 hrs
ABC*m*	81(73.0)	57(50.0)	46(44.7)
ACD*m*	9(8.1)	32(28.1)	34(33.0)
AC*m*	3(2.7)	17(14.9)	18(17.5)
Others	18(16.2)	8(7.0)	5(4.9)
Total	111	114	103

Numerals in the parentheses represent the percentage in the number of cells observed.

cells. On the contrary, the ACD*m*-and AC*m*-type cells, with the passage of time, increased in number. This suggests the replacement of the stem-line by the dominant and more adaptable cell population.

Discussion

Eivdence presented by Makino and his associates (cf. Makino 1957, Sasaki *et al.* 1960 and others) supports the view that a stem-line cell (or cells) dominates in the population for a certain period under both *in vivo* and *in vitro* conditions.

It also has been well accepted that heteroploid transformation of the cells occurs in the course of long-term cultivation in tissue culture. There is some evidence that euploid cells can be maintained for months in the *in vitro* condition (Hayflick and Moorhead 1961, Puck *et al.* 1958, Yerganian and Leonard 1961, and others). Some of the cell lines could not be maintained in their original euploid state either because of changes to the heteroploid condition or poor growth resulting in the extermination of the cells.

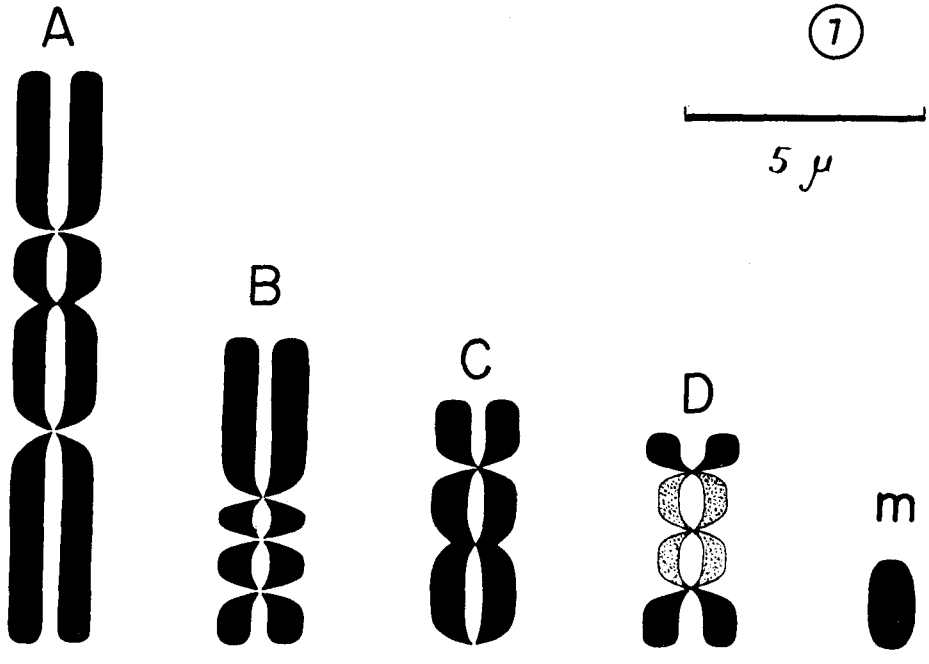

Fig. 7. Schematic representations of marker chromosomes (A, B, C, D and *m*).

The cells in established lines generally have heteroploid chromosome numbers. Using mouse tissues, Hsu *et al.* (1961) have reported that heteroploid transformation may undergo the following 2 patterns: diploidy-tetraploidy-hypotetraploidy and diploidy-subdiploidy-hypotetraploidy. Structural changes of the chromosomes in cell lines were also noted very frequently during this period.

In samples of the McCoy cell line, studied earlier in its history, variation in the range of chromosome numbers fell between 56 to 80, with a modal value at 67 (Hsu and Moorhead 1957). Recent samples investigated by the authors also belonged to the hypotriploid range with a modal number of 66. The latest counts showed further changes in variation of the range of chromosome numbers toward the lower limits. It appears, therefore, very possible that repeated readjustment of genetic properties in the cell population has occurred under the long-term *in vitro* condition.

In the present observation, it was found that completely different cell lines of the same origin could be achieved possibly under different culture conditions. As mentioned previously, the P stock had no distinguishable marker chromosome in the population, while the A stock was characterized by cells having a combination of several marker elements which could be easily distinguished from their fellows. It is, therefore, very possible that a variety of genomes developed in cell population maintained *in vitro*.

Changes in the proportion of several cell types with the markers were demonstrated in the present study by exposure to 400 r irradiation from a cobalt-60 source. This seems to suggest the replacement of a stem-line cell population (or populations) to either a new or more adaptive one with a different genetic composition under certain experimental or environmental conditions.

A similar phenomenon has been postulated by other workers (Hsu 1960, Hsu and Kellogg 1960, Hsu and Merchant 1961, Whitfield and Rixon 1961, Harris and Ruddle 1960, 1961). Since the result of the present observation seemed not to be adequate to determine cell population dynamics, it is hoped that further experiments of this nature will clarify the problem.

Summary

This report deals with the analysis of chromosomes of two different stocks from the same source of the McCoy synovial cell line. Both had the same modal chromosome number of 66. A sample derived from a Philadelphia stock had no marker chromosomes and a rather narrow limit in the variation of chromosome numbers. Another one from an Arizona source was characterized by having several marker chromosomes with a fairly wide range in its number of chromosomes. The markers consisted of two secondary constrictions or a minute element. One of them was a dicentric chromosome of the largest size with a secondary constriction.

The cell population of the Arizona stock was composed of several types of cells with different combinations of markers. These patterns in the population were changed by exposure to 400r of gamma irradiation from a cobalt-60 source. This result suggests the replacement of the stem-line cell by a dominant and more adaptable cell population.

References

Chu, E.H.Y. and N.H. Giles 1958. Comparative chromosomal studies on mammalian cells in culture. I. The HeLa strain and its mutant clonal derivatives. J. Nat. Cancer Inst. **20**: 383–401.

Chu, E.H.Y., K.K. Sanford and W.R. Earle. 1958. Comparative chromosomal studies on mammalian cells in culture. II. Mouse sarcoma-producing cell strains and their derivatives. J. Nat. Cancer Inst. **21**: 729–751.

Earle, W.R. 1943a. Production of malignancy *in vitro*. IV. The mouse fibroblast cultures and changes seen in the living cells. J. Nat. Cancer Inst. **4**: 165–212.

Earle, W.R. and A. Nettleship 1943b. Production of malignancy *in vitro*. V. Results of injection of cultures into mice. J. Nat. Cancer Inst. **4**: 213–227.

Harris, M. and F.H. Ruddle 1960. Growth and chromosome studies on drug resistant lines of cells in tissue culture. Symposium on "Cell Physiology of Neoplasia", University of Texas Press, Austin, Texas (1960), pp. 524–546.

——— 1961. Clonal strains of pig kidney cells with drug resistance and chromosomal markers. J. Nat. Cancer Inst. **26**: 1405–1411.

Hayflick, L. and P.S. Moorhead 1961. The serial cultivation of human diploid cell strains. Exp. Cell. Res. **25**: 585–621.

Hsu, T.C. 1960. Mammalian chromosomes *in vitro*. XIII. Cyclic and directional changes of population structure. J. Nat. Cancer Inst. **25**: 1339–1353.

Hsu, T.C., D. Billen and A. Levan 1961. Mammalian chromosomes *in vitro*. XV. Patterns of transformation. J. Nat. Cancer Inst. **27**: 515–541.

Hsu, T.C. and D.S. Kellogg, Jr. 1960. Mammalian chromosomes *in vitro*. XII. Experimental evolution of cell populations. J. Nat. Cancer Inst. **24**: 1067–1093.

Hsu, T.C. and O. Klatt 1959. Mammalian chromosomes *in vitro*. X. Heteroploid transformation in neoplastic cells. J. Nat. Cancer Inst. **22**: 313–339.

Hsu, T.C. and D.J. Merchant 1961. Mammalian chromosomes *in vitro*. XIV. Genotypic replacement in cell populations. J. Nat. Cancer Inst. **26**: 1075–1083.

Hsu, T.C. and P.S. Moorhead 1957. Mammalian chromosomes *in vitro*. VII. Heteroploidy in human cell strains. J. Nat. Cancer Inst. **18**: 463–471.

Levan, A. 1959. Relation of chromosome status to the origin and progression of tumors: The evidence of chromosome number. Symposium on "Genetics and Cancer", University of Texas Press, Austin, Texas (1959), pp. 151–182.

Levan, A. and J.J. Biesele 1958. Role of chromosomes in cancerogenesis, as studied in serial tissue culture of mammalian cells. Ann. N.Y. Acad. Sci. **71**: 1022–1053.

Makino, S. 1957. The chromosome cytology of the ascties tumors of rats, with special reference to the concept of the stemline cell. In: *International Review of Cytology*, Vol. VI, ed. by. G.H. Bourne and J.F. Danielli, Academic Press, New York, 25–84.

Puck, T.T., S.J. Cieciura and A. Robinson 1958. Genetics of somatic mammalian cells. III. Long-term cultivation of euploid cells from human and animal subjects. J. Exp. Med. **108**: 945–956.

Sasaki, M., S.H. Hori and Y. Hisada 1960. Further studies on tissue culture strains of a methylcholanthrene-induced spindle-cell sarcoma of the rat. La Kromosomo **42–43**: 1409–1420.

Tjio, J.H. and A. Levan 1956. The chromosome number of man. Hereditas **42**: 1–6.

Whitfield, J.F. and R.H. Rixon 1961. Distinctive chromosome markers of normal and radioresistant derivatives of L strain mouse cells. Exp. Cell Res. **23**: 412–415.

Yerganian, G. and M. J. Leonard 1961. Maintenance of normal *in situ* chromosomal features in long-term tissue culture. Science **133**: 1600–1601.