

# Chromosome Number of *in Vivo* and *in Vitro* Cultured Krebs-2 Carcinoma of Mice: The Selective Property of the *in Vitro* Culture Medium

J. O. ELY AND JAMES H. GRAY

(Biochemical Research Foundation, Newark, Delaware)

## SUMMARY

The chromosome number of the Krebs-2 ascites carcinoma maintained in Swiss mice was found to be grouped about a modal number of 78-79. After *in vitro* culture in medium No. 858 (modified) containing 10 per cent calf serum, primary cultures showed a shift to a smaller number of chromosomes. This medium when used for originating single-cell cultures favored the selection of cells with a modal chromosome number of 64-65. The cell lines selected by the medium were, for the most part, stable with respect to maintaining the same modal number both *in vitro* and *in vivo*.

The number of chromosomes of strains of cells cultured *in vitro* for a considerable time usually increases (1, 6, 10). The work of Puck and co-workers (7) in which several strains of human cells were kept in culture for 3-9 months without alteration of the chromosome number, and the work of Saksela *et al.* (9) with heated and unheated serum, suggest that the increase in chromosome number usually observed in cells after culture for a considerable time may be the result of growth under suboptimal conditions. The latter workers found that the great heteroploidy that prevailed in their cultures of malignant cells in a medium containing heated serum was reduced when fresh unheated serum was substituted in the medium for the heated serum. The result suggests that some nutritional element(s) of the serum was inactivated by heating, or that toxic materials were formed, resulting in suboptimal cultural conditions.

Hauschka and Levan (3) found that some *in vivo* stock and clonal cultures of the Krebs and the Ehrlich carcinomas of mice underwent a reduction of chromosome numbers during a period of 3-5 years. It was suggested that the reduction was a result of an adjustment to the environment of the *in vivo* culture conditions.

This is a report on the number of chromosomes of the Krebs carcinoma cells grown in mice and in

primary and clone cultures *in vitro*, and of clone culture cells subsequently returned to mice. The results indicate that the culture conditions exerted a selective influence by favoring the growth of cells with a lower modal number of chromosomes than that which prevailed in the mouse, and that usually the selected lines of cells were rather stable, not only *in vitro* but also *in vivo*.

## MATERIALS AND METHODS

The Krebs-2 ascites carcinoma has been maintained in this laboratory in Swiss mice since 1952, when it was obtained from Dr. T. S. Hauschka of the Institute for Cancer Research, Philadelphia, Pennsylvania.

The synthetic medium No. 858 of Healy *et al.* (4), modified as reported earlier (2), was used with calf serum in the proportion of 9 parts of medium to 1 part of serum. Clone cultures were prepared as described earlier (2).

## PREPARATION OF CELLS FOR CHROMOSOME COUNTS

A. *In vitro*.—Cover-glasses (18 × 15 mm.) were placed on the bottoms of 60-mm. pyrex petri dish-type culture vessels, and 4 ml. of medium were added. Cells in a drop or two of suspension from a culture were distributed over and allowed to settle upon the cover-glasses. After incubation for about 36 hours colchicine in solution was added to the cultures to a concentration of 2 μg. per cent. In-

Received for publication March 24, 1961.

cubation was then continued for 16–18 hr. The cover-glass preparations were then treated with a hypotonic salt solution for 30 minutes, followed by a fixative composed of 3 parts of absolute ethyl alcohol and 1 part of glacial acetic acid for 10 minutes or longer. The preparations were then allowed to dry at room temperature according to the method of Rothfels and Siminovitch (8) and then stained with either aceto-orcein or by a dilute aqueous solution of safranin O.

B. *In vivo*.—A solution of colchicine (10–20 mg. of colchicine/mouse) was injected intraperitoneally into mice 16–18 hours before removing cells for examination. Cell-containing ascitic fluid was lightly centrifuged to sediment the cells; the supernate was discarded, and the cells were dispersed in hypotonic salt solution, which was allowed to act for 30 minutes. The hypotonic salt solution was replaced by the alcohol-acetic acid fixative for 10 minutes or longer. Small drops of the cell-containing fixative were spread on cover-glasses and allowed to dry at room temperature before staining.

#### COUNTING OF CHROMOSOMES

Of each preparation the chromosomes of 100 cells were counted (except mouse #2, from which 99 cells were selected). All cells in which the chromosomes were spread sufficiently for counting were selected. The average error in counting was considered to be 1–2 per cent in cells containing up to 80 chromosomes and 3–4 per cent in cells containing 100 or more chromosomes.

#### RESULTS

The data are shown in Table 1; the following may be observed:

The numbers of chromosomes of cells taken directly from mice were rather uniformly distributed about a modal number of 78–79 (Table 1, 1–6), with a small percentage of the cells containing considerably larger numbers. The chromosome numbers of cells in primary cultures, 21–153 days *in vitro*, shifted to lower values than those which prevailed in cells taken directly from mice. The data are insufficient to indicate whether or not the chromosome numbers of the cell populations of these cultures were uni- or multimodal (Table 1, 151, 157, 159).

The cells of all clone cultures, with one exception, initially had a modal number of 64–65. The exception was culture No. 130S1, which at the 107th day of culture had a modal number of 122–126 chromosomes. The parent cell was large, and as cell division progressed in the early stages of clone development part of the progeny died and

disintegrated; it was doubtful whether the culture would become established. The cells of this culture were large for over 3 months; volume measurements showed that they were twice the size of cells which contained 64–65 chromosomes. Some time after the 107th day in culture the large cells decreased in number until at the 165th day the average size was one-half the original size and equal to those cells containing 64–65 chromosomes. It was then found that the modal number of chromosomes had decreased to 64–65.

Initially the cells of clone culture 85S1 were uniformly small. At the 149th day in *in vitro* culture the modal number of chromosomes was 64–65, after which there was a shift to a larger number, until at the 259th day in culture 60 per cent of the mitotic cells contained approximately twice the original number; 31 per cent contained more than 131, distributed between 132 and 282. It may be observed (Table 1) that there was a gradual return to a modal number of 64–65 during a period of 66 days after these cells were returned to mice.

Cells of clone cultures 117S3 and 117S3S2 maintained modal numbers of 64–65 in both *in vitro* culture and in mice and those of clone 130S1 after shifting from an initially high number of chromosomes to a lower number remained constant in both *in vitro* and *in vivo*.

#### DISCUSSION

The stability of clone culture 85S1 appears to have been less in the *in vitro* medium than that of the other clone cultures, since there was a considerable shift to larger numbers of chromosomes. However, the original selection by the medium probably was of a cell of about 64–65 chromosomes, since earlier the modal number was 64–65 and since there was a return to that value after a short period of growth in mice.

The culture medium appears to have offered a distinct advantage for the growth of cells with a modal number of 64–65 chromosomes, as indicated by (a) the downward shift in the number of chromosomes in primary cultures maintained *in vitro*, (b) the selection of clones with that number of chromosomes (Table 1, Cultures 117S3, 117S3S2, 85S1), (c) the change in the cells of clone 130S1 from a high chromosome number to a modal number of 64–65, and (d) the stability of the selected clones at a modal number of 64–65 in mice, and for the most part in *in vitro* culture.

It seems to be a common experience that it is more difficult to initiate the growth of single cells than of large numbers of cells in culture. “Symbiotic” aid among the cells enables larger numbers to grow under conditions in which single cells fail.

TABLE I  
NUMBER OF CHROMOSOMES OF KREBS-2 CARCINOMA CELLS *in Vitro* AND *in Vivo*

Source of cells	No.	Days		NUMBER OF CELLS CONTAINING THE INDICATED CHROMOSOME COUNTS																								
		In culture		In mice																								
		Primary	Clone	< 60-	60- 61	62- 63	64- 65	66- 67	68- 69	70- 71	72- 73	74- 75	76- 77	78- 79	80- 81	82- 83	113	114- 115	116- 117	118- 119	120- 121	122- 123	124- 125	126- 127	128- 129	130- 131	>	
Mice	1			3	2	2	1	5	4	9	12	10	12	21	7	6												
	2			1	1	1	1	1	1	3	14	20	25	23	5	3												
	3				1	1	1	1	1	5	13	16	13	31	5	8												
	4				3	4	2	11	18	21	25	10	2	10	2													
	5			1	1	2	1	2	5	10	23	36	7	5	6													
	6				2	1	4	5	9	22	30	5	6	1	2													
Primary cultures	159		21		7	7	8	9	10	17	13	15	2	3	2	3												
"	29		29		6	14	10	10	8	2	2	2	2	2	2	1												
157	150		150		4	8	19	12	19	11	6	2	9	2	9													
151	153		153		1	10	24	12	13	6	6	5	1	1	1													
Clone culture no. 130S1	43	107		2	7	15	28	24	14	4	2	1	12	10	16	10	8	2	4	8								
	165	165		3	2	4	24	16	21	12	4	3	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	4
	179	179		11	4	4	43	22	10	6	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	"	"		2	3	7	43	21	12	2	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Clone culture no. 86S1	133	149		6	9	11	47	9	4	6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	259	259		3	1	3	7	5	1	5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	318	318		2	10	2	8	5	1	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	263	263		4	5	6	18	6	4	4	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	"	"		14	8	12	25	13	6	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	46	46		2	5	8	48	17	9	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	66	66		3	3	17	39	15	7	6	5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Clone culture no. 117S3	39	241		3	5	7	22	19	20	13	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	182	182		3	6	5	51	11	9	6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	"	"		12	20	41	8	8	5	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	167	167		7	12	15	29	8	11	8	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Clone culture no. 117S3S2	167	22		6	10	9	44	7	6	6	5	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	167	167		8	21	4	38	12	6	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	214	214		1	2	6	35	17	16	9	1	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	231	231		1	3	9	34	22	18	12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	"	"		1	6	19	33	20	10	6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	120	120		11	4	11	34	19	15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	244	244																										

It is possible that in these experiments the cells with the larger number of chromosomes were less able to survive and grow without the "symbiotic" aid of other cells than were those which contained 64-65 chromosomes. This would account for the selection of clones containing the smaller numbers of chromosomes.

Hsu and Kellogg (5) state that "in a cell population there may be several optimum classes of genomes, each being suitable for one type of environment." Apparently, the medium used in these experiments provided the environment more suitable for cells of the genome with 64-65 chromosomes than for others.

#### REFERENCES

1. CHU, E. H. Y.; SANFORD, K. K.; and EARLE, W. R. Comparative Chromosomal Studies on Mammalian Cells in Culture. II. Mouse Sarcoma-Producing Cell Strains and Their Derivatives. *J. Nat. Cancer Inst.*, **21**:729-51, 1958.
2. ELY, J. O., and GRAY, J. H. *In Vitro* Culture of the Krebs Ascites Carcinoma and the Ehrlich Ascites Carcinoma of Mice. *Cancer Research*, **20**:918-22, 1960.
3. HAUSCHKA, T. S., and LEVAN, A. Cytologic and Functional Characterization of Single Cell Clones Isolated from the Krebs-2 and Ehrlich Ascites Tumors. *J. Nat. Cancer Inst.*, **21**: 77-111, 1958.
4. HEALY, G. M.; FISHER, D. C.; and PARKER, R. C. Nutrition of Animal Cells in Tissue Culture. X. Synthetic Medium No. 858. *Proc. Soc. Exp. Biol. & Med.*, **89**:71-77, 1955.
5. HSU, T. C., and KELLOGG, T. C., JR. Mammalian Cells *in Vitro*. XII. Experimental Evolution of Cell Populations. *J. Nat. Cancer Inst.*, **24**:1067-94, 1960.
6. NAKANISHI, Y. H.; FERNANDEZ, M. V.; MIZUTANI, M. and POMERAT, C. M. On the Chromosome Numbers of Human Amnion Cells in Primary and Strain Cultures. *Texas Rep. Biol. Med.*, **17**:345-53, 1959.
7. PUCK, T. T.; CIECIURA, S. J.; and ROBINSON, A. Genetics of Somatic Mammalian Cells. III. Long-Term Cultivation of Euploid Cells from Human and Animal Subjects. *J. Exp. Med.*, **108**:945-55, 1958.
8. ROTHFELS, K. H., and SIMINOVITCH, L. An Air-Drying Technique for Flattening Chromosomes in Mammalian Cells grown *in Vitro*. *Stain Technol.*, **33**:73-77, 1958.
9. SAKSELA E.; SAXIN, E.; and PENTTINEN, K. Further Studies on the Growth and Controlling Action of Human Serum on Cells Cultivated *in Vitro*. The Chromosomal number of HeLa Cells in Active and Heat-Inactive Serum. *Exper. Cell Research*, **19**:402-7, 1960.
10. VOGT, M. Symposium on Genetic Approaches to Somatic Cell Variation. *J. Cell. & Comp. Physiol.*, **52** (Suppl. No. 1): 271-85, 1958.