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EFFECT OF HETEROLOGOUS TRANSPLANTATION OF H 4-II-E RAT HEPATOMA CELLS INTO THE CHEEK POUCH OF HYDROCORTISONE TREATED HAMSTERS*

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ABSTRACT

A series of transplant-explant experiments using the H 4-II-E rat hepatoma cell line and golden hamsters have been carried out. Tissue culture cells implanted into the cheek pouch of hydrocortisone treated hamsters at a concentration of 10^6 cells per pouch produced tumors which histologically were typical hepatomas. Cells from these tumors were placed back into tissue culture and were transferred twice more into the cheek pouch of hydrocortisone treated hamsters and tissue culture. At each stage of selection, chromosome counts, karyotypes and histological and biological characteristics were determined.

Cytogenetic studies revealed that the chromosome number in both tumors and explanted cultures shifted and became more selected with each succeeding *in vivo* and *in vitro* passage. The cells from the selected cultures had a much more homogeneous karyotype than cells with the same number of chromosomes from the original H 4-II-E culture. Tumors in the third selection had less hemorrhage and necrosis than in the first and second selections and were slower growing and showed lower transptantability than the original tumors produced by implantation of the H 4-II-E cells.

These results indicate that transplantation of tissue culture cells into the cheek pouch of hamsters treated with hydrocortisone results in the selection of cells with a specific chromosomal constitution and altered transplantability and growth rate suggesting that the chromosomal makeup may determine the biological and immunological characteristics of the cell which allow it to grow under these conditions.

INTRODUCTION

There is very little known concerning the effect of abnormal numbers of

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chromosomes on the biochemistry, growth characteristics and morphology of cancer cells. In 1959, Vogt[1] demonstrated a relationship between the chromosome modal number, karyotype and phenotypes of drug resistance and morphology in two HeLa clones. Recently, Bottomley *et al.*[2] reported results on the enzymatic and chromosomal characterization of HeLa variants. They studied seven HeLa lines both at the chromosomal and enzymatic level and showed that the lines differed from each other in both parameters. Higgins *et al.*[3] also presented similar results using the KB cell line. Ghosh *et al.*[4] recently reported studies on two HeLa cell lines and have demonstrated that the differences in growth behavior of these two lines could not be attributed to a numerical difference in their stemline, but were related to differences in the karyotypes of the stemline cells.

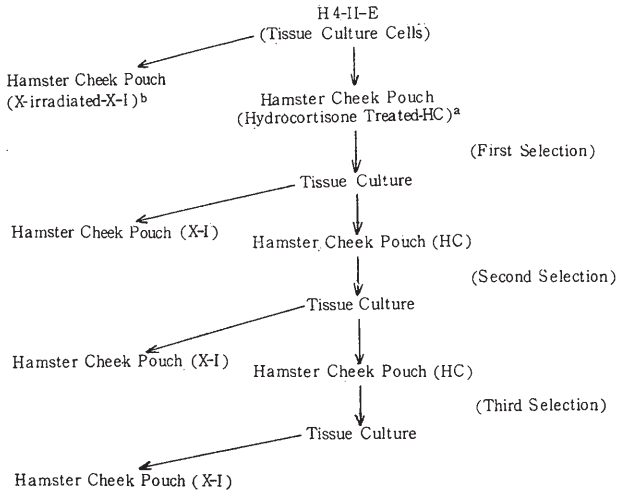
Several investigators have indicated that tumors, transplanted serially in heterologous hosts, retain their cytogenetic and biological characteristics[5][6][7]. Other investigators have reported that after repeated heterologous transfer, some tumors may acquire an accelerated growth in the heterologous host, with simultaneous reduction in their rate of growth in the species of origin, and changes in antigenicity and/or in karyotype have also been reported [8][9][10][11][12]. These changes were ascribed to adaptation of the tumors to the immunologic environment of the new host.

In the present study, heterotransplantation was used to study the relationship of karyotype to phenotype in malignant cells. Serial heterotransplantation and re-culture was carried out three times using hydrocortisone treated hamsters and the H 4-II-E rat hepatoma cell line which exhibits a large number of stable, differentiated functions[13]. Karyotypic and phenotypic characteristics were studied at each stage of selection both *in vivo* and *in vitro*. The purpose of the present investigation was to reveal and chart any chromosomal change in the tumor stemline during the sojourn of the tumor in the foreign host and tissue culture, and to determine possible relationships between cytogenetic variations and biological variations in the cells.

MATERIALS AND METHODS

Experimental design: The experimental design of this study is given in Table 1. 10^6 H 4-II-E hepatoma cells were implanted beneath the mucosa of both cheek pouches of hydrocortisone treated hamsters. Two weeks after implantation animals were killed by decapitation and the tumors were removed. The tumors were then placed back into tissue culture (First selection). The re-cultured subline (first selection, 4th passage generation) was again implanted into the cheek pouches of hydrocortisone treated hamsters. Two weeks later, tumors were removed, and re-culture was carried out (Second selection). This transplant-explant experiment was repeated once again using the 4th passage

TABLE 1. Experimental Design



Conditioning of host:

a. Hydrocorisone acetate (HC). 3 mg, twice a week, subcutaneously starting on the day of implantation.
b. X-irradiation (X-I). Whole body, 400 rads, one day before implantation.

Inoculum size: 10^6 cells per cheek pouch.

This diagram illustrates the passage of cells through three stages of selection in Hydrocortisone treated hamsters and tissue culture and the cross transpiantation of these cell line into irradiated hamsters.

generation explant (Third selection). The original H 4-II-E cells and each re-cultured subline were also implanted into the cheek pouch of X-irradiated hamsters in order to determine the transplantability and tumor growth rate in animals given another type of immunosuppressive treatment.

At each stage of selection, the following phenotypic characteristics were studied: 1) transplantability into hydrocortisone treated hamsters, 2) histological appearance of the tumor, 3) generation time of the explant and 4) transplantability into irradiated hamsters. The cytogenetic characteristics studied included the chromosome distribution and karyotype of both the explant and tumor at each stage of selection.

Animals and conditioning of the host: Female golden hamsters (random bred ENG: ELA-Engle Laboratory Animals, Inc., Farmersberg, Indiana), ranging from 40-60 gm, were employed in these studies. The following treatments were given to two groups of hamsters to suppress the immune mechanism: a) Hydrocrtisone acetate (Hydrocortone, Merck, Sharp and Dohme), at a dose of 3 mg, twice a week, was injected subcutaneously starting on the day of

implantation. b) X-irradiation, in a dose of 400 rads total body was employed at the rate of 60 rads per minute with 230 kvp (General Electric, Maximar 250-III) at 14.5 mA, with 0.25 Cu filter, 24 hours before cell implantation.

Method of culture of rat hepatoma cells: The H 4-II-E cells used in the present study were derived from a minimal deviation rat hepatoma, the Reuber H-35[14], and adapted to growth *in vitro* by Pitot *et al.*[13]. The culture was grown in plastic tissue culture flasks (Falcon #3024) with 5% CO_2 and 95% air. Swim's S-77 medium (GIBCO)[15] was supplemented with 20% horse serum, 5% calf serum, 2 μ moles of L-glutamine/ml, 100 units/ml penicillin and 100 μg/ml streptomycin. This medium was changed every 3-4 days. Cells were grown in monolayers and were subcultured once a week by treating the monolayer with 0.25% trypsin solution (solution A, GIBCO).

Implantation: Tissue culture cell suspension were obtained by replacing the culture fluid of a 4-5 day old culture with a versene-trypsin solution (0.7 mM EDTA plus 0.05% trypsin solution). The cell suspension was centrifuged, washed 2-3 times with fresh medium to remove the Versene-trypsin solution and then redispersed in appropriate volumes of media. After counting in a hemocytometer, the suspension of cells was diluted so that the desired inoculum (1×10^6) was contained in a volume of 0.3 ml of medium. For implantation, hamsters were anesthetized with ether. After implantation, the animals were observed for tumor growth by everting the cheek pouch under light anesthesia, approximately every four days and the tumors were measured at each examination.

Re-culture: The tumors were cut into small pieces with scissors, washed with Hanks's balanced salt solution (HBSS) containing 200 units/ml penicillin and 200 μg/ml streptomycin. The pieces of tumor were placed into a syringe with small screens separated by glass beads, and pushed through the screens into a Petri dish containing HBSS plus 0.05% trypsin solution. The cells were then resuspended in the previously described growth medium and pipetted into plastic tissue culture flasks in the usual manner.

Histological examination: Tumor tissues removed from the animals were examined microscopicallay. They were fixed in 10% neutral formalin and stained with hematoxylin-eosin.

Determination of generation time: Cells were cultured using plastic tissue culture flasks (Falcon #3012). Cell counting was performed on 4 flasks a day for 7 days. Cell viability was determined in a haemocytometer by the exclusion of 0.1% trypan blue[16].

Cytogenetic techniques: Cultures in the log growth phase and cell suspensions obtained directly from tumors as previously described were used for the

preparation of chromosomes. After the addition of colcemid (0.4 μg/ml), the cells were incubated at 37°C for two hours, and chromosome spreads were prepared[2]. One hundred spreads of the original culture and 50 or 100 spreads of each re-cultured subline were examined. Representative spreads of the modal chromosome number from each preparation were photographed. Karyotyping was carried out according to the classification described by Hungerford *et al.*[17)18)19)].

RESULTS

Serial transplantation into the cheek pouch of conditioned hamsters: The original H 4-II-E cells implanted into the cheek pouches of hydrocortisone treated hamsters produced tumors, which grew to maximum size approximately 2 weeks after implantation. Tumors were dark red in color, soft in consistency and round or oval in shape, with necrosis and hemorrhage within the tumors. Appearance of the second and third selection tumors were grossly similar to that of the first selection. Tumors were never found in control animals. The transplantability and average tumor size at each stage of selection is summarized in Table 2. There was a decreased percentage of takes from 80.0% in the first selection to 55.6% in the third selection, although this difference is not statistically significant with the number of animals used in this experiment. There was also a decrease in average tumor size, from 10.0 mm in the first selection to 6.3 mm in the third selection.

To determine possible differences in the types of cells selected by different immunosuppressive procedures, the original H 4-II-E cells, first selection explant

TABLE 2. Transplantability of H 4-II-E and Its Sublines into the Cheek Pouches of Control, Hydrocortisone Treated and X-Irradiated Hamsters

Implanted cells	Untreated	Treated	
		Hydrocortisone	X-irradiation
H 4-II-E	0/34 (0.0%)[a]	16/20 (80.0%) (10.02)[b]	15/20 (75.0%) (8.06)
First selecton explant	0/10 (0.0%)	7/10 (70.0%) (7.04)	1/10 (10.0%) (3.65)
Second selection explant	0/10 (0.0%)	10/18 (55.6%) (6.33)	2/10 (20.0%) (3.78)
Third selection explant	ND	ND	1/10 (10.0%) (2.50)

The ratio of cheek pouches with tumors 2 weeks after implantation is given as the number with tumors over the total number of pouches injected and is expressed as a percentage in parentheses (a). The average size of the tumors at two weeks after implantation is also indicated (b).

(17th generation), second selection explant (11th generation) and third selection explant (4th generation) were implanted into the cheek pouch of X-irradiated hamsters. Results of this experiment are also given in Table 2. These findings demonstrate that cells of each explant have markedly lower transplantability than the original cells in the X-irradiated animals as well as in the hydrocoritsone treated animals.

Re-establishment of cell lines and determination of generation time: Cells derived from hamster tumors could be re-established in tissue cnlture. The re-cultured cells were very similar in appearance to the original H 4-II-E cells, however their shape and size were more homogeneous than the original cells. The mean generation time of the original H 4-II-E cells and explants at each stage of selection are presented in Table 3. The H 4-II-E cells had a mean generation time of 19.0 hours compared with 3.2 days when the clonal strain was first established[13]. The mean generation times of the second and third selection cells were approximately 30 hours, and that of the first selection was between the original and the second selection.

TABLE 3. Mean Generation Time of H 4-II-E and Its Sublines

Culture	Mean generation time (Hours)
H 4-II-E	19.0
First selection explant	26.5
Second selection explant	32.7
Third selection explant	29.4

Histology:

First selection: The tumors showed extensive central hemorrhage with the tumor cells compressed to the periphery (Fig. 1). Within the hemorrhagic areas there were necrotic tumor cells present. The viable tumor cells resembled normal rat liver cells. There was no evidence of bile plugs or pigment in the tumor.

Second selection: The histological appearance of the tumors in the second selection was essentially similar to that of first selection showing extensive hemorrhage and necrosis. The tumor cells formed aggregates or sheets of cells in the hemorrhage and necrotic areas. There was no definite evidence of bile production.

Third selection: As compared with the tumors described above, these tumors showed less hemorrhage and necrosis, and lobules were separated by increased vascular connective tissue (Fig. 2). The tumor cells themselves were essentially similar to those seen in the others, however, the tumor cells were less variable in size and shape. There was no evidence of bile stasis.

It was not possible to differentiate the tumor cells at each stage of selection purely on a morphological basis. It was possible, however, to recognize certain gross histologic differences of these tumors, as previously described. The third selection tumors appeared the most differentiated and thus were most similar to normal rat liver morphologically.

Chromosome distribution of H 4-II-E cells, hamster tumors and tumor explants: The chromosome distribution of the original H 4-II-E cells and the explants at each stage of selection are shown in Chart 1. The cell population of the H 4-II-E cells consisted of a spectrum of hypotriploid cells with a tendency to be bimodal. The mode of the chromosome number was 51 compared with 46 ten years ago[13]. The average chromosome number was 50.25, and showed a wide distribution as is frequently found in an established permanent cell line.

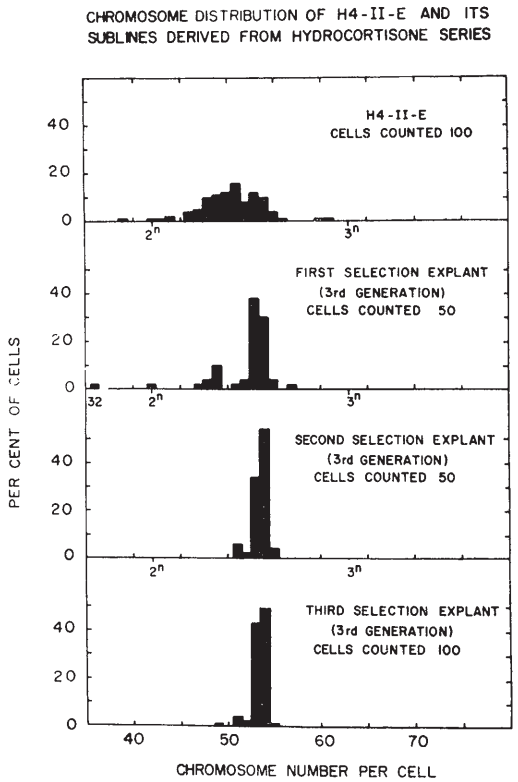


CHART 1. Illustrates the chromosome distribution observed in the original H 4-II-E cell line and the explants from tumors at each selection. 50 or 100 spreads were counted from each culture and the data is expressed as percentage of cells with each chromosome number. The diploid and triploid chromosome numbers are indicated by 2^n and 3^n respectively.

In the first selection, 43 cells were counted directly from the tumor. The modal chromosome number was shifted upwards from 51 to 54, and the distribution was markedly sharpened compared with that of the original cells. Fifty cells were counted from the third passage generation of the explant of the first selection tumors. The result also shows the same tendency toward being selected. The modal chromosome number was 53 and 54 and cells with these chromosome numbers made up 68% of all counted cells as opposed to 22% in the original culture. The average chromosome number was 52.02.

A similar distribution of chromosome number was observed in both the second and third selection. In the second selection, 46 cells from the tumor and 50 cells from the explant were counted. The results showed more narrowing of the scatter of chromosome number in both the tumor and the explant compared with the first selection. The modal chromosome number of the tumor and explant was 54. Cells with 53 and 54 chromosomes made up 89% of the mitoses studied in the tumor and 88% in the primary explant. The average chromosome numbers were 53.33 and 53.48, respectively.

In the third selection, direct examination of the tumor chromosomes failed. Examination of the explant chromosomes showed almost the same result as that of the second selection. Forty nine of 100 metaphase plates counted had 54 chromosomes, the percentage of cells with 53 and 54 chromosomes was 92% and the average chromosome number was 53.37.

Karyotype analysis of the H 4-II-E and third selection explant: Chromosomes were arranged according to the classification used in previous publications [17) 18) 19)] for the normal rat karyotype. Since the individual chromosomes assigned to group 4-10, XY, 11-13 and 14-20 were not always easy to identify, they were pooled together in each group. For karyotype analysis of the H 4-II-E cells, 9 different metaphase plates were chosen to make detailed karyotypes. Moreover, 7 metaphase plates with 53 and 54 chromosomes from the same sample were used to make karyotypes to compare with those of the selected sublines.

A representative karyotype containing 51 chromosomes and the karyotype variation of the original cells are demonstrated in Fig. 3 and Table 4. One marker chromosome that is never found in normal rat karyotypes was observed in 15 of 16 cells. This marker chromosome was the largest submetacentric observed in the material and was clearly distinguishable from other chromosomes. The number of chromosomes in each group varied from cell to cell, even among cells with identical chromosome numbers, except group 1-3, which showed a relatively stable pattern.

For karyotype analysis of the third selection explant, 5 metaphase plates with 53 chromosomes and 4 metaphase plates with 54 chromosomes from the third passage generation explant were used. Fig. 4 and 5 illustrate the

TABLE 4. Variations in Karyotype Observed in the Original H 4-II-E Cells and the Third Selection Explant

Culture	Total number	Group				Marker chromosome
		1-3	4-10, X.Y.	11-13	14-20	
H 4-II-E	44	6	21	7	10	0
	49	7	17	9	15	1
	50	6	19	7	17	1
	50	6	20	8	15	1
	50	6	21	8	14	1
	51	6	22	6	16	1
	51	5	19	8	18	1
	51	7	21	7	15	1
	51	6	18	9	18	1
	53	6	19	9	18	1
	53	6	19	10	17	1
	53	6	19	9	18	1
	54	6	23	7	17	1
	54	6	21	8	18	1
	54	6	21	8	18	1
	54	6	20	9	18	1
Third selection explant	53	6	19	9	18	1
	53	6	19	9	18	1
	53	6	19	9	18	1
	53	6	19	9	18	1
	53	6	19	9	18	1
	54	6	19	10	18	1
	54	6	19	10	18	1
	54	6	19	10	18	1
	54	6	19	10	18	1

representative karyotypes of cells with 53 and 54 chromosomes, respectively. Karyotype variation in this explant is demonstrated in the bottom half of Table 4. The large submetacentric marker chromosome found in the original cells was seen in all karyotypes examined. It is interesting that there are no variations in number observed in any group and the only difference between the cells with 53 and 54 chromosomes is in group 11-13. More detailed examination of the karyotype was attempted especially for group 1-3 and 11-13 of the plates listed in Table 4. The results are presented in Table 5. In group 1-3, they showed exactly the same chromosome arrangement, even among the cells with 53 and 54 chromosomes. In group 11-13, 4 of 5 cells with 53 chromosomes and 3 of 4 cells with 54 chromosomes also had the same arrangement. From these results, the difference between cells with 53 and 54 chromosomes is probably an extra chromosome in group 12. In regard to the genesis of the marker chromosome, since its long arm is nearly equal to group 2, and group 2 has a tendency to be monosomic, it is proposed that the marker chromosome was made from one chromosome in group 2 and one in group 4-10, XY by centromeric fusion.

TABLE 5. Karyotype Variations in the Third Selection Explant

Total number	Group								Marker chromosome
	1	2	3	4-10, X.Y.	11	12	13	14-20	
53	2	1	3	19	2	4	3	18	1
53	2	1	3	19	3	2	4	18	1
53	2	1	3	19	3	2	4	18	1
53	2	1	3	19	3	2	4	18	1
53	2	1	3	19	3	2	4	18	1
54	2	1	3	19	3	3	4	18	1
54	2	1	3	19	3	3	4	18	1
54	2	1	3	19	3	3	4	18	1
54	2	1	3	19	3	4	3	18	1

DISCUSSION

Many previous studies have been concerned with the cell changes occuring in heterotransplanted tomors[5)6)7)8)9)10)11)12)]. These have mainly dealt with changes of transplantability and tumor growth rate after serial heterologous transplantation. Ahlstrom and Ising[9)] reported that a line adapted to a foreign host showed increased virulence in that host and decreased virulence in the original host as compared with the original line following the serial inoculation of Ehrlich mouse ascites cells into the hamster cheek pouch. In 1970, Suciu-Foca *et al.*[12)] also presented alterations in biological behavior using two 2-methylcholanthrene-induced mouse sarcomas and tolerant rats. These observations could generally be explained by the adaptation of the tumor cells to the foreign hosts[9)] or by the assimilation of heterologous antigens by the cells of the donor[20)]. Thus, the accelerated growth in the foreign host after repeated transfer was consistent with previous findings that on serial transplantation a tumor often shows increased virulence[21)22)]. In contrast to the findings described above, an increase in virulence was not observed in our experimental system. The results of the present study demonstrate a decrease in virulence, as expressed by a slower growth rate as well as a decrease in the ratio of takes with each succeeding *in vivo* and *in vitro* passage.

The studies of Foley *et al.*[23)24)25)] demonstrated clearly the relationship between transplantability and the malignancy of cultured cell lines using the cheek pouch of conditioned and unconditioned hamsters. They observed that tissue culture cells derived from malignancies produced tumors in cortisone treated hamsters when as few as 10 cells were inoculated, while cells from non-malignant sources required 1,000 or more cells. Malignant cells produced tumors in the unconditioned hamster when as few 1×10^4 cells were inoculated while cells from normal sources usually required 1×10^6 cells. Thus, the transplantability into the hamster cheek pouch seems an indication of the malignancy of cultured cell lines. If we accept this interpretation, cells with lower malignancy (*i.e.* more differentiated) were selected during the *in vivo*

passages of the present study. This interpretation is supported by the histological appearance of the hamster tumors in each selection and by the generation times of each explant, which were different from those of the original cells, showing a more differentiated pattern and a slower growth rate. Moreover, our observations concerning the phenotypic alterations are also in agreement with previous studies demonstrating that heterotransplantation of cancer cells into hamster cheek pouches results in the selection of cells best able to grow in the new enviroment[10)26)].

In regard to conditioning of the host, tumors were never found in untreated animals, some conditioning being essential for the cells to grow in the hamster cheek pouch. The exact mechanism of the effects of cortisone and X-irradiation are as yet unknown although both result in immuno-suppression. Because the degree and quality of the immuno-suppression differs in these animals it is safe to assume that they represent different *in vivo* transplantation environments. Markedly different transplantability and tumor growth rate of each explant compared with those of the original cells in the irradiated animals do, however, indicate that the explants derived from the hamster tumors have different biological properties which may be characterized as being less malignant.

The main purpose of this study was to determine a possible relationship between cytogenetic variations and phenotypic variations in the cells submitted to serial *in vivo* and *in vitro* selection pressures. Several investigator have been concerned with selective changes in the chromosomal constitution of cell populations after growth in a different species or strain of host[10)26)27)28)29)30)31)]. Under these conditions, selection of cells has been attributed to altered environmental conditions and the selected cells may show increase or decrease in the modal chromosome number[10)26)31)], or significant change in ploidy[27)28)30)]. These studies did not, however, attempt to demonstrate the multiple phenotypic effects of these changes.

The results of cytogenetic examination in the present study indicate that the modal chromosome number was shifted upwards from 51 to 53 and 54 and remarkable sharpening of the distribution was seen with each succeeding *in vivo* and *in vitro* selection. This change was accompanied by the selection of a more homogeneous karyotype. At each stage of selection, the result of the tumor chromosome analysis was almost the same as that of the explant. It is clear from these results that there was selection occurring in the foreign host for a cell with a particular genetic makeup. Since the positive morphologic identification of the species of origin of individual chromosomes is very difficult, intraspecies hybridization can not be completely excluded on the basis of karyotype alone. However, no morphologic differences in the chromosomes between the original H 4-II-E cells and each explant could be observed. Electron microscopic examination of both tumors and explants, which will be

published later, showed no morphologic evidence for the presence of virus. It seems likely, therefore, that the cells studied are from the original H 4-II-E cell line.

The data in the present study, based on detailed karyotype analysis, indicate that the selected cultures derived from hamster tumors consist of a homogeneous karyotypic population. In the third selection explant, more than 90 per cent of the counted cells have 53 or 54 chromosomes, and very little variation is seen in arrangements of their karyotypes in cells containing the modal chromosome numbers. Moreover, the only difference between the cells with 53 and 54 chromosomes is observed in group 12. It appears that this explant consists of only two different cell types cytogenetically. The mechanism by which these cells with a particular genetic makeup were selected in the hydrocortisone treated animals is unknown. The most likely explanation is a biochemical or immunological selection of cells in the new enviroment[26)32)].

In order to discuss the relationship between the phenotype and the genotype of these cells, one must consider some fundamental cytogenetic characteristics of cancer cells. Malignant cells frequently differ cytogenetically from normal cells both with regard to the number and morphology of chromosomes. Cancer cells frequently have pronounced instability in chromosome number. One possible explantion for the difference in transplantability between the original cell line and the selected cell line is that there is a relationship between the karyotype and surface antigenicity and the unstable chromosomal makeup would give cancer cells the capability of altering their antigenic properties, with the possibility of greater adaptability to a foreign immunologic environment. Further, the chromosome number of transplantable tumors shows a wider chromosome distribution[33)34)] than normal adult or embryonic tissues[33)35)]. It is possible that immune surveillance may actually be the mechanism for the maintenance of a stable kayotype in the immunologically intact animal. It therefore appears likely that the uniformity of karyotype and the width of chromosome distribution as well as modal chromosome number and ploidy and morphology of chromosomes may control the biological properties of cancer cells. It is also possible that the antigenicity of cells may relate to their chromosomal constitution especially the homogeneity of chromosome nnmber and karyotype.

CONCLUSION

Based on the data described in the present study, the following conclusions may be drawn; 1) Heterotransplantation of rat hepatoma cells into the cheek pouch of hydrocortisone treated hamsters results in cell selection, 2) cell selection is accompanied by changes in morphology, growth rate, and transplantability, 3) these phenotypic differences are associated with selection of

cells with a more homogeneous karyotype, and 4) it is proposed that chromosomal changes can markedly influence the characteristics of a cell and it is possible that these changes result from complex gene interactions.

REFERENCES

1) Vogt, M., A Study of Relationship Between Karyotype and Phenotype in Cloned Line of Strain HeLa, *Genetics*, **44**, 1257, 1959.
2) Bottomley, R. H., Trainer, A. L., and Griffin, M. J., Enzymatic and Chromosomal Characterization of HeLa Variants, *J. Cell Biology*, **41**, 807, 1969.
3) Higgins, M. L., Tillman, M. C., Leach, K. K., Melnykovych, G., and Leach, F. R., Studies on the Variability of the KB Cell Line, *Texas Reports on Biology and Medicine*, **27**, 1013, 1969.
4) Ghosh, I. and Ghosh, S., Karyological Studies on Two HeLa Lines, *Z. Krebsforsch*, **74**, 103, 1970.
5) Galton, M., Goldman, P. B., and Holt, S. F., Karyotypic and Morphologic Characterization of a Serially Transplanted Human Choriocarcinoma, *J. Natl. Cancer Inst.*, **31**, 1019, 1963.
6) Iverson, H. G., Transplantation from Man to Mouse of Exudates Containing Tumor Cells, *Brit. J. Cancer*, **12**, 210, 1958.
7) Miles, C. P., Chromosomes of Some Heterotransplanted Human Tumors I. H. Emb. Rh. 1, H.S. 1, and ME 1, *J. Natl. Cancer Inst.*, **34**, 103, 1965.
8) Adams, R. A., Flowers, A., and Devis, B. J., Direct Implantation and Serial Transplantation of Human Acute Lymphoblastic Leukemia in Hamsters SB-2, *Cancer Res.*, **28**, 1121, 1968.
9) Ahlstrom, G. C. and Ising, U., Alteration in Growth of Ehrlich Mouse Ascites Carcinoma on Repeated Transfer through Hamsters, *Brit. J. Cancer*, **9**, 582, 1955.
10) Ising, U., Chromosomal Changes in Ehrlich Mouse Ascites Cancer on Repeated Transfer through Hamsters and Rats, *Acta Pathol. Microbiol. Scand.*, **40**, 315, 1957.
11) Ising, U., Chromosome Studies in Ehrlich Mouse Ascites Cancer after Heterologous Transplantation through Hamsters, *Brit. J. Cancer*, **9**, 592, 1955.
12) Suciu-Foca, N., Dumitrescu, V., Lazar, C., and Nachtigal, M., Host and Tumor Modifications Associated with Serial Hetero-transplantation of Tumors through Immunologically Tolerant Animals, *Cancer Res.*, **30**, 1681, 1970.
13) Pitot, H. C., Peraino, C., Morse, P. A. Jr., and Potter, V. R., Hepatomas in Tissue culture Compared with Adapting Liver *in vitro*, *Natl. Cancer Inst. Monogr.*, **12**, 229, 1964.
14) Reuber, M. D., A Transplantable Bile-secreting Hepatocellular Carcinoma in the Rat, *J. Natl. Cancer Inst.*, **26**, 891, 1961.
15) Morse, P. A. Jr. and Potter, V. R., Pyrimidine Metabolism in Tissue Culture Cell Derived from Rat Hepatomas. I. Suspension Cell Cultures Derived from the Novikoff Hepatoma, *Cancer Res.*, **25**, 499, 1965.
16) Marchant, D. J., Kahn, R. H., and Murphy, W. H. Jr., Measurement of Growth, *In* Handbook of Cell and Organ Culture, Minneapolis, Minn., Burgess Publishing Company, 155, 1965.
17) Hungerford, D. A. and Nowell, P. C., Sex Chromosome Polymorphism and the Normal Karyotype in Three Strains of the Laboratory Rat, *J. Morphol.*, **113**, 275, 1961.
18) Nowell, P. C., Ferry, S., and Hungerford, D. A., Chromosomes of Primary Granulocytic Leukemia (Chloroleukemia) in the Rat, *J. Natl. Cancer Inst.*, **30**, 687, 1963.
19) Nowell, P. C., Morris, H. P., and Potter, V. R., Chromosomes of "Minimal Deviation" Hepatomas and Some Other Transplantable Rat Tumors, *Cancer Res.*, **27**, 1563, 1967.

20) Swaen, G. V., A Study on the Antigenicity of a Human Cell Line Propagated in a Heterologous Medium, *Experientia*, **19**, 628, 1963.
21) Foulds, L., The Experimental Study of Tumor Progression: A Review, *Cancer Res.*, **14**, 327, 1954.
22) Snell, G. D., *The Physiopathology of Cancer*, Hoeber, New York, 1953, p. 338.
23) Foley, G. E. and Handler, A. H., Differentiation of "Normal" and "Neoplastic" Cells Maintained in Tissue Culture by Implantation into Normal Hamsters, *Proc. Soc. Exper. Biol. and Med.*, **94**, 661, 1957.
24) Foley, G. E., Handler, A. H., Adams, R. A., and Craig, J. M., Assessment of Potential Malignancy of Cultured Cells: Further Observations on the Differentiation of "Normal" and "Neoplastic" Cells Maintained *in vitro* by Heterotransplantation in Syrian Hamster, *Natl. Cancer Inst. Monogr.*, **7**, 173, 1961.
25) Foley, G. E. and Drolet, B. P., Loss of Neoplastic Properties *in vitro*. I. Observations with S-180 Cell Lines, *Cancer Res.*, **24**, 1461, 1964.
26) Ising, U., Effect of Heterologous Transplantation on Chromosomes of Ascites Tumors: A Contribution to Our Knowledge of Environmental Influence on Tumor Cells, *Acta Pathol. Microbiol. Scand. Suppl.*, **127**, 1, 1958.
27) Hauschka, T. S., Relationship between Chromosome Ploidy and Histocompatibility in Mouse Ascites Tumor, *Cancer Res.*, **12**, 269, 1952.
28) Hauschka, T. S., Kvedar, B. J., Grinnell, S. T., and Amos, D. B., Immunoselection of Polyploids from Predominantly Diploid Cell Populations, *Ann. N.Y. Acad. Sci.*, **63**, 683, 1956.
29) Hauschka, T. S. and Levan, A., Inverse Relationship between Chromosome Ploidy and Host-Specificity of Sixteen Transplantable Tumors, *Exper. Cell Res.*, **4**, 457, 1953.
30) Krishan, A., Raychaudhuri, R., and Flowers, A., Karyotype Studies on Human Leukemic Lymphoblasts *in vitro* and as Serial Transplants in Neonatal Syrian Hamsters, *J. Natl. Cancer Inst.*, **43**, 1203, 1969.
31) Levan, A., Chromosomes in Cancer Tissue, *Ann. N.Y. Acad. Sci.*, **63**, 774, 1956.
32) Kaziwara, K., Derivation of Stable Polyploid Sublines from a Hyperdiploid Ehrlich Ascites Carcinoma, *Cancer Res.*, **14**, 795, 1954.
33) Levan, A. and Hauschka, T. S., Endomitotic Reduplication Mechanism in Ascites Tumor of the Mouse, *J. Natl. Cancer Inst.*, **14**, 1, 1953.
34) Makino, S., A Cytological Study of the Yoshida Sarcoma, an Ascites Tumor of White Rats, *Chromosoma*, **4**, 6491, 1952.
35) Tanaka, T., A Study of the Somatic Chromosomes of Rats, *Cytologia*, **18**, 343, 1953.

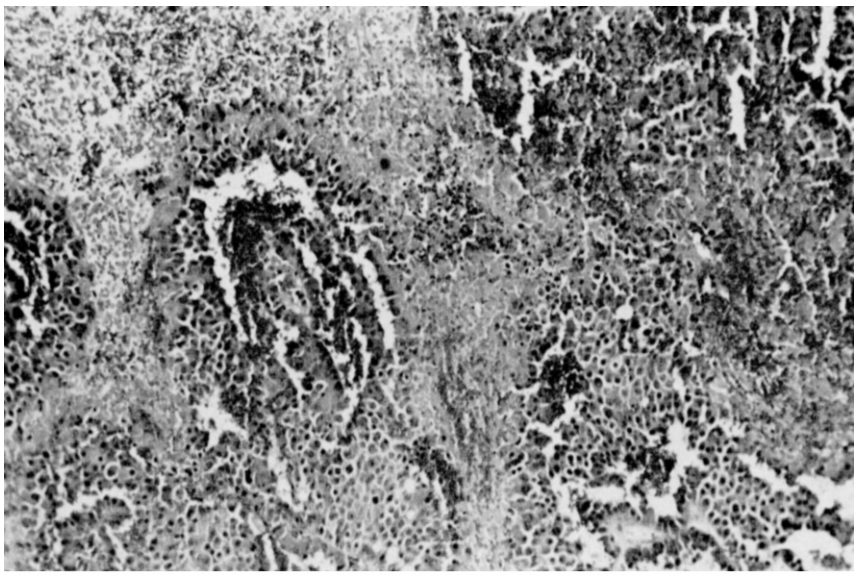


FIG. 1. Illustrates the microscopic appearance of the first selection tumors. Marked hemorrhage and necrosis is evident. The tissue was stained with hematoxylin and eosin. Final magnification is approximately ×200.

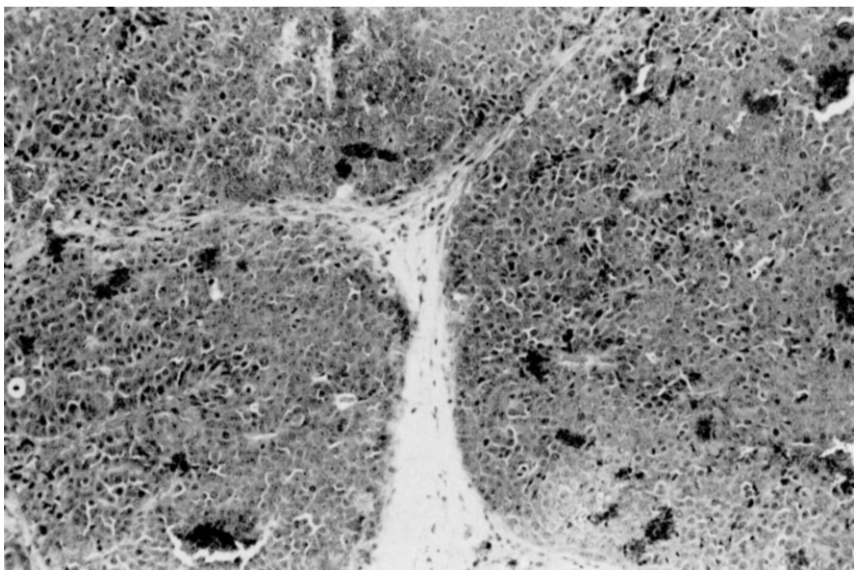


FIG. 2. Illustrates the microscopic appearance of the third selection tumors. A more homogeneous appearance with less hemorrhage and necrosis is evident. The tiesue was stained with Hematoxylin and eosin. Final magnification is approximately ×200.

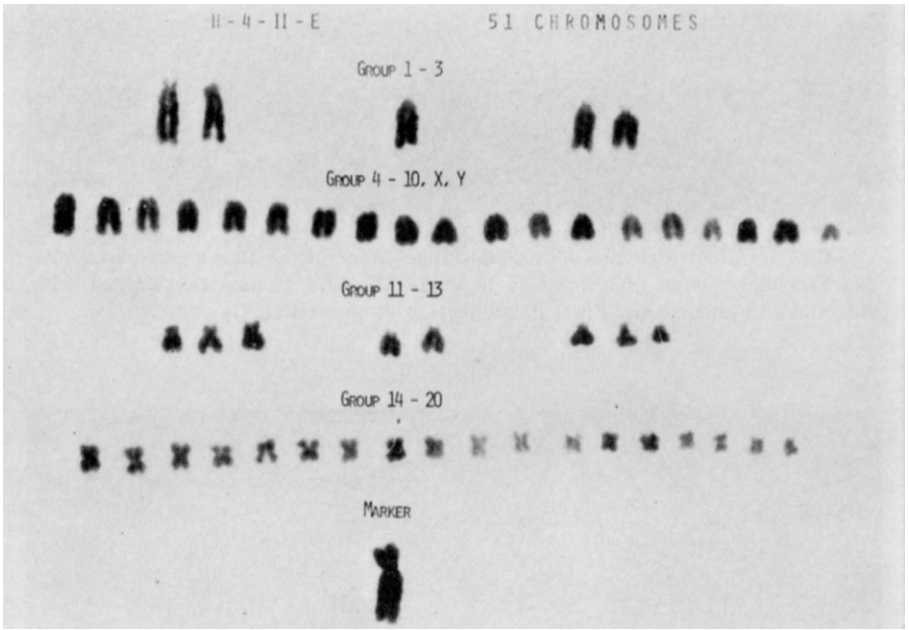


FIG. 3. Shows a representative karyotype from the original H 4-II-E cell line. The cell contains 51 chromosomes including the large marker chromosome.

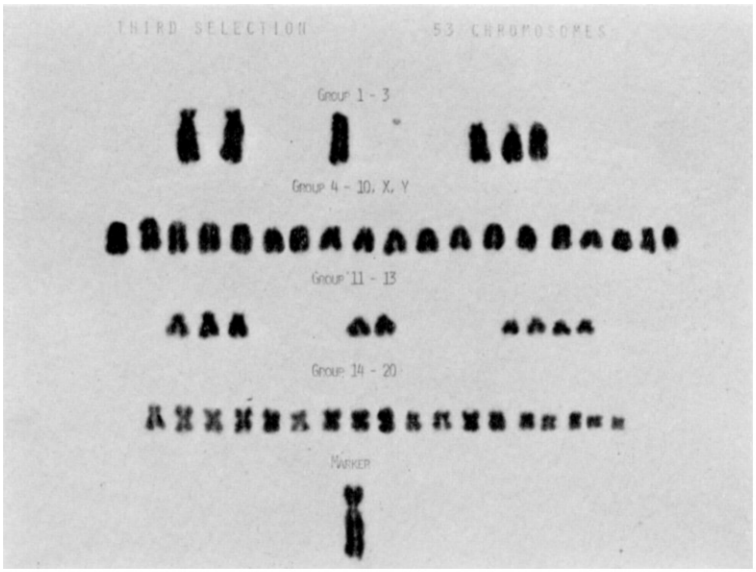


FIG. 4. Shows a cell with 53 chromosomes from the explant of the third selection tumors.

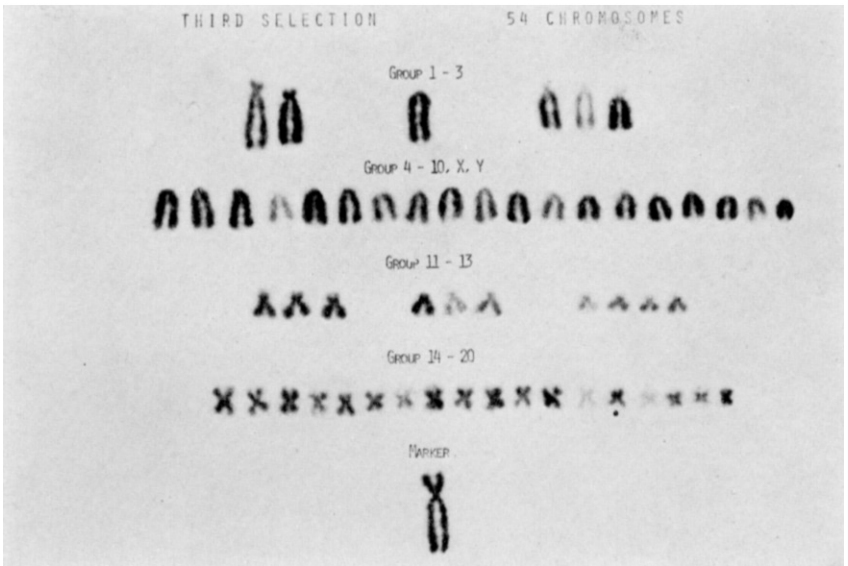


FIG. 5. Demonstrates a cell with 54 chromosomes from the explant of the third selection tumors. This karyotype contains an additional chromosome in group 12 as compared with the cell with 53 chromosomes illustrated in Fig. 4.