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# A Study of a New Human Tumor Cell Line (Rhabdomyosarcoma) (38250)

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A limited number of human rhabdomyosarcomas have been established in culture (1–3) and type C virus have been seen in one of these cell lines (3), but there have been no studies attempting to follow, in a progressive fashion, the transition from a culture to a cell line. Preliminary observations of a human rhabdomyosarcoma cell culture in our laboratory showed a morphologic alteration from a fibroblast to round cells early in the culture. It is our purpose to correlate both morphologic and karyotypic changes that occur throughout this transition from a primary culture to a permanent cell line.

*Materials and Methods.* The tumor was taken from a mass on the lower left posterior chest wall of an 80 year old white female. The tumor was first noted 2 mo before admission. The mass was excised 4 days after admission and the patient was discharged on the 9th day postoperative with no complications at that time. The patient was given no chemotherapeutic drugs prior to excision of the tumor.

The primary culture was established at the University of Kansas Medical Center's Diagnostic Virology Laboratory and the cells were frozen in liquid nitrogen at the 10th cell passage. At this point the cells were grown in Eagles minimum essential medium (EMEM) in Earles salts. We started the present work in October 1971, when the cells were in the 10th passage. The cells were cultured in RPMI 1640 medium, supplemented with 20% fetal calf serum (FCS) both obtained from GIBCO, 3175 Staley Road, Grand Island, NY (4). The cells were transferred at a 1:5 dilution using a 1:500 Versene combined with a 0.05% trypsin to free the cells. Leighton tubes were prepared from selected cultures and stained with a Giemsa stain in order to monitor the morphologic characteristics of the cells. In January 1973, we again cultured the cells, frozen at passage 10, in order to recheck our cellular response and changed the serum to determine if it was a factor in the alteration. At this time, we used RPMI 1640 medium supplemented with 15% calf serum (GIBCO).

During the 25th cell transfer, we had the culture checked for the presence of mycoplasma using the procedure outlined in the P-75 requirements and proposed 9CRF (M-96medium) procedure of the Animal and Plant Health Inspection Service of the USDA. This was accomplished through the cooperation of Dr. M. Field, Cutter–Haver–Lockhart Laboratories, Shawnee, KS. The culture was found to be negative by these tests as well as by electron microscopy.

Certain cells were treated with 5-Iododeoxyuridine (IDU) from nutritional Biochemical Corp., Cleveland, OH. IDU was present in the medium at a concentration of 20 $\mu$g/ml and the cells maintained for 3, 4, 5 or 6 days in continuous contact with IDU. Other cells were treated with 0.1 $\mu$g of 20-methylcholantrene (20-mc)/ml from Sigma Chemical Co., St. Louis, MO. The cells (HUS-2) were seeded at $1.4 \times 10^6$ cells/each 8 oz glass prescription bottle and maintained with RPMI 1640 medium supplemented with 20% FCS containing the IDU at 20 $\mu$g/ml. No virus was seen in the supernatant pellets at day 3, 5 and 6. At this time, the cells had ceased to grow. The experiment was repeated at P-36 but on





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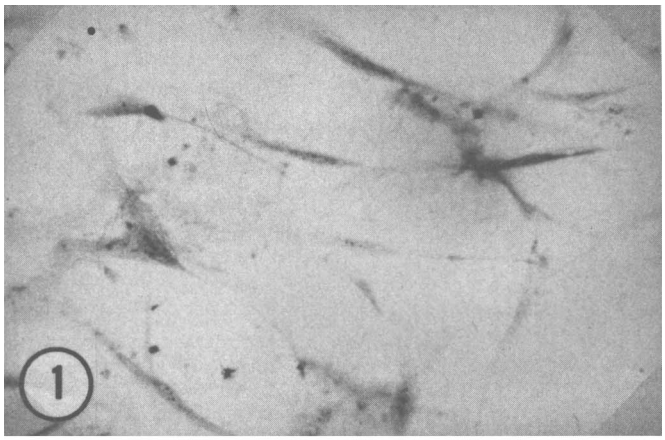
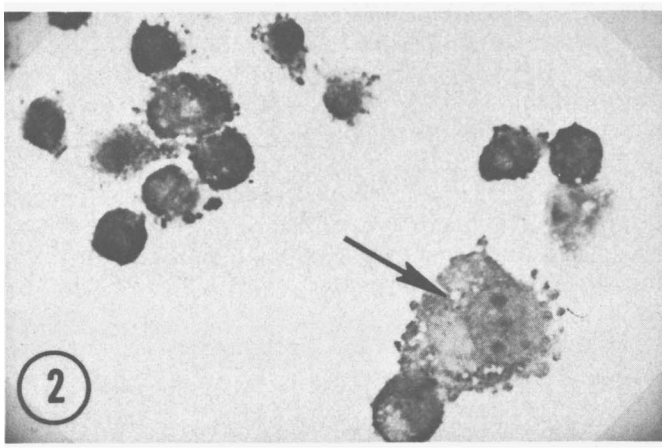
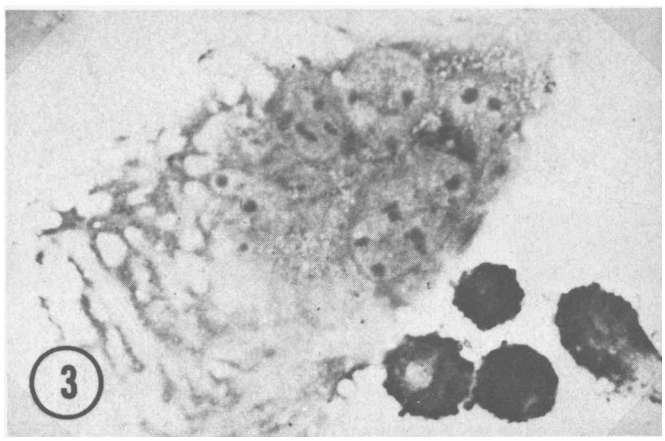



FIGS. 1 and 2. Demonstrate the morphologic alteration between passages 11 and 18 of HUS-2. Figure 1 shows a fibroblastic-like culture at passage 11 (10×); Fig. 2 shows the same culture at passage 18 in which round mononuclear cells and a mononuclear giant cell are shown (50×).

FIG. 3. This shows a detail of 1 of the multinucleated giant cells. All of the light micrographs were stained with a Giemsa stain. (50×).

day 4, the cells were transferred and returned to the conventional medium with IDU. After the next transfer, a part of the cells were pretreated with 20 $\mu$g of DEAE-D and all cells refed and examined at various interval by electron microscopy.

Karyotyping was done using standard procedures and an attempt was made to examine at least 50 metaphase spreads from each passage.

The cultured cells were scraped and the cells centrifuged at 1200–1500 rpm for 20 min, the supernatant fluid decanted and the cell pellet fixed in 2% phosphate buffered glutaraldehyde and postfixed in Os04. The tissues were processed in a routine fashion and examined on an RCA EMU-3G electron microscope.

*Results.* The tumor was described in the pathologist's report as "irregularly arranged round, oval and elongated cells with large oval hyperchromatic or vesicular nuclei, dispersed euchromatin and heterochromatin, prominent nuclei and abundant acidophilic cytoplasm." An occasional giant cell was noted. The final diagnosis was a probable embryonal rhabdomyosarcoma.

The earlier cultures consisted of both fibroblasts (Fig. 1) and occasional multinucleated giant cells. Between passage 12 and 14 the fibroblasts appeared to undergo a progressive morphological change resulting in cells that were now rounded and fusiform (Fig. 2) with giant cells usually containing several nuclei (Fig. 3). The rounded cells tended to float free in clumps. Transfer of these free floating cells apparently free of giant cells, resulted in a culture consisting of attached round cells plus multinucleated giant cells. This suggested that the source for these large cells was from the small mononuclear cells. Examination of the cultures by electron microscopy showed the giant cells to contain fibrillar material,

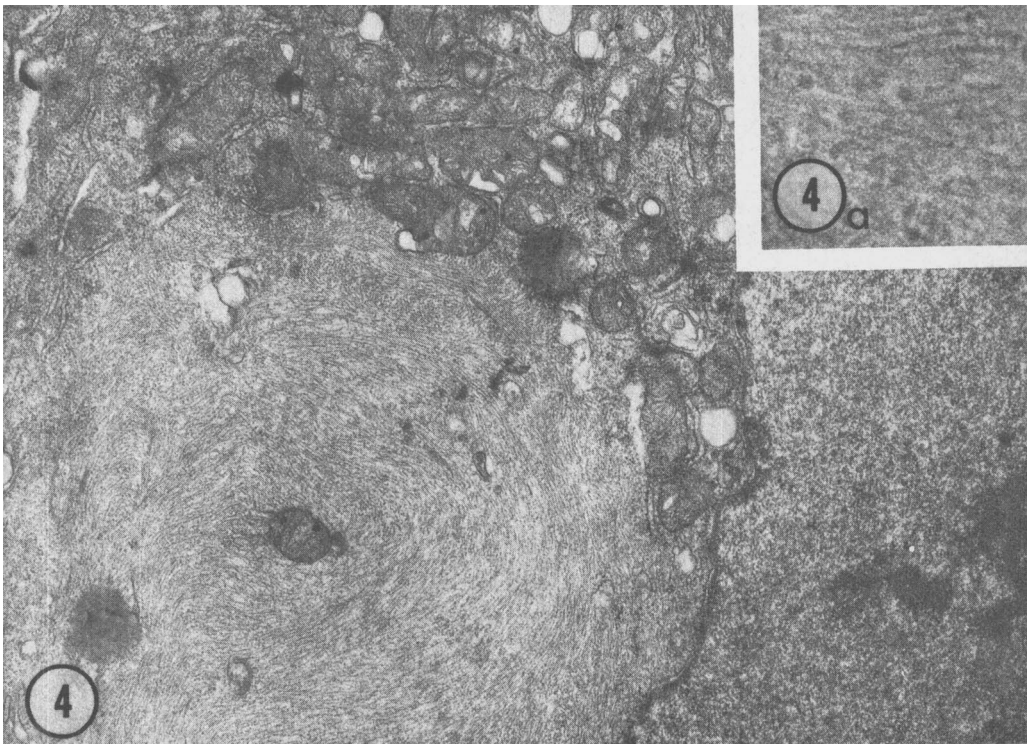



FIG. 4. An electron micrograph of one of the giant cells at passage 13. Note the accumulation of fibrillar material (19,500×). Fig. 4a shows a detail of fibrils. They seem to be of one type and measured approximately 100 Å across (110,000×).

and the fibrils measured 100 Å in diameter (Fig. 4). No other unique feature was seen.

In order to see if this morphological alteration was reproducible, we again removed the cells from liquid $N_2$ at passage 10 (prior to alteration) and designated them as HUS-2B. In order to determine whether a specific serum was required the cells were now maintained on RPMI 1640 supplemented with 15% calf serum. As before, the cells underwent a cultural alteration between the 12th and 14th passage. During this experiment the chromosomal patterns were examined at passages 12–15 and correlated with light microscopic appearance. By passage 15 the cells resembled the previous HUS-2 at the same passage, i.e., consisting of round cells and multinucleated giant cells.

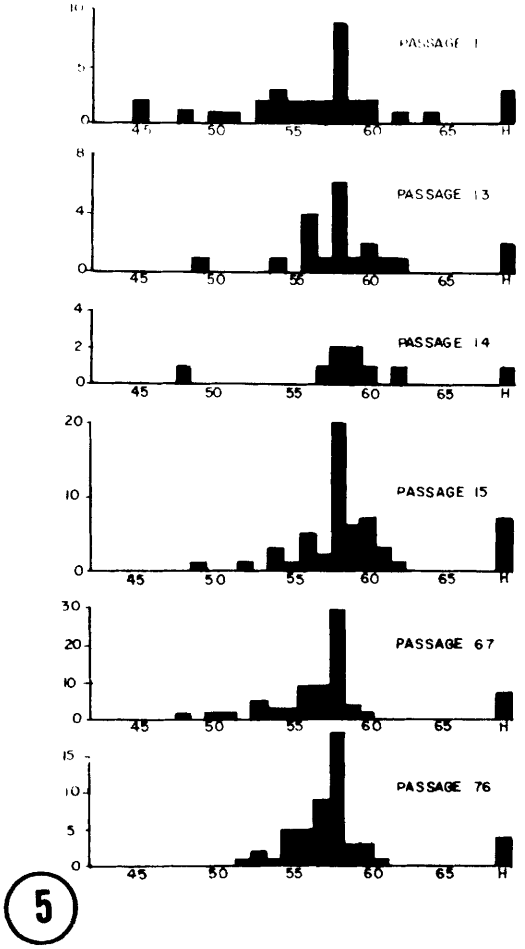
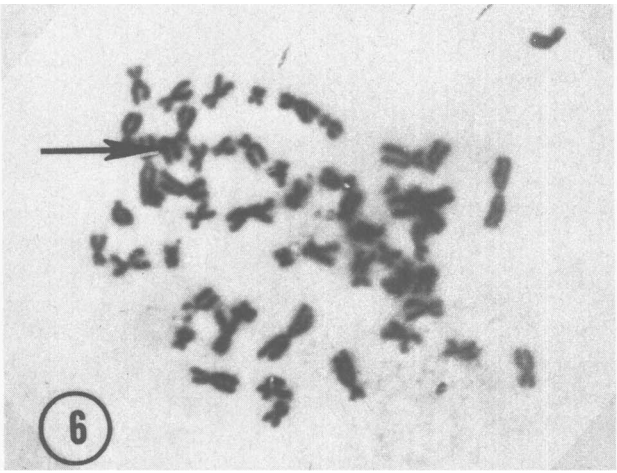



FIG. 5. Histogram to show stability of the modal number (58) before and after the morphologic alteration of the cell type. The number of cells counted is indicated on the ordinate with the number of chromosomes in each cell on the abscissa. The "H" indicated those cells showing hyperploidy with chromosomal numbers exceeding 100.

Examination of the karyotypic pattern of the cells prior to transformation (P-12) showed a characteristic grouping around a modal number of 58. This pattern was maintained throughout the passages 13 and 14 in which the morphologic alteration occurred (HUS-2B) and was present in P-67 and 76 of the original HUS-2 (Fig. 5). About 10% of the cells showed hyperploidy (greater than 100) and in all cases the remnants of only one nucleus was seen. Furthermore, 85%–95% of the cells in all passages showed a chromosomal marker (Figs. 6, 7). We were able to distinguish the marker from the normal D-group chromosomes since it was larger and with few exceptions did not show but 2 arms. Based on these results, we considered the marker chromosome to be telocentric. We found no double minutes in any cell passage as suggested in the literature (1, 5). The idiogram (Fig. 7) shown is typical of the karyotypes obtained during the various passages. Extra chromosomes were found in all Denver groups depending upon the specific cells examined but there was an increased incidence of extra chromosomes in the C and E groups.

We also examined HUS-2 cells that were maintained in continuous culture after treatment with IDU, 20mc and DEAE-D. None of these cultures showed any chromosomal changes when compared with the same passage of untreated HUS-2 cells. Electron microscopy examination of these cell cultures were negative for any virus particles.

*Discussion.* Results from this study have shown a rhabdomyosarcoma cell line derived from an 80 year old female with a stable karyotype and a modal number of 58 chromosomes with one consistent telocentric marker chromosome. White and Cox (1) described the karyotypic change in two successive recurrences of a rhabdomyosarcoma from a 3 year old girl. They found the modal number of around 70 changed to



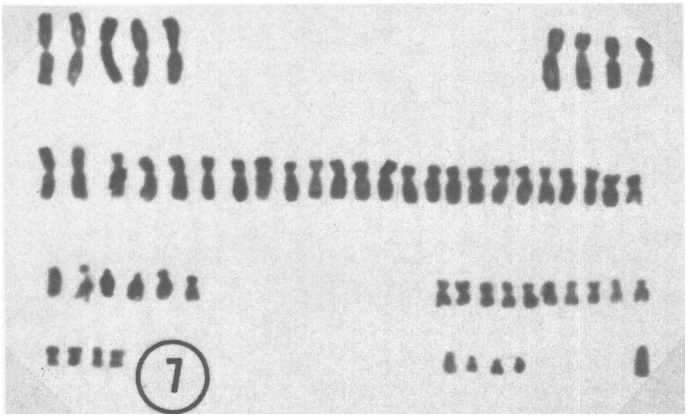


FIG. 6. Metaphase spread at passage 67. The arrow indicates the telocentric marker chromosome.

FIG. 7. Karyotype at passage 67. The marker chromosome is located in the lower right corner.

67–68 in the second recurrence and that in both cases the modal number changed progressively reaching 56–57 after 18 mo in culture. Further, there were continual changes in the number and morphology of marker chromosomes. The second recurrence had been obtained after treatment with x-ray but they were of the opinion that the x-ray was not responsible for the change between the first and second sample. McAllister *et al.* (2) reported the karyotypic pattern of an embryonal rhabdomyosarcoma *in vitro*. They also examined two specimens from a 7 year old girl, the first after treatment with cyclophosphamide and the second after treatment with x-ray and cyclophosphamide. Marker chromosomes were found that were large metacentrics from the C group. The karyotypic stability of our two sublines (HUS-2, HUS-2B) was established although there was a morphologic alteration at passage 12. The fact that a cell can apparently change morphologically without a concomitant change in the karyotype is of interest. This does not however, rule out the possibility of a genetic change not reflected as a chromosomal alteration.

We originally suspected that we were observing a shift in cell population, i.e.,

elimination of "normal fibroblasts" but the constancy of the modal number throughout the morphologic change seemed to disprove this. The question of whether these cells underwent "transformation" at the time of morphologic change is an interesting one. Selected aspects of this problem were discussed by Freeman and Huebner (6). They described the effects of viruses on cellular transformation but did not discuss in any detail the problems of "spontaneous transformation." It is our opinion that no "transformation" took place within our HUS-2 culture and that the morphologic change which occurred was an adaptative cellular response to *in vitro* conditions.

*Summary.* A rhabdomyosarcoma cell culture (HUS-2) was established from an 80 year old female. The cells underwent a morphologic change (at the 12–14 cell transfer) but the karyotypic pattern remained stable throughout. It was our impression that this morphologic change was not an example of transformation but simply an adaptative process of these cells to the culture conditions. The modal number was 58 with a telocentric marker chromosome before and after the morphologic alteration. Treatment with 5-Iododeoxyuridine and 20-methylcholantrene and DEAE-D caused no karyotypic change. Electron microscopy showed masses of fibrillar material in the multinucleated giant cells and no virus particles were seen in any culture examined.

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