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Vulnerability of the Subterminal Chromosomes of Cultured Rat Mammary Cancer Cells to Alterations.* (29721)

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In addition to a pair of sex chromosomes the rat has 20 pairs of autosomes. Unlike the mouse, which has all terminal chromosomes, the rat has 5 *subterminal*, 9 *terminal* (including the sex chromosomes), and 7 *median* pairs of chromosomes. This morphologic division facilitates cytologic study.

Cells from mammary cancers induced in young female Holtzman rats by polynuclear hydrocarbons have been grown in tissue culture. Permanent cell lines from 2 cancers were obtained and chromosome studies of the cultured cells performed. Although many of the cells maintained a diploid number, deviations from a normal karyotype were found frequently. Most of the alterations which occurred were in the group of subterminal chromosomes.

Methods. One of the cell lines (designated #21) originated from a cancer induced by the gastric instillation of 3-Methylcholanthrene(1) and has been in culture since August 1961. The other line (#31) was derived from a cancer induced by feeding 7,12 Dimethylbenz(a)anthracene(2) and this line has been in culture since February 1962. For primary culture, cells were dispersed from the cancers with 0.2% trypsin (Difco 1/250)-0.08 M versene solution. The culture medium was 20% serum (calf serum for the #21 line, horse serum for the #31 line) and 80% Eagle's minimum essential medium containing a doubled concentration of amino acids and vitamins in addition to streptomycin (50 μg/ml) and penicillin (30 units/ml). No other anti-microbial agents were used. The method of Chanock *et al*(3) was used to culture for PPLO (pleuropneumonia-like organisms) in the medium of both cell lines and to date these microorganisms have not been detected.

At the time of chromosome studies, cells of the #21 line had been cultured for 10-15 months with 15-36 transfers and cells of the #31 line had been in culture 7-10 months with 10-23 transfers. The cells were grown on the 30 cm^2 flat bottom Bellco tissue culture tubes containing 3 ml of culture medium. Cells were transferred by detaching and dispersing with 0.2% trypsin, centrifuging, resuspending in fresh culture medium, and inoculating new flasks with 1-2 $\times$ 10^5 cells for each ml of medium in the flask. Later, when the cells were established, the inoculum was reduced to 1-2 $\times$ 10^4 cells per ml medium. For chromosome analyses cells were inoculated into plastic Petri dishes (35 mm diameter) containing a 22 mm square cover slip on the bottom. Near the end of the logarithmic growth phase, chromosome spreads were prepared by the hypotonic procedure of Tjio, Puck and Robinson(4). To arrest mitosis, colchicine in an amount to provide 0.5 μg/ml (1.3 $\times$ 10^{-6} M) was added 4 hours prior to the hypotonic treatment. Cells were stained by the aceto-orcein technique of LaCour(5) with the exception that 2% synthetic orcein in 70% propionic acid was used. The slides were carefully examined and chromosome

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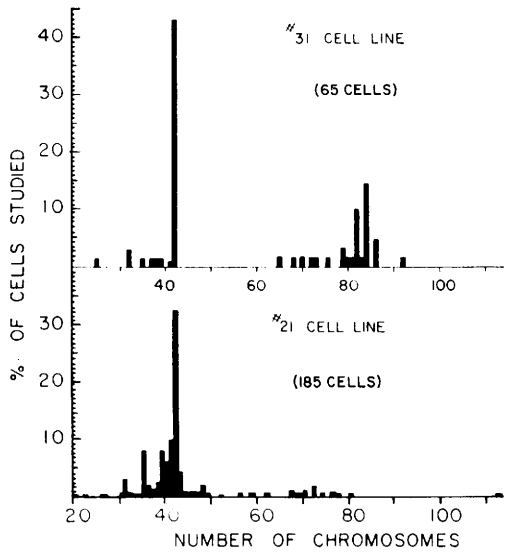

FIG. 1.

counts made. Karyotypes were prepared from microphotographs of suitable spreads from 18 cells enlarged 2100 ×. Chromosome lengths were measured from the photographs with calipers. The idiogram of the Holtzman rat was obtained from karyotypes of 15 bone marrow cells(6) from 5 males (unpublished study, E. D. Rees and J. C. Christian).

Results. Though 38-48% of the cells in the #21 and #31 lines had the normal number of 42 chromosomes (Fig. 1), the #31 line had a substantial portion of tetraploid and hypotetraploid cells and the #21 line tended toward hypodiploidy. Karyotype analyses of 7 cells from the #31 line and 11 cells from the #21 line indicated that the chromosome patterns in all but 2 of the 18 cells were altered. Similar types of deviation from the normal karyotype occurred in both cell lines. Table I summarizes the gross type of alterations. The data for this Table were obtained by comparing the lengths (expressed in terms of % of total chromosome length) of chromosomes from cultured cells with the mean values of the chromosomes of the normal Holtzman rat. Karyotypes of the Holtzman rats corresponded closely to those obtained by Hungerford and Nowell(7) for the Wistar rat, and the X-chromosomes appeared to be the second largest terminal chromosomes. If the length of a given chromosome of a cultured cancer cell was within ± two standard deviations of a normal mean, then chromosomes of the same morphology were considered to correspond.‡ If no chromosome comparable in morphology and size to one in the normal karyotype was present, then that chromosome was considered to be absent. If the morphology was similar but the measurements were not comparable or there was an excess number of chromosomes of a particular size, then the chromosome was considered to be added.

TABLE I. Alterations in Chromosomes from Cultured Mammary Cancer Cells Classified According to the Morphologic Group in Which They Occurred.

Subterminal (180)		Terminal (324)		Median (252)	
Absent	Added	Absent	Added	Absent	Added
46	11	16	22	2	22

Number in parentheses indicates total number of potential chromosomes in each group for 18 normal cells. The added chromosomes either did not correspond in size to a chromosome in the normal karyotype or were supernumerary.

The most striking observation was the absence of many *subterminal* chromosomes. Although the *subterminal* group contains only 10 of the 42 chromosomes and comprises only 27% of the total chromosome length, 46 *subterminal* chromosomes (26% of 180) were missing in the 18 cells studied. The *terminal* group, comprising 18 out of the 42 chromosomes and 48% of the total length, had 16 missing chromosomes (or 5% of 324). In the *median* group, which normally has 14 of the 42 chromosomes and constitutes 25% of the total chromosome length, only 2 chromosomes (1%) were missing. Using missing chromosomes as an index of alterations, a chi square evaluation of the data between the subterminal and terminal groups provides $P<0.001$. In 38 of the 46 instances of absent subterminal chromosomes, members of the 3 shorter of

‡ Material supplementary to this article has been deposited as Document No. 8136 with the ADI Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington 25, D. C. A copy may be secured by citing the Document number and by remitting $1.25 for photoprints, or $1.25 for 35 mm microfilm. Advance payment is required. Make checks or money orders payable to Chief, Photoduplication Service, Library of Congress.

the 5 pairs were involved. In 13 of the 18 cells an abnormally large ("marker") and/or small chromosome was present. Some of the large marker chromosomes were of subterminal morphology.

Discussion. The data above indicate that the subterminal chromosomes are more vulnerable to alterations than are the median or terminal chromosomes. The reason(s) for this differential susceptibility is now known, but it may reflect some property of the subterminal structure or it may be that similar alterations in other chromosomes are less compatible with cell survival. Possibly the difference can be explained by the fact that a subterminal chromosome can become terminal by loss of the upper or lower arms or median by loss of a portion of the lower arms.

Although the distribution of chromosome number of cells in the various sublines corresponded to that of the respective cell line, no distinction between the 2 cell lines could be based on the karyotype changes observed. Morphologic differences in cells and in colonies of the 2 cell lines and sublines have been observed, however. Whether or not the chromosome distribution differences are due to differences in the carcinogen used is a matter for further studies. Studies of cells directly from the cancers are now in progress.

Summary. Permanent lines of cells cultured from a 3-methylcholanthrene and a 7,12-dimethylbenzanthracene induced rat mammary cancer have been obtained. Although the most common chromosome number of cells in both lines was the diploid number, the 3-MC line had many hypodiploid cells and the DMBA line had many tetraploid and hypotetraploid cells. Deviations from the normal rat karyotype were the rule even in diploid cells. The most common alteration was absence of subterminal chromosomes.

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Determination of Serum Amylase by Measurement of Maltose and Other Products from Starch Substrate. (29722)

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Action of serum, urine, pancreatic or salivary α-amylase on starch produces scission of starch molecules into progressively smaller sugar units. This process occurs through a sequence of steps, producing reducing dextrins which then are split into smaller molecules of oligosaccharides with reducing properties, among them being maltotetraose, maltotriose, maltose and possibly maltohexose (1-5). Henry and Chiamori published the results of a study of the saccharogenic method for determination of serum and urine amylase(1) and proposed a modification of Somogyi's method(6) in which conditions of incubation of starch with urine or serum were improved. After 30 minutes' incubation, the reducing capacity is determined using the Folin-Wu(7,8) or Nelson-Somogyi(9) method. Amylase activity in this method is the amount of reducing substances as mg glucose produced in 30 minutes at 40°C per 100 ml serum, which is equal to the number of amylase units. The enzyme activity, therefore, is measured as the difference in reducing substances present before and after incubation with substrate. It would be preferable if one could measure only one or more substances formed in the reaction and not pres-