Genomic Modifications in Cell Line Cultures Chronically Infected With a Myxovirus.* (31587)

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It has been stated(1) that a KB cell line, which has been chronically infected since 1959 with the myxovirus parainfluenzae type 3, had undergone genomic modifications. The principal characteristics of these are a diminution in the modal number of chromosomes, the disappearance of a marker chromosome and the presence of endoreduplication.

The results of a comparative study of 3 KB sublines which have been chronically infected for different lengths of time with parainfluenzae type 3 virus are reported. It was found that there exists a close relationship between the chromosomal alterations and the chronology of the infection.

Material and methods. Both the KB line and the infected KB-EA I subline were obtained from the Institut Pasteur of Paris. Another KB line from the Institut de Microbiologie et d'Hygiene, University of Montreal, was used as a control for the non-infected KB cells.

The KB-EA I, II and III cells were infected with parainfluenzae type 3 virus, strain EA-102 in March 1959, September 1963 and February 1964, respectively. Details relating to these sublines have been described(2).

When the chromosomal study was undertaken, the KB-EA I cells had been subcultured serially 230 times, those of the KB-EA II and III sublines 40 and 30 times, respectively.

Hayflick's medium(3) was used for detection of mycoplasmas in the cells and the supernatant fluid.

Chromosomal analysis was performed on cells cultivated 4 days in Eagle-base medium (4) supplemented with 10% calf serum. Monolayer cultures were incubated 18 hours in the medium containing 0.02 μg/ml of desacetyl-methyl-colchicine (colcemid, Ciba) and harvested by trypsinization. Centrifuged cells were resuspended in diluted (1:8) calf serum, kept 30 minutes at room temperature and, following centrifugation, they were fixed for 20-30 minutes in fresh acetic alcohol (1:3) and 1-2 minutes in 45% acetic acid. Then the cells were spread according to Rothfels and Siminovitch's method(5), and stained with ammoniacal Giemsa. Prints of metaphases photographed with a Zeiss photomicroscope on Agfa Isopan 35 mm film were used for this study. Ten karyotypes were prepared of each of the 3 infected sublines and the 2 KB lines, using the grouping system of Patau(6).

Results. Previous observation(1) indicates that KB cells show a rather limited chromosomal spectrum with modal number of 79 chromosomes (Fig. 1). All cells have 2 atypical chromosomes; the M_1, an acrocentric chromosome with large satellites and corresponding in size to the elements of group D; and the M_2, a large sub-metacentric chromosome as large as those of group A but with the structural characteristics of group B (Fig. 2).

The study of the chromosomal distribution in 78 cells of the KB-EA I subline shows a curve with a maximal amplitude in a very narrow zone corresponding to mitoses with an average of not more than 71 chromosomes (Fig. 1). The karyotypic analysis reveals the disappearance of the M_1 chromosome, the presence of an additional element in group D and a perceptible drop in the number of chromosomes mainly of group C (Fig. 3). In about 4% of the cells, moreover, chromosomes have the paired arrangement (diplochromosomes) which characterize Levan's endoreduplication(7).

The sublines KB-EA II and III are modified but to a much lesser degree than the KB-EA I cells. The modal number of the subline II is 76 and of subline III, 78 (Fig. 1). The marker chromosomes, characteristic of the KB line, could be found in both (Fig. 4). On the other hand, no endoreduplication was ob-

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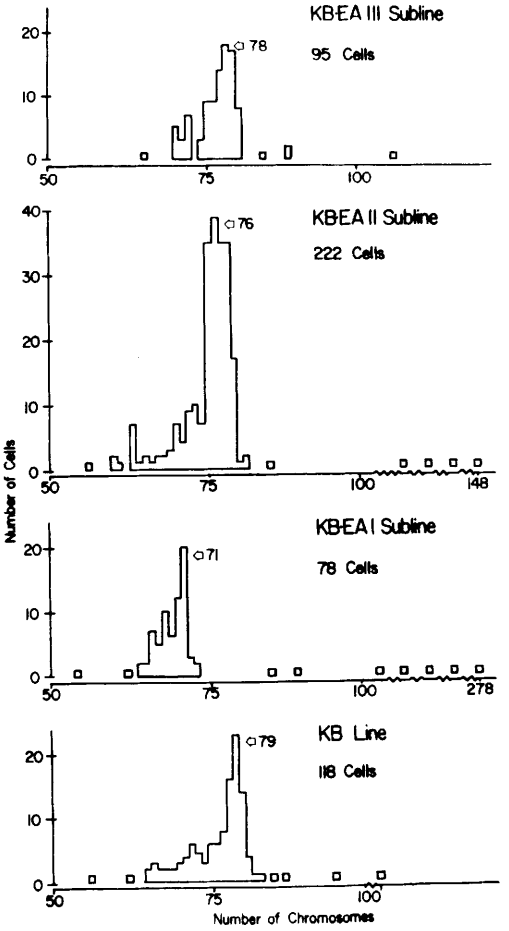

FIG. 1. Chromosome distribution in non-infected KB cell line and the three infected KB-EA sublines. There is a shift to hypoploidy that increases with the age of infection, KB-EA I being the oldest infected subline.

served. Thus, these sublines are not very different from their parent line except for the lesser number of their chromosomes. The degree of variation in these two infected lines increases with the age of the infection. The analysis of variance between the chromosomal distribution of line KB and its infected subline KB-EA III shows that the F value is statistically significant between 1 and 5% level.

Discussion. Following the observation of Hampar and Ellison(8) of chromatid breaks, exchanges and translocations induced by herpes simplex virus in Chinese hamster cell line and the communication of Nichols and coworkers(9) of chromosome breakage in short-term cultures of leukocytes from measles pa-

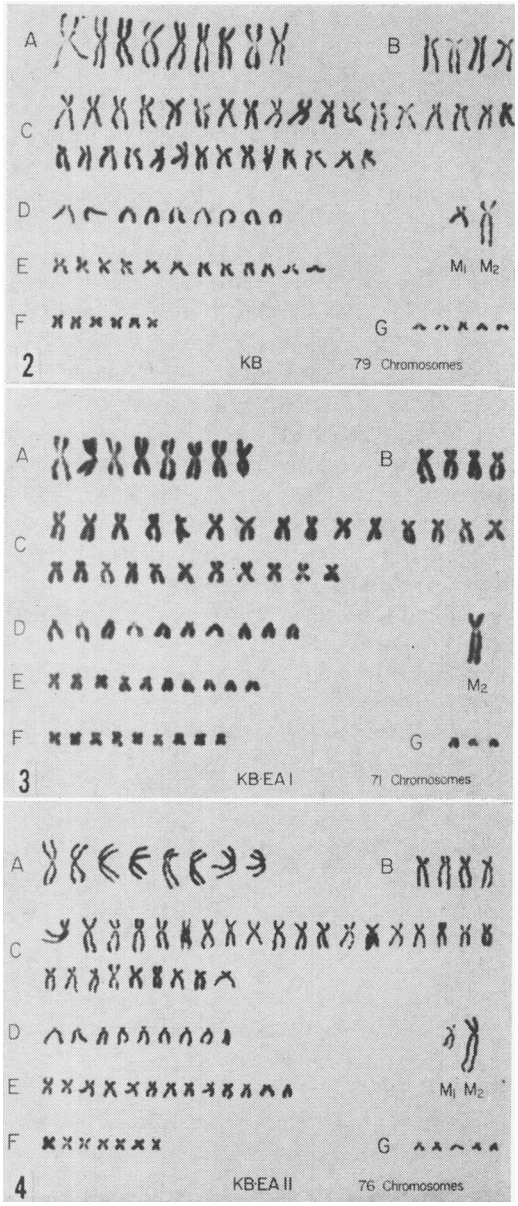

FIG. 2. Karyotype from a non-infected KB cell containing 79 chromosomes. Marker chromosomes M_1 and M_2 were found in all the cells of the Paris and Montreal cultures.

FIG. 3. Karyotype from an infected KB-EA I cell with 71 chromosomes. The marker M_1 is absent and there is a perceptible drop in the chromosomes of group C and, to a lesser extent, in groups E and G.

FIG. 4. Karyotype from an infected KB-EA II cell with a modal number of 76 chromosomes. Note similarity of chromosome distribution with the karyotype of KB cell in Fig. 1. Since the 78-chromosome KB-EA III subline is not slightly different from the parent line, no karyotype is shown.

tients, numerous instances of viral-induced chromosome alterations of tissue cells have been described. However, there exist only isolated reports on karyological disturbances associated with viral infection of chronic duration. Boué(10) has found a high incidence of chromosome breaks in human embryonic diploid cells after 1- and 3-month-old infections with rubella virus. Yerganian(11) has observed karyotypic variations, namely, monosomy, and chromosomal breakage during the 6th to 17th subcultures in 3 lines of SV_{40}-transformed human fetal renal cells. Pirtle (12) has noted deletion of telocentric chromosomes and concomitant appearance of unmatched submetacentric chromosomes in a pig kidney cell line persistently infected for 84 passages with virulent hog cholera virus. Our analyses were performed on cells infected over a period of one to 6 years. No structural modifications characteristic of acute infections were found and the changes were mainly numerical. Although loss of chromosomes has been reported in connection with the SV_{40} by Shein and Enders(13) and Koprowski(14), these karyological modifications affect a lesser number of chromosomes than those described in the KB-EA lines.

Another interesting phenomenon was that the degree of hypoploidy became greater with the age of the infection. Similar findings have been reported by Fogh(15) in the course of infection by mycoplasmas of the human amniotic FL cell line. Since no PPLO were detected in our material, the modifications observed seem to be related to the chronic viral infection.

Radiomimetic effects, lethal in all probability, have been reported in most of the reports concerning the action of viruses. This type of aberration has not been observed in our material. While sublines KB-EA have not been examined during an acute infection, it seems unlikely that the disappearance of chromosomes could be caused by breaks. Structural anomalies of this type were not seen in any material we examined in spite of the progressive numerical alterations. Pirtle(12) who studied the effect of hog cholera virus, a RNA-type virus, on a pig kidney cell line did not find chromosomal breaks either, although karyotype variations were present.

Allison and Paton(16) suggest a different mode of action for RNA type viruses. These authors assume that lysosomal enzymes, in particular the deoxyribonucleases liberated during viral infection, cause chromosomal breaks. Although this hypothesis cannot be discarded *a priori,* the role of a cellular selection has also to be taken into consideration. During the first days following viral infection, a cytopathogenic effect is noticeable in almost all KB cells. The surviving cells may be more resistant on account of their genomic constitution. The chromosomal characteristics of the KB-EA I subline, the first to be infected, differ markedly from those of the KB line; those of sublines KB-EA II and III to a lesser extent.

One might assume that the presence of a hostile agent, the parainfluenzae type 3 virus, would give rise to a genomic selection of the KB cells and that the cells which are most sensitive to cytopathogenic action are exactly the cells farthest from diploidy. The presence of an active biological agent certainly modifies the requirements for immediate survival and only a vigorous genome can have the necessary resistance. This would lead to a progressive selection of the genomes till the appearance of a chromosomal constitution with the characteristics of the most modified cell line, namely, the KB-EA I. It is also possible that the genomic selection could occur at the onset of the viral infection. In such a case the surviving cells would show the karyotypical constitution of the KB-EA III line. The endoreduplication phenomenon and the slowdown of growth in the KB-EA I cells may indicate that a chronic infection may ultimately prove to be lethal.

Summary. Three sublines of human carcinoma KB cell line have been chronically infected with parainfluenzae virus type 3 for a period varying from one to six years. Cytogenetic analysis of the parent cell line and the infected sublines has shown that the modal number, which was 79 for the non-infected KB cells, decreased progressively to 78 in one subline, the most recently infected, to 76 in the second, and to 71 in the third, the longest infected. The variation was noticeable mainly in the C group (Denver's group 6-12) chromosomes. Moreover, one of the two marker chro-

mosomes found in the KB cells was absent in the third subline and some of its cells were endoreduplicated. A hypothesis of genomic selection is discussed.

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On the Synthesis of Taurine from Sulfate by the Chick: I. Influential Dietary Factors.* (31588)

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The sulfate requirements of many animals are met through the degradation of the sulfur amino acids. Some investigations have indicated that young chicks can utilize dietary sulfate in the synthesis of taurine. Lowe and Roberts(1) reported that within 36 minutes after administration of sulfate-S^{35} to a chick embryo, labeled taurine could be detected. Sulfate-S^{35} was not found in cystine, cysteine or methionine in the chick embryo(1), and we have found little or no S^{35} in cystine of the tissues examined in the chick after sulfate dosing(2). Recent reports have shown the universal presence of taurine in practically all tissues and have shown that the functions of taurine in the different tissues were diversified and essential(3,4,5,6,7,8). In view of these reports, the conversion of sulfate to taurine in the animal becomes increasingly significant and of interest.

The over-all purpose of the investigation reported in this manuscript was to study the utilization of sulfate-S^{35} in the chick, specifically the synthesis of taurine from sulfate as it is influenced by the nutritional state of the animal.

Experimental. One-day-old White Leghorn cockerels were maintained on purified basal diets(9) with supplements for a period of time, usually 14 days, as specified in each trial. Groups of 4 or 5 chicks were then orally dosed with 20 microcuries of carrier-free $H_2S^{35}O_4$ by means of a one ml pipette inserted through the mouth into the crop and emptied by gravity flow. The C^{14}-labeled compounds were administered by subcutaneous injection.

Blood, bile and liver samples were taken at various times after dosing. The blood from each group was pooled, allowed to coagulate, then centrifuged. The serum was assayed for total and protein bound S^{35} activity.

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