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# KARYOLOGICAL CHARACTERISTICS OF TWO HUMAN DIPLOID CELL STRAINS ; WI-38 AND MRC-5

by

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INTRODUCTION- According to the definition (1), a cell strain is a population of cells derived from animal tissue, subcultivated more than once in vitro, and lacking property of indefinite serial passage while preserving the chromosomal karyotype, characterizing the tissue of origin. Conversely, a Cell line is a population of cells derived from animal tissue and grown in vitro by serial subcultivations for indefinite periods of time, with a departure from the chromosome number, characterizing its source. Because of the unsuitability of established cell lines with mixoploidy, attempts have been (and still are being) made throughout the world to prepare human as well as non-human primate diploid cell strains, that are seemingly safe and suitable substrates for human virus vaccine productions. Serially-cultured human diploid cell as substrates for the manufacture of virus vaccines was first proposed by Hayflick and Moorhead in 1961 (1), from Wistar Institute, Filadelphia, designated as WI-38. The human diploid cell strain, as developed by these authors was derived from the lung of a female embryo, originaly obtained from Dr. Gard at the Karolinska Institutet, Stockholm, Sweden.

The mother was 32 years old at the time of abortion, and both she and her husband have been known healthy with no history of hereditory disease in either family. The reason why she underwent abortion was her generally weak condition and also her alcoholic husband's being in perison for one and a half years.

Studies performed on WI–38 cells, have shown that these cells have a limited life (4), during their active growth, they retain normal morphology and karyotypic properties and furthermore the cells do not produce tumors in test animals (7).

Another well stablished human diploid cell strain that has proved to be a

very suitable substrate for human virus vaccine production has been developed by Jacobs et al in 1970 (3) at the Medical Research Council Laboratories at Hampstead, London; therefore is designated as MRC–5. The latter cells have been initated from the trypsinized lungs of a 14–week–old male foetus, which has been removed from a 27–year–old woman for psychiatric reasons.

WI–38 cells have been used extensively in this Deptartment for polio vaccine productioon and use has also been made of MRC–5 cell strain to produce large scale measles vaccine made of various virus strains by Mirchamsy et al (4).

The aim of the present communication is to compare the two diploid cell strains WI–38 and MRC–5, based on their chromosome variations in the course of successive passages performed in vitro.

It is also worth mentioning that the cell strains used for virus vaccine productions have to be tested for adventitious agents, haemadsorbing viruses, animal inoculations, and for karyological determinations. Karyology is probably the best documented characteristic of human cell lines. Data collected from control laboratories throughout the world have enabled licensing authorities to establish normal values for cultured human diploid cells of WI–38 line (5).

As far as chromosomal charateristics are concerned, it has been observed that after 40th population doubling level, one is apt to find aneuploidy (5), and chromosome aberration tend to develop mostly between the 45th and 47th levels (6). Based on recent findings, it has been shown that the degree of such abnormalities, are to some extent related to the type of serum used in the culture media (7).

## MATERIALS AND METHODS

a– CHROMOSOME PREPARATION– Cells grown at various passage, and cultured in Basal Medium Eagle (BME) + 5% calf serum, 200U/ml penicilline, and 200 ug/ml streptomycine, were exposed to colchicine (final concentration 10 ug/ml) usually 48 hours after the latest subcultivation. After an exposure time of 2–4 hours at 37° c, the cells were trypsinised and centrifuged at 600 r.p.m. The cells were then suspended in 0.075M KCl solution, and kept in a water bath for 10–15 minutes. The cells were centrifuged and fixed in methanol-acetic acid (3/1), and the fixative was renewed two to three times before slide preparation. One or two drops of the cell suspension were then droped onto clean slides for spreading. (9). Giemsa stained preparations were mounted and preserved for subsequent inspections.

b- KARYOLOGY- This is done, in order to detect gross aberrations in chromosome number or morphology which may indicate (8):

I- Abnormalities intrinsic in the original foetal material.

II- Possibilities of mislabelling and for contamination with other established cell lines.

III- The presence of micro-organisms, such as viruses, mycoplasmas, etc.

IV- The inadvertant exposure of the cell strain to chemical or physical agents which might similarly induce persisting chromosomal changes. Sampling and karyological determinations have been done as follows:

EXACT COUNTS- An exact count of the chromosomes in each of at least 100 metaphase cells were obtained, using low power magnification. All aberrations observed, in view of the total counts, and for the presence of any other aberrations were inspected under "oil immersion" optics. Preparations in which less than 80% of the exact counts fell into the 2n class were rejected, which itself is apparently artifacts of overspreading of the cells in question.

At least a total of 300 divisions have been screened, to consider the number of polyploid cells including tetraploidy and endoredouplication.

**Photographic reconstruction**

One of the metaphase divisions with no type of chromosome aberrations was sellected for each sample of each passage level for the purpose of photographic reconstruction. Individual chromosomes were then paired arbitrarily, and arranged sizewise, and according to general criteria set out by the Denver (10), and London (11) conferences (see Figs. 1 and 2).

In addition to each counts, the diploid cells herewith studied were checked for chromosome abnormalities as stated bellow:

**1- Chromosome Gaps and Breaks:**

What comes under breaks in this study, refers to both open breaks (true break), and achromatic gaps (achromatic lesions), with no disalignment of any fragment, involving either one or both chromatids.

**2- Structural chromosome abnormalities:**

The following classification was followed to divide these abnormalities into two distinct groups:

a) Unstable structural abnormalities, including all aberrations other

than breaks, which may lead to genetic inequalities in subsequent daughter cells (dicentircs, rings, exchange configuration, quadriradials, etc.) whether of the chromatid or chromosome type.

b- Stable structural abnormalities, including so called " marker " chromosomes, stable changes in chromosomal form which represent the result of breakage in a previous division (detetions, inversions, reciprocal translocations).

c- Polyploidy, including tetraploidy and endoreduplication in a population of 300 metaphase spreads.

## RESULTS

As can be noticed from the inspection of tables No. 1 and 2, the findings in view of chromosome abnormalities in either WI–38, or MRC–5 cells agree very well with the latest standards of karyology for human diploid cells (12). Both cells have proved to have similar karyological characteristics. This is in accordance with the studies already made by other investigators who have compared these two cell strains with respect to their chromosome stabilities (13). It has to be stated that a firm conclusion can not be drown from the results of analysis made on WI–38 cell (table 2), because of the fact that these cells have been studied only at 29–30th passage levels. With respect to specific parameters general conclusions can be drown as follows:

1– The frequency of gaps and breaks are not significantly different in the cell strains studied.

2– The mean value for polyploidy and endoreduplication is slightly higher in WI–38 than it is for MRC–5 cells.

3– In the case of MRC–5 cells, no increase in the incidence-of abnormal chromosomes or polyploidy has been observed as the age of this cell strain is increased from 28th to 31st passage levels, a fenomenon which is not in accordance with the existing data (13).

## SUMMARY

Two well stablished human diploid cell strains known as WI–38 (from the Wistar Institute in Philadelphia) and MRC–5 (from Medical Research Council, Hampstead) used as substrates for human virus vaccine production have been compared with respect to their karyological characteristics at various passage levels. It has been shown that both strains have chromosomal characteristics within limits suggested for the diploidy of cell populations of human origin.

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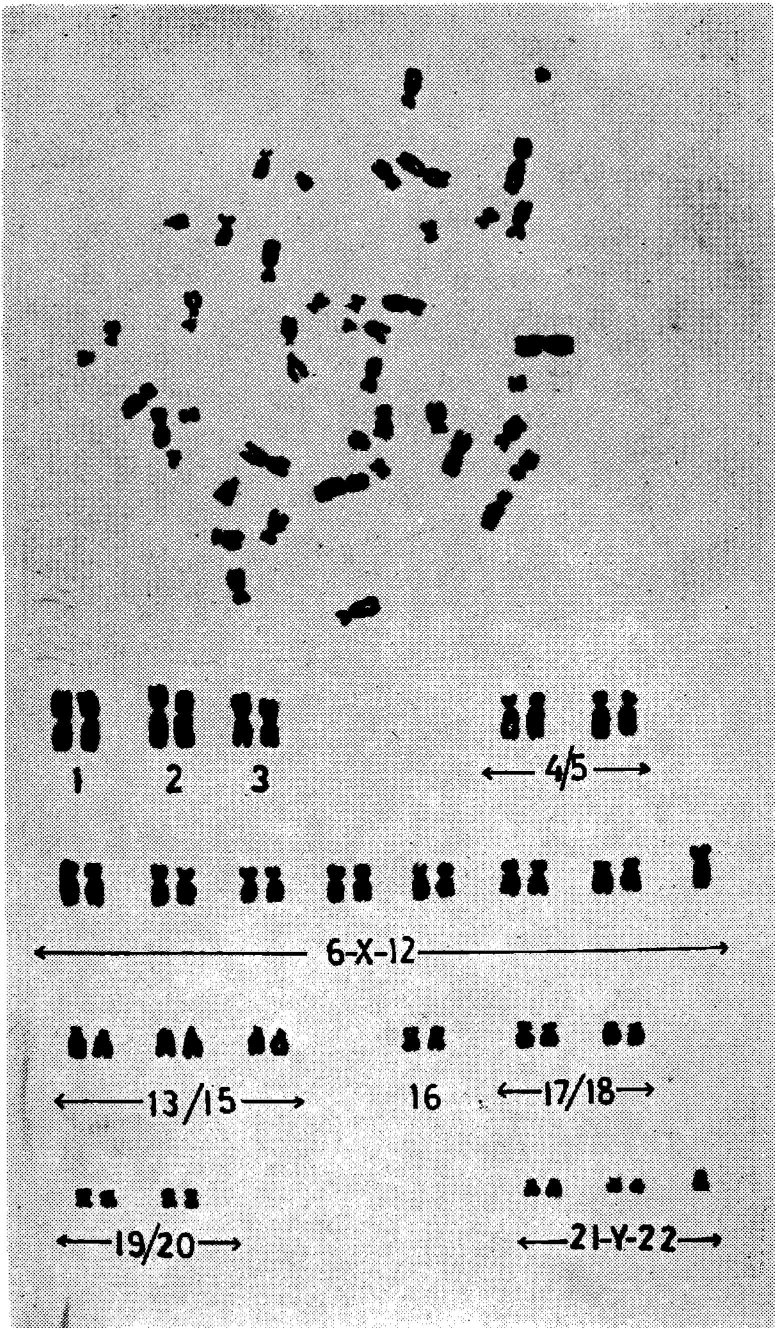


Fig. 1 – Karyotype of Human Diploid cells MRC–5 Lot 54 – 2, Passage 30

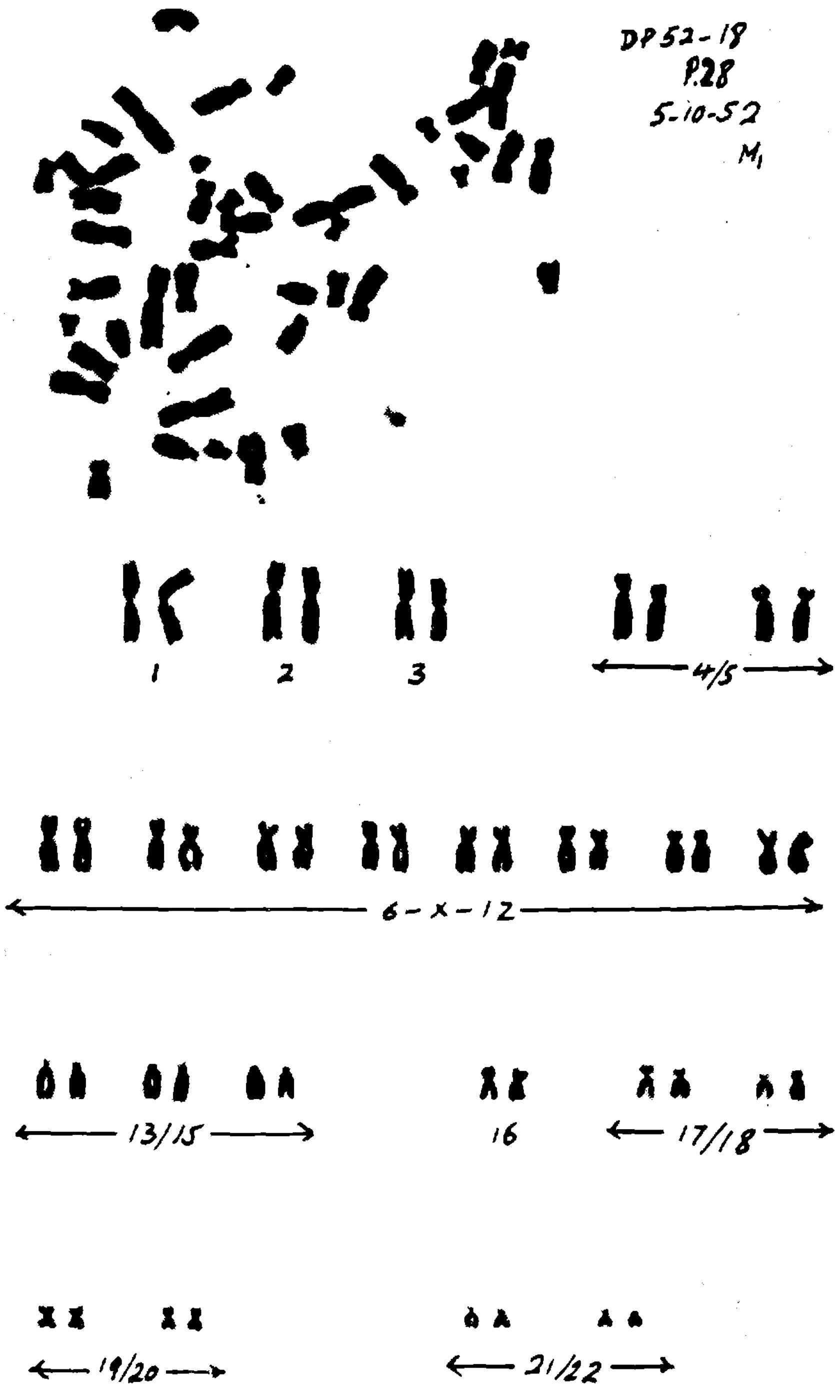


FIG. 2– Karyotype of Human Diploid Cells WI–38 Lot 52–18, Passage 28.

**Table 1: WI-38 Cells Passaged at Levels 30-31**

| Passage Level | P.30 | | | | | | | | P.31 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Lot No. | 54–1 | 51–3 | 52–2 | 52–3 | 52–6 | 52–12 | 52–13 | 52–15 | 51–2 | 52–7 | 52–11 |
| Total | 106 | 107 | 138 | 120 | 117 | 110 | 110 | 106 | 107 | 118 | 130 |
| Normal(Diploid No.) | 97 | 101 | 116 | 99 | 103 | 91 | 99 | 80 | 100 | 99 | 116 |
| Subdiploidy % | 6 | 4 | 10 | 9 | 14 | 6 | 8 | 19 | 3 | 12 | 9 |
| Hyperdiploidy % | — | — | — | — | — | — | — | — | — | — | — |
| Break % | 2 | 3 | 5 | 5 | 8 | 1 | 3 | 3 | 4 | 2 | — |
| Polyploidy % | 3 | 2 | 12 | 12 | — | 13 | 3 | 7 | 4 | 7 | 5 |
| Deletion % | — | 1 | — | — | — | — | — | — | — | — | — |

**Table 2: MRC-S Cells Passaged At Levels 28-31**

| Passage level | P.28 | | P.28 | | P.29 | | P.29 | | P.30 | | P.30 | | P.31 | | P.31 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lot No. | 54–4 | | 54–5 | | 54–4 | | 54–5 | | 54–4 | | 54–5 | | 54–4 | | 54–5 | |
| Duplicates | A | B | A | B | A | B | A | B | A | B | A | B | A | B | A | B |
| Total | 102 | 100 | 106 | 108 | 100 | 105 | 101 | .00 | 101 | 104 | 103 | 106 | 100 | 105 | 100 | 105 |
| Normal (diploid No.) | 91 | 92 | 96 | 98 | 97 | 100 | 97 | 95 | 96 | 99 | 79 | 94 | 88 | 93 | 96 | 94 |
| Subdiploidy % | 4 | 5 | 5 | 7 | 2 | 3 | 3 | 2 | 3 | 2 | 19 | 7 | 9 | 8 | 3 | 6 |
| Hyperdiploidy % | — | — | — | — | — | — | — | — | — | — | 1 | — | — | — | — | — |
| Breaks and Gaps % | 1 | 4 | 5 | 3 | 4 | 4 | 4 | 5 | 8 | 3 | 6 | 3 | 2 | 6 | 3 | 2 |
| Polyploidy % | 7 | 3 | 5 | 3 | 1 | 2 | 1 | 3 | 2 | 3 | 4 | 5 | 3 | 4 | 1 | 5 |
| Minutes % | — | — | 1 | — | — | — | — | — | 1 | — | — | — | — | — | — | — |
| Dicentrics % | — | — | — | — | — | — | 2 | — | — | — | — | — | — | — | — | — |

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