Table 3. ATTEMPTED REVERSAL OF DEPRESSED PFC RESPONSE IN IRRADIATED MICE BY INJECTION OF NORMAL THYMUS OR BONE YARROW CELLS

Group No.	Donor cell inoculum	No. of mice	PFC/ spleen (mean ±s.e.)	Significance levels 1†	2‡
1	10' control spleen cells + SRRC	9	3,187 ± 411	—	P < 0.001
2	10' spleen cells from DMBA-treated mice + SRBC	12	681 ± 147	P < 0.001	—
3	10' spleen cells from DMBA-treated mice + 5 x 10' normal thymus cells + SRBC	7	967 ± 334	P < 0.001	N.S.*
4	10' spleen cells from DMBA-treated mice + 10' normal bone marrow cells + SRBC	5	3,663 ± 885	N.S.*	P < 0.001
5	As in group 4 but with only 2.5 x 10' bone marrow cells	5	3,768 ± 717	N.S.*	P < 0.001
6	5 x 10' normal bone marrow cells + SRBC (no spleen cells)	3	119 ± 44	P < 0.001	—

* Not significant: P > 0.05.
† With respect to group 1.
‡ With respect to group 2.

depleting only a rapidly dividing marrow-derived lympho-cyte population.

We thank Mr G. S. Innes for help with irradiation of the mice, and the Board of Governors of St Bartholomew's Hospital and the British Empire Cancer Campaign for Research for financial support.

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Characteristics of a Human Diploid Cell Designated MRC-5

THE stability and integrity of the human foetal cell strain WI-38, which has been well demonstrated in the past ten years[1-3], and its susceptibility to viruses infective for man[1,4], explain the value of such material for the isolation of viruses and in the development of vaccines[6-8]. We have developed another strain of cells, also derived from foetal lung tissue, taken from a 14-week male foetus removed for psychiatric reasons from a 27 year old woman with a genetically normal family history and no sign of neoplastic disease both at abortion and for at least three years afterwards. The criteria used for characterizing the cells are those recommended internationally[8,9].

The serial propagation of cells was effected essentially according to the method of Hayflick and Moorhead[1]. When the cells reached about the seventh cell doubling, all except a few cultures were harvested and frozen to form a cell stock. Those not subjected to freezing continued to be passaged serially to determine their in vitro

life span and other characteristics. Cells used to prepare a stock were harvested and suspended in a preserving medium which comprised seventy-five parts Eagle's basal medium, seventeen parts unheated calf serum and eight parts dimethyl sulphoxide. The concentration of cells in the final suspension was adjusted so that 1 ml. contained about 3×10^6 cells before being distributed in volumes of 1 ml. to glass ampoules of 1·2–1·5 ml. capacity. Immediately before the cells were distributed and cooled, and before the addition of dimethyl sulphoxide to the medium, samples were removed which were tested subsequently for the presence of microbial contaminants, including mycoplasma. No viable agents were isolated. For storage the cells were cooled at about 2° C per min to −80° C or lower, when they were immersed in liquid nitrogen. A total of 481 ampoules of cells was prepared and stored in this way. Neither the growth medium nor the preserving medium contained antibiotics.

Cells that were cultivated serially without being subjected to freezing and storage attained 48 cell doublings before showing the customary signs of decline exhibited by cells of normal human foetal origin[1]. Later studies on cells reconstituted from the frozen state have shown that storage of the cells in these conditions for about three years does not alter their in vitro life span. Comparative figures for cells propagated serially without being subjected to freezing and cells propagated similarly after restoration from a frozen stock are shown in Table 1.

Table 1. COMPARATIVE NUMBER OF POPULATION DOUBLINGS REACHED BY MRC-5 CELLS BEFORE THE ONSET OF THE STAGE OF DECLINE (CELLS RESTORED FROM STOCK FROZEN AT 'PASSAGE' 7)

Storage time (months)	Number of population doublings
0*	48
1	43
9	42
14	45
18	46
23	44
35	45

* Cultured continuously.

Observations during the concurrent propagation of MRC-5 and WI-38 cells reveal differences in their rates of growth and their sensitivities to minimal environmental (mainly medium) factors. MRC-5 cells replicate more rapidly than WI-38 cells—they attain the same degree of confluency in 3 days as is reached by WI-38 cells in 4 days. Confirmation of this difference in the rate of multiplication has been obtained at comparable time intervals after seeding by doing cell counts. Moreover, MRC-5 cells more readily tolerate environmental changes—in glassware or serum, for example—which can adversely influence the growth of WI-38 cells. For karyological investigation, cells taken from cultures 2 days after subculture, and after 12, 19, 23, 27 and 36 "passages", were arrested in metaphase by adding colchicine and examined for exact chromosome counts and for analysis of karyotype (Table 2).

Table 2. KARYOLOGICAL DATA ON YRC-5 CELLS

Population doubling ("passage")	Structural abnormalities	Frequency of: Breaks or gaps	Aneuploidy	Polyploidy	Hyperdiploidy
12	0/100	5/100 (5%)	5/42 (12%)	1/265 (0·4%)	0/100
19	0/100	5/100 (5%)	5/50 (10%)	4/290 (1·4%)	0/100
23	1/100 (1%) (dicentric)	3/100 (3%)	12/85 (14%)	7/232 (3%)	0/100
27	0/100	5/100 (5%)	8/67 (12%)	6/326 (1·8%)	0/100
36	0/100	1/52 (2%)	4/42 (10%)	8/194 (4·1%)	0/100

Since the completion of these initial studies on the karyology of MRC-5 cells almost 3 years ago, more data have accumulated from the results of analyses on cells reconstituted and propagated after removal from the frozen stock. These long-term results are combined with those obtained earlier (Table 2) in Table 3.

Table 3. CUMULATIVE DATA ON THE KARYOLOGY OF MRC-5 CELLS

Population doubling ("passage")	Structural abnormalities	Frequency of:			
		Breaks or gaps	Aneuploidy	Polyploidy	Hyperdiploidy
12–26	3/2,878 (0·1%)	64/2,878 (2%)	235/1,573 (15%)	283/20,829 (1%)	6/1,573 (0·05%)
27–33	0/400	14/400 (3·5%)	20/186 (11%)	67/2,133 (3%)	0/186
34–40	0/390	8/390 (2%)	27/192 (14%)	105/2,670 (4%)	0/192

These results confirm initial observations and indicate that the karyological properties of MRC-5 cells conform to those required of a diploid cell of human origin to be used for producing viral vaccines intended for human use[e]. The population doublings shown in Table 3 are so arranged because the **WI-38** cells at present used for the manufacture of vaccines are infected with virus up to, but not later than, the thirtieth population doubling and the karyology of the control cells is examined between **27** and **33** doublings. Thus the results for this "middle" group are directly related to cells commonly used in the manufacture of vaccines. The results suggest that with increasing age the karyology of the cells varies only in respect of polyploidy, which seems to increase with age.

Fig. **1** illustrates the characteristic fibroblastic morphology of MRC-5 cells and Fig. **2** shows that chromosomes taken from a metaphase plate have normal appearance.

Cells after 15 to **35** population doublings were used to determine susceptibility to viruses, as shown by cytopathic effects. Rubella virus was grown in MRC-5 and **WI-38** cells and titrated in RK_{13} cells[10]. For comparison, concurrent titrations were set up using **WI-38** cells. All assays were conducted in rolled tube-cultures kept at 37° C, except for the rhinoviruses, when the assays were done at 33° C. Serum-free Eagle's basal medium containing 0·1 per cent $NaHCO_3$ was used, except that for the rhinovirus titrations the initial pH was lowered to 6·8 by reducing the amount of $NaHCO_3$ to 0·02 per cent and adding calf serum (final concentration 2 per cent). Stock suspensions of viruses were used at **0.6** log, steps, with five tubes used at each dilution. Assays were carried out in duplicate using polioviruses, several ECHO viruses, arboviruses, adenoviruses, rhinoviruses and herpes viruses, and those of vaccinia, measles, rubella, and Coxsackie A9. The results show that MRC-5 cells are widely susceptible and that their sensitivity to these viruses is remarkably similar to that of **WI-38** cells.

At several stages during serial propagation we tested for the presence of extraneous agents. Cells and supernatant fluids were taken from cultures after 14, 24, 32

Fig. 2. Chromosomes from an MRC-5 cell showing typical diploid appearance (×1,296).

and **42** population doublings and were used to attempt the isolation of microbial agents *in vivo* and *in vitro*. For the *in vivo* studies, about 10^7 cells were inoculated intramuscularly into each of the following groups of animals: five rabbits, five guinea-pigs, ten adult mice, one litter of suckling mice, one litter of newborn hamsters, and ten embryonated eggs (**11** days old). The animals were observed twice weekly for a period of four weeks and the embryonated eggs were examined after four days. Complementary *in vitro* tests were done to detect the presence of viruses. Cells or spent medium were added to primary cell cultures prepared from patas monkey kidney, human embryo kidney, rabbit kidney, HeLa cells and WI-38 cells. These cultures were incubated at 36°–37° C for 21 days and were examined periodically for any change in morphology. Tests to detect haemadsorbing viruses were done by using red cells from humans, guinea-pigs and chickens. Samples were removed from cultures on days 3–5 and on days 15–21. At the end of the period of incubation, cells were stained and examined for the presence of inclusion bodies. Suitable media were used to test for the presence of bacteria, fungi and mycoplasmas, but no extraneous agents were detected.

To ascertain whether the cells had any neoplastic properties, between 10^6 and 10^7 cells removed from cultures during the log phase of growth were implanted into Syrian hamsters aged six weeks, using both cheek pouches of each animal, and into the lateral thoracic subcutaneous layer. More recently, mice treated with rabbit anti-mouse lymphocyte serum were also inoculated subcutaneously with cells. The cells used were taken from cultures after **16** and **31** population doublings for the test done in hamsters and at the twentieth doubling for those done in mice. All animals were examined at weekly intervals for one year. Animals not surviving the full period were examined post mortem, and observations for neoplastic growth were made on these as well as on those which survived the full term. None of the animals showed evidence of cells which effected the growth of an invasive
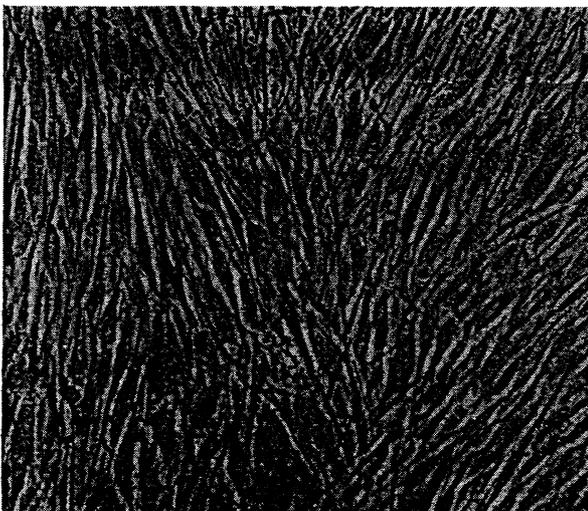

Fig. 1. Normal YRC-5 cells, phase photograph (× 162).