# Characteristics of Attenuated Type 3 Poliovirus Obtained by Alternate Passages in Chick Cell Cultures and Monkey Kidney Cell Cultures

In the previous paper, it was reported that type 3 poliovirus (Saukett strain) could be effectively attenuated by alternate passages in chick embryo cell cultures and monkey kidney cell cultures (Takahashi *et al.*, 1962) and we obtained a highly attenuated strain, designated as the MC strain.

In studying the process of attenuation, it has been found that a gradual change occurs in the rct/40 (the reproductive capacity at the temperature of 40°C) character during these alternate passages (Table 1, Table 2).

Growth of the original Saukett strain and the attenuated MC strain in chick cell cultures were compared. As shown in Fig. 1, no remarkable difference was noted in the growth of the two viruses in these cultures, both strains showing poor susceptibility to chick cell cultures. In studies on thermal inactivation at 37°C,

**Table 1. Passage of Poliovirus (Type 3, Sakett Straiun) in Developing Chick Embryo and Alternate Passages in Monkey Kidney Cell and Chick Embryo Cell Cultures**

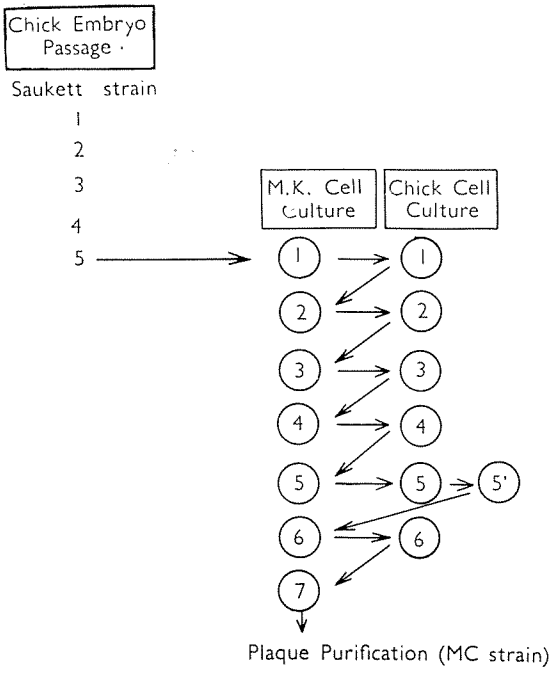

**Table 2. Comparison of Infectivity of Original Saukett and Chick Embryo Cell-Monkey Kidney Cell Passaged Viruses Incubated at 37°C and 40°C**

| Virus | Virus Titer (PFU/0.2 ml) | | Difference in Titer at 37°C and 40°C |
|---|---|---|---|
| | 37°C | 40°C | |
| Original Saukett Strain | $10^{6.8}$ | $10^{6.8}$ | 0 |
| $CE_5$ $MK_1$ * | $10^{6.8}$ | $10^{6.5}$ | $10^{0.3}$ |
| $CE_5$ $MK_3$ $CC_2$ ** | $10^{6.9}$ | $10^{5.3}$ | $10^{1.6}$ |
| $CE_5$ $MK_5$ $CC_4$ *** | $10^{7.0}$ | $10^{4.7}$ | $10^{2.3}$ |
| $CE_5$ $MK_7$ $CC_6$ **** | $10^{7.0}$ | $10^{4.0}$ | $10^{3.0}$ |
| $CE_5$ $MK_7$ $CC_6$ ***** +1 Plaquing (MC Strain) | $10^{6.9}$ | $<10^{1}$ | $>10^{5.9}$ |

* Saukett strain, passaged 5 times in chick embryos and once in monkey kidney.

** Saukett strain, passaged 5 times in chick embryos and alternately twice in MK cells and chick cells and once in MK cells.

*** Saukett strain, passaged 5 times in chick embryos and alternately four times in MK cells and chick cells and once in MK cells.

**** Saukett strain, passaged 5 times in chick embryos and alternately six times in MK cells and chick cells and once in MK cells.

***** Saukett strain, passaged 5 times in chick embryos and alternately six times in MK cells and chick cells and once in MK cells, then once by plaquing.

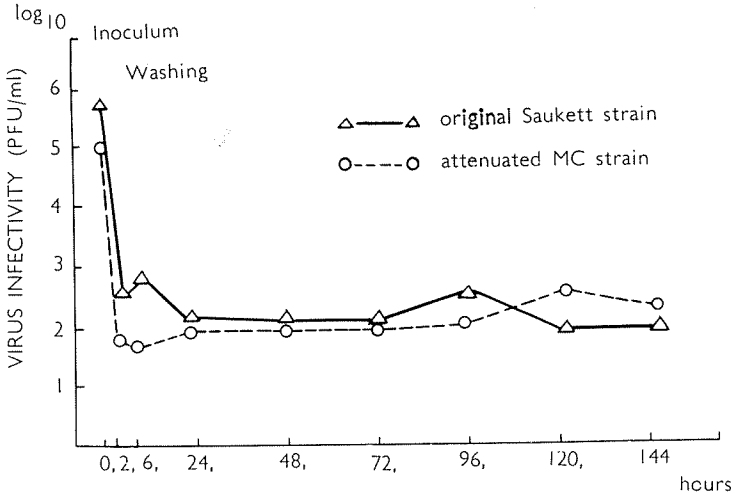


**Fig. 1. Growth of Original Saukett and Attenuated MC Viruses in Chick Cell Cultures**

After a two hour period for virus adsorption, residual virus was removed by repeated washing with Hanks solution. At specified times, bottle was harvested, and after freezing and thawing the contents were centrifuged at 3,000 rpm for 15 min., and the fluid was titrated in MK cells.

**Table 3. Thermal Stability of Original Saukett and Attenuated MC Viruses at 37°C**

| Time (hours) | Infectivity (PFU/ml) | |
|---|---|---|
| | Original Saukett | Attenuated MC |
| 0 | $10^{5.8}$ | $10^{5.3}$ |
| 24 | $10^{6.0}$ | $10^{5.3}$ |
| 48 | $10^{6.0}$ | $10^{4.8}$ |
| 72 | $10^{5.8}$ | $10^{4.3}$ |
| 96 | $10^{5.5}$ | $10^{4.0}$ |
| 120 | $10^{4.5}$ | $10^{3.8}$ |
| 168 | $10^{4.1}$ | $10^{3.5}$ |

no significant difference was obtained either (Table 3). These results suggest that attenuation in this case may not be due to adaptation to chick cell cultures, nor to the advantage of survival at high temperatures in passages in chick cell cultures.

During a few generations passaged in monkey kidney cell cultures, no change in the rct/40 character of this attenuated MC strain was observed. However, on passage in human intestine, most of viruses recovered from the stools was found to have changed from rct/40 (−) to rct/40 (+) and the size of the plaques produced also became as large as before, although a few of viruses recovered remained rct/40 (−). (Table 4).

These results indicate that attenuated MC strain virus like other attenuated strains so far reported is not stable (Melnick and Benyesh-Melnick, 1960; Carp and Koprowski, 1962).

**Table 4. rct/40 Markers of the Human Passage Isolates of Attenuated MC strain**

| Initials of Child | Virus Titer | | Difference in $\log_{10}$ Units | rct/40 Character |
|---|---|---|---|---|
| | 37°C | 40°C | | |
| I. K. | $10^{6.3}$ | $10^{5.4}$ | 0.9 | + |
| N. S. | $10^{6.9}$ | $10^{5.4}$ | 1.5 | + |
| H. S. | $10^{6.8}$ | $10^{6.0}$ | 0.8 | + |
| E. N. | $10^{6.4}$ | $10^{5.8}$ | 0.8 | + |
| H. N. | $10^{6.0}$ | $10^{4.7}$ | 1.3 | + |
| F. K. | $10^{7.2}$ | $<10^{1}$ | >6.2 | − |
| A. S. | $10^{6.5}$ | $<10^{1}$ | >5.5 | − |
| Y. O. | $10^{6.9}$ | $10^{6.0}$ | 0.9 | + |
| U. N. | $10^{6.4}$ | $10^{6.3}$ | 0.1 | + |
| Y. A. | $10^{6.3}$ | $10^{4.3}$ | 2.0 | ± |

## REFERENCES

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