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Quantitative Study on Leucine Incorporation in Multinucleate Cells Induced by Sendai Virus (38362)

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This paper is part of a research program designed to study the biological properties of multinucleate cells. The formation of multinucleate cells *in vitro* can be induced by fusion of cells treated with the Sendai virus (1, 2), or by inhibiting cell cleavage with cytochalasin B (3). To date, heterokaryons (4–6) have been studied more extensively than homokaryons (7). Since multinucleate cells in pathological tissues must be homokaryons, we have chosen homokaryons of human cells for this study. A previous report (8), described RNA metabolism in homokaryons. In the present study leucine uptake by homokaryons was measured quantitatively and the relationship between leucine uptake and cell surface area or the number of nuclei per cell was determined. Results are reported herewith.

Materials and Methods. Cell line and cell fusion. Chang conjunctival cells (9, 10) were cultivated in Eagle minimum essential medium (MEM) supplemented with 10% calf serum. Two to 5 days culture was used for cell fusion, which was induced by ultraviolet-irradiated Sendai virus (HVJ), Z strain, as described in previous reports (2, 11).

Study of leucine uptake. ^{3}H-4, 5-L-leucine (*N*) (sp act 58.1 Ci/m*M*) was purchased from the New England Nuclear Corp. The isotope was diluted to 1 μCi/ml with MEM. The Chang cells grown on coverslips and treated with UV-inactivated HVJ were fed the diluted isotope for one hour and for three hours. The coverslips were washed, fixed, coated with Kodak NTB 2 emulsion, stored at 4° for 10 days, and then developed with Kodak D 19 at 20°. The coverslips were stained with May–Greenwald–Giemsa stain, and the number of grains per cell was counted by the following procedure: The number of grains and nuclei in each randomly selected cell was counted twice under 1250× magnification and the average was used in computation. When grains were confluent, the number of grains was estimated by comparison with the size of an individual grain.

Results. A representative radioautograph is shown in Fig. 1. Grains were evenly distributed throughout the cell including the nucleus; there was little clustering of grains; and the number of nuclei per cell varied considerably. Since cells were randomly selected for grain count, there was an uneven representation of cells with varying degree of nucleation. For example, cells with one to three nuclei were selected more frequently for grain counts than cells with five to ten nuclei.

To analyze the relation between grain counts and nuclear number per cell, the number of grains was plotted against the number of nuclei in each cell. Resulting graphs are shown in Figs. 2 (1 hr exposure to ^{3}H-leucine) and 3 (3 hr exposure to ^{3}H-leucine). Regression equations computed for Figs. 2 and 3 are, respectively,

$$Y = 8.8 + 13.3\,X \qquad [1]$$

and

$$Y = 38.5 + 42.4\,X \qquad [2]$$

(where X and Y are respectively the number of nuclei and grains per cell). In both graphs, the average grain count per level of nucleation is close to the regression line, and the r values were larger than 0.8. We therefore concluded that the number of grains per cell was significantly related to the level of nucleation and the duration

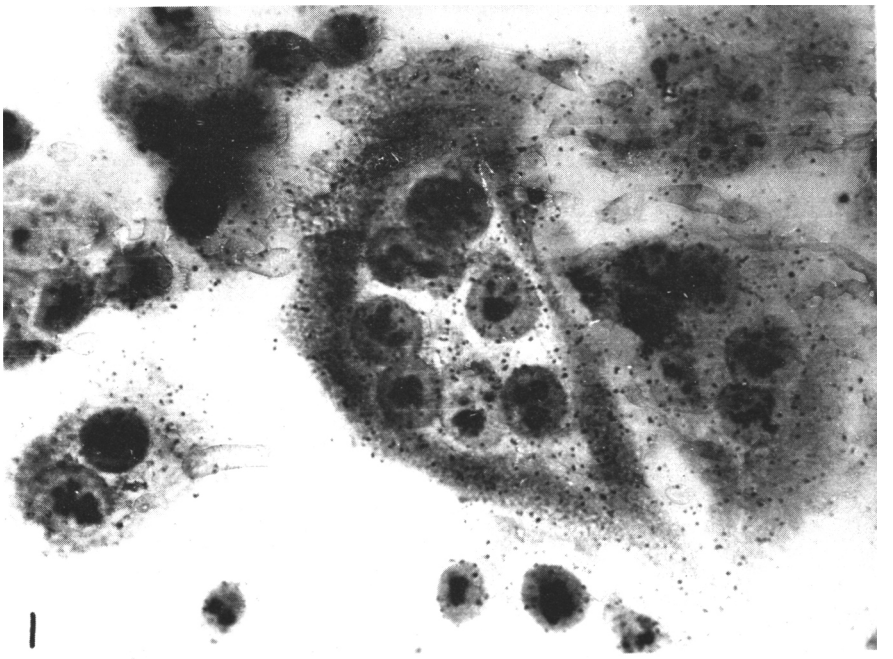

FIG. 1. Radioautograph of mononucleate and multinucleate cells exposed for 3 hr to ^{3}H-leucine. May–Greenwald–Giemsa's stain, ×500.

of exposure to the isotope. It should be noted, however, that grain number in cells exposed to the isotope for 3 hr was larger than three times the grain counts in cells exposed to the isotope for 1 hr.

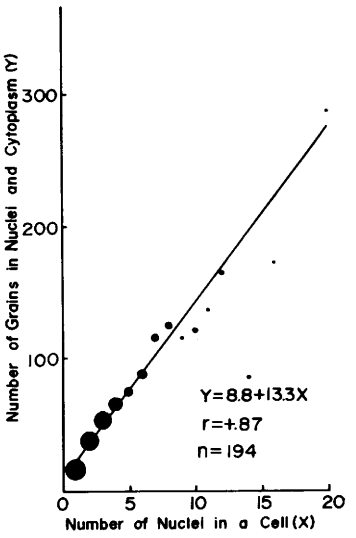

FIG. 2. Regression line of number of grains in mononucleate and multinucleate cells treated 2 days previously with UV-inactivated Sendai virus and exposed to ^{3}H-leucine for 1 hr. The center of spots indicates the mean of grain number in each nucleation. The diameter is shown as square root of the counted cell number and their size is proportional to the counted cell number of each nucleation.

When the experiment (3 hr exposure to the isotope) was repeated using cultures 6 days rather than 2 days after treatment with UV-inactivated HVJ, the following regression equation was obtained:

$$Y = 10.7 + 51.4X \qquad [3]$$

(See Fig. 4). The Y value of eq. [3] was similar to that of [2] for the cells with low level of nucleation. For cells with high level of nucleation, the Y value was larger in [3] than in [2].

Discussion. Under the prescribed experimental condition, the incorporation of leucine into the macromolecules of human multinucleate cells appeared to be directly related to the number of nuclei per cell and the duration of exposure to the isotope. The fact that grain counts per nucleus in cells exposed to the isotope for 3 hr was more than three times the value in cells exposed for 1 hr can best be explained by a lag phase in nutrient uptake.

The finding of a direct relation between leucine incorporation into macromolecules and the number of nuclei per cell is interesting. To appreciate this finding, one must attempt to con-

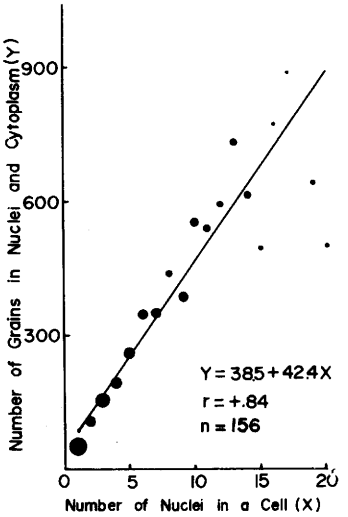

FIG. 3. As Fig. 2 except that the cells were exposed to ^{3}H-leucine for 3 hr.

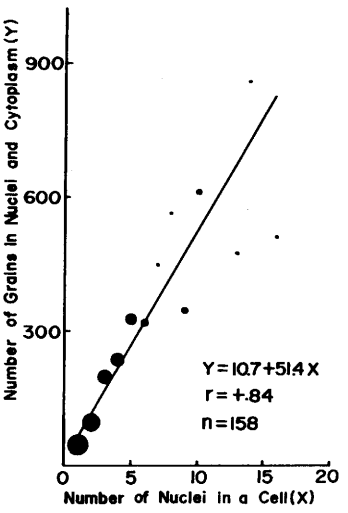

FIG. 4. As Fig. 3 except that the cells were treated with UV-inactivated Sendai virus 6 days previously.

ceptualize the cell fusion process. For example, when eight equal mononucleate spherical cells of unit radius fuse into one spherical octanucleate cell, the initial aggregate cell surface area must be $8 \times 4\ \pi R^2$ or 100.53 and the initial aggregate cell volume must be $8 \times 4/3\ \pi R^3$ or 33.51. If, 2 days after cell fusion, the octanucleate cell continues to maintain its initial surface area and spherical shape, then the cell radius should be 2.83 ($4\ \pi R^2 = 100.53$) and the cell volume should be 94.99 ($\pi 2.83^3 \times 4/3$) rather than 33.51. Since it is reasonable to assume that nutrient uptake is directly related to cell surface area, and since we have found that leucine incorporation was directly related to the number of nuclei per cell, each multinucleate cell appears to have maintained the initial aggregate cell surface. If this is indeed the case, then a multinucleate cell must have greater amount of cytoplasm per nucleus than that of a mononucleate cell; alternately, the surface of a multinucleate cell must be greatly wrinkled in order to maintain a cytoplasm/nucleus similar to that of a mononucleate cell. It is also possible that a multinucleate cell may have more cytoplasm per nucleus and a more wrinkled cell membrane than a mononucleate cell.

Summary. Chang conjunctival cells were treated with UV-inactivated Sendai virus. The resulting mixture of mono- and multi-nucleate cell was exposed to ^{3}H-leucine for 1 and 3 hr. The amount of leucine incorporated, as determined by autoradiographic technic, was nearly proportional to the number of nuclei per cell and to the duration of exposure to ^{3}H-leucine.

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1. Okada, Y., Suzuki, T., and Hosaka, Y., Med. J. Osaka Univ. **7,** 709 (1957).
2. Pan, I. H., J. Formosan Med. Assoc. **67,** 49 (1968).
3. Wright, W. E., and Hayflick, L., Exp. Cell Res. **74,** 187 (1972).
4. Harris, H., Watkins, J. F., Ford, C. E., and Schoefl, G. I., Nature **205,** 640 (1965).
5. Weber, J., and Mak, S., Exp. Cell Res. **74,** 423 (1972).
6. Gledhill, B. L., Sawicki, W., Croce, C. M., and Koprowsky, H., Exp. Cell Res. **73,** 33 (1972).
7. Yamanaka, T., and Okada, Y., Biken J. **9,** 159 (1966).
8. Pan, I. H., and Chen, J. Y., J. Formosan Med. Assoc. **72,** 129 (1973).
9. Chang, R. S., Proc. Soc. Exp. Biol. Med. **87,** 440 (1954).
10. Geyer, R. P., and Chang, R. S., Arch. Biochem Biophys. **73,** 500 (1958).
11. Pan, I. H., Chinese J. Microbiol. **2,** 69 (1969).

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