NOTES

PERSISTENT INFECTION OF FL CELLS BY *RICKETTSIA SENNETSU* (S. *TODAI*)

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Stable cell lines infected persistently in vitro by viruses are known as carrier cultures, and are reported to occur commonly in many kinds of virus-cell systems. However, such a phenomenon has seldom been reported in the field of rickettsiology, although the rickettsiae of epidemic typhus, tsutsugamushi disease, and Rocky Mountain spotted fever have been shown to persist for long periods of time in lymphoid tissues of convalescent persons. This preliminary report describes a rickettsial carrier state established in the *Rickettsia sennetsu* (s. *todai*)-FL cell system in vitro. *R. sennetsu* causes Hyuga fever or Kagami fever in West Japan, clinical features of which are similar to those of infectious mononucleosis (Fukuda, Kitao, and Keida, Med. Biol. Tokyo **32**:200, 1954; Misao and Kobayashi, Tokyo Med. J. **71**:683, 1954).

A monolayer culture of FL cells in a 200-ml bottle was infected by introducing 1 ml of supernatant fluid from a 10% homogenate of spleens and lymph nodes of mice infected with the Nakazaki strain of *R. sennetsu*. This was immediately followed by the addition of medium consisting of 90 parts of Hanks' basal salt solution containing 0.5% lactalbumin hydrolysate and 10 parts of bovine serum. The medium was not changed until the third day after inoculation; thereafter, it was changed once every 3 days with a similar medium containing 2% bovine serum. The cells were subcultured approximately once a month after trypsinization. Detailed records of the subcultures are presented in Table 1, which shows the infectivity titers in mice and the proportion of rickettsia-containing cells, as determined by microscopic examination of Giemsa-stained preparations. The stabilized infectivity measured at the 9th, 10th, and 14th transfers indicates establishment of the carrier system.

TABLE 1. *History of subcultures*

No. of transfer	Days after infection	Rate of infection*	Infectivity for mice†
0	10	24	$>10^{0}$
1	10	82	$10^{5.0}$
2	23	86	$10^{4.5}$
3	49	ND‡	$>10^{0}$
4	73	ND	$>10^{0}$
5	103	22	$10^{3.4}$
6	133	15	$10^{2.5}$
7	166	ND	ND
8	203	7	$10^{1.3}$
9	218	9	$10^{1.8}$
10	232	ND	$10^{1.3}$
11	268	59	ND
12	301	84	ND
13	318	ND	$>10^{0}$
14	346	81	$10^{3.8}$
15	371	64	$>10^{2.0}$

* Percentage of cells containing rickettsiae counted at the time of cell transfers.

† Expressed as LD_{50} per 0.5 ml of infected FL cell culture.

‡ Not determined.

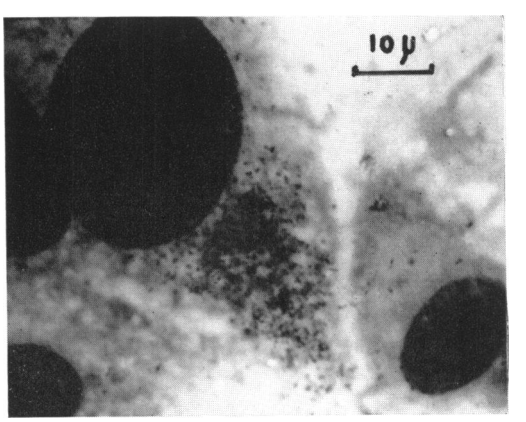

FIG. 1. *Multiplication of Rickettsia sennetsu in FL cell culture at the 12th transfer after infection. Giemsa stain.*

Approximately 2 to 3 weeks of continuous cultivation were necessary before cells became persistently infected with rickettsiae. Although the detachment of cells from the glass surface became prominent during the third week of culture, no cytopathology specific to rickettsial multiplication was observable. Intracytoplasmic localization of the rickettsiae at the 12th transfer of infected FL cells is shown in Fig. 1. Dividing cells in late telophase sometimes showed rickettsiae in both daughter cells; or, only one cell might be involved, the other remaining free from infection. Therefore, this kind of cell division may play a role in the maintenance of the carrier state, together with cell-to-cell infections. Detailed studies of the mechanism of establishment of the carrier state reported here are under way.

In summary, an FL cell strain carrying *R. sennetsu* (s. *todai*) was established by infecting the cells in vitro and subculturing them once a month.

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SITES OF ADENOSINE TRIPHOSPHATASE ACTIVITY IN BACTERIA

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The important role of adenosine triphosphatase in permeability and ion transport was suggested by Whittam (Nature **191**:603, 1961). In higher cells, adenosine triphosphatase activity is predominantly membrane-bound, being located in cell membranes and cristae mitochondriales. Adenosine triphosphatase activity has also been isolated from contractile proteins of dividing cells (*General Cytology*, 3rd ed., W. B. Saunders Co., Philadelphia, 1960). In bacteria, this activity has been demonstrated in particulate fractions of the cytoplasmic membrane, in the cell wall, and in cytoplasmic particles from different species (Hughes, J. Gen. Microbiol. **29**:39, 1962). Georgi et al. (J. Bacteriol. **70**:716, 1955) found adenosine triphosphatase activity in dense granules located in the red layer of fractionated bacteria. Since these granules were membrane-bound, a physical separation of the two structures, granules and adhering membranes, could not easily be achieved, to show whether the enzyme is membrane- or granule-bound, or both (Marr, p. 460. *In* I. C. Gunsalus and R. Y. Stanier [ed.], *The Bacteria*, vol. 1, Academic Press, Inc., 1960). There have been no attempts to apply cytochemical methods, in connection with electron microscopy, for localizing the sites of adenosine triphosphatase activity in bacterial cells.

Three species, *Escherichia coli B*, *Bacillus cereus*, and *Myxococcus xanthus FB* (Voelz and Dworkin, J. Bacteriol. **84**:943, 1962), were chosen for these studies. Cultures of all three species were grown in a liquid medium containing 2% Casitone, 0.1% $MgSO_4$, and 0.01 M K_2HPO_4-KH_2PO_4 (pH 7.2) by shaking at room temperature. Cells were harvested at about the end of their logarithmic phase, washed with phosphate buffer, and fixed in 1% glutaraldehyde buffered with phosphate-saline (Dulbecco and Vogt, J. Exptl. Med. **99**:167, 1954) at pH 7.2 for 10 min at room temperature. Studies on the time kinetics of glutaraldehyde fixation have shown that an exposure of cells of the species investigated to the fixative for up to 30 min does not affect the adenosine triphosphatase activity (Voelz, *unpublished data*). Fixed cells were washed five times in distilled water and suspended in the adenosine triphosphate (ATP) medium of Otero-Vilardebo et al. (J. Cell Biol. **19**:647, 1963). Incubation was for 20 min at room temperature. After incubation, the cells were washed three