are hyphenates with the suffix *zoo* being used to denote the usual source and to emphasize that the variant occurs in a nonhuman subject. The prefix denotes the place of first sampling. Thus Wazoo is found in gorillas at the *Wa*shington *Zoo* and Hyzoo was found in a chimpanzee in the Johns Hopkins University School of *Hy*giene *zoo*logical collection.

5. The observed quantities of hemoglobin Hyzoo and Wazoo present in whole hemolyzates are little more than the 1.6 to 2.0 percent of $A_2$ present in chimpanzees and gorillas, and, moreover, considerably less than the amounts of variant hemoglobin found in almost any of a variety of human hemoglobin heterozygotes (*14*). The source of such scant quantities of Hyzoo and Wazoo seems to lie with impoverished synthesis rather than with premature destruction of abundantly produced hemoglobin. Estimates of the specific activity of purified $\alpha$ and $\beta$ chains from the gorilla Tomoka, after incubation in vitro of bone marrow aspirates (obtained by Dr. S. Charache) with 1 mc of [$^3$H]leucine at 35°C for 100 minutes, indicate that both chains of hemoglobin Wazoo—like those of hemoglobin A from the same animal—are synthesized by marrow approximately in proportion to their final concentration in peripheral blood.
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# Functional Sequences Modulated by Morphological Transitions in Human Lymphoid Cells Grown in vitro

Abstract. *Immunoglobulin-producing cells undergo a series of morphological transitions; each configuration displays specific functional attributes. The life cycle of immunocytes may be visualized as a series of functional compartments expressed by morphological sequences.*

A long-term culture of human lymphoid cells derived from a patient with lymphoma was established in 1966 (*1*). These cells now have been maintained in monolayer cultures for 5 years. Reticuloid fusiform cells and lymphocytoid and plasmacytoid round cells are predominant. Binucleate cells and transitional forms are a frequent finding (Fig. 1). Indirect immunofluorescent studies have demonstrated that these cells synthesize gamma globulin. Cells growing on Leighton cover slips were rinsed in saline and fixed in acetone for 10 minutes. The cells were incubated with unlabeled goat antiserum to human gamma globulin for 30 minutes at room temperature. The cells were then washed twice with a buffer solution (*p*H 7.2) and incubated for 30 minutes with fluorescein tagged rabbit antiserum to goat gamma globulin. The cells, without counterstaining, were examined under an ultraviolet microscope. Positive apple-green fluorescence was easily distinguishable from autofluorescence.

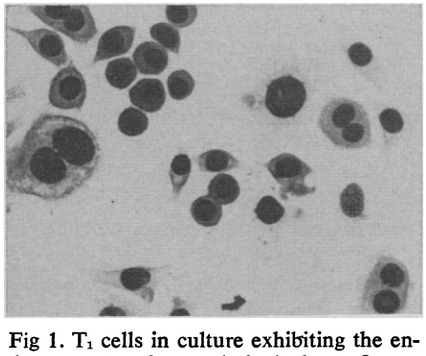

Fig 1. $T_1$ cells in culture exhibiting the entire gamut of morphological configurations (Wright's stain; × 480).

Time-lapse photographic studies were performed with a culture system (*2*). Because of the prolonged doubling time of these cells (52 ± 2 hours), one picture was taken every 30 minutes for 165 hours. Cell pedigrees were generated from enlargements of the negatives for morphologic analysis. Generation times were measured from one cell division to the next daughter-cell division. The median generation time was 36 hours.

Rare cells, still metabolically active as evidenced by mobility and changes in shape, failed to divide during the experiment. Most of the cells undergo changes in shape, from round to fusiform and often back to round. Each of these changes lasts for several hours which allows for morphologic definition. When spindle-shaped cells become round prior to mitosis, they do so very rapidly within a single half-hour interval. Upon division, a fusiform cell can give rise to one elongated and one round daughter cell (Fig. 2) or more commonly, to two fusiform cells. Round cells may also give rise to a morphologically mixed population; sometimes they produce only smaller round cells. Apparently these smaller round cells are terminal because, after a brief period of rapid movement, they become immobile and never divide. Occasionally a cell that remained round for numerous hours will adopt a fusiform shape for a few hours and then start to divide vigorously.

An unusual finding is that two daughter cells may come into close contact and fuse, and a single binucleated cell will emerge (Fig. 3). After several hours this cell may either dissociate into a rapidly mobile round cell and a static spindle-shaped form, or it will divide giving four round daughter cells.

This fusion of cells is different from the mechanisms of emperipolesis (*3*), peripolesis (*4*), and uropodapsis (*5*) because the fusion is long-lived, distinguishable cell boundaries disappear, and the process may sometimes con-

Table 1. All of the cells in the examined fields were arbitrarily assigned to a morphological category according to the prevalent feature and classified as fluorescent or nonfluorescent.

| Cell types | Morphological distribution | | |
|---|---|---|---|
| | Fluorescent cells | Nonfluorescent cells | Fluorescent cells (%) |
| Round | 183 | 45 | 80.5 |
| Intermediate forms | 114 | 93 | 65.5 |
| Fusiform | 32 | 265 | 10.7 |
| Total | 329 | 403 | 44.6 |

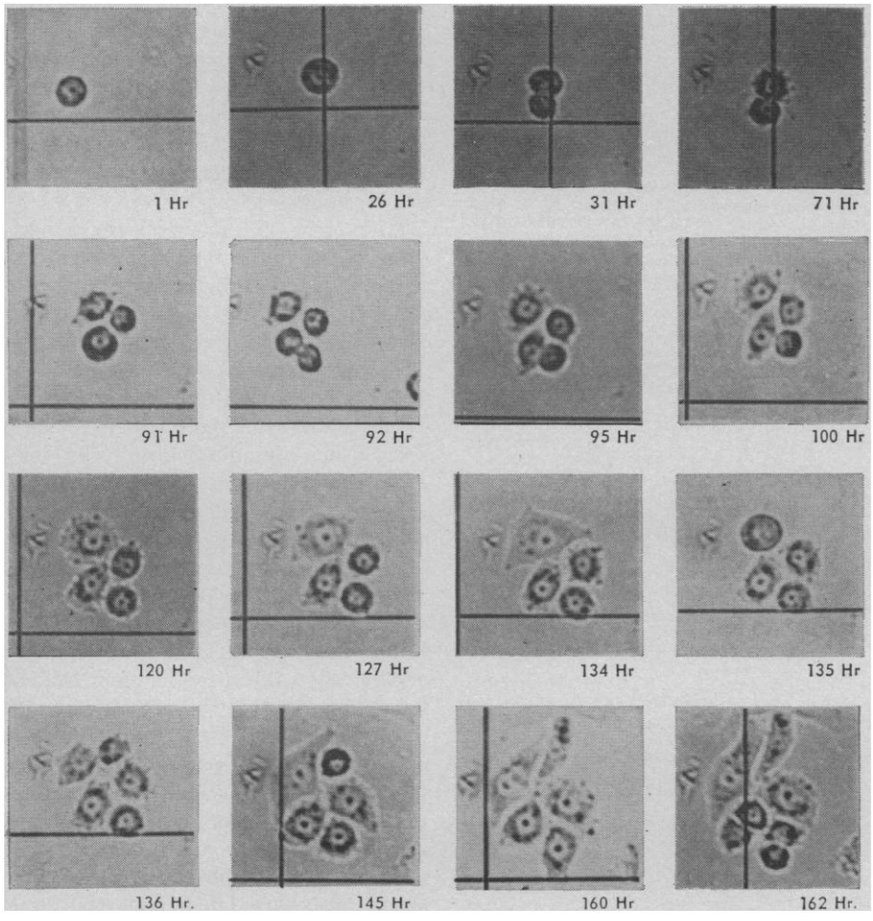


Fig. 2. Time-lapse sequence of morphological transformations undergone by a single cell. There are variations in intermitotic times (× 600).

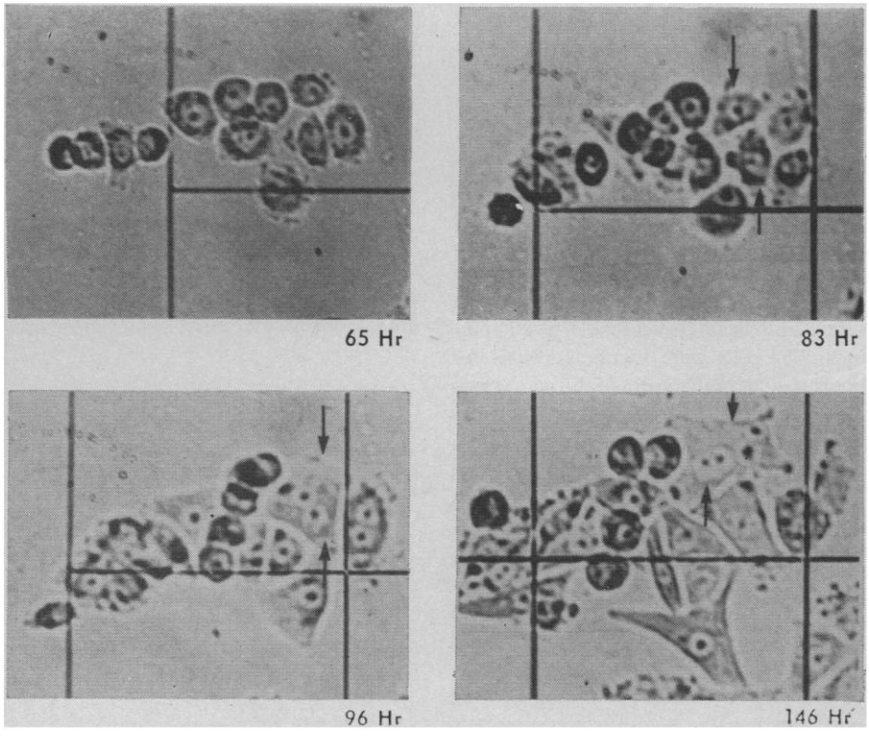


Fig. 3. Film sequence showing the fusion of two daughter cells into a single binucleated cell (× 600).

tinue to the formation of a multinucleated cell. Cell fusion has been proposed as the mechanism by which lymphoma cells can escape the policing action of immune defense mechanisms (*6*).

The morphological changes also have a functional counterpart. Staining with methyl green pyronine demonstrates marked pyroninophilia of the round cells and only traces in the spindloid forms. When exposed to fluorescein-labeled antiserum to human gamma globulin, the round cells show intense fluorescence, whereas only minute quantities, if any, of fluorescent material can be detected in the fusiform cells (Table 1). This indicates that the cells can only synthesize immunoglobulins when they adopt a round form. However, occasionally a large fusiform cell, sometimes multinucleated but more often binucleated, shows strong fluorescence as well as intense pyronin staining properties. Cells with similar shape, also capable of antibody synthesis, have been described as lying amid round cells derived from explants of lymph nodes (*7*). Our data indicate that cultured human immunoglobulin-producing cells undergo "cyclic" changes in shape which are the morphological expression of functional compartmentalization. Therefore, all of the different morphological forms encountered in vivo could be considered as passing events in the life cycle of immunocytes instead of fixed and nontransient classes of cells.

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