Cultivation of Large Cultures of HeLa Cells in Horse Serum

Vernon P. Perry

Tissue Bank, U.S. Naval Medical School, National Naval Medical Center, Bureau of Medicine and Surgery, Bethesda, Maryland

Virginia J. Evans and Wilton R. Earle

Tissue Culture Section, Laboratory of Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

In many fields of experimental biology and virology, cultivation of human cells in media containing no human serum is desirable and even imperative. The increasing usage of the HeLa cells (*1*), isolated originally from a patient with cervical carcinoma and usually grown in culture media containing human serum, suggested this strain as a desirable one for an investigation of the possibility of adapting human cells to rapid proliferation in a medium of which the serum component is of heterologous origin. The desirability of this strain was also indicated because it is known that it does not do well in media that contain horse serum rather than human serum.

HeLa cells obtained from a commercial source (*2*) and routinely cultured on media A (Table 1) for 112 days in Earle's T-60 flasks (*3*) served as the source cells for the present study (*4*). One heavy culture growing in a T-60 flask was subcultured into 11 D-3.5 Carrel flasks in medium A (Table 1) on which they had been maintained for 112 days. At the first fluid renewal, 48 hr after subculturing, the cell cultures were subdivided at random into five groups, and these were respectively exposed to the five different medium combinations shown in Table 1. Cultures on medium A served as the control cultures for those on the other four test media. All cultures were maintained at the usual temperature of 37.5°C. Routine fluid renewals of 2.0 ml were made three times a week.

Table 1. Experimental conditions for cultivation of HeLa cells.

Test media	Number of cultures		Medium components (volume %)			
	Ser. 1	Ser. 2	Chick embryo extract (*6*)	Earle's balanced salt	Pooled human serum	Horse serum
Medium A	1	2	20	50	30	0
Medium B		2	20	50	15	15
Medium C		2	20	50	10	20
Medium D		2	20	60	0	20
Medium E		2	20	40	0	40

Twenty-four hours after the cultures were transferred to the experimental media, a dramatic accumulation of debris appeared in the cultures that were treated with media B, C, and E. The cultures in medium A, the positive control cultures, were free of debris; cultures on medium D showed only a minimum of debris. No attempt was made to wash the debris out of any of the cultures.

By 72 hr the population of all cultures on all media appeared to be increasing. There was a lessening of the debris in cultures on medium D. In cultures on media B, C, and E, in spite of the rinsing brought about by the continued routine fluid change renewals, the amount of this debris present did not lessen.

At the end of 120 hr of cultivation of cultures in media B, C, and E the accumulation of debris was so great that the cultures were discarded. Cultures on medium D at this time were sufficiently heavy to require subculturing. This was done by flushing the cells from the surface of the glass, making a cell suspension in fresh media, and reimplanting the culture in new flasks. These freshly subcultured cells on medium D were transferred at the next fluid change to medium E and were routinely maintained on this medium with fluid renewals three times a week. They were thereafter subcultured whenever the proliferation was adequate as judged by microscopic observation. Initially this was about once every 14 days, since proliferation on medium E was slow. After 2 mo the proliferation rate increased markedly. By approximately 60 days on medium E, they were being subcultured about every 7 to 10 days, whereas those on medium A, the control series of the cultures, were subcultured every 7 days. Fifty days later the cultures on horse serum were being subcultured every 7 days also and were definitely proliferating at a greater rate than the cells of the parent strain maintained on media that contained human serum.

Morphologically no differences could be observed between cells in the two media. At the present time the strain in horse serum is being routinely maintained in T-60 flasks; however, the strain is already being cultured in 1-lit shaker flasks (*5*). This strain of cells cultured in horse serum readily and rapidly readapts to cultivation in the medium containing human serum.

References and Notes

1. G. O. and M. K. Gey, *Cancer Research* **58**, 976 (1954).
2. Obtained through the courtesy of Microbiological Associates, Bethesda, Md.
3. W. R. Earle and F. Highhouse, *J. Natl. Cancer Inst.* **14**, 841 (1954).
4. The opinions and conclusions expressed in this paper are those of the authors and are not to be construed as official or necessarily reflecting those of the Medical Department of the U.S. Navy or of the Naval service at large.
5. W. R. Earle, J. C. Bryant, E. L. Schilling, *Ann. N.Y. Acad. Sci.* **58**, 1000 (1954).
6. J. C. Bryant, W. R. Earle, E. V. Peppers, *J. Natl. Cancer Inst.* **14**, 189 (1953).

6 April 1955.