Cultivation of L Strain (Earle) Mouse Cells in Bacteriological Media Combined with Horse Serum. (22709)

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Research in the development of tissue culture media has been divided into two major areas. The first is the attempt to develop completely chemically defined media(1-8). The second area of research is the attempt to substitute various processed materials for blood serum and tissue extracts currently used in many tissue culture media. These processed materials, which include yeast extract(9), peptone preparations(10-13) and serum fractions(14,15), are sometimes combined with natural body substances(16) or with chemically defined media(1,3,4,17). This paper reports the development of a tissue culture medium which incorporates commercially available bacteriological preparations as supplements to blood serum in the cultivation of Earle's L strain of mouse fibroblasts.

Materials and methods. L cells(18) grown at 37°C for 3 to 5 days were scraped from T-60 Earle flasks and washed twice with Hanks' balanced salt solution (BSS). The cells were suspended in BSS and added to the various media which were then dispensed in 1 ml aliquots to Porter flasks. The concentration of cells in the Porter flasks ranged from 100,000 to 125,000 cells per ml. The following preparations have been tested for stimulation of L cell growth: Bacto Yeast Extract, Bacto Beef Extract, Bacto Casamino Acids, Difco Proteose Peptone, Difco Proteose Peptone #2, Difco Proteose Peptone #3, Difco Neopeptone, Tryptone, Tryptose, and Heart Infusion Broth, all produced by Difco Laboratories; Phytone, and Trypticase, produced by the Baltimore Biological Laboratory and N-Z Case produced by the Sheffield Chemical Co. The preparations to be screened were prepared as a 2% solution in BSS and sterilized by filtration through fritted glass. Concentrations of 2, 1, 0.1, and 0.01% were generally tested. In addition, these concentrations in BSS were tested in combination with 10% horse serum. The standard reference medium consisted of 60% medium 199,* 20% whole egg ultrafiltrate,* and 20% horse serum. Fifty units each of penicillin and streptomycin were added to each flask. All cultures were prepared in triplicate and incubated at 37°C. Various qualitative estimates were made to evaluate the growth rate and the appearance of cells from day to day. In addition to these qualitative estimates, quantitative determinations were made by taking total cell counts on the fourth day. In other short term experiments designed to test for stimulation of growth, a daily count was made for 5 days. The total cell count was made by removing the medium and replacing it with 1 ml of 0.25% trypsin in saline. Cell removal was hastened by shaking the Porter flasks on a New Brunswick rotary-type shaker operating at 100 rpm. The cells thus removed were counted in a hemocytometer. Two total cell counts were done on each Porter flask. The values shown in the figure and in the table represent the average count of triplicate flasks of a single experiment. The results of this experiment are representative of several that were performed. Preparations that showed some enhancement of growth were combined and tested in the presence of various concentrations of horse serum.

Results. Among the products screened, yeast extract and proteose peptone #3 gave the best results. One tenth % yeast extract was found to be the optimum concentration for proliferation of L cells in the presence of horse serum. This agrees with the results of Robertson with Hela cells(9). The optimum concentration of proteose peptone #3 was found to be 0.1%. No multiplication occurred in the absence of horse serum. The growth of L cells in a medium consisting of 0.1% yeast extract and 0.1% proteose pep-

* Obtained from Microbiological Associates, Bethesda, Md.

TABLE I. Multiplication of L Cells in Difco Bacto-Yeast Extract, Proteose Peptone #3, and Horse Serum.*

% yeast extract	% proteose peptone	% horse serum	Total cell count on 4th day $\times 10^5$	Fold increase
.1	0	20	6.00	4.8
.1	0	10	3.44	2.8
.1	0	2.5	1.52	1.2
0	.1	20	4.49	3.6
0	.1	10	3.29	2.7
0	.1	2.5	1.35	1.1
.1	.1	20	6.63	5.3
.1	.1	10	4.99	4.0
.1	.1	5	2.29	1.8
.1	.1	2.5	1.98	1.6
.1	.1	0	.46	.4
Standard reference medium†			3.74	3.0

* Initial inoculum: 1.25×10^5 cells/ml.
† 60% medium 199, 20% whole egg ultrafiltrate, and 20% horse serum.

tone #3 in the presence of various concentrations of horse serum is presented in Table I. The same table shows the growth obtained when these constituents were tested separately in the presence of horse serum. The growth of cells in 0.1% yeast extract or 0.1% proteose peptone #3 in the presence of 20% horse serum exceeded growth obtained in the standard reference medium. Growth in the presence of 10% horse serum was also better than that of the standard reference medium.

Among the combinations tried, 0.1% yeast extract combined with 0.1% proteose peptone #3 in the presence of horse serum gave maximum growth and exceeded that obtained using either alone. This was true at all levels of horse serum tested. There was a 5.3 and 4.0 fold increase in the total cell count in 4 days at the 20 and 10% serum levels respectively, compared to 3.0 fold increase in the standard reference medium. Although the total cell count at serum levels below 10% did not reach that of the standard reference medium, a significant degree of cell multiplication occurred at these low serum levels. There was a 1.8 and 1.6 fold increase in the total cell count at the 5 and 2.5% levels of horse serum.

Fig. 1 shows the growth curve of L cells in these two nutrients when combined with various concentrations of horse serum. The growth rate of L cells in yeast extract and proteose peptone #3 combined with 20 and 10% horse serum exceeded the rate obtained in the standard reference medium, after 5 days incubation.

Fig. 2-4 show photographs of the appearance of L cells grown in 0.1% yeast extract and 0.1% proteose peptone #3 combined with various concentrations of horse serum. The cells are thinner and more sparse in cultures where serum has been omitted.

In preliminary experiments L cells have also been cultivated for 11 days in the standard reference medium and in the yeast extract-proteose peptone #3 combination with horse serum levels of 20, 10 and 5%. The medium was changed twice during this period and the cells were transferred from T-60 Earle flasks to Roux bottles after the seventh day of incubation. The total cell count of these cultures after 11 days incubation had increased 43.7, 47.4, 34.5, and 29.2 fold re-

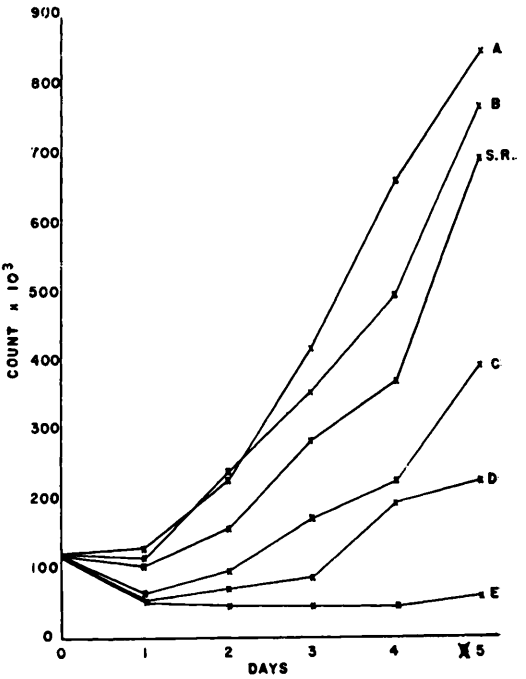

FIG. 1. Growth of L cells in yeast extract (y. e.) and proteose peptone #3 (p-p #3) combined with various concentrations of horse serum (HoS).

A. 0.1% y. e. and 0.1% p-p #3 plus 20 % HoS
B. 10 %
C. 5 %
D. 2.5%
E. 0 %

S. R. Stand. reference medium (60% medium 199, 20% whole egg ultrafiltrate, and 20% horse serum)

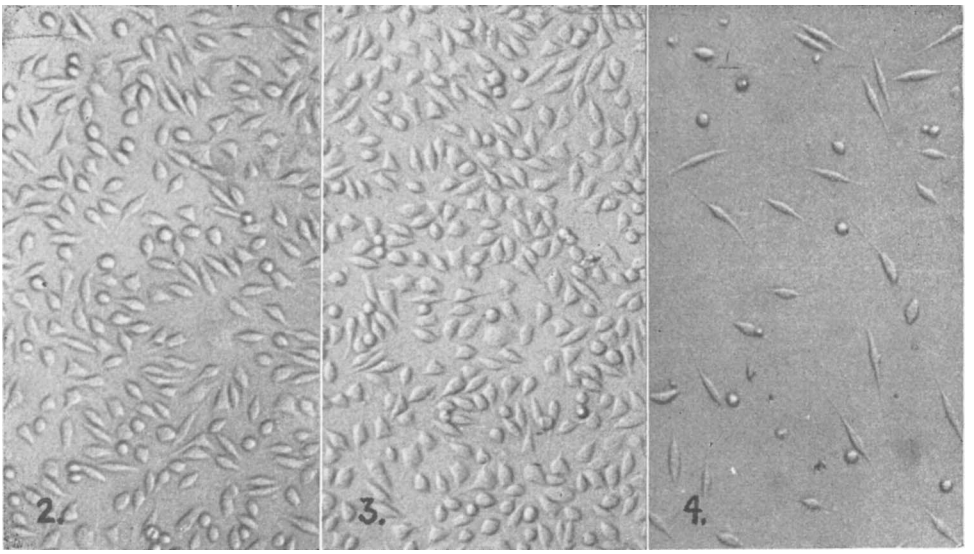

FIG. 2 (left). Earle's L strain cells from the mouse after 4 days in the standard reference medium (60% medium 199, 20% whole egg ultrafiltrate, and 20% horse serum). 100×.

FIG. 3 (center). L cells after 4 days in 0.1% Bacto-yeast extract and 0.1% proteose peptone #3, Difco, with 20% horse serum. Growth with 10% horse serum was equally good. 100×.

FIG. 4 (right). L cells after 4 days in 0.1% Bacto-yeast extract and 0.1% proteose peptone #3, without horse serum. 100×.

spectively. Under these conditions, the yeast extract-proteose peptone #3 combination in the presence of 20% horse serum produced slightly more growth than did the standard reference medium.

The medium reported here supported the growth of Gey's MBIII strain (mouse lymphoblasts)(19), Maben strain (a human epithelium-like cell derived from an adenocarcinoma of the lung)(20), and a strain of guinea pig lung fibroblasts that was isolated in this laboratory. Eighteen serial passages of L cells have been made in this medium containing 10% horse serum during a period of 17 weeks. The MBIII strain, Maben strain, and the guinea pig lung fibroblasts have been subcultured 13, 5, and 13 times during a 12, 6, and 18 week period respectively. The growth of these cell lines is undiminished and their appearance is normal.

The yeast extract-proteose peptone #3 medium, without horse serum, can be prepared as one solution and autoclaved.

Discussion. The present findings are consistent with the earlier reports that yeast extract and peptones support the proliferation of cells when combined with serum. The rapid multiplication of washed cells in the yeast extract-proteose peptone #3 medium reported here suggests that this medium contains growth factors that supplement blood serum and promote the growth of several lines of mammalian cells. Yeast extract is known to be an excellent source of B vitamins, and peptones are a rich source of nitrogenous compounds. The possibility that other unidentified growth factors are present in these preparations can not be ruled out.

It is significant to point out that the growth obtained in this medium, combined with horse serum, was slightly better than the growth obtained in a synthetic medium supplemented with whole egg ultrafiltrate and horse serum. The medium described here contains commercially available products that are cheap, reproducible, and convenient to handle. Their use in the large-scale cultivation of cells is therefore advantageous.

Summary. The combination of 0.1% yeast extract and 0.1% proteose peptone #3 in Hanks' balanced salt solution plus 20% horse serum stimulated the rate of growth and produced a higher total count of L cells at the end of 5 days than did the standard reference

medium (60% medium 199, 20% whole egg ultrafiltrate, and 20% horse serum). Lowering the serum content in the new medium to 10% still permitted better growth than the standard reference medium. The yeast extract-proteose peptone solution could be sterilized by autoclaving without affecting its growth-stimulating properties.

1. Morgan, J. F., Morton, H. J., and Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 1.
2. White, P. R., and Waymouth, C., *Ann. N. Y. Acad. Sci.*, 1954, v58, 1023.
3. Healy, G. M., Fisher, D. C., and Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 71.
4. Eagle, H., *Science*, 1955, v122, 501.
5. ———, *J. Biol. Chem.*, 1955, v214, 839.
6. ———, *J. Exp. Med.*, 1955, v102, 37.
7. ———, PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 362.
8. White, P. R., *J. Nat. Cancer Inst.*, 1955, v16, 769.
9. Robertson, H. E., Brunner, K. T., and Syverton, J. T., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 119.
10. Carrel, A., and Baker, L. E., *J. Exp. Med.*, 1926, v44, 503.
11. Baker, L. E., and Carrel, A., *ibid.*, 1928, v48, 533.
12. Waymouth, C., *Anat. Rec.*, 1955, v121, 478.
13. Ginsberg, H. S., Gold, E., and Jordan, W. S., Jr., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 66.
14. Sanford, K. K., Waltz, H. K., Shannon, J. E., Jr., Earle, W. R., and Evans, V. J., *J. Nat. Cancer Inst.*, 1952, v13, 121.
15. Sanford, K. K., Westfall, B., Fioramonti, M., McQuilkin, W., Bryant, J. C., Peppers, E., Evans, V. J., and Earle, W. R., *ibid.*, 1955, v16, 789.
16. Fischer, A., *Acta Physio. Scandinav.*, 1941, v3, 1954.
17. Evans, V. J., Bryant, J. C., Sanford, K. K., and Earle, W. R., *Ann. N. Y. Acad. Sci.*, 1954, v58, 1012.
18. Sanford, K. K., Earle, W. R., and Likely, G. D., *J. Nat. Cancer Inst.*, 1948, v9, 229.
19. Owens, O., Von, H., Gey, M. K., and Gey, G. O., *Ann. N. Y. Acad. Sci.*, 1954, v58, 1039.
20. Frisch, A., Jentoft, V., Barger, R., and Losli, E., *Am. J. Clin. Path.*, 1955, v25, 1107.

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Evaluation of a Rotating Disc Type Reservoir-Oxygenator.* (22710)

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The historical development of various pump-oxygenators is well covered in the literature(1,2,3). Many of the early machines, utilized both experimentally and clinically, bypassed but one side of the heart, this being a simpler solution to the overall problem(4, 5,6). However, Gibbon(7) and Dennis(8) have continually stressed the necessity of total cardiac bypass as a requirement for an entirely satisfactory pump-oxygenator. Lillehei and associates(9,10,11,12) hastened the reality of direct vision intracardiac surgery through the use of controlled cross-circulation, oxygenated blood reservoirs, and biological oxygenators, as temporary expedients before the final utilization of a pump-oxygenator. They also have stressed the concept of the low flow principle which has been satisfactory in their hands for shorter periods of bypass, and which possibly has simplified certain problems inherent in the development of effective pump-oxygenators.

It was the purpose of the present study to evaluate a rotating disc type reservoir-oxygenator, which has been used successfully for open intracardiac surgery experimentally and clinically.

Description of apparatus. The essential components of the present pump-oxygenator are a model TS Sigmamotor pump and a rotating disc type reservoir-oxygenator. (Fig. 1 and 2). The basic mechanism of the oxygenator is a series of 59 plastic coated stain-

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