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Waymouth C. Rapid proliferation of sublines of NCTC clone 929 (strain L) mouse cells in a simple chemically defined medium (MB 752/1).
J. Nat. Cancer Inst. **22**:1003-17, 1959.
[Roscoe B. Jackson Memorial Laboratory, Bar Harbor, ME]

This early defined medium was among the first to permit continuous culture of mouse cells—here for over 300 days, 30 passages—without reduction in rate of proliferation. The study established that proteins are not indispensable components of a culture medium. [The *SCI*® indicates that this paper has been cited in over 300 publications since 1959.]

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My search for chemically defined media for animal cells began at the Jackson Laboratory in 1952, in collaboration with Philip R. White, who had started to apply to animal systems his experience in defining media for plant tissue cultures. Earlier, in Albert Fischer's laboratory in Copenhagen, I had gained appreciation of the role of amino acids. The importance of vitamins, especially the B-vitamin complex, was evident from many studies made in the 1930s and 1940s.[1,2] Contributions to the study of salt balance, both major and minor ions, had been made by W.H. and M.R. Lewis, G.O. Gey, J.H. Hanks, W.R. Earle, and others.[1,2] By 1959, several laboratories had published defined media, of varying degrees of completeness, for growing several cell types. Some supported maintenance and proliferation of selected cells. Most required supplementation with variable biological products, such as serum, plasma, or tissue extracts.

Proteins were among many nutrients that these supplements supplied. Some of us had tried to replace these, e.g., with partially digested proteins. The success of the 1959 paper was perhaps achieved because of our demonstration that proteins or peptones were not required for continuous proliferation of Earle's Strain L, clone 929, mouse cells—as we now know, a rather unfastidious strain—but that amino acids, especially fairly high levels of basic amino acids, could substitute for polypeptides.

Additional data from the past 25 years have made us aware that a simple formulation, such as MB752/1, is the "model T" of defined media. Its simplicity has lured many to use and cite it. But adding serum to defined media, even those nutritionally much more complete than MB752/1 and other early models (e.g., medium 199, F10, or F12), remains a common practice. When the medium supplemented is designated "minimum" or "basal" (i.e., incomplete), this may be justifiable. It can no longer be justified when early models from many laboratories—including my own[3-5]—have been superseded by nutritionally and functionally more effective formulations, designed for specific cell types. Addition of serum defeats the purpose of defining a medium, which is to enable cells to express their functions—growth, differentiation, or product synthesis—under experimentally reproducible conditions. Medium MB752/1 was a beginning. We now know that every cell type has unique requirements, e.g., for salts, trace elements, amino acids, vitamins, hormones, lipids, energy sources, pH, gas phase, osmolality, and temperature.[6] We know the negative effects, in the absence of proteins, of antibiotics on some cells. Short cuts, using incomplete media and undefined, highly variable supplements, retard the progress of cell biology.

There is a wealth of information on carefully designed and tested media for *specific* cells and *specific* functions.[3,6] The "model T" media are no longer appropriate and ought to be abandoned. This is also true of the so-called "general purpose" media. There is no such thing, because no single formulation is likely to be optimal for all cells. The task remains to define what each cell needs for its unique metabolism and functions. Nothing less ought to be acceptable.

1 **Waymouth C.** Construction and use of synthetic media (Willmer E N, ed.) *Cells and tissues in culture* London Academic Press, 1965. Vol 1. p. 99-142
2. ----------. Construction of tissue culture media. (Rothblat G H & Christofalo V J, eds.) *Growth nutrition and metabolism of cells in culture* New York. Academic Press, 1972 Vol 1 p 11-47
3. ----------. Nutritional requirements of cells in culture, with special reference to neural cells (Fedoroff S & Hertz L, eds.) *Cell, tissue and organ culture in neurobiology* New York Academic Press, 1977 p 631-48
4. ----------. Autoclavable medium AM77B *J Cell Physiol* 100 548-50, 1979
5 **Waymouth C, Ward P F & Blake S L.** Mouse prostatic epithelial cells in defined media (Sato G H, Pardee A B & Sirbasku D A, eds.) *Growth of cells in hormonally defined media* Cold Spring Harbor, NY. Cold Spring Harbor Laboratory, 1982 Book B p 1097-108.
6 **Waymouth C.** Preparation and use of serum-free culture media (Barnes D W, Sirbasku D A & Sato G H, eds.) *Methods for preparation of media, supplements, and substrata for serum-free animal cell culture Cell culture methods for molecular and cell biology* New York Alan R Liss, 1984 Vol 1. p 23-68