Leibovitz A. The growth and maintenance of tissue-cell cultures in free gas exchange with the atmosphere. *Amer. J. Hyg.* 78:173-80, 1963.
[Sixth US Army Medical Laboratory, Fort Baker, CA]

Tissue cell cultures grew in free gas exchange with the atmosphere when the bicarbonate buffer was replaced by the free base amino acids, especially L-arginine. Glycolysis of the medium was significantly reduced when glucose was replaced by galactose, sodium pyruvate, and DL-alpha alanine. [The *SCI*® indicates that this paper has been cited over 270 times since 1963.]

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"As an Army microbiologist, I was assigned to establish a diagnostic virology laboratory at the Fifth US Army Medical Laboratory in St. Louis. This was accomplished in 1958. Most commercially available human tissue cell lines were 'altered' cell lines, as HeLa or HEp II. In closed systems (screw-capped test tubes) they grew luxuriantly in such glucose-bicarbonate buffered media as Eagle's MEM, but several problems were obvious. These cell lines were highly glycolytic and unless the media were changed at least three times a week, the sharp drop in pH destroyed the cell monolayer. If the screw caps did not fit the test tubes properly, carbon dioxide escaped into the atmosphere and the residual sodium carbonate raised the pH to toxic levels. Maintenance of inoculated cultures was labor intensive; the technicians felt like 'apes' in feeding the cultures and in blind passing those that became toxic.

"The classical studies of Eagle and coworkers established the minimal nutritive requirements of tissue cells *in vitro*. Analysis of his data, especially his studies with the HeLa cell line,[1] revealed that the essential amino acids could be incorporated in media at much higher concentrations and that he utilized the basic amino acids as hydrochloride salts. My studies determined that the basic amino acids, in their free base form, could replace bicarbonate as a buffer. L-arginine, 3 μM/ml, yielded a pH 7.6 medium that permitted tissue cell growth in free gas exchange with the atmosphere.

"In 1960, I was transferred to the Sixth US Army Medical Laboratory in the presidio of San Francisco. Soon thereafter, I resumed my research to solve the glycolysis problem. Eagle *et al.*[2] and Chang and Geyer[3] noted that glycolysis was significantly reduced when galactose was substituted for glucose. Growth was haphazard, however, unless such carbohydrate saving agents as pyruvate and DL-alpha alanine were incorporated. Various combinations of galactose, sodium pyruvate, and DL-alpha alanine were tested and by my fifteenth formulation, the desired medium was attained.

"In collaboration with Charlotte John (unpublished observations), medium L-15 was used to establish cell cultures from normal human tissues. Such primary cultures are significantly less glycolytic than HeLa or HEp II and could be maintained at least 30 days without refeeding. Thus, the labor intensive side of diagnostic virology was eliminated, resulting in a significant savings in man-hours, media, and material. This proved most valuable in a collaborative study with the Walter Reed Army Institute of Research Virology Division in the development of adenovirus vaccines. In this nationwide study, not having to refeed inoculated cultures resulted in the savings of $50,000 the first year in just pipettes as well as thousands of man-hours of labor. I received an Army award and the Legion of Merit.

"I am naturally pleased that medium L-15 is being used internationally in a variety of fields including the establishment of cell lines from cold-blooded animals such as fish and amphibians. Since retiring from the Army, I have been engaged in the establishment of cell lines from human solid tumors using medium L-15 with certain additives."[4]

1. **Eagle H.** The specific amino acid requirements of a human carcinoma cell (strain HeLa) in tissue culture. *J. Exp. Med.* 102:37-48, 1955.
2. **Eagle H, Barban S, Levy M & Schulze H O.** The utilization of carbohydrates by human cell cultures. *J. Biol. Chem.* 233:551-8, 1958.
3. **Chang R S & Geyer R P.** Propagation of conjunctival and HeLa cells in various carbohydrate media. *Proc. Soc. Exp. Biol. Med.* 96:336-40, 1958.
4. **Leibovitz A, Stinson J C, McCombs W B, McCoy C E, Mazur K C & Mabry N D.** Classification of human colorectal adenocarcinoma cell lines. *Cancer Res.* 36:4562-9, 1976.