that determine cell division. The meaning of these numbers has yet to be clarified. But the Monte Carlo method affords a means for estimating them from data on various aspects of individual cell life.

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Nonbicarbonate Buffers in Cell Culture Media

The use of cell cultures in studying fundamental problems in many fields of experimental biology has increased considerably in the last few years. It is frequently desirable to employ cells that have been cultivated under conditions that are as standardized as possible. From the beginning of "tissue culture," cells of mammalian and avian origin have been cultivated *in vitro* in media composed of a balanced salt solution containing sodium bicarbonate as the principal buffer, supplemented with embryo extract and serum. Adequate control of the *p*H of such cultures introduces a number of technical problems, for it is necessary to maintain a specified carbon dioxide tension in the atmosphere by employing gassing procedures and closed culture flasks. In an attempt to circumvent some of these difficulties, studies were initiated to test the possibility of growing cells in media containing a fixed buffer instead of the conventional bicarbonate system. In addition to facilitating the control of *p*H and reducing the labor required for maintaining cultures, fixed-buffer media have a number of obvious applications in mass culture techniques and in metabolic and viral studies with cultured cells.

The following cell cultures were employed: fibroblasts derived from normal human foreskin, testicle, human embryonic kidney, and lung, as previously described (*1*); epithelial cells, strain HeLa (*2*); mouse fibroblasts, strain L (*3*). All cultures were grown directly on glass. The medium for foreskin, embryonic kidney, lung, and testicle cells consisted of mixture No. 703 (*4*) supplemented with 5-percent beef embryo extract and 10- to 20-percent horse serum. Human serum (20 percent) was employed instead of horse serum for HeLa cultures, and the L cells were grown on mixture No. 703 supplemented with 10-percent beef embryo extract.

The sodium bicarbonate of mixture No. 703 was replaced with tris(hydroxymethyl)aminomethane (5, tris) at concentrations of 2.0 to 3.6 g/lit, and the *p*H was adjusted to the desired value by the addition of hydrochloric acid. This mixture was supplemented with embryo extract and serum in the same concentrations as were employed for the stock cultures. Foreskin, embryonic kidney, lung, and testicle cultures grew as well as the controls (bicarbonate medium) with all levels of tris when the initial *p*H of the nutrient fluid is 7.8 to 8.1. All cultures were propagated serially with weekly subcultures. Foreskin and testicle cells have been maintained for the longest period and continue to do well after 3 mo. These cultures have been maintained in both stoppered flasks and in flasks closed only by means of cotton plugs or loose-fitting metal caps. A number of differences between cells grown in tris and those grown in bicarbonate have been observed. For example, it appears that the number of cells required per flask is a more critical factor in tris fluids than it is in bicarbonate fluids. The only change in the appearance of cells cultured in tris fluids noted to date is a small increase in the number of intracellular granules. The results of a limited number of experiments indicate that prolonged cultivation of cells in fluids containing tris does not alter their susceptibility to infection with polio virus or the yield of virus. In contrast with the growth rate of normal human fibroblasts, the growth rate of HeLa and L cells was somewhat inhibited with higher levels of tris. The HeLa cells were smaller and more granular than those in the control cultures and there was a tendency for the cells to grow in clumps rather than as a continuous monolayer. The L cells also exhibited changes in morphology when they were cultured in media containing tris. Despite alterations in morphology and decreased rate of proliferation, both strains have been maintained through 14 subcultures and continue to proliferate at a constant rate.

The results of short-term experiments indicate that glycylglycine is less toxic than tris for HeLa cultures. Under these conditions, the cells grew rapidly without any appreciable alteration in morphology. Cultures of foreskin cells, however, did not proliferate as well in glycylglycine as they did when tris was employed as the principal buffer.

Although the results obtained to date with tris are particularly promising with cultures of normal human fibroblasts, factors such as concentrations of tris, optimal *p*H, and the effect of other constituents of the medium require more detailed study before a final evaluation can be made. Several organic and inorganic compounds with a pK_a in the range of 6.5 to 8.5 that are compatible with other components of cell culture media are now under investigation in an attempt to replace tris in cultures where it is toxic or where the optimal *p*H for growth is below the effective limit of this buffer system.

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Substrate Specificity of Peroxidase

A current view of enzyme specificity is that compounds of certain specific configurations can be bound by the enzyme and can lead to formation of the activated complex. The substrate specificity of catalases and peroxidases for hydrogen peroxide has been recognized for some time, and Theorell has recently suggested the term *hydroperoxidases* for this class of enzymes (*1*).

Such a definition of specificity includes reactions with homologs of the substrate, methyl or ethyl hydrogen peroxide, which have been shown to react in a second-order fashion with peroxidase and to form the same series of intermediates as does hydrogen peroxide (*2*). But the possibility of forming these reactive intermediates by a wide variety of inorganic oxidizing agents, recently pointed out by P. George (*3*), would seem to vitiate current ideas of formation of the enzyme-substrate compound and to favor the idea that the spectroscopically detectable peroxidase intermediate (complex I) is only the product of some rapid, unobserved oxidation reaction caused by a great variety of nonspecific oxidants.

This possibility lead George to the view that peroxide would react by a similar mechanism and would not therefore be a component of the structure of complex I, nor would it, per se, be required for