carbamyl phosphokinase of *Neurospora* normally serves only the arginine pathway. A pyrimidine-specific system of forming carbamyl phosphate or other carbamyl compound (presumably lacking in *pyr-3a*) may therefore be postulated (*1*, *4*). If this system does exist, it is probable that carbamyl phosphate is its product, since aspartate transcarbamylase of *Neurospora* utilizes pure carbamyl phosphate. This is further supported by the observation that the product of the carbamyl phosphokinase reaction may be used by aspartate transcarbamylase in vitro, and, according to the gene interaction studies mentioned above, in vivo. The segregation of the postulated CAP-forming systems in intact, wild-type cells will not be clearly understood until a pyrimidine-specific system and its substrates have been demonstrated.

Some of these experiments are similar in methodology to those of Gorini and Kalman (*14*), which indirectly implicate only one carbamyl phosphokinase in wild type *Escherichia coli*. It is probable, in comparing the experiments, that *Neurospora* and *E. coli* differ in the multiplicity of CAP-forming systems. However, the present results may be of significance in a similar problem of carbamyl phosphate synthesis in pigeon livers, mentioned in a recent article by Jones (*15*). Arginine synthesis is presumed not to take place in pigeon liver, and ornithine transcarbamylase activity is not demonstrable. However, no system that can synthesize carbamyl phosphate is demonstrable either. On the basis of substantial aspartate transcarbamylase activity, pyrimidine synthesis presumably does prevail, and it may be dependent upon a pyrimidine-specific carbamyl-forming system, as yet undetected. A multiplicity of enzymes that catalyze CAP formation, and a segregation of their products, is best understood in terms of independent feedback control of the arginine and pyrimidine pathways (*16*; *17*).

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Ethylene Oxide Sterilization of Tissue Culture Media

Abstract. *Liquid ethylene oxide, which kills bacteria, yeasts, and molds, was added to a tissue culture medium instead of the usual antibiotics. With some limitations, the use of ethylene oxide sterilization of tissue culture medium has been shown feasible. Data indicate that this method can be used in experiments involving the sterilization of test chemicals and components of the medium used for mammalian cells in culture.*

Antibiotics, when added to tissue culture media prevent the growth of contaminating bacteria, but usually have no effect on molds and other fungi, which also are common contaminants of such media. Futhermore, penicillin has been shown to cause various contaminating bacteria in tissue-culture media to change to pleuropneumonia-like organisms which invade the tissue cells and multiply within them (*1*). The need for a lethal agent with a wider range of effectiveness than antibiotics, prompted us to experiment with liquid ethylene oxide and to develop a routine method for sterilizing tissue culture media.

Ethylene oxide kills bacteria, fungi, and viruses, but is not an inhibiting or bacteriostatic agent (*2*). Sterilization by the gas is essentially a function of concentration, exposure time and temperature (*3*). As a chemical agent, ethylene oxide is recognized as being toxic in both its liquid and vapor phases, and when prepared as a solution. Hollingsworth (*4*) has reviewed the effects of ethylene oxide vapor on animals. Fraenkel-Conrat (*5*) reported studies of ethylene oxide on proteins, amino acids and vitamins. Wilson and Bruno (*6*) used ethylene oxide for the sterilization of bacteriological culture media, milk, and serum. The dangers inherent in the use of ethylene oxide should not be overlooked; personal hazards that may be encountered have been reviewed (*7*).

In preliminary single-cell plating experiments with HeLa and Chang liver cells, after the method of Puck (*8*), varying amounts of liquid ethylene oxide were added to the standard tissue culture medium (*9*) 30 minutes prior to inoculating the medium with the cells. The liquid ethylene oxide was applied directly from the deep-freezer in which it and the sterile applicator were kept. Results indicated that the ethylene oxide destroyed the HeLa cells; evidently, 30 minutes was not enough time for vaporization of the ethylene oxide from the medium to occur, so, in subsequent experiments, 24 hours were allowed to elapse before inoculation of the medium with the mammalian cells. This procedure apparently provides a routine method of sterilization of the tissue culture medium used for the growth of the mammalian cultures investigated.

The persistence of toxic effects after sterilization with ethylene oxide has been reported (*2*) but adequate aeration, evacuation, and dilution (*10*) seemed sufficient to remove any residual inhibitory properties of the ethylene oxide solution.

As shown in Table 1, the presence of some toxic residue of ethylene oxide is evident from the lower plating efficiency which resulted when 100 μl of ethylene oxide was used. Decreasing plating efficiencies were obtained with increasing concentrations of ethylene oxide, but Chang liver cells were not as sensitive to the toxic effects of ethylene oxide as were the HeLa cells.

Experiments were performed to determine whether the liquid ethylene oxide could be diluted so as to prevent the medium from remaining toxic. Plating efficiencies of 95.8 percent to 98.9 percent were obtained with HeLa cells, with ethylene oxide dilutions of 1 : 1000 parts by volume up to 1 :

1,000,000 parts. Ethylene oxide diluted 1 : 100 was also tested, but the results were inconclusive.

After the method of Hsu (*11*), mitotic indices were taken as a criterion of damage to the HeLa cells, caused by ethylene oxide. There was no significant difference in the percentages of mitotic phases between HeLa cells grown in a control medium, and HeLa cells grown in a medium which had been treated with 0.4 percent or 0.5 percent ethylene oxide.

Since sterilization with ethylene oxide is a function of concentration, exposure time, and temperature, it is difficult to specify a concentration of ethylene oxide which is 100 percent effective for sterilization. In our laboratory, under environmental conditions of 60 percent to 80 percent relative humidity and a temperature of 37°C, and for an exposure time of at least 24 hours, 1 percent by volume of ethylene oxide killed large inoculums of *Aerobacter aerogenes, Escherichia coli,* and *Bacillus megaterium* bacteriophage, which were added to the tissue culture media, as well as a yeast-like organism isolated previously from a contaminated stock bottle of HeLa cells; but sterilization of large amounts of heavily contaminated materials, or residual microbial toxins injurious to cells, was not attempted.

It is recommended that tissue culture media should be sterilized with ethylene oxide 24 hours before the media are to be used, although practical applications may depend on what cell lines are to be cultured. From the results obtained in this laboratory, it would seem entirely feasible to sterilize test chemicals or any chemical agents added to tissue culture media which cannot be sterilized by heat or which are ordinarily filtered, especially if these substances are generally used in very small quantities.

Table 1. The effects on a tissue culture medium of ethylene oxide, added 24 hours prior to inoculation of Chang liver cells or HeLa cells.

Ethylene oxide (concn.)	Plating efficiency (%)	
(μl/5ml of medium)	HeLa cells	Chang liver cells
0	47.2*	24.3*
25	63.1	85.9
50	56.3	95.8
75	23.3	74.2
100	22.0	

*These figures represent the absolute plating efficiency which was calculated from the original inoculum of cells per plate. All other relative plating efficiencies shown were calculated from the number of colonies that developed on the experimental plates, compared with the number of colonies that developed on the controls.

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Culture of Small Barley Embryos on Defined Media

Abstract. *Barley embryos, measuring 90 μ in length, grew and became differentiated on a phosphate-enriched White's medium which was fortified with glutamine and alanine as major sources of organic nitrogen, and lesser amounts of five other amino acids. The pH of the medium was a critical factor in differentiation. On this medium, excised barley embryos developed more rapidly than embryos remaining in vivo, or embryos grown on media containing coconut milk.*

In order to study many aspects of the growth and development of living embryos of vascular plants, it is necessary to excise them from surrounding parental tissues and culture them on a suitable medium. A number of investigators have successfully cultured larger, differentiated plant embryos, but cultures of small, undifferentiated or slightly differentiated embryos have rarely succeeded, and much remains to be discovered about their nutritional requirements.

In studies of embryogenesis in barley, difficulties have been experienced in the culture of embryos smaller than 0.5 mm (*1, 2*). The best results have been obtained (*3*) with media containing coconut milk, but reproducible results are difficult to obtain. Therefore, we attempted to devise a satisfactory medium of entirely known, or defined, composition.

The analysis of the organic constituents of coconut milk reported by Tulecke *et al.* (*4*) served as a starting point. No attempt was made to duplicate coconut milk in its known entirety; rather, those constituents found previously to be active in embryo cultures were used. For example, the reports of Sanders and Burkholder (*5*), that combinations of amino acids promoted the growth of small *Datura* embryos, and of Rijven (*6*) that glutamine and alanine were especially effective in promoting growth of *Capsella* embryos, suggested that these substances should be used in trial media. Other substances, such as sorbitol, inositol, indoleacetic acid, gibberellic acid, various purines and pyrimidines, and certain vitamins also were tested. Some of these were ineffective; others produced peculiar morphogenetic effects and were not included in our final medium. In addition, we studied the effects of dif-

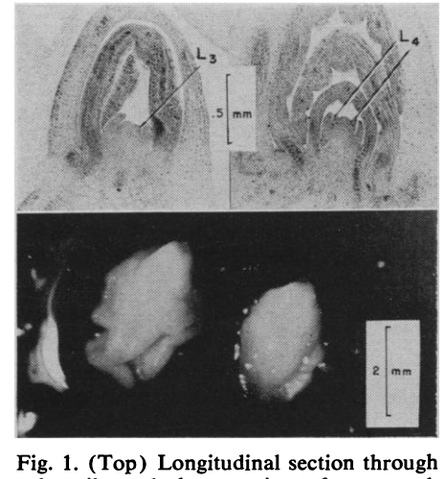

Fig. 1. (Top) Longitudinal section through coleoptile and shoot region of a normal, full-term barley embryo (left), and an embryo which was initially 0.3 mm in length, after it was cultured for 10 days on the medium defined in Table 1 (right). Number of leaf primordia in each indicated by arrows and symbols. (Bottom) Embryo cultured for 10 days on the defined medium (left) is compared with normal, full-term embryo (right).