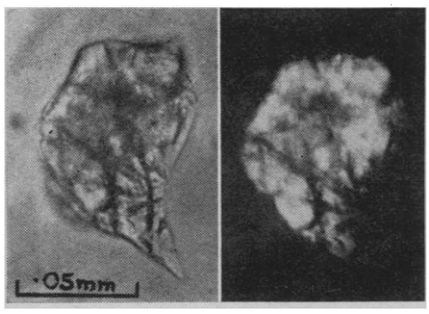

Fig. 1. (Left) Mineral grain in layered Muong Nong-type indochinite from Nong Sapong, Thailand, in plane-polarized light. (Right) The same mineral grain between crossed-nicols remains light, showing that it is crystalline. The streaks which remain dark between crossed nicol prisms may be fractures filled by isotropic glass.

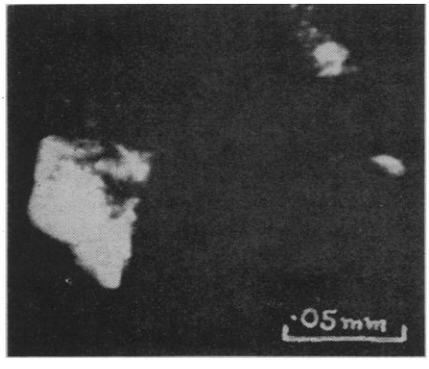

Fig. 2. Crystalline mineral grains in layered Muong Nong-type indochinite from Nong Sapong, Thailand, between crossed nicol prisms. The angularity of the larger grains shows that the melt was relatively cool when the grain was incorporated.

Nong Sapong, showed scarce to abundant detrital mineral grains in five of the plates (Fig. 1). Most of the mineral grains range in size between 0.01 and 0.1 mm. Several tested samples have birefringence and uniaxial-positive interference figures typical of quartz, and two grains isolated from crushed tektite have the refractive index of quartz. It is likely, therefore, that most of the included detrital mineral grains are quartz.

That the mineral grains were incorporated into the tektites when the melt was still very hot is apparent from their crackled appearance and hazy birefringence. The grains occur in bubbly layers rich in tiny lechatelierite particles and frothy lechatelierite (*1*), and these layers alternate with layers which are free of mineral grains and contain scant lechatelierite and bubbles. Thus the grains appear to have entered the melt while it was in the process of flowing, cooling, and becoming layered. The angularity of one of the larger grains (Fig. 2) indicates that at the time it was incorporated the melt had cooled below the point at which rounding of grain edges could take place.

Previously I described detrital mineral grains along faults in layered indochinites from Kan Luang Dong, Thailand, about 40 km south of Nong Sapong (*2*). These grains, as well as those included in tektite layers at Nong Sapong, are all the size of silt and finest sand. The country rock in the vicinity of these tektite localities is silty and sandy clay, and angular quartz grains up to 0.25 mm (rarely as much as 0.4 mm) are very abundant in the sandy clay and overlying soil. Such a correspondence in size and angularity between grains in layered tektites and grains in the material on which they lie strongly implies that layered tektites are locally derived.

By whatever manner the mineral grains of silt and fine-sand size were introduced along the faults, it is likely that dust of similar size was thrown into the air at the time of the faulting. If the dust settled on flowing melt it would have been incorporated, and the association of silica particles which had been subjected to different degrees of heating would thus be explained. The quartz particles which settled while the melt was hottest would have become lechatelierite, those which fell while the melt was at an intermediate temperature would have become frothy lechatelierite, and those particles incorporated after the temperature dropped below the melting point of quartz would have retained their crystallinity.

The supposed absence of transitional phases between tektites and earth materials has been considered as evidence against the terrestrial origin of tektites. This discovery of abundant detrital mineral grains in some layers of Muong Nong-type indochinites indicates the presence of this type of transitional phase (*3*).

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Neurospora Mutant Lacking an Arginine-Specific Carbamyl Phosphokinase

Abstract. *An arginine-requiring mutant of* Neurospora *lacks the carbamyl phosphokinase activity present in other strains. Although carbamyl phosphate, the product of this enzyme, is required in both arginine and pyrimidine synthesis, the enzyme appears to serve only the arginine pathway. A pyrimidine-specific mode of carbamyl phosphate synthesis is inferred, although it has not yet been demonstrated.*

Previous work in this and other laboratories (*1–3*) has indicated that some *Neurospora* mutants requiring arginine and others requiring pyrimidines are deficient in the synthesis of carbamyl phosphate (CAP), which is a precursor of both compounds. In the pathway of arginine synthesis, it is used in the carbamylation of ornithine to form citrulline, a reaction catalyzed by ornithine transcarbamylase (OTC). Early in the orotic-acid pathway of pyrimidine synthesis, it is used in the carbamylation of ornithine to form ureidosuccinate, a reaction catalyzed by aspartate transcarbamylase (ATC).

The mutants presumed to be deficient in carbamyl phosphate synthesis are the nonallelic *arg-2* (33442) and *arg-3* (30300) strains, and certain members of a series of allelic *pyr-3* mutants, represented by *pyr-3a* (37301) (*4*). The two arginine mutants grow on arginine or citrulline, but not on ornithine; this phenotype is not the consequence of an ornithine transcarbamylase deficiency. The *pyr-3a* mutant is blocked in the synthesis of ureidosuccinate, but has normal aspartate transcarbamylase activity (*5*). These observations suggest a pyrimidine-specific mode of carbamyl phosphate synthesis, under the control of the *pyr-3* gene, and an arginine-specific mode, under the control of the *arg-2* and *arg-3* genes (*1, 3*). Such a hypothesis is supported by studies of gene interaction. A strain carrying both *pyr-3a*, and a second mutation imposing an ornithine transcarbamylase deficiency, has no pyrimidine requirement (*1*). Similarly, strains carrying either *arg-2* or *arg-3*, plus a second mutation imposing an aspartate transcarbamylase deficiency, are relieved of their arginine requirement (*2, 6*). The data indicate that the specific source of carbamyl phos-

phate remaining in the *pyr-3a, arg-2,* or *arg-3* mutants could be shunted to the other pathway by an impairment of the transcarbamylase in the normal route.

The hypothesis that there are arginine- and pyrimidine-specific modes of CAP synthesis is supported by work, described in this preliminary report, initiated with the finding that *arg-3* lacks completely a carbamyl phosphokinase (CPK) activity demonstrable in wild type strains. This enzyme occurs in lower organisms; it utilizes adenosine triphosphate (ATP) and carbamate, formed nonenzymatically or enzymatically from bicarbonate and ammonia, to form carbamyl phosphate.

Mycelia of various genetic constitutions were grown in 700-ml shake cultures (*7*). Samples were withdrawn at regular intervals, acetone-dried, and used for enzyme measurements and dry-weight determinations. Assays for ATC and OTC have been described (*1, 5, 8*); specific activities are reported as micromoles of product (ureidosuccinate or citrulline) per milligram of protein per hour in reactions proceeding at *p*H 9.1. The standard carbamyl phosphokinase reaction mixture was designed to couple CAP synthesis from radioactive bicarbonate to the OTC or ATC reaction. Because bicarbonate, carbamate, and CAP are acid-labile, CAP formed from bicarbonate was measured after its further conversion to citrulline or ureidosuccinate, which are acid-stable. The transcarbamylases were present or were added greatly in excess. The standard reaction mixture (1 ml) contained 10 μmole of $MgCl_2$; 20 μmole of NH_4Cl; 8 μmole of ATP; 100 μmole of tris-HCl, pH 8.3; 5 μmole of L-ornithine hydrochloride; 18 units of *Neurospora* OTC (*8*); an aliquot of dialyzed extract; and 20 μmole of C^{14}-$KHCO_3$ (0.05 μc/μmole). After 30 minutes, 0.4 ml of the reaction mixture was plated into acid on an etched glass planchet, dried, and counted in a Nuclear-Chicago D-47 gas-flow detector with window (*9*). Specific activities of CPK are given as counts per minute for the acid-stable derivative per milligram protein per hour. The rate of the reaction was linear for several hours under the conditions described; substrate concentrations and *p*H were optimal.

The carbamyl phosphokinase reaction was identified by the following criteria:

1) It was completely dependent upon HCO_3^-, NH_4^+, Mg^{++}, ATP and either aspartate or ornithine. Incorporation by whole extracts of wild type was the same whether aspartate or ornithine was used as a transcarbamylase substrate to trap the CAP formed.

2) Carbamyl phosphate was indicated as an intermediate because, upon addition to reaction mixtures, it depressed completely the aspartate- or ornithine-dependent incorporation of radioactive bicarbonate to an acid-stable form. Further, when neither aspartate nor ornithine was present, an acid, radioactive compound with the stability of CAP accumulated in reaction mixtures.

3) Ureidosuccinate and citrulline were identified by column chromatography of the acid-stable products, and by their low rate of formation when extracts deficient in ATC or OTC activity were used.

4) Glutamine, a substrate (*9*), and acetylglutamate, a cofactor (*10*), of alternate modes of carbamyl phosphate formation in other organisms, did not stimulate or otherwise affect the reaction. Carbonic anhydrase, known to facilitate the formation of carbamate from bicarbonate and ammonia (*11*), did not stimulate the reaction. The replacement of bicarbonate and ammonia with "ammonium carbonate" (containing carbamate ion) did not significantly influence the reaction (*12*). It is possible that extracts have carbonic anhydrase activity sufficient to sustain optimal rates of carbamate formation, and that carbamate is the true substrate of carbamyl phosphate synthesis.

Under conditions where extracts of all other strains (including *pyr-3a*) displayed carbamyl phosphokinase activity, those of *arg-3* displayed none (less than 0.1 percent). This deficiency could not be reversed by additions of carbonic anhydrase or ornithine transcarbamylase. Moreover, *arg-3* extracts had no effect when added to reaction mixtures containing extracts of wild type. It is concluded that *arg-3* lacks a single carbamyl phosphokinase enzyme demonstrated in wild type. (*Arg-2,* the unlinked mutant with similar nutritional requirement, displayed carbamyl phosphokinase activity.)

The arginine-specific nature of the carbamyl phosphokinase lacking in *arg-3* was indicated by four criteria:

1) The *arg-3* mutant, despite its lack of CPK, does not have a pyramidine requirement for growth. In fact, uridine actually eliminates the small amount of growth of *arg-3* seen in the absence of arginine (*3*).

2) There is a possibility that arginine used for growing *arg-3* is in some way utilized for pyrimidine synthesis. If this were so, starvation for arginine would also lead to starvation for pyrimidines. Because arginine starvation leads, in other arginine mutants, to increased ("derepressed") ornithine transcarbamylase activity (fivefold normal), and pyrimidine starvation of pyrimidine mutants leads to increased aspartate transcarbamylase activity (tenfold normal) (*4*), it is possible to infer whether or not pyrimidine synthesis is impaired in *arg-3* grown on limiting arginine concentrations. It was found that OTC activity of *arg-3* increased from the normal 35 μmole of citrulline per milligram of protein per hour in unlimiting arginine, to 114 in limiting arginine. Aspartate transcarbamylase activity of *arg-3,* on the other hand, was normal on all levels of arginine supplementation. This indicates that the carbamyl phosphokinase deficiency of *arg-3* is significant only to arginine synthesis.

3) The arginine specificity of the carbamyl phosphokinase is further supported by its increased activity—from 2400 to 10,000 count/min of citrulline —when an arginine mutant lacking ornithine transcarbamylase (*13*) is starved for arginine. Enzyme activity is normal, however, in pyrimidine mutants starved for pyrimidines.

4) The last, and perhaps most compelling, evidence in favor of the arginine-specificity of carbamyl phosphokinase was gained by using the *pyr-1* mutant, 263. This mutant, because of a mutational block between ureidosuccinate and orotic acid in the pathway of pyrimidine synthesis, accumulates ureidosuccinate in the mycelium when it is starved for pyrimidines. If ureidosuccinate synthesis were dependent upon the CPK missing in *arg-3,* the double mutant *pyr-1, arg-3* would not be expected to accumulate ureidosuccinate. This double mutant, isolated from a mating of the two strains, had, as expected, no carbamyl phosphokinase activity. A comparison of *pyr-1* and the double mutant showed that both accumulated ureidosuccinate: *pyr-1* yielded 87 μmole/g (dry weight), the double mutant yielded 97 μmole/g. Wild type, on the other hand, yielded only 6 μmole/g.

These results suggest strongly that the

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 :