Urine as A Nitrogen Source For Photosynthetic Gas Exchangers

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ABSTRACT

The ability of human urine and individual urinary nitrogen compounds to support growth of *Chlorella pyrenoidosa* has been determined. To achieve maximum growth rates with urine, addition of inorganic salts is necessary. Urea is an ideal nitrogen source for algal growth as only slight pH changes occur during its utilization. Purines and the amino acids related to the Krebs urea-ornithine cycle and the citric acid cycle are utilized. Creatinine and hippuric acid are not utilized. Accumulation of these compounds during urine recycling is discussed. Conversion of urea to ammonia is undesirable for a closed environmental control system.

UNICELLULAR GREEN algae have been considered as one component of a biological regenerative environmental control system in long-term space flights. By the process of photosynthesis, algae transform carbon dioxide into cellular components and liberate oxygen as a waste product. An active photosynthetic algal system, operating for more than a few days, requires a continual synthesis of new cells. A source of nitrogen is necessary for the formation of essential cell components such as proteins, nucleic acids, and the photosynthetic pigment, chlorophyll.

The nitrogen waste products of man, found primarily in urine, are a possible continuous and convenient source of nitrogen in a space system. The ability of human urine and individual urinary nitrogen compounds to support growth of the green alga, *Chlorella pyrenoidosa*, has been determined. The utilization, accumulation and toxicity of these compounds as related to a closed ecological system has been investigated and evaluated.

EXPERIMENTAL

Individual nitrogen compounds were added in amounts of 0.025 to 0.1 gm. nitrogen per liter to a basal inorganic salt medium containing no nitrogen compounds.[1] Their purity was tested by melting point, infrared and ultraviolet absorption, and paper chromatography analyses. Urine as collected, immediately sterilized by ultra filtration, and added in amounts of 0.1 to 0.5 gm. nitrogen per liter to the basal inorganic salt medium or to distilled water. The cultures were grown in Roux flasks on a shaking light table with air flowing through the flasks. An actively growing, unialgal suspension of *Chlorella pyrenoidosa* (Starr #252 obtained from Carnegie Institute of Washington, Department of Plant Biology, Stanford, California) was used as the inoculum. All experiments were performed under sterile conditions, since extraneous microorganisms might be responsible for competitive utilization or chemical transformation of the nitrogen compounds.

Samples were removed aseptically for chemical analyses. The amount of algal growth was determined by measuring the chlorophyll concentration of the algal suspension. The algae were extracted with boiling 80 per cent ethanol and the absorption of the clear extract measured at 665 mu. Utilization of each nitrogen compound was determined by analysis of the culture medium after removal of the algal cells. Amino acids were determined using a ninhydrin color reaction, creatinine by reaction with alkaline-picrate, ammonia by nesslerization, urea by conversion to ammonia with urease followed by nesslerization, purines and hippuric acid by specific absorption in the ultraviolet. Paper chromatograms of the growth medium were examined to detect possible chemical transformation of a nitrogen compound without its utilization for growth. Controls included a flask having no nitrogen present and another flask having urea as a nitrogen source.

RESULTS

Urine can serve as a satisfactory growth medium for *C. pyrenoidosa*. Figure 1 presents data showing the comparative growth rates in culture medium containing

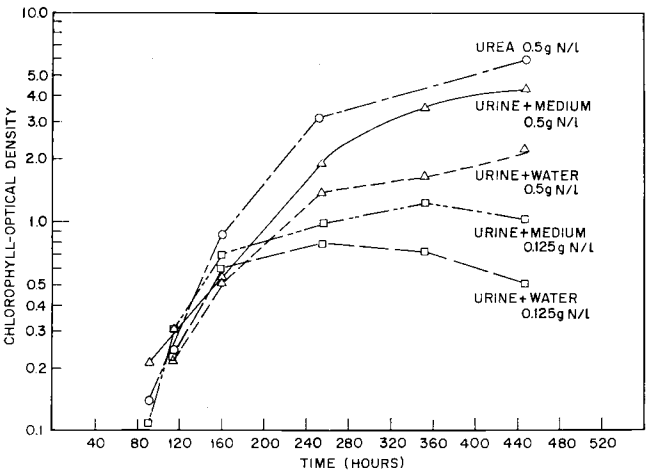

Fig. 1. Growth of *C. pyrenoidosa* with urea or urine as the nitrogen source. - — - Urea (0.5 gm. nitrogen per liter) plus basal inorganic salt medium. —— Urine (0.5 gm. nitrogen per liter) plus basal inorganic salt medium. — — — Urine (0.5 gm. nitrogen per liter) in distilled water. - - — - - Urine (0.125 gm. nitrogen per liter) plus basal inorganic salt medium. — — Urine (0.125 gm. nitrogen per liter) in distilled water.

From the Lockheed Missiles & Space Co., Research Laboratories, Palo Alto, California.

urea and the basal inorganic salt nutrient, urine plus a basal inorganic salt nutrient and urine in distilled water. *Chlorella* grows on urine supplemented with inorganic salts at a rate comparable to that of the complete median of urea and inorganic salts. However, for unsupplemented diluted urine, the growth rate of *Chlorella* is decreased. Therefore, dilute urine supports growth of *Chlorella* but is deficient in some inorganic salts necessary for maximum growth. In a practical photosynthetic gas exchanger, urine would always be used in a dilute form. Theoretical calculations show that the concentration of urea in urine is so concentrated that complete removal of nitrogen from undiluted urine is improbable. Under optimum growth conditions in the presence of excess urea, 1 mg. of urea is converted into 50.2 mg. of algal cells. If a concentration of 18 mg. of urea per ml. of urine is assumed,[3] one ml. of urine could yield 0.9 gm. of algae. Since algae have a density of approximately one, a culture growing under these conditions would contain 90 per cent of algal cells and only 10 per cent culture fluid. Considering other limiting factors in a photosynthetic gas exchanger, such as light penetration and CO_2 availability, urine would never be used undiluted. The practical approach to urine utilization is a slow addition of urine to a large volume of algal culture at a rate sufficient for maximum oxygen production. The ideal operational design of such a system would be a feeding rate that achieves maximum removal of nitrogen from the urine with maximum conversion of carbon dioxide to oxygen.

The results of growth experiments on individual nitrogen compounds are summarized in Tables I and II.

TABLE I. UTILIZATION OF NITROGEN COMPOUNDS BY *CHLORELLA PYRENOIDOSA*

Growth	No growth
Urea	Allantoin
Ammonia	Creatinine
Nitrate	Hippuric acid
Uric acid	βalanine
Adenine	
Guanine	
Xanthine	
Hypoxanthine	

TABLE II. UTILIZATION OF AMINO ACIDS BY *CHLORELLA PYRENOIDOSA*

Growth	No growth
Glycine	α amino butyric acid
l-alanine	Norvaline
l-serine	l-valine
l-glutamine	l-leucine
l-aspartic acid	l-isoleucine
l-asparagine	l-lysine
l-arginine	l-phenylalanine
l-ornithine	l-tyrosine
l-cirulline	l-tryptophane
	l-histidine
	l-cysteine
	l-glutamic acid

Urea, the major nitrogen compound in human urine, is an excellent source of nitrogen for *C. pyrenoidosa*. For long-term, high-density growth systems, urea is a more ideal nitrogen source than the two commonly used inorganic nitrogen sources, ammonium ion and

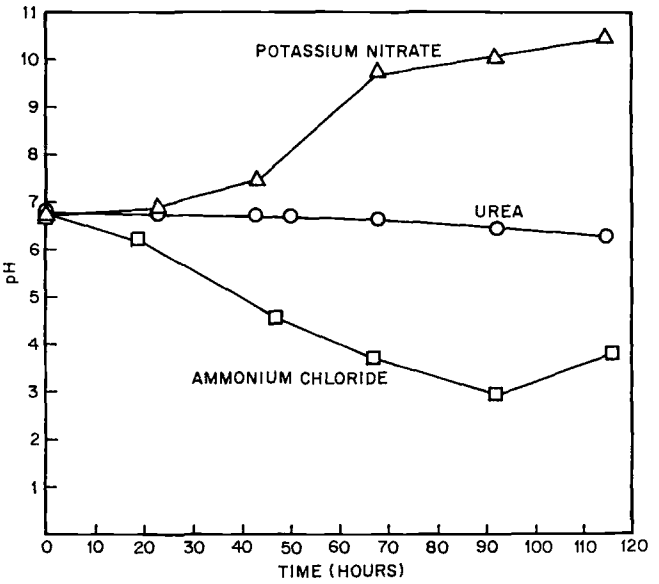

Fig. 2. pH changes in culture during growth of *C. pyrenoidosa*. Initial nitrogen cocentration – 0.005 moles per liter.

nitrate. Large pH changes occur during growth when the latter two are used (Fig. 2). Constant adjustment of the pH is required to maintain a favorable growth environment. Utilization of urea produces only minor changes in pH of the growth medium and no adjustment of the culture medium is required.

Ammonia is utilized by *C. pyrenoidosa*. Urine, as excreted, contains approximately 0.5 mg. ammonia per ml. This concentration of ammonia is not toxic to algae. They can grow and utilize ammonia when present in this amount. Under spacecraft conditions if the urine is stored, the possibility exists that urea will be decomposed by bacteria to yield ammonium carbonate. Under conditions of complete conversion, there would be approximately 30 mg. ammonium carbonate per ml. of urine. Growth of *Chlorella* with ammonium as a nitrogen source was tested (Fig. 3). At concentrations up to

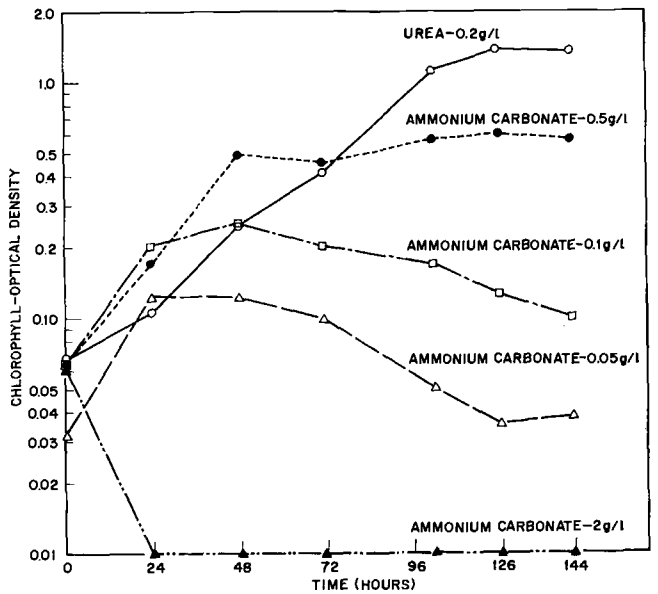

Fig. 3. Growth of *C. pyrenoidosa* with ammonium carbonate as a nitrogen source. Concentration expressed as grams of nitrogen compound per liter.

0.5 gm. per liter, growth was rapid as long as ammonia nitrogen was available in the culture medium. At high concentrations of ammonium carbonate (2 gm./liter), no growth was obtained, although a favorable hydrogen ion concentration, pH 8,[2] existed for penetration of ammonia into the cell. The growth obtained on ammonium carbonate was low when compared with that on urea. This is partially explained by loss of ammonia from solution caused by aeration during the growth period. One fifth of the total nitrogen was lost to the gas stream as ammonia in 24 hours from a control culture solution containing 0.5 gm. per liter of ammonium carbonate.

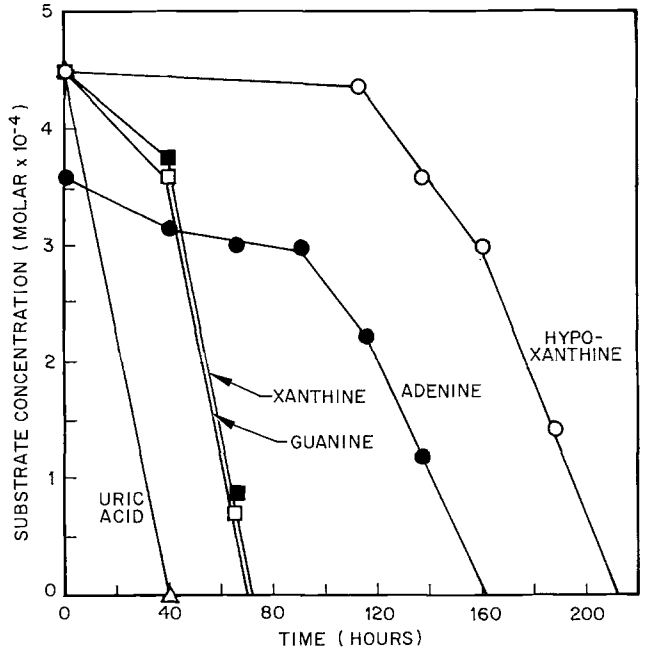

Fig. 4. Utilization of purines by *C. pyrenoidosa.*

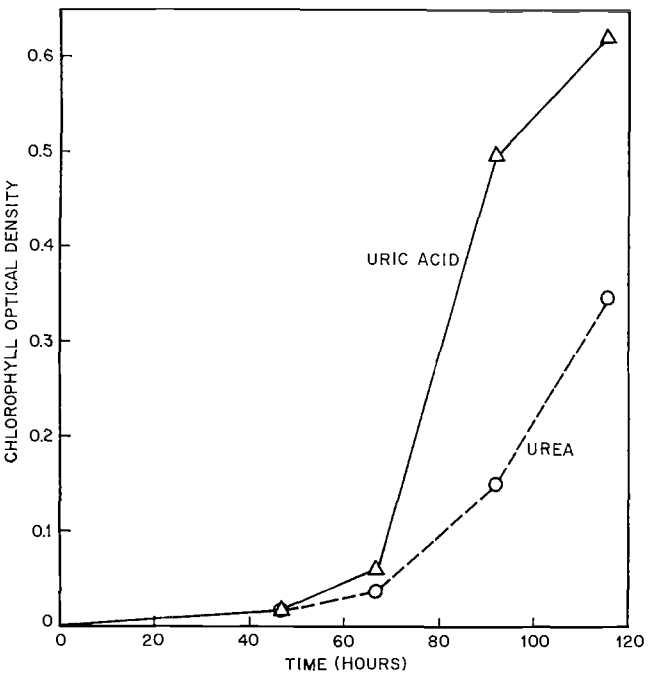

Fig. 5. Comparative growth of *C. pyrenoidosa* with uric acid or urea as sole nitrogen source.

These results indicate that conversion of urea to ammonium carbonate during urine storage should be prevented. The possibility exists that concentrations of ammonia toxic to the algae in the photosynthetic gas exchanger could be reached under conditions of complete hydrolysis. Conversion of urea to ammonium carbonate also increases the possibility of free ammonia escaping into the environment of the astronaut. This would place an extra burden on the cabin air purification system.

Purines, breakdown products of nucleic acid metabolism, are present in low concentrations in urine. Uric acid, guanine, adenine, xanthine, and hypo-xanthine can be used as a sole nitrogen for *Chlorella* (Table I, Fig. 4). Growth on uric acid shows an accelerated rate as compared with urea (Fig. 5). A closely related breakdown product of purines, allantoin (which is also present in urine), is not used as a nitrogen source. Allantoin may not be permeable to the cell wall of *C. pyrenoidosa,* since metabolic studies show that allantoin is an intermediate in purine metobolism of *Chlorella.*

Amino acids show wide differences in their ability to support growth (Table II). Figure 6 is presented to

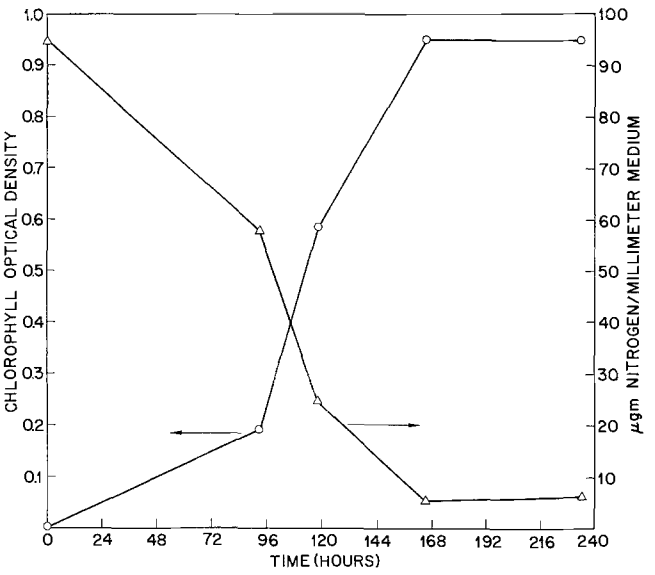

Fig. 6. Utilization of arginine by *C. pyrenoidosa.* Removal of arginine from the culture medium and production of algal cells expressed as chlorophyll optical density.

show the correlation between utilization of an amino acid, arginine, and synthesis of new cell material. As the algal culture grows as measured by increase in chlorophyll, there is a corresponding removal of arginine from the growth medium. When the arginine has been completely utilized, chlorophyll synthesis stops.

Amino acids which are active in metabolic cycles are utilized by the algae. The amino acids of the Krebs urea-ornithine cycle, arginine, ornithine and citrulline, are used as a nitrogen source for complete cell synthesis. Those amino acids that are closely related to the citric acid cycle are also utilized as a nitrogen source. Glycine, alanine, serine, and aspartic acid were all observed to support growth.

The amides of aspartic and glutamic acid, asparagine and glutamine, were also used as nitrogen sources. Both the amide nitrogen and amino nitrogen were used for growth. Figure 7 presents the data for glutamine utili-

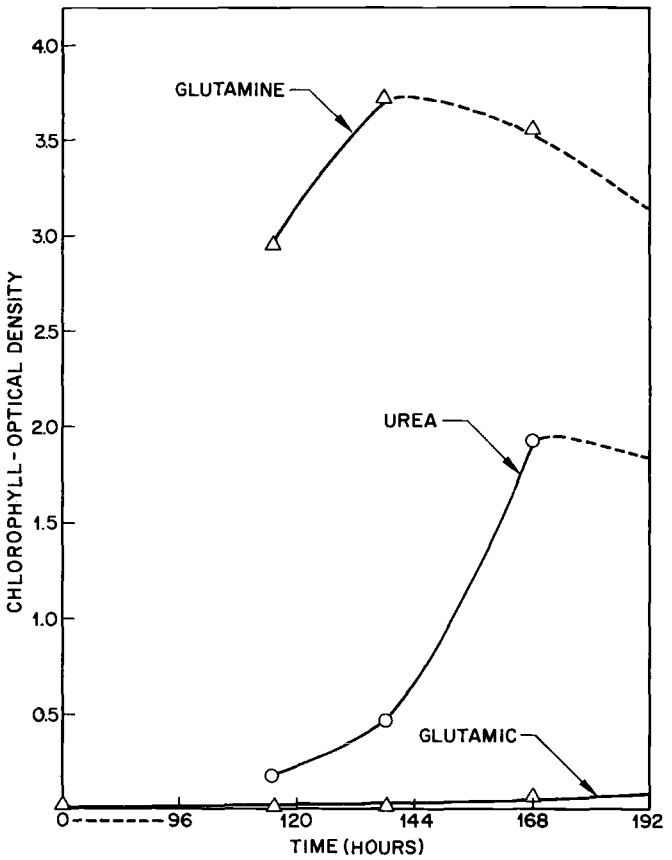

Fig. 7. Growth of *C. pyrenoidosa* with glutamine and glutamic acid as nitrogen source. Glutamic acid — 0.1 gm. nitrogen per liter, urea — 0.1 gm. nitrogen per liter, glutamine — 0.2 gm. nitrogen per liter.

zation. In these experiments, glutamine was added to the culture medium to give twice the nitrogen concentration as the control urea flask. The maximum growth obtained on glutamine was twice that on urea. Since both urea nitrogens are utilized for growth, it can be concluded that both glutamine nitrogens are also used for growth. Similar results were obtained with asparagine.

Glutamic acid is not used by *C. pyrenoidosa* under growth conditions where complete utilization of its amide is observed. The non-utilization of added glutamic acid may be related to its inability to penetrate through the algal cell wall under the environmental test conditions. The possibility also exists that glutamic acid is not an intermediate in the utilization of glutamine.[3]

As shown in Table II, many of the essential amino acids for man are not used by the alga. These cannot be used as a nitrogen source for growth. During recycling operations of urine feeding, these amino acids would accumulate in the culture medium. A recovery process for these essential amino acids might be desirable when they accumulate to a significant amount.

Creatinine and hippuric acid are not utilized by *Chlorella pyrenoidosa* for growth. Algae, growing on another nitrogen source such as urea, do not remove these compounds from the culture medium. Therefore, they will accumulate and, because of their relative high concentration in urine, might reach toxic levels during recycling of urine. Figure 8 shows that concentrations

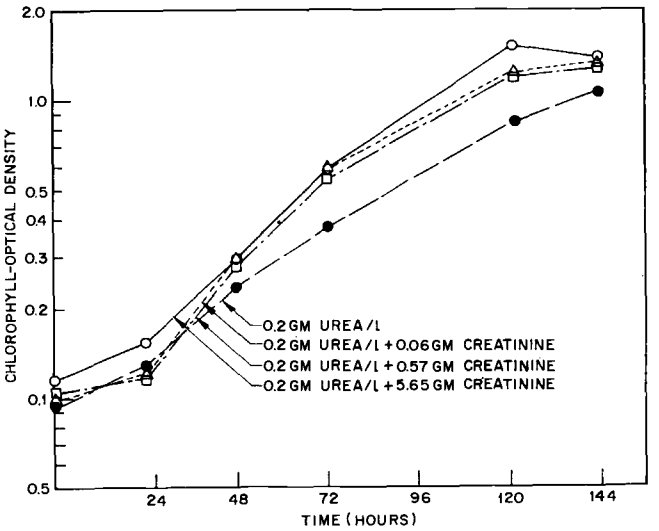

Fig. 8. Growth of *C. pyrenoidosa* on urea in the presence of creatinine.

of creatinine as high as 5.65 mg./ml. do not inhibit growth. Hippuric acid is not inhibitory at amounts up to 1.38 mg./ml. (Fig. 9). These levels are equivalent to

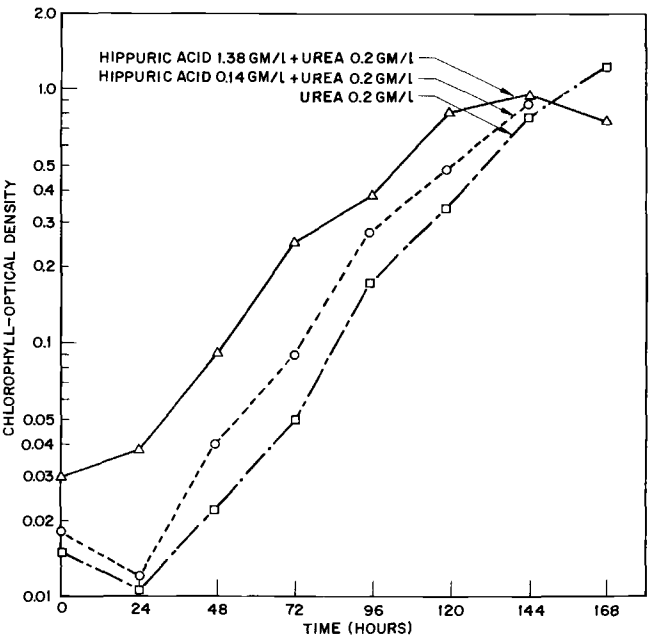

Fig. 9. Influence of hippuric acid on the growth of *C. pyrenoidosa* using urea as a nitrogen source.

100 recyclings for creatinine and 50 recyclings for hippuric acid when urine is used in a one-to-twenty dilution. Therefore, the accumulation of these compounds

in the medium after many recyclings will not inhibit growth of the algae.

Non-utilization of creatinine and hippuric acid is important from another consideration. Approximately four per cent of the urinary nitrogen is contained in creatinine and hippuric acid. If a truly closed system is attempted with urine as the sole nitrogen source, within a period of approximately 17 days 50 per cent of the originally available nitrogen will be trapped in these two compounds. Therefore, methods of converting creatinine and hippuric acid to usable forms must be developed or additional nitrogen added to the system.

CONCLUSIONS

Urine is a good source of the nitrogen compounds necessary for growth of algae. Ninety to ninety-five per cent of the urinary nitrogen compounds are utilized. However, in a long-term truly closed system, the unused nitrogen compounds become important and means of converting them to usable compounds should be developed.

Proper storage of urine from the time of collection to the time of addition to the algal culture is important. Conversion of urea to ammonium carbonate by bacterial action is undesirable because of the possibility of ammonia escaping from the algal system into the environmental system of the astronauts.

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