Table 1. Spectral data for the urinary substance and for 5-ribosyluracil from yeast ribonucleic acid (from *3*).

*p*H	$\lambda_{max.}$ (mμ)	$\lambda_{min.}$ (mμ)	Ratios			
			250/260	280/260	290/260	300/260
			Yeast Ribonucleic Acid			
2	263	234	0.74	0.43	0.07	
12	286	245	0.64	2.09	2.16	1.22
			Urine			
2	263	233	0.75	0.43	0.08	
12	287	246	0.65	2.09	2.19	1.29

the course of an investigation of nucleosides occurring in urine in man (*7*). The urine of two adult males was studied. One was a normal subject; the other was gouty. Both abstained from consumption of tea and coffee for the period of study. The urine specimens were adjusted to *p*H 9 with ammonium hydroxide and were filtered. The filtrate was allowed to percolate through an amount of Dowex 2 (8×, 20-50 mesh) ion-exchange resin in acetate form equivalent to one-sixth of its volume. After washing-in with ammonium acetate buffer (*p*H 9) and water, the column was eluted with water. The aqueous eluate was collected in ⅓ column-volume fractions until the initial $E^{1cm}_{260m\mu}$ of 15.0 decreased to a value of 0.3. The combined fractions were evaporated to dryness under reduced pressure and at temperatures below 50°C. The residue was extracted with 60 percent aqueous ethanol. The extract brought to dryness *in vacuo* was dissolved in a minimum amount of water and applied to Whatman 3 MM filter-paper sheets for descending two-dimensional chromatography (*8*). A solvent system composed of *n*-butyl alcohol and 0.6*M* ammonium hydroxide (6:1) (*9*) was used in the long dimension of the sheet for 58 hours, and a system composed of *n*-butyl alcohol, formic acid, and water (77:11:12) (*10*) in the second direction for 17 hours. A spot which strongly absorbed ultraviolet light, located nearest the origin of the chromatogram, contained the uridine isomer. An extract of this spot gave the absorption spectrum shown in Fig. 1. The close correspondence of the spectral data with those reported for the uridine isomer derived from yeast ribonucleic acid by Yu and Allen (*3*) is indicated in Table 1.

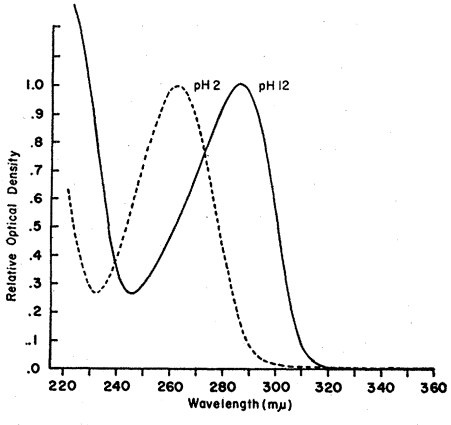

Fig. 1 Absorption spectra of the urinary uridine isomer.

A comparison of the chromatographic mobilities of the two compounds in four solvent systems was made (*11*). The relative mobility values of the spot extract coincided with those of authentic 5-ribosyluracil. Thus, in solvent system A [isobutyric acid, ammonia (0.5) (10:6)], the relative mobilities of the authentic sample and of the urinary product were 0.88 and 0.85, respectively (mobility of uridine = 1.0); in solvent system B [isopropanol, acetic acid, water (6:3:1)], 0.66 and 0.60; in solvent system C [tertiary amyl alcohol, water, formic acid (6:3:1)], 0.76 and 0.73; in solvent system D [butanol, water (86:14)], 0.53 and 0.48.

With three solvents (A, B, and C) the urinary product appeared homogeneous. The fourth solvent (D), however, revealed a small residual spot at its origin, indicating some impurity. We therefore used solvent D for further purification of a larger sample by band chromatography, obtaining a microcrystalline product.

A rough estimate of the amounts of the uridine isomer excreted in 24 hours was obtained by applying the figure for $\varepsilon_{max} = 7.5 \times 10^3$ at *p*H 2 given by Yu and Allen (*3*) to the spot extracts. Values of 18 mg/24 hr for the urine of the normal subject and 41 mg/24 hr for the urine of the gouty subject were obtained. Further studies are in progress to determine whether these differences in the gouty and nongouty subjects are consistent. In any event, both figures are in excess of the quantities of free purine bases reported previously in human urine (*8*).

The possibility that the uridine isomer found in the urine is derived from the diet is not excluded, although foods rich in nucleic acids, as well as coffee and tea, were omitted by the two subjects investigated. An endogenous origin is considered more likely, however. This would imply that the new uridine isomer occurs in human tissue, and this is in accord with the reported presence of the isomer in dog pancreas (*12*). It may belong to the class of naturally occurring "additional" nitrogenous constituents of ribonucleic acid which thus far are known to include 5-methylcytosine, various methylated purines, and thymine (*13*).

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Production of Large Amounts of Plant Tissue by Submerged Culture

Abstract. The growth rate of plant tissue cultures is substantially increased through the use of a large-volume carboy system. Aeration is considered to be the most important factor. With this system, yields of a pound or more of tissue can be obtained within 2 weeks.

The methods of plant tissue culture have suggested a way to put the metabolic systems of plants to work under controlled conditions. A prerequisite for the most efficient use of this idea would be the large-scale production under submerged conditions of the tissue or tissues involved. Although the growth of microorganisms under these conditions is now commonplace, and advances in this direction with animal cells and tissues have been made (*1*), no such work has been reported for plants.

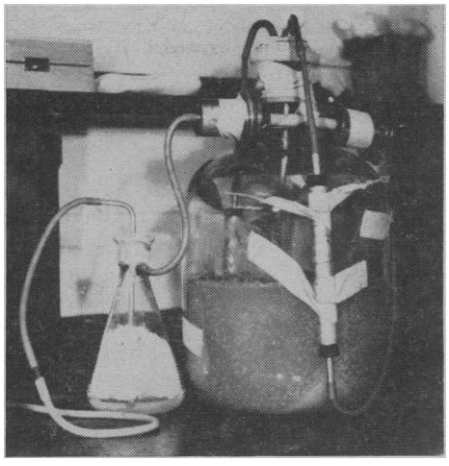

Fig. 1. The carboy culture apparatus. Aeration and agitation are accomplished by the introduction of compressed air (on the left) through a side-arm trap and then through two Seitz filters, which sterilize the air.

Since this approach constitutes part of the tissue-culture program in this laboratory, some of our results on the large-volume growth of plant tissue cultures are presented here.

The principal tissue used for large-volume culture was one obtained from Paul's Scarlet rose stems. This tissue was selected because of its rapid growth rate on solid media and the fact that it grows well in liquid culture. The medium used for carboy studies was made up of White's basal medium (*2*), 2, 4-dichlorophenoxyacetic acid (6 parts per million), yeast extract (0.1 percent), and malt extract (0.1 percent).

The inoculum for the carboy is prepared by taking the tissue culture from a test tube and placing it in liquid medium in a 300-ml erlenmeyer flask. This culture is then agitated on a New Brunswick gyrorotatory shaker. After 1 week's growth, the contents of one flask (the culture in 100 ml of medium) is transferred to a Fernbach flask containing 900 ml of medium. This flask is also aerated and agitated by shaking. After an additional week's growth, the Fernbach culture (1000 ml) is inoculated into a 20-liter carboy containing 9 liters of medium. This is the final stage of this particular scale-up. For purposes of aeration and agitation of the culture during growth, the carboy is fitted with stainless-steel tubing to receive compressed air, which is sterilized by passage through two Seitz filters (Fig. 1). In addition, appropriate connections are included for an air outlet, the addition of medium, and sampling. In all of these manipulations, aseptic procedures must be followed.

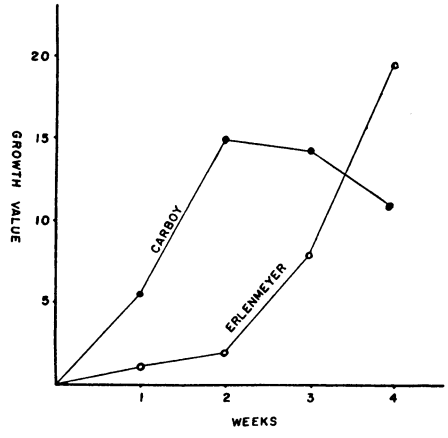

Fig. 2. Growth curves for Paul's Scarlet rose stem tissue grown in carboy culture and in erlenmeyer flasks. The growth value equals the ratio (final fresh weight): (initial fresh weight).

Samples of the carboy culture were made at weekly intervals and compared with results obtained from the same tissue grown on a New Brunswick gyrorotatory shaker in 300-ml erlenmeyer flasks containing 100 ml of culture. These results, shown in Fig. 2, indicate an accelerated rate of growth in the carboy for the first 2 weeks. Growth values (final wet wt./initial wet wt.) of 5.5 and 14.9 are attained by the tissue grown in the carboy system after 1 week and 2 weeks, respectively, as compared to growth values of 1.1 and 1.9 for the same periods with the shaker cultures.

The difference in growth rates between these two systems during the first week is due to the method of aeration. At the end of the first week of growth, 2 liters of medium were added to the carboy; the effect of this supplementation is an increase in rate of growth during the second week. When no further additions are made, the rate of growth drops off sharply, as shown by the growth values for the third and fourth weeks.

Other tissues also grow well in this carboy system of culture. The data available thus far for tissues from *Ilex* stem, *Lolium* endosperm (*3*), and *Ginkgo* pollen (*4*) indicate growth increases similar to the increase found with the Paul's Scarlet rose stem. The *Ginkgo* pollen tissue, for example, yielded 2 lb of cells after 4 weeks in carboy culture.

Other factors recognized as important in increasing the growth rate of plant tissue cultures are those well known to microbiologists working with large-volume cultures. Besides the necessity for an adequate nutrient medium, which itself may be improved in numerous ways, a number of physical factors are involved. In addition to the method and efficiency of aeration, which have already been stressed, the temperature of incubation and the means of agitation are important. More dependent on the tissue culture itself are such things as the age of the inoculum and the particular strain of tissue selected for growth. Moreover, the maintenance of a maximum growth rate rests on such things as timely addition of medium supplements, minimizing of foam production, and proper buffering of the hydrogen ion concentration.

From a practical standpoint, the carboy system of culturing plant tissues is a step in the direction of decreasing the lag phase of growth and increasing the ultimate yield. Our results emphasize the importance of timely nutritional supplements to the medium and of adequate aeration for the rapid growth of certain plant tissue cultures. The aeration factor receives major consideration in microbial "fermentations," but investigations with plant material have not been concerned with the growth of large amounts of material. In the roller-tube technique employed by Steward. Caplin, and Millar (*5*) and the variable-speed shaker technique used by Muir, Hildebrandt, and Riker (*6*), aeration has been studied, but the aim has not been the production of large yields (*7*). Another difference is that in both of these systems the agitation provides the aeration, whereas in the carboy system the aeration provides the agitation and at the same time furnishes a continuous flow of air.

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Pipecolic Acid in Leaves of Strawberry Plant as Influenced by Treatments Affecting Growth

Abstract. Growth inhibition due to maleic hydrazide spray or to an unsatisfied chilling requirement caused young fully expanded leaves of the strawberry to accumulate pipecolic acid to a very striking degree. It is postulated that the accumulation resulted from blockage in normal metabolic conversion to other intermediary nitrogenous compounds.

Pipecolic acid is a naturally occurring cyclic imino acid that has been isolated from several plants and identified chromatographically in many others (*1*, *2*).