Certain —SS— compounds also gave protection against oxidant toxicity; both dimethyl disulfide and hydrogen polysulfide significantly protected intraperitoneally injected mice, and dimethyl disulfide and *tert*-butyl disulfide protected via inhalation. It is noted that purified H_2S provided less protection than that shown in Table 1 for a technical grade. This difference in protective ability is attributed on infrared evidence to the presence of —SS—, possibly hydrogen polysulfide, in the technical H_2S, and inasmuch as addition of —SS— to purified H_2S reinforced its protective ability.

The antagonism of oxidant toxicity by sulfur compounds is physiologic and not the result of chemical combination prior to inhalation to form less active compounds. Analyses of chamber atmosphere for oxidant gas showed essentially no change upon addition of sulfur compounds. Moreover, protection was conferred by the injected compounds. Histologic examination of pulmonary tissue showed degrees of tissue change that paralleled the protective effect. Pulmonary edema and cellular infiltration, characteristic responses of oxidant exposure, were markedly inhibited in the protected groups.

The mechanisms involved in the protection are, as yet, unknown. The functional unit appears to be —SH or —SS—, or both, but not —S—; dimethyl-sulfide and thiophene were ineffective. Significantly, —SH and —SS— are characteristic of compounds conferring protection against ionizing radiation (*9*). Brinkman and Lamberts (*10*) have called attention to the possible radiomimetic properties of O_3 by showing that O_3 and irradiation produced the same defect in oxygen consumption of the skin of the finger; also cysteamine was equally protective against the effects of both agents. Likewise, Fetner (*11*) has shown a similar capacity of O_3 and x-rays to produce chromosomal aberrations in *Vicia faba*. The action was presumably mediated through the OH and HO_2 radicals formed from the aqueous decomposition of O_3; the separate effects of O_3 plus irradiation were fully additive.

Single injections of MEG (2-mercaptoethylguanidine-HBr), highly protective against radiation at 200 mg/kg, gave modest protection against NO_2 (mortality: 8/20 S treated, versus 12/20 for NO_2 alone) and O_3 (13/20 versus 19/20). When given in a series of three injections (100 mg/kg on each of 3 days), however, MEG provided greater protection (2/20 versus 10/20 for NO_2 alone). Similarly, 0.04 mg/kg of BAL (2,3-dimercaptopropanol) provided some protection to mice exposed to NO_2 (6/22 versus 12/22 for NO_2 alone) but did not decrease O_3 toxicity (13/25 versus 12/25 for O_3 alone). Thus, there is similarity of action of protective compounds which favors the view that the mechanism of action of the oxidants O_3 and NO_2 may be in part similar to that of x-irradiation.

Another means of protecting against the lethal effects of NO_2 and O_3 was achieved by still other sulfur compounds, α-naphthylthiourea (ANTU) and phenylthiourea (PTU). Mice exposed to 5.8 ppm of O_3 the day following the last of 3 intraperitoneal injections of ANTU (10 mg/kg every other day) and rats which received 128 ppm of NO_2 18 days after administration of ANTU (8 mg/kg) showed marked tolerance to the oxidants. Comparative mortalities were: mice, 0/15 versus 12/15 for controls; rats, 1/6 versus 6/6 for controls. Similarly, PTU (8 mg/kg) given in four injections every other day and exposed to either O_3 or NO_2 3 days after the last injection produced good protection (4/10 versus 9/10 for O_3 alone, and 4/10 versus 8/10 for NO_2 alone). Although the mechanism presumably still involves reactive sulfur constituents, the pathway is obviously different from that mediated by —SH and —SS— compounds because the tolerance afforded by ANTU and PTU persists, a condition not met by the simple —SH and —SS— compounds. Also, unlike —SH and —SS—, simultaneous treatment with oxidant and ANTU (itself a potent producer of pulmonary edema) produces additive toxicity, instead of protection. It has been demonstrated that rats develop a marked tolerance to ANTU (*12*) and also to O_3 (*1*). The cross tolerance between ANTU and oxidant indicates that the basic mechanisms may be similar. Furthermore, it appears that the tolerance mechanism is related to the edema mechanism, and the latter to sulfur balance, inasmuch as —SH (cysteine) blocks the lethal effects of ANTU (*13*), whereas —SH or —SS—, or both, reduces oxidant toxicity.

It is felt that the antagonism to oxidants displayed by certain sulfur compounds is highly significant for the insight it provides concerning possible mechanisms for protection against air pollutants, and also toward elucidation of the hitherto unexplained toxic action of oxidants (*14*).

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Uridine Isomer (5-Ribosyluracil) in Human Urine

Abstract. A substance isolated from human urine by anion exchange absorption and paper chromatography was found to correspond in its ultraviolet absorption spectrum and chromatographic mobilities with a uridine isomer (5-ribosyluracil) recently described as a component of yeast ribonucleic acid.

In 1957 Davis and Allen (*1*) reported the isolation from yeast ribonucleic acid of a new nucleotide ("the fifth nucleotide") and described its physical and chemical properties, notably a characteristic bathochromic shift in the ultraviolet absorption spectrum at alkaline *p*H. The corresponding nucleoside, prepared by the action of intestinal phosphatase, was subjected to hydrazinolysis, and the carbohydrate component was identified as D-ribose. Cohn (*2*) also obtained from yeast ribonucleic acid an "apparently modified uridylic acid" identical with the "fifth nucleotide" of Davis and Allen.

The structure of this new nucleotide proved to be of unusual interest. Recent investigation by Yu and Allen (*3*) revealed that the nitrogenous component is uracil and that the D-ribose, which has the furanose configuration, apparently is attached to the pyrimidine ring at the 5 position. Methylation studies by Scannell, Crestfield, and Allen (*4*) also indicated the structure to be 5-ribosyluracil. Further evidence for this formulation was obtained by Cohn (*5*) by means of periodate oxidation and nuclear magnetic resonance spectra. The C—C ribosyl linkage reported for the uridine isomer is unique thus far for components of nucleic acid, but C—C glycosyls occur in certain natural products (*6*).

A compound identical in spectral and chromatographic properties with this isomer of uridine was encountered in

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.

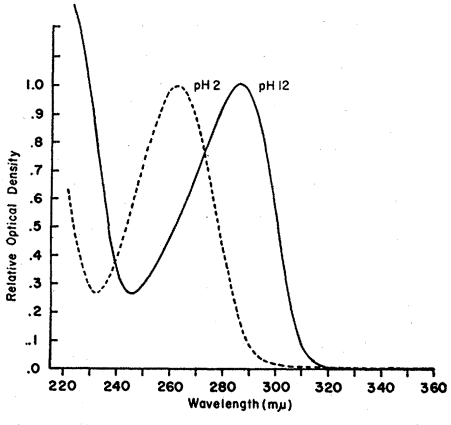

Fig. 1 Absorption spectra of the urinary uridine isomer.

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.