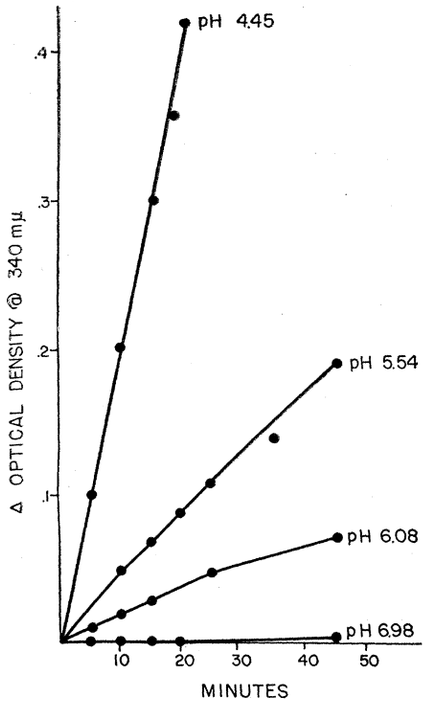

Fig. 1. Acid-catalyzed oxidation of DPNH.

kinetics at a given acidic *p*H. The *p*H dependency of the reaction rate is indicated in Fig. 1 for DPNH in phosphate buffers. The decrease in optical density is recorded as positive integers.

When the oxidative rates are compared for equivalent concentrations of DPNH and TPNH at constant acidic *p*H, the rate for TPNH oxidation is approximately 3 times that for DPNH oxidation. This is shown in Fig. 2.

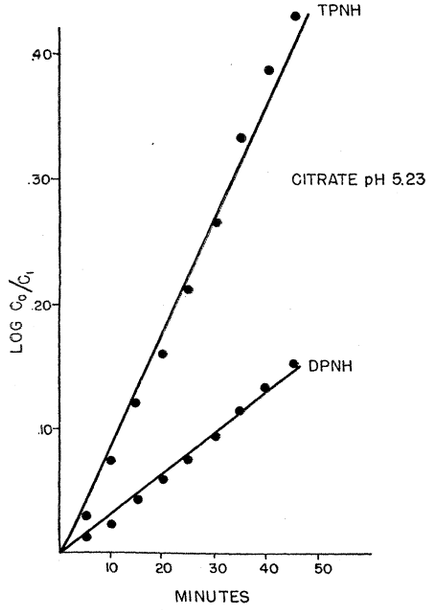

Fig. 2. Acid-catalyzed oxidation of TPNH and DPNH.

Preliminary experiments suggest that the reaction does not involve heavy metal catalysis, for no inhibition is observed with 1×10^{-2} m*M* NaCN; nor is there any curtailment of activity noted in a metal-free buffer prepared from "tris" (tris-hydroxymethylaminomethane) and HCl. However, this aspect is being investigated more fully, since oxidative catalysis without heavy metal participation is indeed a singular event. Evidence thus far points to the pyridine moiety as being the sole locus of oxidation. The purine component remains unaltered, as evidenced by measurements at 260 mμ.

The biochemical importance of the reaction becomes apparent when it is recalled that TPNH exists in considerable excess over triphosphopyridine nucleotide in all tissues (*2*). The interpretation generally given is that the oxidation of TPNH is a rate-determining step in the various biosynthetic reactions in which it is linked (*3*). This is probably true for normal cells, which function in the *p*H range of 7.0 to 7.4. However, for malignant tumor cells, which metabolize in decidedly acidic *p*H (*4*), primarily because of accumulated lactic acid (*5*), the oxidation of TPNH would not be rate-limiting. This reaction therefore represents a means by which a tumor cell exercises metabolic superiority over the normal cell. It also explains the so-called "nitrogen trap" mechanism usually associated with tumors, for the accelerated TPNH oxidation would result in the sparking of biosynthetic reactions.

The reaction may also play a key role in wound healing and muscle contraction, processes in which there is a local sharp drop in *p*H (*6*). In wound healing, the injured tissue, aided by the nonenzymic oxidative catalysis, would emphasize the synthetic reactions of repair until the *p*H returns to neutrality. Lactic acid formed in muscle contraction would stimulate the TPNH-linked synthesis of glycogen. The difference between the malignant tumor and these two processes is that there is no return to neutrality in the case of the tumor (*7*).

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References and Notes

1. DPNH (Sigma), enzymatically reduced diphosphopyridine nucleotide, 90 percent pure; TPNH (Sigma), enzymatically reduced triphosphopyridine nucleodtide, 96.5 percent pure.
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7. I acknowledge with gratitude the assistance of Marian Berman in preparing the figures.

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L-Tyrosine Oxidase System in Tuber of Nutsedge

Abstract. In the nutsedge, *Cyperus rotundus* L., the oxidation of L-tyrosine follows steps similar to those reported for mammalian liver and *Blattella conjuncta*. The system is activated by α-ketoglutarate, L-ascorbic acid, glutathione, and pyridoxal phosphate. Folic acid markedly inhibits the reaction.

Previous studies (*1*) have shown that the nutsedge tuber has an active tyrosinase. Ascorbic acid activated 3,4-dihydroxyphenylalanine (DOPA) oxidase, but ascorbate inhibited the complete oxidation of this compound to hallochrome. The dormant tuber contains large quantities of ascorbic acid (115 mg/100 g of fresh weight), and it is evident that this vitamin undoubtedly plays a vital role in the metabolism of the tuber. The function of ascorbic acid in the L-tyrosine oxidase system has been shown for mammalian liver (*2, 3*) and *Blattella conjuncta* (*4*).

The purpose of the present study was to follow the oxidation of L-tyrosine by nutsedge tuber tissue as influenced by α-ketoglutarate and certain cofactors.

Dormant nutsedge tubers were taken from greenhouse pots. The tubers were homogenized at 2°C in a chamber of the Omni mixer which contained 100 ml of 0.2*M* potassium phosphate buffer at *p*H 6.8. The suspension was strained through one layer of cheesecloth, and the filtrate was centrifuged at 1300*g* for 10 min at 2°C.

Activity was determined by conventional Warburg methods at 25°C. The vessels contained the cofactors and substrates in a total volume of 2.4 ml of 0.2*M* potassium phosphate buffer. A 0.6-ml portion of the homogenate was placed in the side arm. The reaction was initiated by tipping the side arm. The final *p*H for the treatments was

Table 1. Effects of α-ketoglutarate and other components on the tyrosine oxidase activity of a homogenate of nutsedge tubers.

Components added to homogenate	Activity $Q_{O_2}^{O_2}$ (N)
None	17
α-ketoglutarate (α-K)	26
α-K + folic acid	3
α-K + ascorbate	31
α-K + pyridoxal phosphate	36
α-K + glutathione	56
α-K + glutathione + ascorbate	37
α-K + glutathione + ascorbate + pyridoxal phosphate	68
None + glutathione + ascorbate + pyridoxal phosphate	31
α-K + glutathione + ascorbate + pyridoxal phosphate + folic acid	18
None + glutathione + ascorbate + pyridoxal phosphate + folic acid	30

7.0. Activity measurements were taken for 1 hr.

The final concentrations of L-tyrosine and α-ketoglutarate were $6.8 \times 10^{-3}M$ and $5 \times 10^{-2}M$, respectively, while the final concentration of the cofactors was: ascorbic acid, $5 \times 10^{-2}M$; pyridoxal phosphate, $5 \times 10^{-6}M$; reduced glutathione, $3.3 \times 10^{-3}M$; folic acid, $5 \times 10^{-4}M$.

Table 1 shows results of a typical experiment. Activity was stimulated by α-ketoglutarate alone, or in combination with either ascorbic acid, pyridoxal phosphate, or glutathione, but the greatest effects were obtained with a single combination of these. Folic acid with only α-ketoglutarate or in combination with the afore-mentioned cofactors markedly inhibited activity. These results are contrary to the stimulation by folic acid reported for this system in mammalian tissue (*3*) and the cockroach (*4*). Preliminary studies (*5*) indicate that folic acid is required for the oxidation of L-phenylalanine to L-tyrosine in the nutsedge homogenates.

It appears that the oxidation of L-tyrosine by homogenates of nutsedge follows a pathway in which tyrosine is deaminated by a transamination reaction with α-ketoglutarate, which requires pyridoxal phosphate (*6*) and yields *p*-hydroxyphenylpyruvic acid (Fig. 1). This acid is oxidized to homogentisic acid and carbon dioxide. This system in nutsedge evolved 115 $Q_{CO_2}^{O_2}$ (N) with the combination of cofactors in Table 1 that gave the greatest activity. The intermediate, 2,5-dihydroxyphenylpyruvic acid, is involved in the process which requires ascorbic acid and glutathione.

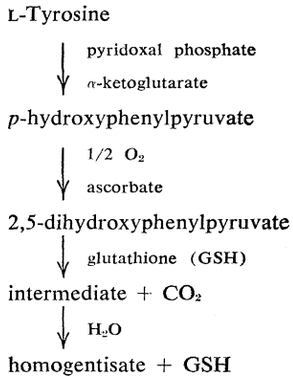

Fig. 1. A proposed scheme for the oxidation of L-tyrosine in *Cyperus rotundus* L.

The present study establishes that L-tyrosine is oxidized by a pathway similar to that reported in mammals and the cockroach (*7*).

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Correlation of Nuclear Volume and DNA Content with Higher Plant Tolerance to Chronic Radiation

Abstract. The causes of extreme differences in radiosensitivities of different plant (or animal) species have long puzzled radiobiologists. Our investigations show that, for diploid species, the larger the nuclear volume, the more radiosensitive the organism. Correspondingly, species with large nuclei have more deoxyribonucleic acid (DNA) per nucleus than those possessing small nuclei. Our data now make it possible to predict fairly accurately the tolerance of plant species to ionizing radiation on the basis of average nuclear volume and DNA content. The same correlations are expected to hold for some microorganisms and for animals and may explain differences in sensitivities of different cell types in many living organisms.

It has been noted in previous investigations (*1–3*) that the chromosome size characteristic of a species is related to the amount of radiation required to produce a given effect. In view of the tedious nature of accurately determining chromosome size or volume, no really quantitative data have been presented. We assumed that differences in chromosome size or volume in different species would be reflected in comparable differences in size or volume of interphase nuclei (*4*). On this basis, average nuclear volumes of a number of plant species of known radiation tolerance have been determined.

A summary of preliminary information concerning the tolerance of higher plants to chronic gamma irradiation from cobalt-60 was given by Sparrow and Christensen (*2*). In a later report 79 species of plants were grouped according to daily dose rates required to produce a comparable degree of morphological deformity or growth inhibition. The dose rates required varied from 30 to 6000 r/day. The most resistant plants were polyploid (*3*). In most of our tolerance experiments accurate measurements of growth inhibition were not made, but the row (or group) of plants which appeared to have shown not more than about 10 to 20 percent of the normal growth was chosen as the critical dosage level. Thus, the end point used was an approximation which can be assumed to be the daily dose rate which would essentially stop further cell division. In our studies the critical dosage level as determined above seems sufficiently valid because the possible error for each species is relatively small compared to the variation between species. By using the degree of growth inhibition as a measure of radiation damage, we have found that plant species ranging from algae to higher plants differ in their tolerance by a factor of at least 5000. The tolerance difference was still at least 100-fold when algae and known polyploid species were eliminated.

Collections for nuclear volume studies were made from growing plants before floral transition. Shoot apices were killed, fixed in Craf III, dehydrated, and infiltrated with paraffin by the use of a tertiary butyl alcohol series. Sections were cut at 10 μ and stained with safranin-fast green. The diameters of interphase nuclei in the tunica layer(s) and outer corpus of the vegetative meristems were measured with a Zeiss ocular micrometer. Ten nuclei on each of two slides were measured for each species, and average nuclear volumes were calculated. The deoxyribonucleic acid (DNA) was extracted from root-tip material with sodium chloride, a modification of the Schmidt-Thannhauser procedure (*5*), and the amount of DNA was estimated by the