Catalase Photoinactivation

Abstract. *The enzymatic activity of catalase is lost during exposure to sunlight in the presence of oxygen. A simultaneous decline occurs in the absorption peak at 405 nanometers.*

Carotenoids accompany chlorophyll in all aerobic photoautotrophs able to survive in nature (*1*). Carotene protects chlorophyll from photodestruction. Eyster (*2*), working with another porphyrin, reported that the catalase activity of carotenoid-deficient seedlings was much lower than the catalase activity of normal seedlings. Evidence of the photodestruction of catalase by visible light is reported here.

Crystalline catalase from beef liver (Nutritional Biochemicals, Inc.) was suspended in 0.01*M* potassium phosphate, *p*H 7.0, the concentration during the subsequent exposure to light being 0.01 mg of enzyme per milliliter. The source of light was the sun. Intensities obtained by direct radiation were 99,000 to 110,000 lu/m^2. A Wratten filter (2c) was used to cover the solution during exposure. This filter absorbed virtually all radiation below 390 nm. Effects of oxygen during illumination were evaluated by leaving the samples open to the atmosphere. To obtain relatively oxygen-free conditions, samples were alternately flushed with nitrogen containing no oxygen and evacuated three times. Catalase was assayed according to the procedure of Chance and Maehly (*3*) with the following dilution modifications. Nine milliliters of 0.057*M* H_2O_2 in 0.01*M* potassium phosphate buffer, *p*H 7.0, was combined with 1 ml of enzyme preparation at zero time, and the reaction was allowed to proceed for 3 minutes. At the end of the reaction time, 1 ml of the mixture was placed in 10 ml of 0.4*N* H_2SO_4. This acid-denatured enzyme-H_2O_2 solution was then titrated with 0.002*M* $KMnO_4$ to determine the residual amount of H_2O_2.

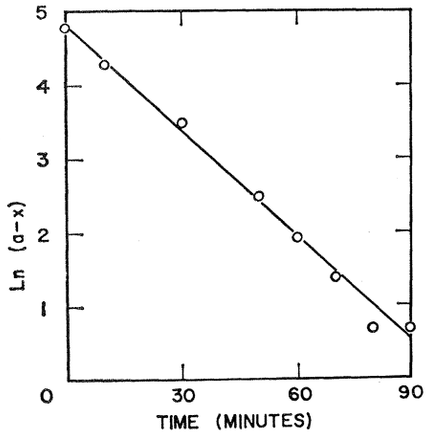

Fig. 1. Rate of catalase inactivation with increasing illumination time; *a*, initial concentration of enzyme in μg/10 ml; *x*, amount of catalase destroyed at time *t*.

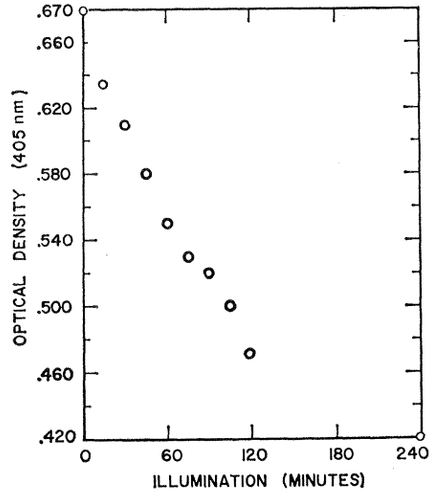

Fig. 2. The optical density decrease at 405 nm during a 240-minute interval.

Under vacuum, 92 percent of the activity of the catalase enzyme was retained after 30 minutes of exposure to sunlight. Aerobic samples lost all their activity from this sunlight exposure.

The rate of enzyme inactivation follows that for a first-order reaction (Fig. 1). Concomitant with the activity loss, the optical density of catalase at 405 nm (its visible absorption peak) declines as indicated in Fig. 2. The decrease in optical density follows a different kinetic pattern from that which describes the loss of enzymatic activity (*4*). We postulate that the enzyme was inactivated through alteration of one of the four porphyrin moieties within a catalase molecule, even though the remaining three retained their normal absorption spectrum. With prolonged illumination, all porphyrins would be altered.

Inactivation of catalase thus occurs in visible light in the presence of oxygen. There was a simultaneous decline in enzyme activity and absorption at the 405 nm peak during illumination.

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Polonium-210 in Leaf Tobacco from Four Countries

Abstract. *Tobaccos grown in the United States, Rhodesia, South Africa, and New Zealand, have been measured for their polonium-210 content. Details of the method of measurement are given and the results are listed. A mean of 0.15 picocurie per gram has been found in New Zealand tobacco, compared with 0.49 picocurie per gram in United States tobacco. The concentration in South African tobacco was approximately the same as in United States tobacco, but the level in Rhodesian tobacco appeared to be significantly higher.*

The naturally occurring, alpha-emitting radioelement polonium-210 is found in plants generally, and its presence in tobacco is of special significance because this radioelement is volatile at the temperature of the burning cigarette, attaches itself readily to smoke particles, and thereby gains direct access to the lung. The report by Radford and Hunt (*1*) on the amounts of Po^{210} in four brands of American cigarettes and their assessment of the significance of inhaled Po^{210} as an initiator of lung cancer is of special interest.

Tobacco is normally aged for a period of 1 to 2 years between harvest and manufacture. This delay allows 138-day Po^{210} to approach equilibrium with its parent once removed—22-year lead-210 present in the leaf. The lead-210 in the plant may be derived from the soil or deposited on the leaves as "natural fallout" resulting from the decay of atmospheric radon-222. Any Po^{210} unsupported by lead-210 which might be present in the plant at harvest would largely disappear by radioactive decay during the ageing period.

Preliminary measurements of Po^{210}

in a few tobacco products manufactured in New Zealand were made by the procedure of Radford and Hunt (*1*). The results indicated that the Po^{210} in the New Zealand products was less than half that reported for the American products. For a more thorough survey, samples of locally grown and imported tobaccos used in the manufacture of tobacco products sold in this country were obtained. On receipt, each sample was finely macerated in a high-speed blender, thoroughly mixed in a rotary mixer, and stored in a glass jar.

The hot concentrated HCl-digestion procedure (*1*) used for solution of the cigarettes resulted in much undigested material, and even when HNO_3 was substituted there were indications that complete oxidation had not been obtained, even though solution was complete. The following "wet-ashing" technique was therefore used on all samples. Two grams of air-dried sample was digested for 1 hour with 50 ml of concentrated HNO_3 in a 400-ml beaker with cover. The solution was evaporated to low volume, and 3 ml of concentrated H_2SO_4 was added. Evaporation was continued until the solution darkened. After cooling, 2 ml of concentrated HNO_3 was added and the solution evaporated until white fumes started to evolve. Two-milliliter portions of HNO_3, HCl, and H_2O_2 were then added and the solution was evaporated to fumes. This step was repeated. Then HNO_3 was eliminated from the digest by evaporating to fumes twice with 2 ml of HCl and twice with water. The solution was neutralized with saturated NaOH solution; it was diluted to 200 ml in 0.5*M* HCl, and 100 mg of ascorbic acid was added.

The spontaneous plating of polonium on silver foil at 95°C was initially done in the modified plating cell made from a commercial baby nursing bottle with the bottom removed (*2*). When the plating cell was placed in the water bath, however, expansion of the screw cap holding the silver disc caused loss of sample. This problem was not solved with nursing bottles available here and it was soon evident that an improved plating cell would consist simply of the original beaker containing the digest solution with a holder for the silver disc resting on the bottom of the beaker.

The holder (Fig. 1) consists of a base and screw top machined out of polytetrafluoroethylene (Teflon) and presents only one surface of the silver disc to the solution. The entire plating apparatus consists of a hot plate to accommodate four beakers. The beakers are fitted with polyethylene covers with central holes for the glass stirring rods which are driven by a top drive assembly. Plating is for 5 hours at incipient boiling point, the temperature being controlled by means of an energy regulator. Apart from simplicity, this procedure has the advantage, particularly for carrier-free activities, that all operations are carried out in the same beaker. Glassware and disc holders were decontaminated by scrubbing with detergent and rinsing in concentrated HCl and finally distilled water. Silver discs were prepared for plating by scrubbing with a mild abrasive cleaner, rinsing in concentrated HCl, and degreasing in acetone.

After plating, the discs were mounted with ZnS on Mylar and the alpha activity was measured by scintillation counting. The counters were calibrated by plating and counting portions of an Amersham standard solution of radium D, E, and F in equilibrium. Complete recovery of Po^{210} from the standards was obtained in 5 hours' plating, the supernatants yielding no detectable net activity when replated on fresh discs. The efficiency of the counters was 50 percent within the limitations imposed by the uncertainty of the standard which has an "effective standard deviation" of less than 5 percent. To check the overall recovery of the method, tobacco samples were spiked with aliquots of the standard, wet ashed, and plated. After making allowances for the original Po^{210} activity of the sample, when significant, the recoveries were 98.0 ± 2.0 percent, indicating no significant loss with the method. The mean of several reagent blank determinations was 5.0 ± 0.3 counts per hour and this has been subtracted from all sample counts. Included in this blank activity is the activity of the silver foil and ZnS phosphor, approximately 3 counts per hour. Counter stability was checked by frequent control runs with a radium source and also by observations of the decay of the standards which had been plated at different times during the course of these measurements. All observations of decay made on standards and samples have closely conformed to the Po^{210} half-life of 138 days. The precision of the measurement was good as shown by the reproducibility of results from several duplicate determinations.

Table 1 gives the results of the measurements; the error term given is the overall standard deviation including the uncertainties in the counting

Table 1. Polonium–210 radioactivity in leaf tobacco.

Sample No.	Tobacco type*	Crop year	Radioactivity (pc/g)
		Georgia	
1		1963	0.700 ± .043
2		1963	.375 ± .026
3		1963	.640 ± .040
		Eastern North Carolina	
4		1963	.573 ± .036
5		1963	.411 ± .028
		South Carolina	
6		1955	.500 ± .029
7		1955	.511 ± .031
8		1963	.372 ± .022
9		1963	.453 ± .029
10		1963	.516 ± .032
		Virginia	
11		1961	.348 ± .024
12		1961	.353 ± .021
13		1963	.463 ± .030
14		1963	.641 ± .038
15		1963	.494 ± .031
16		1963	.616 ± .038
17		1963	.463 ± .030
		South Carolina – Virginia (1 : 1)	
18		1961	.455 ± .028
		Rhodesia	
19		1963	.842 ± .051
20		1963	.750 ± .046
21		1963	.670 ± .042
		South Africa	
22		1963	.343 ± .023
23		1963	.363 ± .024
24		1963	.532 ± .034
		New Zealand	
25		1962	.105 ± .009
26		1962	.075 ± .009
27		1962	.129 ± .011
28		1962	.134 ± .009†
29		1963	.150 ± .011
30		1963	.078 ± .008
31		1963	.149 ± .011
32		1963	.221 ± .015
33		1963	.224 ± .015
34		1963	.145 ± .012
35		1963	.134 ± .011
36		1963	.200 ± .015
37		1963	.152 ± .011
38		1963	.122 ± .010
39		1963	.162 ± .012
40		1963	.145 ± .012
41		1963	.166 ± .012
42		1963	.181 ± .014
43	Cutters	1964	.183 ± .015
44	Middle leaf	1964	.093 ± .009
45	Top leaf	1964	.217 ± .015

* Samples are mainly flue-cured tobacco with some air-cured samples. † Burley.

rates, blanks, calibration, and recovery. These results show that the average Po^{210} activity in the New Zealand grown leaf samples tested is 0.15 pc/g, compared with 0.49 pc/g in the United States samples. Our results for United States leaf are somewhat higher than the mean value 0.37 pc/g for 15 samples of post-1950, flue-cured, United States tobaccos recently reported (*3*). Although fewer samples were analyzed for the remaining two countries, it would appear that the level in South African tobacco is approximately the same as, or a little less than, United States tobacco, and the level in Rhodesian tobacco is significantly higher than that in United States tobacco. These comparisons are in approximate agreement with Marsden's values (*4*) for total alpha activity of tobacco.

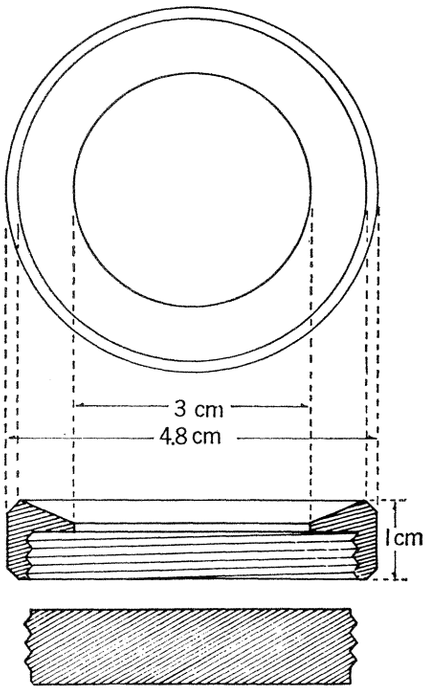

Fig. 1. Polytetrafluoroethylene holder for silver foils.

Table 2. Polonium–210 radioactivity in manufactured tobacco products.

Product	Radioactivity
I. Tobacco blends	(pc/g)
Cigarette blends	
Blend A	0.370 ± .021
Blend B	.386± .024
Blend C	.457 ± .029
Blend D	.339 ± .022
Blend E	.228 ± .015
"Roll your own" blends	
Blend F	.174 ± .013
Blend G	.108 ± .010
Blend H	.222 ± .015
Pipe tobacco blends	
Blend I	.123 ± .011
Blend J	.228 ± .016
Blend K	.192 ± .015
II. Manufactured cigarettes	(pc/cigarette)
Brand A	0.428 ± .027
Brand B (filter)	.319 ± .021
Brand C (filter)	.452 ± .028
Brand D (filter)	.344 ± .023
Brand E	.235 ± .016
Brand F	.235 ± .016
Brand G	.252 ± .016
Brand H (filter)	.236 ± .015

Tobacco manufacturers here are required to use a minimum of 30 percent New Zealand leaf in their overall production. In practice about 50 percent New Zealand leaf is used. They are free to blend this as they wish, however, and they may make a brand of all New Zealand tobacco or all imported tobacco if they so desire. About two-thirds of the manufactured products listed in Table 2 have been obtained directly from the manufacturer and have been made from the leaf samples listed in Table 1. The measured results agree with calculated results in those cases where information on leaf types and proportion has been made available to us. The remainder of the manufactured products listed have been purchased from normal retail outlets. The lower level of Po^{210} in New Zealand leaf is reflected in the results for those manufactured products, notably "roll your own" and pipe tobacco blends, where a relatively high proportion of New Zealand leaf is used. It may be interesting to note that the popularity of "roll your own" cigarettes has been a characteristic of New Zealand smoking habits.

The reason for the lower level of Po^{210} in New Zealand tobacco is not fully understood. The main tobacco growing area is Nelson, at the northern end of the South Island, relatively near the coast. A possible explanation is that much of the radon diffusing from the land's surface is dispersed over the sea. The resulting natural fallout of the lead-120 precursor of Po^{210} may well be significantly less over a country with a predominantly insular climate.

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5. I thank the director of the laboratory, G. E. Roth, for encouragement; H. J. Yeabsley, for valuable guidance and helpful discussions; the Tobacco Research Station of the Department of Scientific and Industrial Research and the tobacco companies, who kindly supplied samples of leaf and manufactured products; and Dr. D. P. Kennedy, Director-General of Health, for permission to publish.

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Release of Dissolved Amino Acids by Marine Zooplankton

Abstract. *Marine net zooplankton release dissolved amino acids into the water. Release rates are positively correlated with temperature and can be estimated by the equation: Release rate (milligrams of alpha-amino nitrogen per gram dry weight of zooplankton per day) = 1.0 × temperature (°C) − 5.9. Release rates appeared to be independent of the taxonomic composition of the test samples, which were variously dominated by copepods, salps, chaetognaths, coelenterates, or radiolarians. These amino acids constitute an important source of dissolved organic matter in the sea.*

The origins of dissolved organic matter in sea water have been the subject of much recent research and discussion (*1–4*). A number of workers have reported the release of dissolved organic matter by phytoplankton, and it is this source that has received most attention (*2*). Duursma (*3*), on the other hand, believes that dissolved organic matter in the North Sea "mainly originates from particulate matter con-