(*17*) who found that a tryptic peptide mixture derived from human basic protein was encephalitogenic.

Our results reveal that the encephalitogenic activity of the bovine A1 protein is primarily due to a short, linear sequence of amino acids surrounding the single tryptophan residue. The requirements for encephalitogenic activity are precisely determined within a framework of nine amino acids or less which are represented by the common sequence found within peptic peptides E and E1, tryptic peptide T27, and synthetic peptide S1, the only synthetic peptide which was active. This sequence is:

Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Lys

The question arises concerning the number of regions in the A1 molecule which can independently induce EAE. Is the nine-residue tryptophan region the only encephalitogenic determinant and the remaining 95 percent of the molecule superfluous? It appears that the region defined by the amino acid sequence around the tryptophan residue is the major encephalitogenic determinant because (i) specific chemical modification of the tryptophan residue with 2-hydroxy-5-nitrobenzyl bromide greatly reduces the encephalitogenic activity of the A1 protein (Table 2) and (ii) the only encephalitogenic peptides derived from the peptic and tryptic digests of the A1 protein come from the tryptophan region. Kibler *et al.* (*18*) have reported that a peptide of 45 residues, derived from bovine spinal cord, is encephalitogenic in rabbits at doses of 50 μg per animal. We have now derived (*8*) the identical peptide from a peptic digest of the A1 protein; it occupies a portion of the polypeptide chain near the NH_2-terminal region and does not overlap the tryptophan region. Therefore two independent encephalitogenic sites may exist in the A1 protein, and considerable species variability may exist in response to these regions. It is not clear why the bovine peptide of Kibler *et al.* (*18*) is not encephalitogenic in guinea pigs (*19*), whereas the bovine A1 protein is highly encephalitogenic.

The delayed skin test (Table 2) with the encephalitogenic peptides in guinea pigs sensitized with the A1 protein was negative in each case. However, the HNB-A1 protein, which is nonencephalitogenic, nonetheless gives a delayed-type skin reaction equivalent to that of the A1 protein (*1*). Thus, the skin test, which has been correlated with induction of EAE (*1, 3*), can be differentiated from the disease process; this suggests that more than one site of the A1 protein molecule may induce the delayed-skin response.

E. H. EYLAR
JUANITA CACCAM
J. J. JACKSON
Salk Institute,
San Diego, California 92112
FRED C. WESTALL
Department of Chemistry,
University of California, San Diego
ARTHUR B. ROBINSON
Department of Biology,
University of California, San Diego

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Photochemical Oxidants: Effect on Starch Hydrolysis in Leaves

Abstract. *Starch-filled leaves of plants which have been subjected to low dosages of naturally occurring photochemical oxidants, ozone, or peroxyacetyl nitrate hydrolyze their starch more slowly when placed in the dark. Delayed hydrolysis occurs irrespective of whether the oxidants were applied during the light or dark period. Occasionally this effect is evident only in the intervenal areas.*

In conjunction with research on tobacco mosaic virus (TMV) at Arcadia, California, P. C. Cheo found that starch in normal appearing, inoculated cucumber cotyledons failed to disappear as it had from those similarly treated in studies conducted at Wenatchee, Washington. The formation of starch lesions is a critical determining feature in the TMV assay (*1*). In this assay the virus concentration is proportional to the number of leaf spots which fail to translocate starch due to interference by the infecting virus. Failure of starch hydrolysis in the uninfected leaf portions made the assay useless. Because we suspected that air pollution (photochemical oxidants) was causing this problem, we installed activated carbon filters in the greenhouse. Starch disappearance, as expected, then occurred after the cotyledons were held in the dark. We conducted further studies into the effect of photochemical air pollutants on starch hydrolysis in leaves.

We observed that starch retention occurs over the entire leaf blade when plants are exposed to low dosages of naturally occurring airborne oxidants such as ozone or peroxyacetyl nitrate (PAN). Starch normally accumulates in plant leaves during the daylight hours when photosynthesis exceeds the rate of translocation of products of photosynthesis from the leaves. The following night this starch undergoes hydrolysis and is exported to areas of growth and storage. Photochemical oxidants somehow block or retard certain steps of the starch hydrolysis–translocation process.

Seeds of cucumber *Cucumis sativa*, bean *Phaseolus vulgaris* cv. "Pinto," *Cassia occidentalis*, and *Mimulus cardinalis* were grown in greenhouses equipped with activated carbon air filters to remove atmospheric oxidants. The 3-week-old seedlings were then exposed for varying lengths of time to

Table 1. Starch remaining in 20-day-old *Cucumis sativa* cotyledons and foliage leaves of *Cassia occidentalis, Phaseolus vulgaris,* and *Mimulus cardinalis* after exposure to gaseous oxidants at Riverside, California, or to ambient oxidants (air) at Arcadia, California, and subsequent placement in darkness. Relative starch concentrations are comparative photometric readings on a scale of 0 to 160 after the leaves were cleared and stained with I_2-KI. The differences between control (unexposed) and exposed tissue are all statistically significant ($P < .01$).

Oxidant	Exposure (hours)	Concentration (pphm)	Dark (hours)	Comparisons (No.)	Relative starch concentration	
					Control	Exposed
			Cucumis sativa			
Ozone	4	5	36	24	46.3	72.5
Ozone	2	5	24	6	42.3	56.7
Ozone	6	5	12	12	41.0	47.2
Ambient	72		16	6	35.0	78.0
			Cassia occidentalis			
Ambient	72		16	5	90.2	137.4
Hexene-ozone	6	3–4	16	5	44.0	87.4
Ozone	6	5	16	26	63.0	72.8
Ozone	6	5	12	34	42.8	117.3
			Phaseolus vulgaris			
Ozone	4	5	36	10	54.5	75.7
			Mimulus cardinalis			
Ozone	6	5	12	8	41.1	80.5

Table 2. The effect of ozone on starch hydrolysis in *Cucumis sativa* cotyledons. Starch retention is noted only for tissue that exhibited no visible damage. All fumigations were carried out in light except for the 40-hour exposure noted in row 7 below.

Exposure (hours)	Concentration (pphm)	Severity of visible damage	Starch retention
4	40	+	No
22	30	++	No
35	30	+++	No
8*	20		Yes
10*	20		No
12*	20	+	Yes
40	20	±	Yes

* Cumulative time; actual exposure was divided equally among four consecutive days (for example, 2 hours' exposure on each of 4 days yields 8 hours' exposure).

ambient ozone or PAN. The source of ambient oxidants was the air at Arcadia, California. The ozone used in the experiments at Riverside, California, was produced by an electric arc generator, and that at Arcadia was produced by a series of ultraviolet lamps. At both locations concentration of oxidants was measured by the neutral potassium iodide bubbler technique (*2*). The concentration of PAN was measured by gas chromatography with an electron-capture detector (*3*).

Starch concentrations were determined by (i) staining with I_2-KI the leaves from which chlorophyll had been extracted with hot 80 percent alcohol, placing them between a light source and a Densichron photometer fitted with a mask to record light received from only a very small area, and scanning them systematically to obtain relative starch concentrations or (ii) by the anthrone method in which the starch is extracted with perchloric acid, and hydrolyzed with sulfuric acid, and the hydrolyzate is then reacted with anthrone reagent and assayed photometrically (*4*).

Numerous comparisons confirmed that exposure of cucumber, bean, *Cassia occidentalis,* and *Mimulus cardinalis* to oxidants delayed starch disappearance in comparison with nonexposed leaves when both groups were given an immediate treatment of 12 to 36 hours of darkness after exposure (Table 1). Later experiments involving exposure of cucumbers and beans to controlled fumigations with either PAN or ozone at 5 parts per 100 million (pphm) for 1 to 4 hours usually confirmed these findings, but the "starch effect" was not always present. Under certain conditions exposure to ambient oxidants, to PAN, or to ozone will significantly reduce subsequent starch hydrolysis in the dark, whereas in other as yet undetermined conditions no effect is evident. Because light intensity, temperature, and relative humidity greatly influence the damage a given oxidant will produce on a plant (*5*), the reason for inconsistent results may be due to insufficient control of these variables.

Additional tests were conducted on *Cucumis sativa* to determine whether the "starch effect" could be demonstrated with higher concentrations of ozone. Ozone dosages which are just short of inducing visible damage or which induce only slight damage will cause starch retention in undamaged areas when the leaves are subsequently excised and placed in darkness (Table 2). Occasionally such ozone-treated leaves hydrolyzed their starch in the vicinity of veins but retained it elsewhere. These hydrolysis patterns were never observed in control leaves.

Ambient oxidants, PAN, and ozone under certain conditions can reduce starch hydrolysis in the dark. The fact that the treated leaves will lose their starch if left in darkness for 3 or 4 days demonstrates that the effect is a lag in starch hydrolysis rather than a permanent inhibition. This starch-retention effect bears certain similarities to accumulated starch found in spots or "halos" around necrotic areas induced by localized infections of several viruses, bacteria, and fungi. In these infected areas the starch is retained after the remainder of the leaf blade has undergone starch hydrolysis during a dark period (*6*). Perhaps these organisms somehow act to inhibit starch hydrolysis as suggested by Tanaka and Akai (*7*). We suggest that photochemical oxidants behave similarly. Low dosages of ozone that failed to induce visible damage to tobacco leaves did inhibit respiration in a manner similar to known metabolic poisons whereas dosages high enough to produce visible damage stimulated respiration (*8*). This agrees with our observations of the starch effect—visible damage was obvious in many of our experiments in which we could not demonstrate starch retention.

GEORGE P. HANSON
WILLIAM S. STEWART*
Los Angeles State and
County Arboretum,
Arcadia, California 91006

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* Present address: Pacific Tropical Botanic Garden, P.O. Box 758, Koloa Kauai, Hawaii 96756.

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