Propanal May Be a Precursor of Ethylene in Metabolism

Abstract. *Propanal, a product of the decomposition of peroxidized linolenate, is a very effective precursor of ethylene in the copper-catalyzed ethylene-forming model system. This aldehyde also stimulates production of ethylene in slices of tissue of mature green tomatoes. We suggest that propanal is a precursor of ethylene in metabolism.*

We have postulated two pathways for biosynthesis of ethylene in plant tissues (*1, 2*): one involves the degradation of methionine; the other is associated with the breakdown of peroxidized linolenic acid or its methyl ester. Both pathways originally derived from studies using model reaction systems catalyzed by cuprous ions in phosphate or acetate buffers at 25° to 30°C (*3*).

Table 1. Production of ethylene by various substances in the copper-catalyzed model system. System consisted of 400 μmole of acetate or phosphate buffer at *p*H 4.5 and 7.0, respectively; 5 μmole of precursor substance (except where acetate is indicated as the precursor substance in acetate buffer); 5 μmole of copper; and 50 μmole of ascorbate in a total volume of 5 ml. These substances were incubated for 1 hour at 30°C in a closed system. Blank value for system is about 20 μl C_2H_4 in acetate buffer and virtually zero in phosphate buffer. Ethylene was detected by gas chromatography in a manner described (*1*). In the absence of the catalyst there is no spontaneous breakdown to ethylene of the compounds used as precursors.

Precursor substance	Ethylene (× 100 μl/hr) from buffer at:	
	*p*H 4.5	*p*H 7.0
Alcohols		
Methanol	5	5
Ethanol	160	70
1-Propanol	240	140
1-Butanol	150	40
Aldehydes		
Acetaldehyde	5	5
Propanal	10	530
Butanal	5	25
Pentanal	15	5
Hexanal	15	15
Heptanal	15	20
Acids		
Acetic	20	10
Propionic	7	5
Butyric	4	12
Epoxides		
Ethylene oxide	10	3
Propylene oxide	2	5
Butylene oxide	3	2
Ethers		
Ethyl ether	175	85
Proply ether	200	150
Butyl ether	25	10
Related unsaturated and carbonyl compounds		
Acrolein	12	105
Acrylic acid	7	5
Acetone	8	5
Pyruvate	108	8

The general mechanism by which methionine is converted to ethylene in the model system has been described, and the conversion has also been demonstrated in tissues (*1*). However, the mechanism of formation of ethylene from peroxidized linolenate is not clear, and there is little direct evidence of its involvement in tissues. We now report the identity of a breakdown product of peroxidized linolenate that forms ethylene very efficiently in the copper-catalyzed model system, and also stimulates production of ethylene in slices of tomato tissue. This substance is propanal, a major product in the dismutation of hydroperoxides formed during the peroxidation of linolenate (*4*); it has been identified as a metabolic product in fruit tissues (*5*).

Since it is known that hydroperoxides formed in the autoxidation of linolenate readily decompose to aldehydes and various carbonyl compounds (*6*), we tested the effectiveness of several substances as precursors of ethylene in our copper-catalyzed ethylene-forming model system. Among the compounds tested, propanal was outstanding as a precursor of ethylene (Table 1). Furthermore propanal was detected as a major product of the oxidative breakdown of linolenate (*7*).

Slices of tissue of mature green tomatoes, incubated with propanal, were stimulated in their production of ethylene by about 60 percent in comparison with the control (*8*). Stimulation by propanal was much greater than that produced by methionine (20-percent increase), which had been shown to be a precursor of ethylene in tissue slices (*1*).

Although definitive evidence is not yet available, our evidence is sufficient to suggest that propanal, which derives from the dismutation of peroxidized linolenate, can be a precursor of ethylene in metabolism. A related aldehyde, methional, also has been implicated in the methionine ethylene-forming system (*1*). However, current evidence does not support the possibility that propanal is an intermediate between methionine and ethylene (*9*). It now appears that the linolenate-propanal-ethylene system and the methionine-methional-ethylene system are independent pathways.

MORRIS LIEBERMAN
ALICE T. KUNISHI

Market Quality Research Division, Agricultural Research Service, Beltsville, Maryland 20705

References and Notes

1. M. Lieberman, A. T. Kunishi, L. W. Mapson, D. A. Wardale, *Biochem. J.* **97**, 449 (1956); *Plant Physiol.* **41**, 376 (1966).
2. M. Lieberman and L. W. Mapson, *Nature* **204**, 343 (1964).
3. The model systems produced methane, ethane, propane, and higher homologues of hydrocarbon gases, both saturated and unsaturated, in addition to ethylene.
4. H. T. Badings, *Neth. Milk Dairy J.* **14**, 215 (1960).
5. R. E. Henze, C E. Baker, F. W. Quackenbusch, *J. Agr. Food Chem.* **2**, 1118 (1953); D. F. Meigh, *J. Sci. Food Agr.* **7**, 396 (1956); ———, H. K. Pratt, C. Cole, *Nature* **211**, 419 (1966).
6. A. M. Gaddis, R. Ellis, G. T. Currie, *J. Amer. Oil Chem. Soc.* **38**, 371 (1961).
7. In the model system, the yield ratio of ethylene from peroxidized linolenate and from propanal is 1 : 51,600 (300 μmole of peroxidized linolenate, which consumes 290 μmole of oxygen, produces 0.1 mμmole of ethylene per hour; 5 μmole of propanal produces 86 mμmole of ethylene per hour).
8. Ten grams of tissue slices (0.5-cm squares) were incubated in 10 ml of 0.1*M* phosphate and 0.1*M* citrate (*p*H 4.5; previously found to be an optimum buffer for ethylene production with slices of green tomato tissue), with and without addition of propanal ($10^{-3}M$) or *l*-methionine ($10^{-3}M$). These samples yielded 184, 296, and 220 mμl of ethylene per hour per gram of tissue for control, propanal-treated, and methionine-treated samples, respectively. The tissue was incubated for 5 hours at 30°C in 100-ml Warburg-type flasks containing 1 ml of 15-percent KOH, with filter-paper wicks in the side arms. Propanal, acrolein, and propyl ether do not stimulate production of ethylene in slices of green tomato tissue.
9. In the model system, rates of production of ethylene from methional are considerably greater than from propanal.

21 September 1967

Audiogenic Seizure Susceptibility Induced in C57B1/6J Mice by Prior Auditory Exposure

Abstract. *Pronounced susceptibility to audiogenic seizures was produced in highly resistant C57B1/6J mice after earlier exposure to a loud electric bell. There is a critical period between initial acoustic presentation and subsequent testing for susceptibility; this suggests a minimum age and a minimum lapse of time during which this "priming" is effective.*

Many studies of audiogenic seizures with inbred strains of mice have attributed differences in susceptibility to specifically defined genetic backgrounds. Mice of the C57B1/6J strain are highly resistant to sound-induced convulsions, and those of the DBA/2J strain are extremely susceptible to them (*1*). This behavior has been ascribed to differences between these two strains in (i) oxidative phosphorylation and adenosine triphosphatase which are involved in energy metabolism (*2*); (ii) concentration in the brain of norepinephrine and serotonin, which

have been proposed as neural transmitter hormones (*3*); and (iii) phenylalanine hydroxylase activity, the liver enzyme that degrades the amino acid phenylalanine (*4*). Simple treatments (prior exposure to a loud noise) which make C57B1/6J mice nearly as susceptible as their DBA/2J cousins should be of importance for two reasons. (i) Any early behavioral condition of such a transient nature that yields so dramatic an aftereffect as a severe convulsion followed by death may be useful in the analysis of normal neural-behavioral development and of the pathology of seizure. (ii) The physiological correlates which used the C57B1/6J strain as a nonsusceptible standard should be reevaluated. There have been reports of these procedures (*5*).

The C57B1/6J-strain inbred mice, offspring of parents obtained from the Jackson Laboratory, were tested for audiogenic seizures when either 21 or 28 days old. The age groups contained 255 and 355 mice, respectively. They were placed individually in a glass chromatography jar (45 cm high and 30 cm wide) and exposed for 30 seconds to an electric bell (103 decibels relative to 2×10^{-4} dyne/cm^2) mounted directly overhead. Occurrence of the successive stages of the audiogenic seizure syndrome (wild running, myoclonic convulsion, myotonic convulsion, and death) was recorded for each animal. All animals were kept under conditions of 12 hours of light followed by 12 hours of darkness and were weaned at age 21 days into separate cages in groups of five. All subjects were tested between the 4th and 6th hour of the daily 12-hour light cycle, in view of the circadian nature of audiogenic seizures. The mice were tested under either an X-21 or an X-28 schedule, the X designating the age in days at which they were initially exposed to the bell for 30 seconds (constituting the priming condition) and the second number designating the age in days at which their response to auditory stimulus was observed. Of the 580 mice tested, only 27 exhibited wild running during the initial exposure, mostly at ages 14 and 15 days, and none exhibited clonic convulsions, tonic convulsions, or deaths. This supports the conclusions of earlier reports which describe this strain as nonsusceptible. The results (Table 1) show that this technique is differentially effective, depending upon the ages at which both priming and testing occur; the age of 19 days is optimum for priming mice to be tested at 28 days of age, whereas the age of 16 days is most effective for priming mice to be tested at 21 days. Acoustic priming appears to be ineffective before the age of 14 days, corresponding to the normal onset of hearing in mice (*6*). To be effective, priming requires a minimum number of days; this period is shorter in the 21- than in the 28-day-old mice. An orthogonal polynomial analysis (*7*) of the severity scores in Fig. 1 showed that the quadratic component accounted for 76.2 percent of the treatment sum of squares [ratio of variances (F) = 190, degrees of freedom (df) = 1, 154] for the X-21 schedule, and 56.4 percent of the treatment sum of squares (F = 232, df = 1, 238) for the X-28 schedule. The same analysis of only those data that show a treatment effect (from 13 to 20 days for priming 21-day-old mice, and from 13 to 25 days for 28-day-old mice) showed that the quadratic term accounted for 99.01 and 82.14 percent of the treatment effect for the X-21 and X-28 schedules, respectively. Figure 1 also shows the least-squares quadratics (*8*) superimposed over the severity scores. These statistical results indicate that the quadratic curve best describes, for this experiment, the ages at which acoustic priming is most effective. Further support is obtained from a study in which 11 strains of

Table 1. Audiogenic seizure profile for mice (of the C57B1/6J strain) primed and tested at various ages. On the X-21 schedule, the age at the second acoustic presentation was 21 days; on the X-28 schedule, this age was 28 days. Priming age, age in days at initial acoustic presentation. Fifteen mice were used for each priming age.

Priming age (days)	Mice (%) exhibiting four components of audiogenic seizure:			
	Wild running	Convulsions: Myoclonic	Convulsions: Myotonic	Death
X-21 Schedule				
21	0	0	0	0
20	7	0	0	0
19	87	20	20	20
18	87	80	67	33
17	93	80	80	47
16	100	80	80	60
15	80	80	80	47
14	60	40	40	33
13	0	0	0	0
12	0	0	0	0
X-28 Schedule				
28	7	0	0	0
27	0	0	0	0
26	0	0	0	0
25	0	0	0	0
24	20	0	0	0
23	73	13	0	0
22	67	7	0	0
21	80	47	13	0
20	100	40	27	13
19	100	73	53	47
18	100	100	20	13
17	93	80	13	13
16	87	73	7	0
15	67	53	7	0
14	20	0	0	0
13	7	0	0	0
12	7	0	0	0

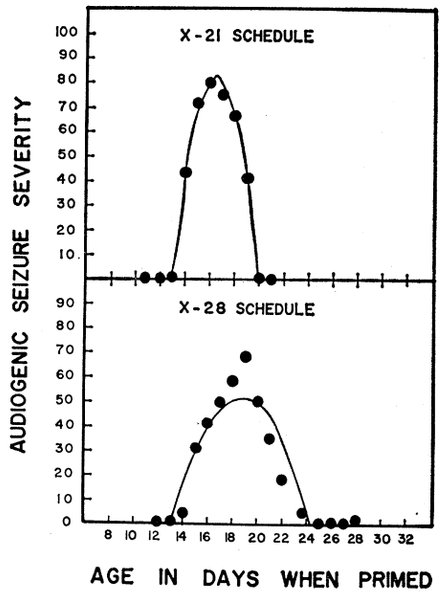

Fig. 1. Severity of audiogenic seizure for various priming and testing ages. This represents an average of the scores for wild running, clonic seizure, tonic seizure, and death.

Table 2. Effects of different treatments (at 16 days of age) on audiogenic seizures of 21-day-old mice of the C57B1/6J strain. Doses of Nembutal (sodium pentobarbital) for 30 mice were 12.5 mg per 10 g of body weight; acoustic priming for 15 of these animals occurred 10 minutes after injection. The 30 mice anesthetized with ether were exposed for 30 seconds to atmosphere containing 5 ml of ether per 1 liter of air; half of these were acoustically primed 10 seconds later. Fifteen mice were used for each priming age.

Treatment	Seizure behavior (%)			
	Wild running	Clonic seizure	Tonic seizure	Death
Nembutal	0	0	0	0
Ether	0	0	0	0
None	0	0	0	0
Nembutal plus bell	93	87	87	53
Ether plus bell	100	100	100	67
Bell only	100	80	80	60

mice were tested at 21 days of age and tested again later (*9*). The four "least susceptible" strains, including the C57B1/6J strain, did not show seizure at the initial acoustic presentation but did so when tested again 7 days later. Fuller and Sjurnsen noted this in their tables but did not discuss it in the text.

These data indicate that this is not a transient sensitization effect; instead, it involves a longer-term neural change—perhaps an increase in auditory sensitivity or a decrease of neural inhibition. Acoustic priming can occur during wakefulness or under anesthesia by ether or sodium pentobarbital (Table 2); this suggests that neither the brainstem reticulum nor a conscious mechanism is involved. Because the C57B1/6J strain is homozygous nondilute (its chromosomes do not carry the mutant genes responsible for a lighter coat pigmentation and reduced liver phenylalanine activity; the dilute condition has been used by some as an analog of the human phenylketonuric mental deficient condition), its susceptibility to audiogenic seizure rules out the dilute genetic locus as a necessary condition for seizures, as some have suggested (*10*). The priming technique may provide a more useful means of analyzing audiogenic seizures than comparison of strains that differ at many loci does, in that it permits an experimental rather than a correlative approach. Biochemical examinations of primed and nonprimed mice may reveal whether differences in oxidative phosphorylation, concentration of norepinephrine and serotonin in the brain, or liver phenylalanine hydroxylase activity are associated with changes in audiogenic seizure susceptibility. Whether auditory priming affects other behaviors—chemoconvulsive and electroshock seizures, learning, and emotionality—is still unknown. The phenomenon in this study is analogous in some respects to Lorenz' descriptions of imprinting; both have a critical period early in life during which a relatively brief stimulus can exert a profound, long-lasting effect on later behavior (although acoustic priming may not constitute as natural a situation as that which occurs when a duckling imprints its behavior to the first moving object it sees, accepts it as its mother, and models later behavior after this relationship). This technique may be useful in the investigation of behavioral development and of musicogenic seizures in humans.

KENNETH R. HENRY
Regional Primate Research Center, University of Wisconsin, Madison 53706

References and Notes

1. C. S. Hall, *J. Hered.* **38**, 2 (1947); G. M. Witt and C. S. Hall, *J. Comp. Physiol. Psychol.* **42**, 58 (1949); J. L. Fuller and W. R. Thompson, *Behavior Genetics* (Wiley, New York, 1960), pp. 154–156.
2. L. G. Abood and R. W. Gerard, in *Biochemistry of the Developing Nervous System*, H. Waelsch, Ed. (Academic Press, New York, 1955), p. 467.
3. K. Schlesinger *et al.*, *Life Sci.* **4**, 2345 (1965).
4. D. L. Coleman, *Arch. Biochem. Biophys.* **91**, 300 (1960); S. D. Huff and J. L. Fuller, *Science* **144**, 304 (1964).
5. K. R. Henry, unpublished thesis, Univ. of North Carolina, Wilson Round Library (1966); W. B. Iturrian and G. B. Fink, *Fed. Proc.* **26**, 736 (1967).
6. B. R. Alford and R. J. Ruben, *Ann. Otol. Rhinol. Laryngol.* **72**, 237 (1963).
7. R. A. Fisher and F. Yates, *Statistical Tables* (Hafner, New York, 1953).
8. G. W. Snedecor, *Statistical Methods* (Iowa State Univ. Press, Ames, 1956).
9. J. L. Fuller and F. H. Sjursen, Jr., *J. Hered.* **58**, 135 (1967).
10. S. D. Huff and R. L. Huff, *Science* **136**, 318 (1962); D. L. Coleman and K. Schlesinger, *Proc. Soc. Exp. Biol. Med.* **119**, 264 (1965).
11. I thank R. E. Bowman and G. E. Harding for their assistance. Supported by grant FR-0167 from NIH to the Wisconsin Regional Primate Research Center.

25 September 1967

Stimulus Generalization as Signal Detection in Pigeons

Abstract. *Pigeons that were reinforced for pecking at a single-wavelength responded to nearby wavelengths with lower rates. Response rates indicated the pigeons' certainty that the reinforced stimulus was present. The ratings yielded receiver operating characteristic functions that approximated straight lines on a double probability plot.*

Modern psychophysics may clarify our thinking about the stimulus control of animal behavior (*1*). Conversely, animal subjects might supply data necessary to test psychophysical hypotheses. Unfortunately, most experiments with animals are concerned with transient phenomena, whereas psychophysics deals with the steady state; many animal experiments yield response rates to prolonged stimuli, while psychophysics favors choice responses to brief stimuli. This experiment effects a compromise by putting the response rate of an animal in a form suitable for signal detection analysis. A pigeon's response during a 30-second trial shows its "degree of certainty" that a reinforced stimulus was present on that trial. From such ratings, existing procedures yield relatively well-defined receiver-operating characteristic (ROC) functions (*2*).

Three White Carneaux pigeons with a long history of discrimination training were the subjects. Each pigeon, in a darkened chamber, pecked at a plastic disk upon which appeared a bright spot (0.95 cm in diameter). A 250-mm grating monochromator supplied light for this spot through a fiber-optics light guide. The stimulus assumed one of 12 wavelengths (570 to 592 nm in 2-nm steps), each with a half-width dispersion of 6.6 nm. The stimuli were uncorrected for brightness; over this range, brightness probably varied little for the pigeons (see *3*).

After several weeks of training, experimental data were collected for 28 days. Each daily session comprised a 2-hour series of 30-second presentations of the stimulus with 3-second dark periods between these presentations. The key also went dark during reinforcement and for 0.6 second after each peck. On some trials, with 582 nm on the key, pecks intermittently brought reinforcement of 3-second access to mixed grain. Such reinforced trials were mixed with unreinforced "test trials" in a semirandom sequence as follows. Each session began with four reinforced trials followed by 13 stimulus sequences presented serially. The 16 stimuli in each sequence included four reinforced trials of 582 nm and 12 test trials in random order, with each test wavelength appearing once. The data below came from responses made in the 12 test trials on the last 12 stimulus series. Although 582 nm was the reinforced stimulus, it also appeared as one of the unreinforced test stimuli. Responses to reinforced trials and to the first complete series each day are omitted from this analysis.

A LINC digital computer (*4*) controlled the experiment and recorded responses. One reinforcement was delivered, on the average, on each reinforced trial. A peck produced reinforcement only if it followed the preceding peck by an interval least frequently emitted by the pigeon. This schedule is designed to generate a moderate, stable rate of response (*5*). In two birds, performance over the 28