Ozone and Vitamin E

Abstract. *Vitamin E deficiency in rats is associated with a greater susceptibility to lethal levels of ozone. Exposure of rats to sublethal ozone concentrations produces an accelerated decline in serum vitamin E levels. These findings are consistent with the possibility that lipid peroxidation is a mechanism of ozone toxicity.*

We have suggested that an important cause of ozone toxicity is the peroxidation (*1*) of unsaturated fatty acids (*2*). According to this hypothesis, a deficiency of the lipid antioxidant vitamin E (tocopherol) would render animals more susceptible to ozone toxicity. Furthermore, exposure to ozone would be expected to lead to a more rapid utilization of this antioxidant. The following studies are in support of these suppositions.

Half of a group of 16 inbred laboratory strain rats were placed on a vitamin E–deficient diet (*3*). The remaining rats received a normal diet and served as controls. Hemolysis with the hemolyzing agent dialuric acid (hemolysis was performed three times weekly) was measured as an index of vitamin E deficiency (*4*). When better than 80 percent hemolysis was observed in the vitamin E–deficient rats, all 16 animals were simultaneously exposed in the same chamber to ozone. The average ozone concentration was 10.4 parts per million (ppm). The eight vitamin E–deficient rats died of pulmonary edema after 360 to 410 minutes of exposure (mean, 380 minutes). However, all of the control animals were still alive after 440 minutes of exposure, at which time the experiment was terminated. The animals given the vitamin E–deficient diet weighed somewhat less than the animals in the control group but there was a great degree of overlap (vitamin E–deficient rats: average weight, 262 g; range, 221 to 293 g; control rats: average weight, 282 g; range, 255 to 328 g).

This experiment was repeated with a different group of 16 rats. This time we used higher concentrations of ozone during the exposure period (average concentration, 14.0 ppm) and a different source for the vitamin E–deficient diet (*5*). The eight control and eight vitamin E–deficient rats were simultaneously exposed in the same chamber to ozone; the vitamin E–deficient animals expired after breathing ozone for 240 to 310 minutes (mean, 265 minutes) and the control rats expired after 315 to 405 minutes (mean, 372 minutes). A difference in weights with a substantial overlap was again found (vitamin E–deficient rats: average weight, 264 g; range, 236 to 299 g; control rats: average weight, 288 g; range, 264 to 314 g). The degree of overlap in the weights of the animals precludes the possibility that size was the sole reason why all the vitamin E–deficient rats in these two studies died prior to any of the control animals after exposure to lethal levels of ozone.

Vitamin E utilization was investigated in 24 rats randomly divided into four groups. Rats in groups A and B were given a vitamin E–deficient diet (*3*); rats in groups C and D were given a standard rat diet. After 1 week the rats in groups A and C were exposed to 3.5 ppm ozone generated in filtered room air for 4 hours daily on ten consecutive days. The animals in groups B and D were exposed in a similar manner to filtered room air. Twenty-four hours after the last exposure period vitamin E concentrations were measured in the serum of each animal (*6*). As shown in Table 1, the concentration of α-tocopherol in the serum of rats in group A was significantly lower than that in any other group (*7*). This concentration is lower than would appear to be expected on the basis of the summation of the effects of ozone (group C) or vitamin E–deficient diet (group B) alone. However, the relative differences in vitamin E concentrations in serum may not quantitatively reflect changes in total body stores.

Tocopherol deficiency has been shown to render tissues more susceptible to oxidation, presumably owing to the ability of this vitamin to prevent lipid peroxidation (*8*). The finding that vitamin E–deficient rats suffer fatal pulmonary edema during exposure to high concentrations of ozone more rapidly than control animals do suggests that the breakdown of unsaturated fatty acids may contribute to mortality. Increased utilization of vitamin E at sublethal levels of ozone is consistent with our earlier suggestion that ozone results in lipid peroxidation (*2*).

Lipid peroxidation is cited as a cause of biological damage in various pathological states. In vitro peroxidation of lipid in vitamin E deficiency in numerous animal tissues has been reported; however, there is a paucity of definitive in vivo studies (*9*). The reaction of ozone with carbon-carbon double bonds of unsaturated fatty acids has been known for many years. Previous studies have given evidence of lipid peroxidation in red cells exposed to ozone in vitro, and in lungs of mice exposed to 0.7 ppm of ozone in vivo (*2*). Similar evidence of lipid peroxidation in the lungs of rats exposed to ambient levels of nitrogen dioxide, another photochemical air pollutant, has been reported by Thomas *et al.* (*10*).

It has been suggested that lipid peroxidation is a central mechanism of the aging process (*11*). Animals chronically exposed to ozone have shown signs of premature aging (*12*). Studies designed to determine whether current ambient levels of ozone and other photochemical air pollutants can cause lipid peroxidation in the lungs of man are necessary before it can be speculated that long-term exposure to oxidants in urban air leads to an increase in chronic respiratory disease by causing an acceleration in lung aging.

Table 1. Vitamin E concentrations (mean ± 1 standard deviation) in the serum of rats (in micrograms per 100 ml).* Group A, rats exposed to ozone during induction of vitamin E deficiency; group B, rats exposed to room air during induction of vitamin E deficiency; group C, rats exposed to ozone after a standard diet regimen; group D, rats exposed to room air after a standard diet regimen.

Group	Vitamin E concentration
A	45 ± 17
B	238 ± 83
C	255 ± 22
D	295 ± 57

* Group A versus groups B, C, or D: $P < .01$; groups B or C versus group D: $.20 > P > .10$.

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References and Notes

1. We use the term "lipid peroxidation" rather than "lipid ozonization" for a number of reasons. The radiomimetic characteristics of ozone suggest that it may produce free radicals which could then cause lipid peroxidation. The ozonization of unsaturated fatty acids may itself conceivably lead to free radical formation, thus causing peroxidation of nearby unsaturated fatty acids. It is difficult to distinguish peroxides from ozonides chemically, and their biological implications may be similar. Furthermore, the term "lipid peroxidation" is more commonly found in the biological literature.
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Nervous Control of the Heart during Thoracic Temperature Regulation in a Sphinx Moth

Abstract. *Heating the thorax of the sphinx moth,* Manduca sexta, *evoked pulsations of the heart in the abdomen. These pulsations were of relatively high rate and amplitude, and traveled from the abdomen into the thorax. While heat was continuously applied exclusively to the thorax, thoracic temperature often stabilized and abdominal temperature increased sharply. Thoracic heating of moths with transected nerve cord, however, did not evoke these responses. It is inferred that the heart in the abdomen responds to overheating of the thorax through neural influence.*

Descriptive knowledge of the insect circulatory system dates back to Harvey (*1*) and has recently been reviewed (*2, 3*). This circulatory system is an open one, although blood is pumped by a pulsatile vessel, the heart. In some insects, the heart is segmentally innervated from the ventral nerve cord (*4*), but that of saturniid moths, for example, is thought to be exclusively myogenic (*5*).

The heart is a muscular tube extending along the middorsal line of the abdomen into the thorax (Fig. 1). The so-called alary muscles are laterally attached. Segmentally arranged pairs of ostia allow inflow of blood, and ostial valves prevent outflow (*6, 7*).

In the sphinx moth, *Manduca sexta,* blood circulation is involved in the stablization of thoracic temperature during free flight over a wide range of ambient temperatures (*8*). It was not known if thoracic temperature was regulated, or if stabilization was achieved automatically by the pumping of the heart in the abdomen at its own temperature-dependent rate. This rate might be rapid when the abdomen is hot and slow when the abdomen is cool, as it is at low ambient temperature. I now report, however, that the pumping of the heart in the abdomen is modulated in response to thoracic temperature.

The moths used in this study were supported by their wings from a frame of balsa wood so that their bodies were freely suspended. Heat was directed onto the thorax with a narrow beam of light from an incandescent lamp. During thoracic heating, both the heart pulsations and the body temperatures were simultaneously and continuously recorded. The latter were measured with 40-gauge copper-constantan thermocouples implanted in the thorax, as well as in the second and fifth abdominal segments (Fig. 1). The leads from the thermocouples were connected to a multichannel potentiometric recorder.

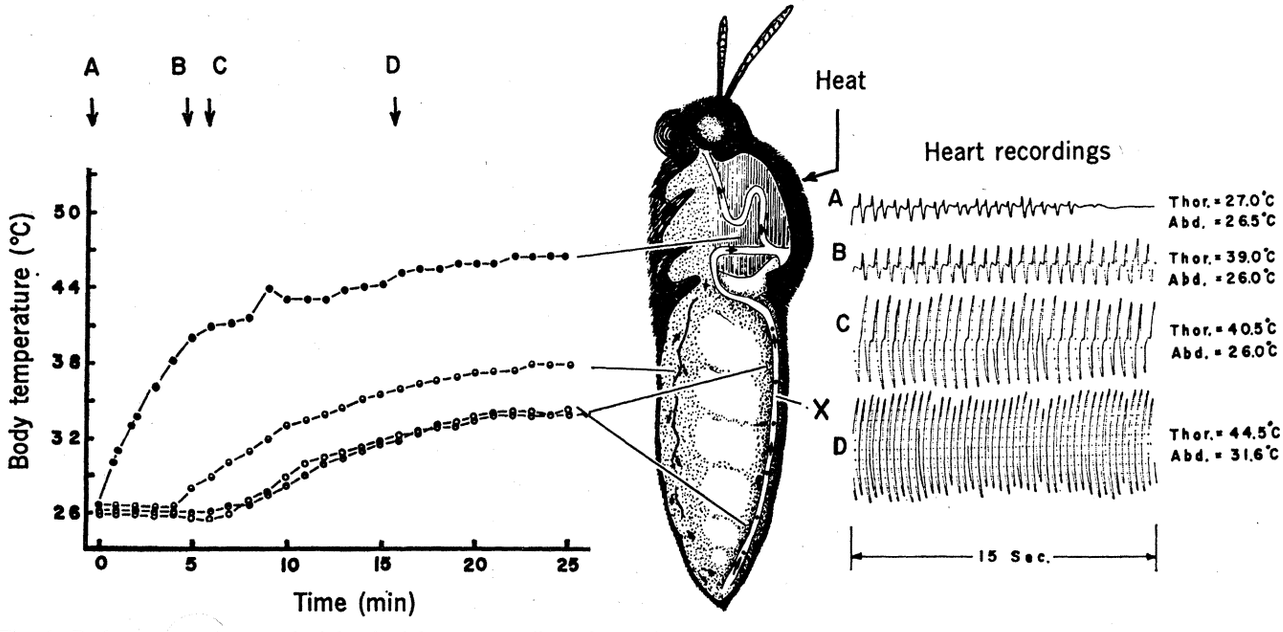

Fig. 1. Body temperatures and abdominal heart recordings from the same run during the application of heat exclusively to the thorax of a moth. Heating of the thorax was initiated at minute 0. Lines on the sagittal view of thorax and abdomen point from the positions of the implanted thermocouples to the respective temperatures recorded from that area during the 25 minutes of heat application to the thorax. The approximate position of the electrodes, which were implanted laterally along the abdominal heart to record heart pulsations, is indicated by X. The specific times at which the abdominal heart pulsations were recorded are indicated by *A, B, C,* and *D*. The same moth, when dead, was again heated in a similar manner. The initial rate of temperature increase in the thorax was the same as in the live moth; however, after 12 minutes of thoracic heating, the dead moth had a thoracic temperature of 53°C; abdominal temperature did not exceed 26°C during this time.