reveals that there are no hemorrhages, necrotic foci, or areas of gliosis. The main damage in all lesions is a narrow band of total destruction and, for our lowest radiation dose, this band is the only striking evidence of destruction in Nissl preparations, when such sections are examined under relatively low power.

Production of lesions at appreciably greater depth will require the use of particles of higher energy. Deuterons at 20 Mev may be expected to produce lesions at any desired depth within the cortex.

Production of narrow lesions of total destruction, sharply bordering on normal or nearly normal tissue, promises to be of great value as a research tool for studies of connections and discharge patterns of cortical neurons. Studies on standardization of this technique preliminary to such work are now in progress.

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2. We wish to express our appreciation to C. P. Baker and L. E. Farr of Brookhaven National Laboratory, who made the initiation of this work possible. We also thank S. Feitelberg, director of the André Mayer department of physics, Mount Sinai Hospital, New York, for his stimulating and valuable suggestions in the planning of these studies.

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Reversal of Inhibitory Effects of Ozone on Oxygen Uptake of Mitochondria

There is evidence to suggest that ozone may be present in the Los Angeles atmosphere in sufficient concentrations to damage plants and affect animals. It has been reported that concentrations of ozone of from 25 to 50 parts per 100 million by volume (pphm) that existed for long periods of time were lethal to guinea pigs, and particularly so when the animals were infected with tuberculosis (*1*). It has been reported that a noticeable irritation to human beings results from concentrations of 20 pphm (*2*). Middleton *et al.* (*3*) found that ozone at concentrations as small as 20 pphm produced visible leaf damage to pinto beans after a 2-hour exposure.

Renzetti (*4*) has reported that concentrations of ozone of up to 35 pphm have been recorded on days of heavy smog in Pasadena, Calif. Littman and Marynowski (*5*) found that from 30 to 45 percent of the total oxidant in the Los Angeles atmosphere resembles ozone. Oxidant levels of as high as 90 pphm have been recorded for short periods in Los Angeles (*6*), and smog levels in the basin reach a point where they cause appreciable irritation on about 70 days of each year (*7*).

Small concentrations of ozone have been shown to affect the respiration of plant cells (*8*). An important part of the respiratory activity in certain plant and animal cells has been shown to be associated with the mitochondria. Because of this, an understanding of the effects of ozone on mitochondria could be important for the prevention and control of air-pollution damage to organisms.

Ozone, produced by passing oxygen through a high-voltage discharge tube, was diluted with filtered air through rotameters to give the desired concentration. The air in every case was filtered through activated charcoal. The concentration of ozone for each experiment was continuously recorded with an ultraviolet photometer (Harold Kruger Instruments, model 52) and was also determined by iodometric titrations.

Mitochondrial preparations from cabbage and spinach were obtained by methods that have been described previously (*9*). The methods of Kielley and Kielley (*10*) were used for the preparation of the cow's liver mitochondria. Mitochondria isolated from 700 g of plant tissue or from 50 g of liver were suspended in 48 ml of 0.6*M* sucrose, 0.1*M* potassium phosphate buffer at *p*H 7.0. Aliquots of the mitochondrial suspensions of 6 ml each were swirled with ozone-free air or the desired concentration of ozonated air in 2-lit volumetric flasks which had previously been flushed with either the ozone-free air or the ozonated air mixtures. The volumetric flasks were rotated uniformly in an ice bath to maintain a thin layer of suspension on the walls of the flasks as well as to maintain the low temperature required to preserve the mitochondria.

Table 1. Effect of ozone on plant and animal mitochondria. All values reported are average values from duplicate flasks. These duplicates did not vary from each other by more than 5 percent. Where no figures are given, no analysis was made.

Additions (μmole)	Oxygen taken up (μmole)		Reduced compounds remaining (μmole)	
	Treatment		Treatment	
	Air	Ozone	Air	Ozone
Cabbage	*4.5 μmole O_3/flask*		*Ascorbic acid*	
None	11.6	3.3	0.19	0.04
GSH, 16	11.2	4.9		
AA, 20	10.2	8.5	7.5	5.8
GSH, 16, + AA, 20	9.3	9.6	6.4	4.9
Spinach	*5 μmole O_3/flask*		*Glutathione*	
None	10.7	7.1	0.07	0.01
GSH, 48	10.8	9.1	35.5	12.0
Cow liver	*7 μmole O_3/flask*			
None	3.2	1.9		
GSH, 20, + AA, 20	5.8	5.2		

After the air or ozone treatment, 2 ml of the mitochondrial suspensions were added, with 1 ml of cofactors, to each Warburg flask. The usual Warburg techniques (*9*) were used to test for citric-acid-cycle activity. A concentration of 0.006*M* potassium citrate was used as the substrate for the mitochondria. Ascorbic acid (AA) and reduced glutathione were added directly to the Warburg flasks. Conventional methods were used for the analysis of the ascorbic acid (*11*) and the sulfhydryl compounds (*12*) at the end of the experiments.

The results in Table 1 indicate that when reduced glutathione and ascorbic acid were added together to the ozone-treated mitochondrial suspensions, an apparent reversal of the ozone inhibition took place. Partial reversal of the ozone inhibition was obtained when the two compounds were added separately. This reversal of ozone inhibition has been repeated more than 13 times in separate experiments. Data for both oxygen uptake and CO_2 evolution verified the effect.

The mitochondria were treated with ozone before the substrates, cofactors, and reducing compounds were added. These mitochondria were therefore initially changed by the treatment with ozone. Later addition of ascorbic acid or reduced glutathione apparently overcame part of this change and, in some cases, brought the oxygen uptake completely back to that of the air control.

When 15 μmole of hydrogen peroxide

was added to an untreated cabbage mitochondrial preparation, there was an immediate evolution of oxygen, which continued for some time. This was presumably attributable to the presence of catalase. It took 76 μmole of hydrogen peroxide per flask to cause a 30 percent inhibition in rate of oxygen uptake. This 30 percent inhibition was reversed with 16 μmole of ascorbic acid plus 20 μmole of reduced glutathione. These experiments indicated that the action of ozone did not involve the intermediate formation of hydrogen peroxide, since the relatively large amounts of hydrogen peroxide required for inhibition could not have been formed from the smaller amounts of ozone that were added. The results also suggested that ozone and hydrogen peroxide might have been acting in a similar manner, since reversal could be obtained in both cases. If this were the case, then the action of these agents could have been attributable to simple oxidation, and other strong oxidizing agents (such as peroxyacids and ozonides) could perhaps produce a similar reversible inhibition.

The addition of excess cofactors—sodium ethylene-bis(dithiocarbamate), sodium bisulfide, cysteine, 2,3-dimercapto-1-propanol—to ozone-treated mitochondria did not reverse the ozone inhibition. In many cases, the addition of these compounds inhibited oxygen uptake. These experiments suggested that some type of specificity existed for the reversing agent.

The results presented here suggest that the effects of small amounts of ozone on cabbage, spinach, and liver mitochondria were reversed by ascorbic acid, reduced glutathione, and ozone-affected enzymes that were associated with the mitochondrial citric-acid-cycle activity.

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Effect of Lysergic Acid Diethylamide on Absolute Visual Threshold of the Pigeon

There have been many recent reports that human subjects receiving small doses of lysergic acid diethylamide (LSD) tend to behave in some ways like psychotic patients. These reports have stimulated efforts at careful specification of the psychological and physiological effects of LSD. Prominent among the effects found thus far have been disturbances of visual functions, including apparent changes in visual sensitivity. E. V. Evarts (*1*) recently reported that monkeys recovering from large doses of LSD were active but behaved as though they were blind. Carlson (*2*) has noted a slight rise in the absolute visual threshold of human subjects following intravenous administration of 100 μg of LSD. In a related neurophysiological study (*3*), LSD markedly reduced the postsynaptic response in the lateral geniculate nucleus to stimulation of the optic nerve of the cat.

Such findings suggest that elevation of the absolute visual threshold is characteristic of the action of LSD. The present study (*4*) uses a recently devised technique (*5*) to measure this effect in the pigeon. The method is rather complex and its restatement here will be brief. The pigeon stands in a light-tight box and views a stimulus patch fixed in the wall. It pecks one response key when the stimulus patch is visible and another key when the patch appears dark. These pecks, operating through automatic control circuits, cause the intensity of the stimulus to vary up and down across the pigeon's absolute threshold. A recorder charts the stimulus intensity, indicating the bird's threshold through time. The automatic controls provide the pigeon with periodic rewards of food for correct responses.

The subjects were three male domestic pigeons (White Carneaux). The bird to be tested first was dark-adapted for at least 1 hour in the experimental box. The stimulus patch was then illuminated by a light beam of 500-mμ wavelength from a Bausch and Lomb grating monochromator. The bird responded to this stimulus for at least 30 minutes, or until its threshold appeared to be stable. Then the experimental box was opened in darkness, and a dose of water or LSD solution (100 or 300 μg/kg) was administered either orally or by intraperitoneal

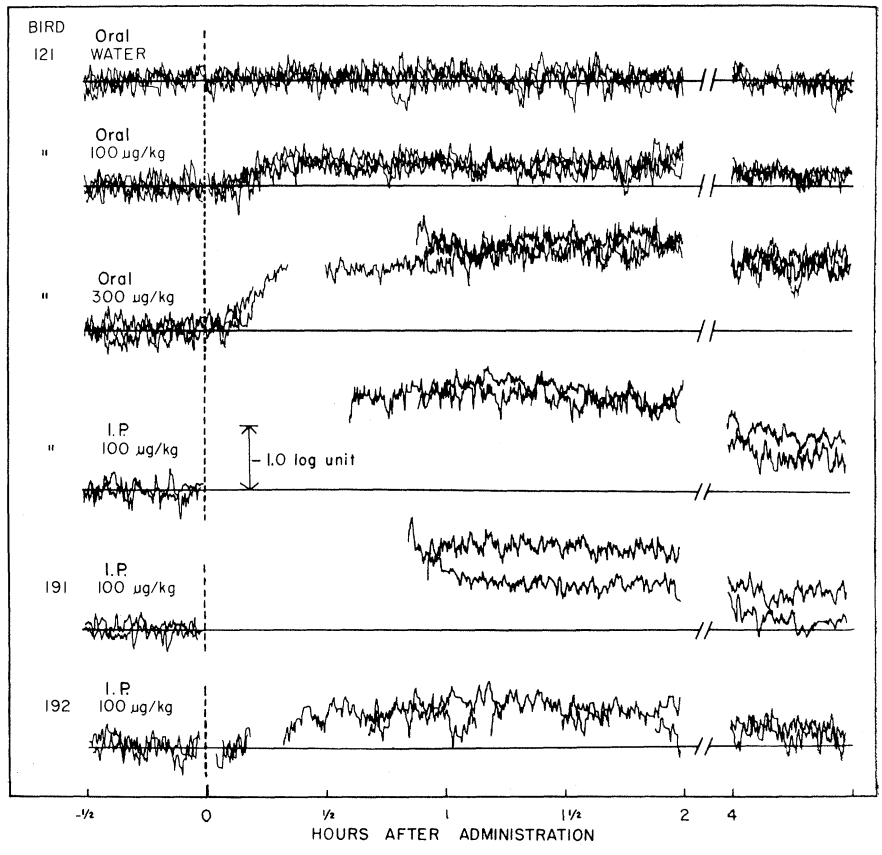

Fig. 1. Effect of LSD on the pigeon's absolute visual threshold. Vertical shifts in the curves represent changes in the brightness of a "just visible" stimulus patch. A single oral dose amounted to about 4 ml of solution (10 ml/kg). A single intraperitoneal dose amounted to about 0.4 ml of solution (1 ml/kg).