original tumor fluid, thus indicating no significant loss of activity during dialysis.

By the slow addition of solid ammonium sulfate to tumor fluid, a cumulative saturation series was carried out at 10-percent intervals. This was done at 4°C. No precipitate occurred at 10-percent saturation. Inhibitor activity was present in the precipitates at 20- and 30-percent saturations. No further amount of inhibitor was precipitated at 40-percent saturation or thereafter.

It appears that the inhibitor may be an enzyme. Efforts to identify it are continuing. It is of interest to speculate whether or not there is a connection between the inhibitor and the fact that, on serial passage in the tumor, the IHD-E virus loses the capacity to produce hemagglutinin (*3*).

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Production of Laminar Lesions in the Cerebral Cortex by Heavy Ionizing Particles

One of the difficulties in studying the connections and functional capacity of cortical neurons results from the fact that no technique thus far employed permits selective destruction of a single cortical layer. It appears that heavy ionizing particles of high and nearly equal energies may be used to produce such destruction. The steep rise in linear energy transfer near the end of the particle's range, the sharp cut-off in energy transfer at the end of the range, and the relatively straight trajectory are properties of heavy ionizing particles which are useful in this connection. High-energy protons and deuterons have already been extensively employed by Tobias and his coworkers (*1–3*) for irradiation of the pituitary.

In our pilot experiments (*2*) production of laminar lesions was attempted with 10-Mev protons. The cyclotron at Brookhaven National Laboratory was used. The beam passed from vacuum into air through a brass foil about 0.025 mm thick. After it has passed through a circular defining aperture 0.5-cm in diameter, the beam was measured by an ionization chamber consisting of two sheets of aluminum foil (each 0.024 mm thick), spaced 1.27 cm apart. The beam traversed a total air path of about 10 cm before it reached the exposed cortex of a deeply anesthetized cat. Under the experimental conditions used, the protons entered the tissue with a residual range of about 0.9 mm and caused maximal destruction at a depth of about 0.8 mm.

In two cats, five lesions were produced in the lateral and suprasylvian gyri of the cerebral cortex. After irradiation, the animals were permitted to survive 10 weeks. They were then sacrificed, and their brains were cut serially. The sections were stained with thionine. The intensity of the beam (number of particles per unit area, per unit time) was held constant, and exposure times were varied for different lesions. Figure 1 shows the typical appearance of one irradiated cortical field. The dosage here was intended to be 5000 rad at the peak of the energy-transfer curve. This estimate, however, may be in error by a factor of 2 or more because of nonlinearity of the ion chamber that was used for measurement. It will be noticed that in the middle of the cortical field there is a band (marked by an *x*) in which all the nerve cells disappear. The small cells visible in this strip are glia cells. The limits of the destructive lesions are sharp, and the total width of the destroyed cortex measures about 100 μ. The lesion covers a circular area, the diameter of which approximates closely the size of the aperture (0.5 cm).

It is noteworthy that, apart from the sharply demarcated destroyed zone, the rest of the cortex appears normal. The first, second, and third cortical layers above the lesion display a substantially intact cytoarchitectonic structure even though these layers were traversed by the proton beam. If these layers are damaged, the damage is apparently minor by comparison with that in the zone of total destruction. Likewise, below the lesion, the fourth, fifth, and sixth layers appear intact. A survey of serial sections

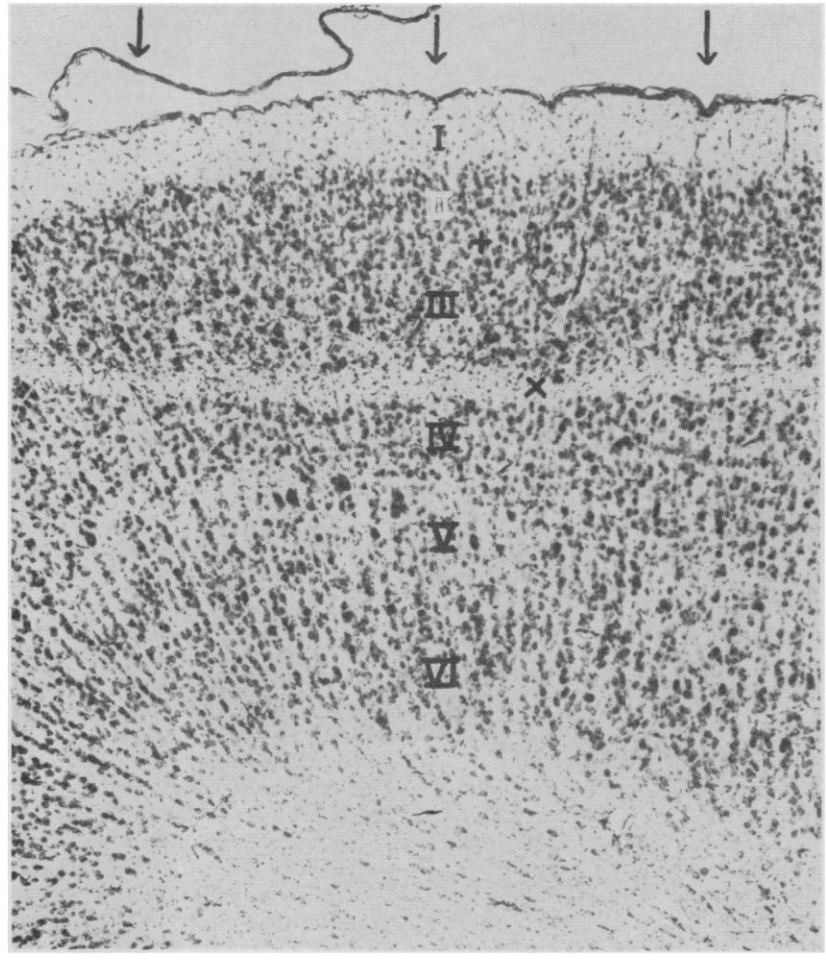

Fig. 1. Cortex of the lateral gyrus of the cat, showing a laminar lesion produced by 10-Mev protons. Arrows indicate the direction of the beam. Roman numerals denote cortical layers, and *x* indicates the zone of destruction. Note the sharp borders of the destructive lesion and the normal cytoarchitecture of the remaining cortex. Dura was not opened during irradiation. (Cat 2, section 330, magnification × 68.) Shrinkage of the tissue owing to imbedding is about 30 percent.

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