540 mμ. These are the results expected if a cyanmethemoglobin is formed (by the reaction of cyanide with methemoglobin), with spectral properties resembling the normal compound. If this solution is now oxidized with potassium ferricyanide and again treated with sodium cyanide, a curve with a single maximum, in the visible region, at 535 mμ results. (When the zone-A eluate was similarly treated, the maximum was found to be at 545 mμ. In addition, the maximum for the zone-C pigment was broader than that for zone A. As was noted in the case of the oxyhemoglobin spectra, such differences cannot be considered significant at the present time.) From this it is concluded that all of the pigment in the oxidized eluate of zone C is reactive with cyanide and, hence, presumably is methemoglobin. On the basis of these findings, it may be stated that the original eluate from zone C is a mixture of methemoglobin and oxyhemoglobin.

We have as yet no direct proof that the pigment of zone B is formed from the oxyhemoglobin of zone C, since we have not succeeded in separating them sufficiently to study their interconversion in detail. The relatively greater cathodic migration of the zone-B pigment suggests this relationship however. If the two pigments differ only in the valence of the iron of the heme group (+2 for the oxyhemoglobin and +3 for the methemoglobin), then the methemoglobin would be expected to migrate cathodically slightly in advance, since it would have the greater positive charge. If we assume that these two pigments are so related, the spectral curve of the oxidized eluate of zone C (Fig. 1) is that of pure methemoglobin M. The absence of a maximum at 630 mμ explains the failure to detect methemoglobin by the method of Evelyn and Malloy (*4*), since this test is dependent on the disappearance of this maximum after chemical treatment. The finding of a hemoglobin derivative with spectral characteristics in the visible region differing from those of the normal compound is unique, since the location of maxima is nearly identical, even when hemoglobin derivatives from different vertebrate species are compared (*7*).

In summary, a second family with methemoglobinemia of the Hörlein and Weber type has been discovered. Both normal oxyhemoglobin and an abnormal oxyhemoglobin, designated *hemoglobin M*, have been identified in the hemolyzate from one affected individual. An abnormal methemoglobin differing in its visible spectral characteristics from normal methemoglobin is also present. It is tentatively concluded that the abnormal oxyhemoglobin is the precursor of the abnormal methemoglobin, and in consequence the term *methemoglobin M* has been applied to the latter pigment. From the available family history, it is expected that hemoglobin M will show the dominant inheritance pattern usually found with the abnormal hemoglobins. The individual described herein then represents the hemoglobin-M trait.

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3 June 1957

Nonspecific Inhibition of Vaccinia Hemagglutinin

Knowledge concerning the occurrence of nonspecific (nonantibody) inhibitors of viral hemagglutinins in body fluids is essentially limited to influenza virus. The present report (*1*) concerns the hemagglutinin of vaccinia virus. The hemagglutinin inhibitor to be described was found in the plasma of the Lettré, hyperdiploid line of the Ehrlich ascites carcinoma of the mouse. The procedure used to measure the inhibitor was the same as that described in a previous paper (*2*), except that calcium chloride was omitted from the diluent. Five agglutinating doses of the IHD-E strain of virus (*3*) were employed in the hemagglutination inhibition tests. The method of inoculating mice has been described previously (*2*).

Variation of inhibitor concentration with the age of the tumor is shown in Table 1. The concentration is highest in the older tumors. That the inhibitor is not antibody is apparent from two facts: (i) it is not present in the serum of tumor-bearing mice; and (ii) it is inactivated by heating at 60°C for 30 minutes. Of additional interest is the observation that it has no neutralizing action on the infectivity of vaccinia virus.

A sample of the ascites tumor was disintegrated by shaking with glass beads (*4*). The supernatant of this preparation, following centrifugation, did not show an increase in titer over that of the cell-free fluid from an untreated control specimen. This was equally true of both the 5- and 13-day tumors. Disintegrated preparations made from washed cells, which had been resuspended in 0.85-percent NaCl, yielded no inhibitor at all.

When cell-free tumor fluid was mixed with an equal volume of packed, agglutinable fowl cells, and specimens were held at room temperature and at 4°C for 1 hour, the inhibitor was not adsorbed from the fluid in either case. A 1-percent suspension of fowl cells was prepared in tumor fluid, and samples were held at room temperature and at 4°C for 1 hour. The cells were subsequently washed with 0.85-percent NaCl and used to determine the hemagglutination titer of a suspension of vaccinia virus. The titers were the same as those of control suspensions that had not been in contact with tumor fluid. The evidence suggests that the inhibitor acts against the hemagglutinin, and not against the fowl cells.

Inhibition was also observed with other strains of vaccinia virus. In comparative tests in which five agglutinating doses of the IHD-E, Nelson, and Levaditi strains were employed, inhibition occurred with all three, and to the same titer.

Upon the dialysis of cell-free tumor fluid against distilled water at 4°C for 24 hours, the inhibitor was not removed. However, it was found only in the precipitate in the dialyzing bag. When the volume in the bag was adjusted to what it had been prior to dialysis, it was found that the precipitate represented 7 percent of the original volume. A sample of the thoroughly resuspended contents of the dialyzing bag was brought back to solution by adding NaCl. The titer of this sample was the same as that of the

Table 1. Vaccinia hemagglutinin inhibitor concentration in tumors of different ages. Titers were determined individually on the cell-free tumor fluids obtained from each of three mice which were sacrificed daily.

Age of tumor (day)	Titer		
	Mouse No. 1	Mouse No. 2	Mouse No. 3
3	< 10	10	< 10
4	20	20	20
5	20	40	10
6	40	40	80
7	80	80	80
8	80	20	40
9	40	40	40
10	160	160	80
11	40	80	40
12	160	160	160
13	320	160	80
14	160	160	160

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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* These studies were carried out during the tenure of a Lederle Medical Faculty award.

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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

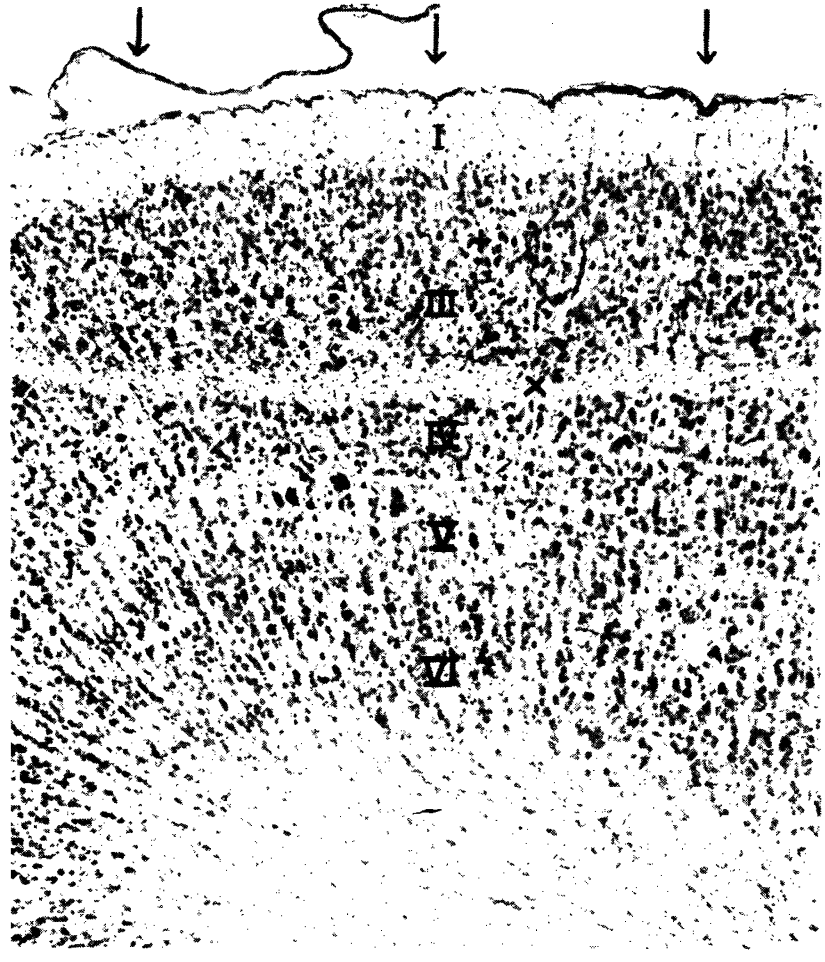

Fig. 1. Cortex of the lateral gyrus of the cat, showing a laminar lesion produced by 10-Mev protons. Arrows indicate the direction of the beam. Roman numerals denote cortical layers, and *x* indicates the zone of destruction. Note the sharp borders of the destructive lesion and the normal cytoarchitecture 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.