appearance of muscular after-discharges for longer intervals than on the untreated side; we further found an increase in duration of cortical after-discharges and a decrease of about 30 percent of the voltage necessary to produce movement. This observation may be of interest in connection with McCulloch's recent findings(3) that eserine and atropine which generally sensitize and inhibit central acetylcholine effects and which have been found to prolong and to shorten respectively the period of cortical facilitation, do not produce any change in the cortical threshold to electrical stimulation.

Local application of an epinephrine solution 1/10,000 as well as that of isotonic saline, was ineffective in our control experiments.

Conclusions. 1. On the basis of these results, it may be concluded that epinephrine stimulates neuronal aggregates in the cerebral cortex. 2. The local effect is marked by an increase in duration and intensity of the period of over-excitation subsequent to a stimulus eliciting movement, and it is characterized by the appearance of after-discharges of cortex and muscle after stimuli just sufficient to produce movement.

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Experimental Poliomyelitis. Studies in Mice with Human Gamma Globulin. (20342)

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Recent preliminary evaluation of gamma globulin as a prophylactic agent for poliomyelitis in children by Hammon, Coriell, Wehrle, Klimt, and Stokes(1), demonstrated significant temporary protection. Experimental study of gamma globulin to determine dosage, time of administration, and duration of passive protection, becomes a matter of considerable importance.

The data in the present report deal exclusively with studies in mice which offer a convenient though limited tool for investigating certain quantitative aspects of the problem. Bodian(2) protected cynomolgus monkeys with doses of gamma globulin as small as 0.1 ml/kg of body weight when the poliomyelitis virus was fed immediately after the administration of gamma globulin. Adams and his co-workers(3,4) found that when cynomolgus monkeys were tonsillectomized, much larger doses were required for protection. A dose of 2.0 ml/lb inoculated 24 hours prior to the feeding of virus gave complete protection. Rhodes and Clark(5) reported an *in vitro* neutralization titer of approximately $10^{-3.5}$ for gamma globulin tested against Lansing virus in mice. Hammon, Cheever, and Sather(6) obtained some protection against poliomyelitis in suckling mice with gamma globulin in 0.05 ml/lb dose.

Methods. A rodent-adapted Y-SK strain* of poliomyelitis virus was employed. After 4 intracerebral passages through mice, virus-infected brains and spinal cords of 7 mice were harvested aseptically and emulsified in distilled water to a 10% suspension. This suspension was titrated by intracerebral inoculation of white mice. The LD_{50} dose calculated at 21 days according to the Reed-Muench(7) formula, was $10^{-3.35}$. The 10% suspension

* Kindly provided by J. R. Paul.

was stored in a deep freeze with dry ice and was used as source of virus for all experiments in this report. Samples were thawed as needed and appropriately diluted in sterile distilled water to contain 50 LD_{50} doses of virus in a 0.03 ml intracerebral inoculum, as estimated by titration at the start of this series of experiments. Each experiment was controlled for virus potency by a group of mice inoculated with virus alone. Mice were examined for paralysis or death daily for a period of at least 5 weeks. The gamma globulin employed was a concentrate of pooled human plasma gamma globulin provided by the American National Red Cross (Immune Serum Globulin Human 160 mg of gamma globulin per cc Squibb). *In vitro* neutralization tests were performed in the usual way(8). Constant amounts of virus were mixed with an equal volume of dilutions of gamma globulin in sterile saline. The mixtures were kept at 37°C for 1 hour and in the refrigerator overnight, then inoculated intracerebrally into groups of 8 white mice.

Results. In vitro neutralization. The concentration of gamma globulin required for 50% survival at 30 days, calculated according to the Reed-Muench method of accumulation totals, was $10^{-3.05}$. Since the mice in this experiment averaged 22.5 g weight, 0.015 ml of this concentration of gamma globulin (1/1130) corresponded to a dosage of 0.0006 ml/kg body weight.

In vivo neutralization. In this experiment, gamma globulin in varying amounts was inoculated intraperitoneally into mice 1 day prior to the intracerebral test dose of virus. A divided dose was used only in the group receiving the largest amount of gamma globulin, where the second injection was given 48 hours later. The 30-day results are shown in Table I. The gamma globulin dosage was computed on the basis of average mouse weight of 22.5 g in this series. The 50% neutralization dose of gamma globulin, when given 1 day before virus inoculation, was calculated as 8.5 ml/kg. This was more than 14,000 times the 0.0006 ml/kg dose which sufficed for 50% neutralization of the same virus dose *in vitro*.

Duration of protection with gamma globulin.

TABLE I. Protective Effect of Gamma Globulin *In vivo* when Given One Day before Poliomyelitis Virus.

	Gamma globulin (ml/kg)						
	50	20	10	5	.5	.05	0
Mice dead or paralyzed at 30 days	1/7	4/8	6/8	4/8	7/8	8/8	6/8
Cumulative mortality index*	.047	.243	.461	.633	.930	1.000	

* Inclusion of 2 additional doses of gamma globulin between the critical values of 5 ml/kg and 50 ml/kg introduced the factor of unequal log-dose intervals. Irwin's generalization of the Reed-Muench method was therefore used to calculate the 50% neutralization dose of gamma globulin(9).
50% neutralization dose = 8.5 ml/kg.

The purpose of this experiment was to determine the optimal interval for administration of gamma globulin prior to injection of virus. Use of an excessively high dose of gamma globulin was to be avoided therefore. Three-week-old mice were divided into 11 groups. All groups except the control received 0.09 ml of gamma globulin intraperitoneally but on a staggered schedule. When all mice were injected simultaneously with the constant challenge dose of virus 2 months later, the interval between the prophylactic and infective inoculations varied from 2 months to one-half hour in the different groups as indicated in Table II. When the amount of gamma globulin was referred to average body weight of 22 g on the day of infection, the dosage was calculated as 4.1 ml/kg. Table II shows the results at different preinfection intervals. Since there was no single correct date for terminating the experiment, Table II records the results obtained at 21, 30, and 38 days after infection. After 38 days, new cases among the mice had virtually ceased. At 21 days after infection, the groups which had received gamma globulin up to 4 days prior to infection, all showed survival of one-half or more mice. None of the other groups showed significantly greater survival than did the untreated controls (17%). At 38 days, only the 4-day pretreated group continued to show any large number of survivors (5 of 8). The chi-square test for significance of the difference between the 4-day group and control group, gave a

TABLE II. Protective Effect of Gamma Globulin* *In vivo* when Given at Varying Intervals before Poliomyelitis Virus.

Days after infection	Mice dead or paralyzed	Time gamma globulin preceded virus — Days										Control: no gamma globulin
		60	46	32	14	7	4	3	2	1	½ hr	
21	No.	4/4	4/4	6/9	8/9	9/11	1/8	2/9	5/10	4/10	5/12	10/12
	%	100	100	67	89	82	13	22	50	40	42	83
30	No.			9/9	8/9	10/11	3/8	5/9	7/10	5/10	9/12	11/12
	%			100	89	91	38	56	70	50	75	92
38	No.				8/9	10/11	3/8	8/9	7/10	6/10	10/12	11/12
	%				89	91	38	89	70	60	83	92
		Repeat experiment										
21	No.			9/15†	8/13	5/10	2/10	4/10	3/10	4/10	9/11	8/13
	%			60	62	50	20	40	30	40	82	62
30	No.			13/15	10/13	5/10	4/10	5/10	3/10	5/10	10/11	10/13
	%			87	77	50	40	50	30	50	91	77

* 0.09 ml intraper. † This group received gamma globulin 28 days before virus.

"P" value of 0.02-0.05 (Yates' correction for small numbers). The significance of this result is increased by the observation that none of 15 control groups which had received the same virus suspension and dose in the course of these experiments, showed more than 25% survival at 30 days. In the total of 129 control mice, mortality at 30 days was 91%. Especially noteworthy was the complete absence of protection in the 7-day pretreated group. Three additional groups of 12 mice received the same dose of gamma globulin 1, 4, or 7 days after virus inoculation. The first two groups showed no protection; the last showed only equivocal protection, and are not included in the table.

Repetition of previous experiment. To test the validity of the observations of the last experiment, a similar protocol was carried out. In this experiment, average mouse weight on day of infection was 32.2 g. The same gamma globulin dose of 0.09 ml intraperitoneally was, therefore, equivalent to 2.8 ml/kg. The virus suspension was inoculated in 1/45 dilution as before. The results are also recorded in Table II. At 21 days after infection, the groups which had received gamma globulin, 4, 3, 2, or 1 days before infection, all showed lower mortality rates than did the control group. Amongst these, the 4-day group again appeared to have an advantage.

Plotting the curves of mortality rate against time for each group, did not reveal more than a transitory advantage of questionable significance for the 4-day group as against the 3, 2, 1-day groups. The 7-day pretreated group showed mortality rate curve in close parallel to the control group for the first 20 days. The results then diverged, indicating some possible protection in this group. The 14-day, 28-day, and ½-hour pretreated groups showed mortality rates equal to or greater than the control group throughout the period of observation.

Application of the chi-square test to the 30-day readings in Table II, indicated in both experiments a probability of 0.01-0.02 that the observed variations in mortality might be due to chance alone. These data also showed a statistically significant reduction in mortality for the combined 1, 2, 3 and 4-day groups, contrasted with all other groups. Combined mortalities were 54% vs 90%, and 42.5% vs 77.4% ($P < 0.001$ in both series).

Discussion. The findings of several investigators have suggested that passive immunity to poliomyelitis obtained with practicable doses of gamma globulin was only of few weeks' duration(1,10-13). In our experiments on duration of passive protection, all groups were infected simultaneously with the identical virus suspension, and special effort was made to determine the endpoint of the protection period. The 0.09 ml dose of gamma globulin was equivalent to about 3-4 ml/kg body weight, yet the mice showed complete loss of protection against intracerebral challenge within the brief space of 7 days in one experiment, and within 2 weeks in the second experiment.

It is a matter of some interest that gamma globulin given intraperitoneally in mice may require as much as four days to achieve maximal protection. The explanation for the lag-period is not entirely clear. Kramer(11) found that when convalescent serum was inoculated into mice intraperitoneally, the level of poliomyelitis neutralizing antibody in the circulation was high the next day and fell sharply within the following 3 days. However, Morgan, Schlesinger, and Olitsky(14) and Kramer(11) presented evidence that the concentration of neutralizing antibody in the central nervous system—the *locus minoris resistentiae*—was a more important criterion of immunity to intracerebral inoculation of virus than was circulating antibody. Appearance of antibody in the brain and spinal fluid lagged behind antibody in the blood(14).

It is important then to consider that experiments on passive protection with gamma globulin or immune serum administered a customary 24 hours before intracerebral virus challenge, may not constitute a test under conditions of maximal prophylactic effect.

Summary. 1. The dosage of gamma globulin required to protect mice against poliomyelitis virus intracerebrally was several thousand times the amount which was effective for *in vitro* neutralization. 2. The prophylactic effect of gamma globulin was maximal with a dose of 4 ml/kg intraperitoneally when given 4 days before inoculation of virus intracerebrally; at 7 to 14 days protection had apparently disappeared.

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Electrophoretic Plasma Protein Patterns in Families with Hemophilia.* (20343)

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The appearance of an electrophoretic anomaly in a group of hemorrhagic diseases has been described in an earlier paper(1). This anomaly, which was called α_x-globulin, represents one of the few instances in which the electrophoretic patterns are typical for a given disease or for a certain group of diseases. The detection of this anomaly was made possible by the thorough morphologic study of the electrophoretic patterns(2) rather than by

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