TABLE I. Volume of Distribution and Rate of Destruction of Antipyrine in the Dog.

Sex	Wt, kilo	Antipyrine space, %	Decay rate, %/hr
♀	10.4	55.2	31
♂	8.2	60.8	28
♀	17.3	60.1	23
♀*	13.3	75	29
♀*	18.4	79.5	46
♀	14.8	53.9	31
♀*	4.2	76.5	30
♀	16.9	56.8	21
♂	24	67.9	60
♂	10	59.1	24
♂	18.5	66, 66.4†	20

* Very lean or emaciated dogs.
† Repeat determination after a 3 week period.

to total body mass in obese animals and men. The results in 11 dogs are given in Table I.

Summary and conclusions. 1. Intravenously injected antipyrine is destroyed in the dog at the rate of 20 to 60% per hour. 2. Nevertheless, antipyrine space may be used for the determination of total body water in the dog, because of the fact that the distribution of antipyrine appears to be essentially complete after 10 minutes. 3. Dogs in adequate nutritional status are found to have antipyrine spaces of 53.9% to 67.9%. The average value obtained from 8 normal dogs was 59.9%. 4. Inadequate nutrition and emaciation result in an increase in the antipyrine space, a result consistent with the idea that body water is related more closely to lean body mass than to body weight.

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Passive Immunity in Poliomyelitis. IV. Protection of Rhesus Monkeys Against Cerebral Challenge.* (19400)

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During the last 20 years, several workers have investigated the protective properties of immune serum from human and animal sources in experimental poliomyelitis. The significance of much of this earlier work is difficult to assess, as technics for the accurate titration of virus pools and immune sera had not been developed, the existence of distinct immunologic types of virus was unsuspected, and often the number of animals used was too small for the results to have statistical value. The experiments of Kramer(1,2), however, indicated that human convalescent serum exerted a significant degree of protection in mice challenged cerebrally with the Lansing strain. Interest in this subject was revived when it was shown that gamma globulin prepared from human plasma protected mice against the Lansing strain(3). Bodian(4) demonstrated the presence in this material of antibody to all three immunologic types of poliomyelitis virus. Later he showed that some protection against an intramuscular challenge was achieved in rhesus monkeys inoculated intramuscularly with 2 ml of gamma globulin per kilogram body weight(5). We have shown that mice can be protected against a cerebral challenge of Lansing mouse virus by the inoculation of monkey immune serum, as well as plasma and placental gamma globulins(6). The present paper describes 3 passive pro-

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tection experiments in monkeys, the virus challenge being given intracerebrally with the object of paralyzing 90-100% of controls, and thus making easier the evaluation of any sparing effect. In each experiment the virus challenge was given at a time when large amounts of circulating antibody were present. It is possible that the mechanism of protection depends on the neutralization of virus at the site of inoculation, because of the leakage of antibody from ruptured vessels. We consider, however, that this possibility does not detract from the theoretical value of the results.

Materials and methods. Monkeys. Rhesus monkeys were used in all experiments. They weighed from 5 to 11 lbs and were obtained from Okatie Farms, South Carolina. All were shown to be tuberculin negative. *Sera and gamma globulin.* Plasma gamma globulin used in the preliminary experiments was supplied by the American National Red Cross; the 50% neutralizing titer determined in mice for the Lansing strain was 3.5 logs ($10^{-3.5}$). Placental gamma globulin was generously provided by Lederle Laboratories Division, American Cyanamid Company, and contained at least 16% gamma globulin. The average 50% neutralizing titer against Lansing virus was 3.5; this material exerted a powerful protective action in mice against intracerebral challenge of Lansing virus(6). The pooled immune monkey serum was obtained from rhesus monkeys injected every 2 weeks by the intramuscular route with Lansing virus incorporated either in distilled water or adjuvants (7). The 50% neutralizing endpoint of the serum pool used in Experiment 2 was 4.5 and in Experiment 3, 3.86. No Lansing antibody was detected in the pools of normal monkey serum. *Virus pools.* Lansing virus monkey pool No. 9 was used throughout for the challenge of monkeys(7). It was prepared from the cord enlargements of monkeys harvested on the first day of paralysis following inoculation of a $10^{-2.0}$ dilution of monkey cord pool No. 8. A titration of pool No. 9 was carried out shortly after its preparation, by inoculation in 5 groups of 10 monkeys, and the PD_{50} was found to be 5.2. *Histologic examination of monkeys.* Histologic examination was carried out on all test animals, and on those control animals in which typical paralyses were not observed. The brain and cord were removed from each animal, fixed in formalin, and sections of cortex, basal ganglia, brain stem, cerebellum, and several levels of spinal cord were examined microscopically.

TABLE I. Lansing Antibody Levels in Monkeys after 20 ml (Intravenous Injection) of Plasma Gamma Globulin Approximately 4 ml/lb body wt.

		50% neutralizing titer of monkey serum at following times*			
No.	Wt	Before inj.	24 hr	48 hr	72 hr
A242	5 lb, 2 oz	0	2.65		
A243	5 15	0	2.72	1.95	
A244	5 3	0	2.79	2.72	1.9

* Sera tested with 17 LD_{50} of Lansing virus.

TABLE II. Lansing Antibody Levels in Monkeys after 20 ml (Intramuscular Injection) of Plasma Gamma Globulin Approximately 3 ml/lb body wt.

		50% neutralizing titer of monkey serum at following times*				
			Days			
No.	Wt	Before inj.	4	7	10	14
A293	7 lb, 8 oz	0	2.76			
A294	6 14	0	3.1	2.72		
A296	7 12	0	2.65	2.72	2.16	1.67

* Sera tested with 17 LD_{50} of Lansing virus.

Preliminary experiments in monkeys. Certain preliminary experiments were carried out in order to estimate the neutralizing titers likely to be obtained in monkey serum shortly after the intravenous or intramuscular injection of gamma globulin. In Table I are shown the Lansing virus antibody levels in 3 monkeys at various intervals after the intravenous injection of approximately 4 ml of plasma gamma globulin per pound of body weight.

In Table II are shown the Lansing virus antibody levels in the serum of 3 larger monkeys after the intramuscular injection of approximately 3 ml of plasma gamma globulin per pound of body weight. It will be seen that antibody titers in the region of 3.0 were obtained 4 days after the inoculation of 3 ml of gamma globulin per pound of body weight intramuscularly, whereas such a level was only found 24 hours after an injection of approximately 4 ml per pound of body weight intravenously. A serum antibody titer of 3.0 was considered by Morgan(8) to represent the

TABLE III. Passive Protection Experiment with Placental Gamma Globulin Intravenously (Experiment 1).

No. of animals and designation	Avg wt of monkeys	Prophylactic admin. intrav. day before challenge	Challenge inoculum (thalamic)	Deaths from causes other than poliomyelitis	Poliomyelitis rate
10 control	5 lb, 14 oz	0	160 PD_{50} (.8 ml of 10^{-3} dilution of Lansing cord pool #9)	0/10	10/10
10 test	5 14	5 ml (.8 ml/lb body wt)		0/10	9/10
10 "	6	25 ml (4 ml/lb body wt)		1/10 (meningitis)	8/9

TABLE IV. Passive Protection Experiment with Immune Monkey Serum Intramuscularly (Experiment 2).

No. of animals	Avg wt of monkeys	Monkey serum admin. intramusc. day before challenge	Challenge inoculum (thalamic)	Deaths from causes other than poliomyelitis	Poliomyelitis rate	Avg incubation days
20 control	6 lb, 8 oz	5 ml normal serum per lb body wt	50 PD_{50} (.8 ml of $10^{-3.5}$ dilution of Lansing cord pool #9)	1/20 (meningitis)	18/19	7
20 test	6 6	5 ml immune serum per lb body wt		4/20 (3 meningitis, 1 diarrhea)	4/16	14

minimum level in vaccinated animals to be associated with resistance to cerebral challenge; our preliminary experiments suggested that such a level could be achieved by parenteral administration of gamma globulin or immune serum of high titer.

Protection experiments in monkeys. In these experiments monkeys were assigned to the test and control groups at random. The prophylactic agent or normal control serum was given intramuscularly. Both test and control groups were challenged thalamically with Lansing virus on the day after serum inoculation, and were observed daily for a period of 6 weeks or until the onset of paralysis. Animals were killed only when there was obviously no hope of recovery; and histologic examination was performed on all test animals, and on control animals if the diagnosis appeared doubtful. Rectal temperatures were recorded daily on the test animals, and small samples of blood were taken for antibody study, from both test and control animals, 4 days after injection of the gamma globulin or serum, and at weekly intervals for 6 weeks.

Exp. 1. Thirty monkeys were used in this experiment. Ten animals in the control group received no injection of gamma globulin, 10 animals in the test group received 5 ml, and another 10 animals 25 ml of gamma globulin intravenously on the day before thalamic challenge with 160 PD_{50} of Lansing virus. The results of this experiment are recorded in Table III, from which it will be seen that all 10 controls succumbed to poliomyelitis. Two of 19 animals injected with gamma globulin failed to develop paralysis, and as one paralyzed monkey in the test group receiving 5 ml survived for 6 weeks, 3 of 19 monkeys in the test groups escaped a fatal paralysis.

Exp. 2. In this experiment, 20 control and 20 test animals were used, the former receiving 5 ml of normal monkey serum per pound body weight, and the latter immune monkey serum (titer 4.5) in the same dosage. All monkeys were challenged the following day with 50 PD_{50} of Lansing virus. Table IV shows the results of this experiment. It will be noted that 18 out of 19 control monkeys contracted poliomyelitis, whereas only 4 out of 16 test animals did so; there was also a striking contrast in the average incubation periods of the 2 groups. Twelve animals in the test group remained healthy for 6 weeks and histologic

TABLE V. Passive Protection Experiment with Immune Monkey Serum Intramuscularly (Experiment 3).

No. of animals	Avg wt of monkeys	Monkey serum admin. intramusc. day before challenge	Challenge inoculum (thalamic)	Deaths from causes other than poliomyelitis	Poliomyelitis rate	Avg incubation days
20 control	9 lb, 11 oz	3 ml normal serum per lb body wt	100 PD_{50} (.8 ml of $10^{-3.2}$ dilution of cord pool #9)	0/20	20/20	5.9
20 test	9 15	3 ml immune serum per lb body wt		2/20 (1 trauma, 1 brain abscess)	3/18	17.3

examination revealed no poliomyelitis. The survival of 12 out of 16 monkeys points to a high degree of protection (75%) afforded by the high titer homologous immune serum. Even if it is assumed that the 4 animals dying from other causes might have acquired poliomyelitis if they had survived long enough to become infected, the survival rate is still 12 out of 20 (60%).

Exp. 3. The design of this experiment was identical with that of Experiment 2, except that an immune serum pool of slightly lower titer was employed (3.86) and 3 ml per pound of body weight were given. A slightly greater challenge (100 PD_{50}) was employed. The results are recorded in Table V, where it will be seen that all 20 control animals developed poliomyelitis while only 3 of 18 test animals contracted the infection. There is again a marked difference in the average incubation periods in the 2 groups. Fifteen out of 18 test animals survived for 6 weeks, and histologic examination revealed no evidence of poliomyelitis infection.

Neutralization tests are at present being carried out on the sera obtained in Exp. 2 and 3 in order to correlate resistance to cerebral challenge with serum antibody level. The results of these tests will be reported separately.

Summary. 1. Lansing virus serum antibody titers in the range of $10^{-2.0}$ to $10^{-3.0}$ can be achieved in monkeys shortly after the administration of gamma globulin intramuscularly or intravenously. 2. Gamma globulin injected intravenously failed to protect a significant number of monkeys against an intracerebral challenge of 160 PD_{50} of Lansing virus. 3. In 2 further experiments, the majority of monkeys receiving high titer immune monkey serum were protected against an intracerebral challenge of 50 and 100 PD_{50} of Lansing virus respectively. In one experiment only 4/16 test animals became paralyzed as compared to 18/19 controls. In the other experiment 3/18 test animals developed poliomyelitis as compared to all of 20 controls.

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