ing an amino acid imbalance has been investigated. The hypothesis that threonine inhibits the conversion of tryptophan to tissue pyridine nucleotides via 3-hydroxyanthranilic acid is not strongly supported by our results. The results indicate, however, that excess dietary threonine markedly interferes with the digestion and absorption of the ration and that this is probably a better explanation for the action of excess threonine in producing growth inhibition in the rat.

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Gamma Globulin Passive Protection Tests in Mice Injected Intraperitoneally with MEF1 Poliomyelitis Virus.*† (19552)

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Human gamma globulin prepared from pooled adult plasma has been shown to possess substantial amounts of antibody against the Lansing strain (Type 2) of poliomyelitis virus (1). Similar observations have been made in connection with the other known types of poliomyelitis virus(2). Human gamma globulin when administered intraperitoneally to mice has been shown to afford some protection against the subsequent intracerebral inoculation of Lansing virus(3). More recently Bodian(4) demonstrated that in the case of monkeys 2.0 ml of gamma globulin per kg of body weight gave significant protection against the intramuscular inoculation of Lansing virus. The globulin and the virus were inoculated into the calf muscles of opposite legs. A protective effect could be demonstrated if the globulin was inoculated as much as 3 weeks before or 3 days after the injection of virus. Amounts of antibody sufficient to protect against the intramuscular injection of active virus did not interfere with the subsequent development of active immunity.

The recent demonstration that the suckling mouse-adapted MEF1 strain of poliomyelitis virus(5) can infect and cause the death of a significant proportion of mice aged 5-15 days when inoculated by the intraperitoneal route (6) has made available another laboratory animal which may be used for determining the efficacy of human gamma globulin in furnishing protection against the peripheral inoculation of a rodent-adapted strain of poliomyelitis virus. The present paper deals with the results of such an experiment.

Materials and methods. Two viruses belonging to the Lansing group (Type 2) were employed. The suckling mouse-adapted MEF1 strain (53rd passage material) was kindly furnished to us by Dr. Casals. The classical Lansing strain was obtained originally from Dr. Armstrong. Suckling mice of the CFW strain were used. The human gamma globulin was processed by E. R. Squibb (Lots No. 116-1 and No. 157-3) from pools of human plasma provided by the American National

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TABLE I. Protective Effect of Gamma Globulin Against Intraperitoneal Inoculation of MEF1 Virus in 5-6-day-old Mice.

Group	Protective inoculation γ globulin	Mortality Exp. 1	Exp. 2	Exp. 3	Total	%	Diff./ S.E. diff.
I	0	18/35*	22/38	20/30	60/103	58.3	
II	.02 ml/lb	—	16/24	6/12	22/36	61.1	.3
III	.1	3/13	6/22	3/14	12/49	24.5	4.3
IV	.5	—	1/24	1/12	2/36	5.6	8.5
MEF1 inoculum:		Pool					
Passage No.		54, 55, 56	59	59			
Titer (i.c.)		$10^{-4.4}$	$10^{-3.7}$	$10^{-3.5}$			

* Numerator: No. of mice dying. Denominator: No. of mice inoculated.

TABLE II. Protective Effect of Gamma Globulin Against Intraperitoneal Inoculation of MEF1 Virus in 6-13-Day-Old Mice.

Group	Protective inoculation	Mortality Exp. 1	Exp. 2	Total	%	Diff./ S.E. diff.
I	0	45/57*	41/49	86/106	81.2	
II	Gelatin	26/32	—	26/32	81.2	.0
III	.02 ml/lb G.G.†	20/31	—	20/31	64.5	1.8
IV	.1 ml/lb G.G.†	7/29	10/35	17/64	26.6	8.1
V	.5 ml/lb G.G.†	9/35	—	9/35	25.7	6.7
MEF1 inoculum:						
Passage No.		61	61			
Titer		10^{-4}	$10^{-3.6}$			

* Numerator: No. of mice dying. Denominator: No. of mice inoculated.
† Gamma globulin.

Red Cross. In the first series of experiments litters of suckling mice 5-6 days old were pooled and redistributed at random after the average weight of the animals had been determined. Three groups of mice were injected intramuscularly with varying dilutions of gamma globulin so that the groups received 0.5 ml/lb, 0.1 ml/lb and 0.02 ml/lb of body weight, respectively. The dilutions were so adjusted that each mouse received the same volume (0.03 ml) of fluid. A fourth group received no injection and served as a control. Twenty-four hours later all mice were challenged by the intraperitoneal inoculation of 0.1 ml of a 10% suspension of MEF1 virus. Mice were inspected daily for 21 days for the presence of paralysis or death. In the second series of experiments 6-13-day-old suckling mice were used. Three groups received 0.5 ml, 0.1 ml, and 0.02 ml of gamma globulin per lb of body weight, respectively, while a fourth group served as uninoculated controls. An additional control group of mice inoculated with 0.03 ml of 1.5% gelatin solution was employed. Twenty-four hours later all the mice were challenged with MEF1 virus. Furthermore, the possible effects of the preliminary inoculation *per se* upon subsequent mortality was investigated by permitting corresponding groups of mice to go through the observation period unchallenged. In the third series of experiments 8-10-day-old mice were employed and the amounts of gamma globulin injected were 0.0125 ml/lb, 0.025 ml/lb, 0.05 ml/lb, and 0.1 ml/lb, respectively, for the 4 experimental groups. A fifth group received no protective inoculation. All mice in these 5 groups were challenged with MEF1 virus 24 hours later. A final group of mice received no preliminary inoculation and was not challenged with MEF1 virus.

Ten days after the close of each experiment of Groups I and II the surviving mice were challenged by the intracerebral inoculation of approximately 30 LD_{50} of Lansing virus in order to detect any evidence of the development of active immunity.

Results. Gamma globulin in concentrations

TABLE III. Effect of Injection of Gamma Globulin or Gelatin upon Subsequent Mortality Among 6-13-Day-Old Mice Not Injected with Virus.

Group	Protective inoculation	Mortality Exp. 1	Mortality Exp. 2	Mortality Total	Mortality %	Diff./S.E. diff.
I	0	13/51*	8/35	21/86	24.4	
II	Gelatin	11/31	—	11/31	35.5	1.1
III	.02 ml/lb G.G.†	7/32	—	7/32	21.9	.3
IV	.1 ml/lb G.G.†	5/31	1/29	6/60	10	2.4
V	.5 ml/lb G.G.†	7/30	—	7/30	23.3	.1

* Numerator: No. of mice dying. Denominator: No. of mice inoculated.
† Gamma globulin.

of 0.5 ml/lb and 0.1 ml lb appeared to furnish significant protection in the 5-6-day-old mice employed in the first series of experiments (Table I). The respective mortalities for these groups was 5.6% and 24.5% as compared to 58.3% for the control group. No protection was observed in the group receiving 0.02 ml of gamma globulin per lb.

The second series of experiments employing mice 6-13 days of age gave similar results (Table II). Significant protection was conferred by gamma globulin injected in concentrations of 0.5 ml/lb and 0.1 ml/lb as compared to the controls. Mice receiving 0.02 ml of gamma globulin per lb showed a lower mortality rate as compared to control mice but the difference was not statistically significant. As was expected gelatin conferred no protection. No evidence was obtained that the protective inoculations alone increased the mortality in the injected mice as compared to the uninjected ones (Table III). In one instance (mice receiving 0.1 ml of gamma globulin per lb) the injected mice showed a significantly lower mortality rate as compared to the control mice. The explanation for this finding probably lies in non-specific factors affecting individual litters.

The third series of experiments employing mice 8-10 days old demonstrated again that 0.1 ml of gamma globulin per lb gave a high degree of protection. Half as much gamma globulin (0.05 ml/lb) conferred significant but less marked protection, while smaller amounts had no demonstrable protective effect.

In Tables V and VI are given the mortality rates of the various groups of survivors challenged by the intracerebral inoculation of Lansing virus one month after the first injection of virus, gamma globulin or gelatin. No protection was conferred by any of the injections of gamma globulin or gelatin alone. The mortality rate for each group was over 90%; the average for all mice was 94.1%. The control mice which had survived the injection of MEF1 virus although unprotected by gamma globulin showed a significantly reduced mortality (47.6%) as was expected. A significant reduction in mortality was observed also among the survivors in the group receiv-

TABLE IV. Protective Effect of Gamma Globulin Against Intraperitoneal Inoculation of MEF1 Virus in 8-10-Day-Old Mice.

Group	Protective inoculation	Virus	Mortality No.	Mortality %	Diff./S.E. diff.
I	0	+	44/47*	93.7	
II	.0125 ml/lb G.G.†	+	53/59	89.8	.74
III	.025 ml/lb G.G.†	+	52/58	89.7	.75
IV	.05 ml/lb G.G.†	+	39/55	70.9	3.2
V	.1 ml/lb G.G.†	+	21/54	38.9	7.3
VI	0 (controls)	0	15/47	31.9	8.1

MEF1 inoculum:
Passage No. 61
Titer (i.c.) $10^{-3.8}$

* Numerator: No. of mice dying.
Denominator: " " " inoculated.
† Gamma globulin.

TABLE V. Challenge of Control Mice with Lansing Virus.

Group	Protective inoculum	Mortality Ratio	Mortality %
I	0	62/65*	95.4
II	Gelatin	20/21	95.2
III	.02 ml/lb G.G.†	24/25	96
IV	.1 ml/lb G.G.†	47/52	90.4
V	.5 ml/lb G.G.†	22/23	95.7
Total		175/186	94.1

* Numerator: No. of mice dying.
Denominator: " " " inoculated.
† Gamma globulin.

TABLE VI. Challenge of Control Mice and MEF1 Inoculated Mice with Lansing Virus.

Group	Protective inoculation	Prev. MEF1 challenge	Mortality Ratio	Mortality %	Diff./ S.E. diff.
I	Controls	0	175/186*	94.1	
II	0	+	19/36	47.6	5.9
III	Gelatin	+	1/6		
IV	.02 ml/lb G.G.‡	+	6/10	60	—†
V	.1 ml/lb G.G.‡	+	25/47	53.2	5.5
VI	.5 ml/lb G.G.‡	+	21/26	80.8	1.7

* Numerator: No. of mice dying. Denominator: No. of mice inoculated.
† Numbers too small for comparison. ‡ Gamma globulin.

ing 0.1 ml/lb of gamma globulin prior to the intraperitoneal inoculation of MEF1 virus. The mortality rate among the survivors of the group receiving 0.5 ml of gamma globulin per lb was lower than that of the control group, but the difference was not statistically significant. The survivors among those receiving 0.02 ml of gamma globulin were too few to permit significant comparison.

Discussion. These results demonstrate that human gamma globulin administered to suckling mice in amounts as small as 0.05 ml/lb will confer significant protection against the intraperitoneal injection of the suckling mouse-adapted MEF1 virus 24 hours later. The duration of this passively conferred immunity has not been ascertained. In Bodian's observations in monkeys it was observed that an amount of gamma globulin adequate to protect against paralytic disease did not interfere with the development of active immunity. Our experiments confirm this when using 0.1 ml gamma globulin per lb, but although the number of mice involved was small the results suggest that 0.5 ml per lb may have prevented the development of an active immunity in some of the animals.

It will be noted that the mortality rate in unprotected mice challenged intraperitoneally with MEF1 virus varied from 58% to 93%. Conversely, a mortality rate of 24% to 32% was noted among the uninoculated control mice. These two extremes limit the present usefulness of this method as a means of testing the efficacy of biological or chemical substances in the prophylaxis of otherwise fatal infections with the MEF1 virus in suckling mice. Serial intraperitoneal passages of the agent in suckling mice, now in progress, may increase the virulence of the virus when inoculated by this route. The excessive mortality among uninoculated control mice can probably be reduced somewhat by more meticulous care of the individual litters. By such means there might be effected some reduction in the relatively large number of mice required in these experiments in order to obtain statistically significant results.

Summary. Suckling mice 5-13 days old were protected against the intraperitoneal inoculation of suckling mouse-adapted MEF1 virus by the intramuscular inoculation of human gamma globulin in amounts as small as 0.05 ml/lb 24 hours prior to the virus challenge. Human gamma globulin in an amount (0.1 ml/lb) adequate to protect against the intraperitoneal inoculation of the suckling mouse-adapted MEF1 virus did not prevent the development of active immunity against the subsequent intracerebral inoculation of Lansing virus.

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