TABLE II.
Summary of Results of Neutralization Tests in Mice with Serums from Rabbits Inoculated with Various Materials.

Material inoculated	No. tested	No. showing neutralization	Results*
Nil (normal)	16	2	(+) (±)
Noninfective	4	4	+ + ± (±)
Human lung and sputum	7	4	+ + + +
Cotton rat or hamster lung	7	2	± (±)
Chick embryo tissue	3	1	+
Mouse lung (influenza)	4	1	±

* + and ± represent definite and incomplete neutralization respectively. Appearance of neutralizing antibodies between first and subsequent bleedings was demonstrated except where results are in parentheses to indicate that neutralization was obtained with the earliest serum specimen available.

ing the indicated inoculations are presented.

Summary and Comment. Fourteen rabbits in a total of 28 developed neutralizing antibodies to the pneumonia virus of mice (PVM) either before or during the time they were under experiment in this laboratory. The appearance of these antibodies could not be correlated with the kind of material injected or with the development of antibodies to the atypical pneumonia virus[5] or to other agents. The incidence of antibodies to PVM was definitely higher in rabbits which had been kept in the laboratory for 2 to 10 months, and thus exposed to this agent carried by other rodents in active or latent form, than in normal rabbits newly received from an outside source or in those under experiment for a short interval of time. An agent related to PVM has been isolated from the lungs of apparently normal cotton rats, but as yet no attempt has been made to isolate a similar agent from rabbits. Human serums frequently contain neutralizing antibodies to PVM,[2] but an increase in these antibodies has not been observed. Although these results do not exclude the possibility that a virus antigenically related to PVM may be carried in the human respiratory tract, they do suggest that previous experiments[3] have failed to establish the relationship of such an agent to the virus isolated in our laboratory from persons with atypical pneumonia.[5]

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Influence of Riboflavin on Susceptibility of Mice to Experimental Poliomyelitis.*

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In previous studies on the relation of nutrition to resistance to infection, we have described the effect of thiamine and of pantothenic acid deficiencies on the susceptibility of mice to the poliomyelitic viruses.[1,2,3] The

* These studies were aided by a grant from the National Foundation for Infantile Paralysis, Inc.

We are indebted to Merck and Co., Rahway, N.J., for the crystalline vitamins, and to Abbott Laboratories, North Chicago, Ill., for the halibut liver oil.

1 Rasmussen, A. F., Jr., Waisman, H. A., Elvehjem, C. A., and Clark, P. F., *J. Bact.*, 1943, **45**, 85.

2 Rasmussen, A. F., Jr., Waisman, H. A., Elvehjem, C. A., and Clark, P. F., *J. Inf. Dis.*, 1944, **74**, 41.

3 Lichstein, H. C., Waisman, H. A., Elvehjem, C. A., and Clark, P. F., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 3.

TABLE I.

Series No.	Diet	Virus	Route of inoc.	Days on exp. when inoculated	No. paralyzed / No. inocul.	% paralyzed
8*	Riboflavin-Low 203	Theilers F.A.	IP	5	14/27	52
	203 + 200 γ riboflavin/100 g	0.5 ml 10% susp.		5	14/27	52
	203 + 5 mg " "			5	12/24	50
16*	203	Theilers F.A.	IP	14	6/22	27
	203 + 200 γ " "	0.5 ml of 10% susp.		14	5/20	25
	203 + 5 mg " "			14	5/23	22
18	203	Theilers F.A.	IP	17	9/25	36
	203 + 200 γ " "	0.5 ml of 20% susp.		17	16/25	64
	203 + 5 mg " "			17	14/27	52
18a	203	Lansing	IC	107	7/13	54
	203 + 200 γ " "	.03 ml of 20% susp.		107	7/10	70
25	203	Lansing	IC	23	21/33	64
	203 + maintenance riboflavin inj. every other day	0.03 ml of 2% susp.		23	23/33	69
	203 + 200 γ riboflavin/100 g			23	24/29	83
25a	203 + 30 γ " "	Lansing	IC	60	6/20	30
	203 + 200 γ " "	0.03 ml of 2% susp.		60	18/27	67
38	203	Theilers GD VII	IC	23	14/33	42
	203 + 300 γ " "	0.03 ml of 0.1% susp.		23	12/35	35
	203	Lansing		23	20/34	59
	203 + 300 γ " "	0.03 ml of 5% susp.		23	26/35	74
38a	203	Theilers GD VII	IC	46	10/13	77
	203 + 300 γ " "	0.03 ml of 10% susp.		46	11/14	78

IP = Intraperitoneal.
IC = Intracerebral.
* Mice from outside source; all others from our own colony.

present report is concerned with similar experiments on riboflavin.

The riboflavin-low diet (203) used throughout these studies was composed of the following parts per 100: sucrose, 73; purified-vitamin-free casein,† 18; mineral salts,[4] 4; corn oil, 5; thiamine, 300 μg; pyridoxine, 300 μg; nicotinic acid, 5 mg; calcium pantothenate, 2 mg; choline, 300 mg; inositol, 100 mg; para-aminobenzoic acid, 100 mg; biotin, 5 μg. Each mouse received adequate quantities of halibut liver oil by mouth twice weekly. The Swiss mice obtained from our own colony ate approximately 2-3 g per day at the beginning of the experiment; after 3 to 4 weeks the riboflavin-deficient animals still consumed about 2 g per day, but began to lose weight after 5 weeks. In contrast, thiamine-deficient mice[2] ate very little at the low point of that deficiency. Aside from loss in weight, the riboflavin-deficient mice showed thinning of the fur coat, slight bleeding about the ears, and in roughly a third of the animals, some scabs over the body. To the best of our knowledge, the animals were supplied with all necessary food substances except riboflavin, since mice on this diet plus riboflavin at a 200 μg per 100 g level, appear sleek, well nourished and in apparent good health. In the several hundred mice in our thiamine series[2] no scabs or signs of hemorrhage were observed. The thiamine-low diet and the riboflavin-low diet were identical except that the respective vitamin was omitted.

The viruses employed were Lansing strain poliomyelitis and both FA and GD VII strains of Theiler's encephalomyelitis; the FA strain was given intraperitoneally, while Lansing and GD VII strains were injected intracerebrally. Mice were inoculated when signs of riboflavin deficiency appeared in the majority of the animals, usually after 14 to 24 days on the deficient diet, so as to obtain maximum paralysis at the peak of the deficiency. Various concentrations of the viruses were used, and the resulting paralysis was directly related to the particular preparation and route

† S. M. A. Corporation, Chagrin Falls, Ohio.

[4] Phillips, P. H., and Hart, E. B., *J. Biol. Chem.*, 1935, **109**, 657.

of inoculation. A concise summary of the experiments is given in Table I.

In series 8 and 16, mice from outside sources were used and were inoculated intraperitoneally with 0.5 ml of a 10% suspension of Theiler's FA virus. The period of depletion prior to inoculation was 5 days in series 8, and 14 days in series 16. In these 2 series, no difference was noted in the percentage of paralysis in any of the groups.

In series 18, mice from our own colony were employed after 17 days of depletion; they were inoculated intraperitoneally, with 0.5 ml of a 20% suspension of Theiler's FA strain. The deficient group showed 36% paralysis (9/25), as compared to 64% (16/25) in the optimum, and 52% (14/27) in the group with marked excess vitamin intake.

The results in series 18a, which included the uninoculated control mice from series 18, indicate that age does not influence the susceptibility of riboflavin-deficient mice to Lansing strain poliomyelitis. When the initial signs of deficiency were observed in the control mice (107 days after the start of the experiment, at which time the animals were 128 days old), 0.03 ml of a 20% suspension of Lansing virus was injected intracerebrally in both deficient and optimum groups with only slight difference (16%) in the incidence of paralysis. Later, in series 25a with Lansing strain, and in series 38a with Theiler's GD VII, the age factor proved to be of no significance.

In series 25, with Lansing virus used intracerebrally, the incidence of paralysis was approximately the same for those mice on a deficient diet (64%) as for those kept on a maintenance level by intraperitoneal injections of 1-3 μg of riboflavin per day (69%). On the other hand, the difference between the deficient mice and those on an optimum level, was 19%, not marked but worthy of note, in view of results in later series. The uninoculated adult mice, control groups of this series (age 81 days) were subsequently depleted of riboflavin and then placed on a suboptimum level (25a) (30 μg per 100 g of diet with an average individual daily intake of less than 1 μg per day). The resulting incidence of paralysis in the deficient group was 30% (6/20) and 67% (18/27) in the optimum group, a difference in this instance of 37%.

Theiler's GD VII strain of encephalomyelitis virus and Lansing virus were used intracerebrally in parallel groups in series 38. The Theiler series showed no significant differences whereas the optimum and deficient groups inoculated with Lansing virus showed a 15% difference in favor of the deficient animals. Uninoculated controls of this series were subsequently injected with the Theiler's GD VII strain 46 days after the start of the original experiment (38a), but the incidence of paralysis was essentially the same both for the deficient and optimally fed groups.

Discussion. Our own experiments with thiamine and pantothenic acid[1,2,3] and those of Foster *et al.*[5] with thiamine deficiency and gross inanition,[6] showed that deficient mice tended to be more resistant to poliomyelitis than were well nourished controls.

Riboflavin deficiency results in a decreased resistance against pneumococcus infection in mice,[7] and against typhus in rats.[8] In our riboflavin experiments, no significant differences have been observed with the FA and GD VII encephalomyelitis viruses. With the Lansing strain poliomyelitis virus, the differences are slight and in one series only (25a), statistically significant.‡ The trend, however, is in the same direction in all 4 experiments and therefore merits consideration. It should also be noted that the riboflavin and the pantothenic acid-deficient mice, unlike the thiamine-deficient animals, continue to eat well so that reduced caloric intake can hardly be the important explanation of the observed differences in resistance. Furthermore, in pantothenic acid-deficient mice a definite increased resistance to Theiler's virus was observed, with no change in susceptibility to Lansing virus under identical conditions. In

[5] Foster, C., Jones, J. H., Henle, W., and Dorfman, F., *Science*, 1943, **97**, 207.

[6] Foster, C., Jones, J. H., Henle, W., and Dorfman, F., *J. Exp. Med.*, 1944, **79**, 221.

[7] Wooley, G., and Sebrell, W. H., *U. S. P. H. R.*, 1942, **57**, 149.

[8] Pinkerton, H., and Bessey, O. A., *Science*, 1939, **89**, 371.

‡ We are indebted to Professor J. H. Torrie for the statistical study.

our riboflavin series, on the other hand it is the Lansing strain which shows the differences between the optimum and the deficient groups while the Theiler's viruses demonstrate no consistent changes. May not these points indicate definite differences in the metabolism of the several viruses?

Summary. Mice fed an adequate diet, save for various levels of riboflavin, showed no consistent differences to infection with Theiler's encephalomyelitis viruses. In four similar series (totaling 234 mice) injected with Lansing strain poliomyelitis virus, the differences were slight but the greater resistance in each series was in the deficient group.

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A Rickettsia-like Organism Recovered from Guinea Pigs.*

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In the summer of 1942 there appeared an unusual febrile disease at Fort Bragg, North Carolina. This mild disease was called "pretibial fever" and was an apparently new clinical entity, as described by Daniels and Grennan.[1] The disease was characterized by 3 to 5 days of fever, splenomegaly, a maculopapular eruption typically limited to the legs, and rapid return to normal health. All of the cases were quartered in the same limited area of the post, a fact of probable epidemiological significance. An army commission which studied the 1942 epidemic was unable to establish the etiology or mode of transmission of the disease. In the summer of 1943, there reappeared a small outbreak of a clinically identical disease in which the cases came from the same area of the reservation as those of the preceding year. A rickettsia-like organism was recovered from 3 of 5 guinea pigs injected with blood from one of these latter patients. Attempts have been made to show that this agent was concerned in the etiology of the disease, but it is not possible at the present time to be sure of its origin. The purpose of this paper is to record the known characteristics of the organism in the hope that future developments may clarify its significance.

Method of Isolation. Defibrinated blood was drawn from the last case of pretibial fever appearing in the 1943 outbreak and was injected intraperitoneally into 5 guinea pigs in 4 to 6 ml amounts. After incubation periods varying from 3 to 5 days, 3 of these guinea pigs became febrile. Three independent passage lines were established by the intraperitoneal transfer of pooled brain and spleen on the third or fourth day of fever and the same organism was recovered from each. After a few transfers, the agent became lethal for guinea pigs; a titration of infected spleen from the fourth passage showed deaths through the 10^{-3} dilution of a 10% emulsion.

The Organism. Poorly staining, gram-negative rods were seen in impression smears of guinea pig tissue or of egg yolk sac. When stained by the Machiavello method,[2] the material was shown to contain numerous bright red, somewhat pleomorphic, small bacillary forms, tending to be paired and often clumped

* This work was supported in part through the Commission on Acute Respiratory Diseases, Board for the Investigation and Control of Influenza and Other Epidemic Diseases, Preventive Medicine Service, Office of the Surgeon General, U. S. Army, and in part by a grant from the National Foundation for Infantile Paralysis, Inc.

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‡ Fellow in the Medical Sciences of the National Research Council.

1 Daniels, W. B., and Grennan, H. A., *J. A. M. A.*, 1943, **122**, 361.

2 *Virus and Rickettsial Diseases*, Harvard University Press, 1940, p. 896.