TABLE II.
Bactericidal Action of Streptothricin on Mycobacteria.

	Units per ml						
	0	1.0	1.5	2.0	3.0	4.0	5.0
M. tuberculosis (var. hominis 607)	+	+	+	+	—	—	—
M. leprœ P.	+	+	+	+	+	±	±
M. smegmatis I.M.	+	+	+	+	+	—	—
M. smegmatis Tol.	+	+	+	+	+	—	—

+ = growth upon subculture on Long's agar.
± = poor growth upon subculture on Long's agar.
— = no growth upon subculture on Long's agar.

much as 1,000 Oxford units per ml. Further studies revealed that this resistance probably is due to the abundant formation of a penicillin-destroying enzyme, presumably penicillinase. Details of this are reported elsewhere.[3]

A study of the inhibitory action of streptothricin for 5 *Mycobacterium* cultures is included in Table I. Since streptothricin is stable, a single addition of the antibiotic was sufficient to maintain the desired unitage over the period of 6 to 8 weeks necessary for growth of some strains of mycobacteria. Streptothricin was particularly effective in Long's medium; this is probably the result of the alkalinity of the medium which is known to enhance the *in vitro* activity of streptothricin.[4] The inhibitory range of 0.1 to 1.0 unit of streptothricin per ml places these *Mycobacterium* cultures among the most sensitive organisms to streptothricin. *M. phlei* previously was found sensitive.[5]

A rough indication was obtained for the bactericidal action of streptothricin upon mycobacteria. Long's medium containing various quantities of streptothricin was inoculated heavily with 4 *Mycobacterium* strains. After 14 days' inhibition, portions of each culture were streaked on Long's agar to determine viability of the cells in the inoculum. Table II indicates that streptothricin was definitely bactericidal in low concentrations.

These experiments, placing the mycobacteria among the streptothricin-sensitive organisms, as well as the known therapeutic effectiveness of streptothricin against certain infections in guinea pigs and mice,[1,2] suggest the desirability for detailed *in vivo* tests of streptothricin as a possible agent in the therapy of tuberculosis.

[3] Woodruff, H. B., and Foster, J. W., *J. Bact.*, in press.

[4] Foster, J. W., and Woodruff, H. B., *Arch. Biochem.*, 1943, **3**, 241.

[5] Waksman, S. A., and Woodruff, H. B., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 207.

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Demonstration in Cotton Rats and Rabbits of a Latent Virus Related to Pneumonia Virus of Mice.*

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A previous report from this laboratory[1] described the isolation from Syrian hamsters

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation and in cooperation with the California State Department of Public Health.

[1] Pearson, H. E., and Eaton, M. D., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 677.

(*Cricetus auratus*) of a virus which produced fatal pulmonary consolidation in these animals, and which, on the basis of cross immunity tests, was shown to be antigenically related to the pneumonia virus of mice (PVM) described by Horsfall and Hahn.[2] Subsequent observations have shown that cotton rats may carry a similar agent.

A group of 6 cotton rats was inoculated intranasally with a 10% suspension of lung from an apparently normal animal of the same species. Four of the 6 rats died between 3 and 13 days later, with bright red, patchy, oedematous pulmonary consolidation. The agent causing the pneumonia was directly transmissible to mice and produced death with characteristic lung lesions in the first passage. Neutralization tests in mice using mixtures of the cotton rat lung or mouse lungs of the first passage and the serum of a rabbit immune to PVM showed specific neutralization. The cotton rats surviving inoculation with the above-mentioned normal lung had antibodies to PVM in their serum, as demonstrated by neutralization tests with a mouse-adapted strain. At various times during the past 2 years lesions similar to those described above have been seen in the lungs of cotton rats and hamsters receiving intranasal inoculations of broth or normal chick embryo tissue.

Interest in the possible occurrence of latent viruses related to PVM in other animals was aroused by the apparent production of antibodies to this virus in rabbits after the injection of plasma from persons with atypical pneumonia and of chick embryo tissues derived from serial passages started with human throat washings or plasma.[3†]

Twenty-eight rabbits from which serial bleedings were obtained had been under study from September, 1942, to December, 1943. Some of these had been inoculated subcutaneously or intraperitoneally with samples of lung tissue or sputum from persons having atypical pneumonia; some with cotton rat lung, hamster lung, or chick embryo tissue derived from serial passages initiated with lung or filtered sputum from persons having atypical pneumonia; and others with the lungs of mice infected with influenza virus. Serums from the same rabbits before inoculation and from rabbits which had been kept in the laboratory, but were inoculated only with non-infectious material, were also available.

All of these rabbit serums were tested for their ability to neutralize PVM. In these tests the mouse-adapted strain Wa, isolated in our laboratory from normal mice, was used. This strain is antigenically related to the strain used by Horsfall and his associates.[3] A 1% suspension of infected mouse lung representing 10 to 100 M.L.D. was mixed with an equal volume of rabbit serum in all tests. After incubation at 37°C for 30 minutes each of the serum-virus mixtures was inoculated intranasally under light ether anesthesia into 6 Swiss mice 4 to 6 weeks of age. The mice were observed for 12 days, at which time all survivors were sacrificed. All animals were examined for lung lesions, and the results were recorded as percentage scores[4] to indicate the degree of consolidation in each group.

In Table I details of some of the positive experiments with rabbit serums are presented. The lesion scores in mice receiving PVM plus the rabbit serum under test and in control mice receiving virus plus horse serum are given in the 2 columns to the right. Where the percentage score in the second column from the right was less than 1/3 of that in the controls (last column) definite neutralization was considered to have occurred. Similarly ratios of 1/3 to 1/2 between the lesion scores were taken as evidence of incomplete neutralization. Four rabbits were inoculated with suspensions of lung from persons who had

[2] Horsfall, F. L., Jr., and Hahn, R. G., *J. Exp. Med.*, 1940, **71**, 391.

[3] Horsfall, F. L., Jr., Curnen, E. C., Mirick, G. S., Thomas, L., and Ziegler, J. E., *Science*, 1943, **97**, 289.

† After our studies had been completed an unpublished report of the occurrence of antibodies to PVM in normal rabbits, cotton rats, and hamsters was presented by Dr. E. C. Curnen in a round table discussion on primary atypical pneumonia at the 45th general meeting of the Society of American Bacteriologists, New York, May 3, 1944.

[4] Horsfall, F. L., Jr., *J. Exp. Med.*, 1939, **70**, 209; Eaton, M. D., *J. Immunol.*, 1940, **39**, 43.

TABLE I.
Details of Positive Neutralization Results with Rabbit Serums.

Rabbit No.	Material injected	Case	No. of injections	Date of bleeding	Interval, days*	Lesion scores in mice: Test serum, %	Lesion scores in mice: Controls, %
56	Human lung	Bl	5	11/16/42	10	100	
56	" "	De	5	2/23/43	17	3	95
61	Mouse lung	Influenza	1	3/29	21	100	
61	Human "	Ln	1	7/23	23	13	83
69	Nil		0	3 /9	—	93	
69	Human lung†	Lt	5	6/28	80	23	70
70	Nil		0	3 /9	—	73	
70	Human lung	De	11	1/ 8/44	88	7	70
71	Nil		0	3/12/43	—	66	
71	Chick embryo	De	8	7/23	17	10	83
66‡	Mouse lung	Influenza	1	3/26	28	90	
66	" "	"	1	9 /8	194	3	83
68‡	Mouse lung	Influenza	1	3/26	28	100	
68	" "	"	1	9/27	209	13	83
65	Nil	—	0	2/26	—	17	70
75	Rabbit serum ex 65	—	6	10/26	50	100	
75	" " " "	—	6	12/27	112	30	83

* Interval since last inoculation.
† This suspension of lung tissue was heated to 60°C for 2 hours before injection.
‡ Rabbits 66 and 68 received 6 intramuscular injections with carbon tetrachloride in August, 1943.

pneumonia. A virus described elsewhere[5] was isolated from patient De, and presumptive evidence of the same agent was found in patient Bl. No similar agent was isolated from patients Ln and Lt, and the material from the latter patient was heated to 60°C for 2 hours before being injected into rabbit 69.

Rabbit 56 after inoculation with the suspension of lung from patient Bl failed to develop neutralizing antibodies to PVM, but both this animal and rabbit 70 had neutralizing antibodies after inoculation with the lung suspension from patient De. Rabbit 71, which received chick embryo tissues from passages initiated with the same specimen of human lung also developed antibodies to PVM. Two other rabbits inoculated with chick embryo passages of other strains of the atypical pneumonia virus had antibodies to the homologous virus, but none to PVM. Rabbits 66, 68, and 75 were under experiment during the same period as those just discussed, but did not receive inoculations of any infectious material during the period indicated. They also developed antibodies to PVM. Rabbit 65 was found to have antibodies to this agent soon after arrival at the laboratory.

A summary of these and other neutralization tests with rabbit serums is presented in Table II. It is of some interest that only 2 out of 16 normal rabbits had neutralizing antibodies to PVM on arrival at the laboratory. These animals were obtained from a breeder who also raised mice but who kept his rabbits in a separate building. The rabbits receiving cotton rat, hamster, or mouse lung also showed a relatively low incidence of neutralizing antibodies. Most of the cotton rat and hamster material was from passages initiated with sputum or lung from persons having atypical pneumonia, and many of these bleedings were obtained between September, 1942, and March, 1943, or earlier than those in Table I. The mouse lung was infected with influenza virus carried at passage intervals of 3 to 4 days. Most of the rabbits counted as normal in Table II also appear under another category where results with bleedings follow-

5 Eaton, M. D., Meiklejohn, G., van Herick, W., and Talbot, J. C., *Science*, 1942, **96**, 518; Eaton, M. D., Meiklejohn, G., and van Herick, W., *J. Exp. Med.*, 1944, **79**, 649; Meiklejohn, G., Eaton, M. D., and van Herick, W., submitted for publication.

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

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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.