Summary. Cultures on 30% ascitic fluid agar of material obtained by swabbing the eyes, nose, and throat of rheumatic and non-rheumatic children and a few adults failed to reveal any pleuropneumonia-like colonies. No success was encountered in additional attempts to isolate microörganisms of the pleuro-pneumonia group from the blood of children in the febrile phase of acute rheumatic fever or Still's disease, from the joint fluid during the first attack of rheumatic polyarthritis, and from rheumatic pericardial, myocardial, and valvular tissues obtained at necropsy. Cultures of 58 pairs of excised tonsils, however, yielded in 3 cases peculiar microscopic colonies ("X" colonies) which were 20 to 40 μ in size and strikingly similar to those of certain members of the pleuropneumonia group. The "X" colonies could not be passaged beyond the first generation, and their nature remains unknown.

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Pathogenic Pleuropneumonia-Like Microörganisms in Tissues of Normal Mice and Isolation of New Immunological Types.

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That normal mice can be carriers of a distinct group of pathogenic pleuropneumonia-like microörganisms has already been demonstrated in an investigation of 3 different stocks of animals in New York.[1] Previous studies have established that their natural habitat was the conjunctiva and nasal mucosa,[1] although at least one strain was found in the brain of a normal mouse.[2] They have also been isolated from the lungs of mice which had received nasal instillation of various materials under ether anaesthesia[1, 3] and in the brains of mice which had been used for passage of various other infectious agents.[2, 4] Three distinct immunological types—A, B, and C—which vary in their pathogenicity and tissue affinities as well as in their antigenic make up, have now been described.

1 Sabin, A. B., *Science*, 1939, **90**, 18.

2 Sabin, A. B., *Science*, 1938, **88**, 575; *ibid.*, 1939, **89**, 228.

3 Sullivan, E. R., and Dienes, L., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 620.

4 Findlay, G. M., Klieneberger, E., MacCallum, F. O., and Mackenzie, R. D., *Lancet*, 1938 (Dec. 31st), 1511.

The purpose of the present study was to determine (a) to what extent these microörganisms were present in other tissues of carrier mice, (b) whether the carrier state persisted throughout life or was limited to a special age group, and (c) to investigate further the multiplicity of immunological and biological types that make up the mouse pleuropneumonia group. The mice used in the present studies came from an albino stock that had been inbred in Ohio for about 50 years. A preliminary investigation of the nasal mucosa and conjunctiva of 6 mice, yielded 5 new strains from 3 mice, all of which produced the neurotropic exotoxin and were immunologically type A. The distribution of the microörganisms in various tissues of carrier mice was studied in 10 animals which were 3 to 4 weeks old. The eyes, nose, trachea, lungs, heart, blood, liver, spleen, kidney, brain, and intestinal contents were cultured on 30% ascitic fluid agar. The intestinal contents were taken up in physiological salt solution, centrifuged at about 2000 rpm for 30 minutes, and the supernatant liquid was used for cultivation. Microörganisms of the pleuropneumonia group were obtained from 7 of the 10 mice (Table I). With the exception of the eyes and the upper respiratory tract they were isolated from the brain of 3 of these mice. Only a

TABLE I.

Pathogenic Pleuropneumonia-like Microörganisms in Various Tissues of Carrier Mice.

Mouse No.	Tissues cultured									
	Eyes	Nose	Trachea	Lungs	Heart blood	Liver	Spleen	Kidney	Intestinal contents	Brain
1	0	+ (A)*	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	+ (A)
4	0	+	0	0	0	0	0	0	0	0
5	+	+ (A,D,E)	+	0	Uns.	Uns.	Uns.	Uns.	0	0
6	+ (A)	+	0	0	0	0	0	0	0	+ (A)
7	+	0	0	0	0	0	0	0	0	0
8	+ (A)	0	0	0	0	0	0	0	0	+ (A)
9	0	0	0	0	0	0	0	0	0	0
10	0	Uns.	0	0	0	0	0	0	Uns.	0
11	0	+ (D)		+ (D)		0	0			0
12				0		0	0			+ (D)

*Letters in parentheses refer to the immunological type of the strain that was isolated.

Uns.—culture unsatisfactory.

few colonies were present in the cultures from the brain (there were no ordinary bacteria) and all 3 strains proved to be neurotropic exotoxin producing Type A's. Because the culture obtained from the nose of mouse 5 behaved peculiarly in tests for pathogenicity and agglutination, it was plated out and 3 different kinds of pleuropneumonia-like colonies were observed. Isolation and passage of single colonies revealed that the original culture was a mixture of 3 immunologically distinct types—A, D, and E. The new types D and E produce a progressive chronic arthritis but no neurotropic exotoxin and are immunologically different not only from one another but also from types A, B, and C of the mouse group and L_3 and L_4 of the rat group of pleuropneumonia-like microörganisms. Mice 11 and 12 (Table I) were sacrificed several days after the intravenous injection of bacteria, and the 3 strains of pleuropneumonia-like microörganisms which were isolated from the nose, lungs, and brain all belonged to the new type D.

Cultures from the conjunctiva and nasal mucosa of 10 old mice (6 months or older) yielded 6 strains from 4 mice, suggesting that the carrier state is probably not a transitory phenomenon. Two of these strains were typed and the one from the eye was a type A and that from the nose a type B. While type A as well as other types have been encountered in various tissues, the strains which have thus far been isolated from the eyes have all been type A. That the carrier state probably develops after birth by contact infection is suggested by a preliminary study of 5 mothers and their offspring. While 4 of the 5 mothers were carriers (nose, eyes, or both), no such microörganisms were found in 13 of their offspring at 3 days of age and were present in the nose of only 1 out of 20 at 5 days of age.

Summary. Pathogenic microörganisms of the mouse pleuropneumonia group in addition to being present in the conjunctiva and nasal mucosa may often be found in the brain and occasionally also in the trachea and lung of normal carrier mice. They were not found in the heart blood, liver, spleen, kidneys or in the intestinal contents. The carrier state is probably the result of contact infection and has been demonstrated as early as the 5th day of life and later than 6 months. Two new, immunologically distinct types (D and E) have been isolated; they produce arthritis but not the neurotropic exotoxin which thus far has been found to be elaborated only by the type A strains.