advantages offered by the chorioallantoic membrane of the developing egg. The chorioallantoic membrane of a 10-day-old egg was removed, washed in saline, and placed in 10 cc of serum ultrafiltrate in a 50 cc Erlenmeyer flask. The cultures were incubated at room temperature and the supernatant fluid only was used for passage and titration. The virus titered up to 10^{-4} after six passages and up to 10^{-5} after nine and ten generations. With this type of culture, only very fresh preparations were used, as, on storage, acid accumulates which must be neutralized by alkali in order to maintain a constant pH over a long period of time. The standardization of this type of culture is under consideration at present.

Conclusions. The virus of St. Louis encephalitis may be grown in a medium containing embryonic mouse or guinea pig brain in ox serum ultrafiltrate. Cultures of organs from adult mice fail to support growth of the virus. Incubation at room temperature produces higher titers (10^{-5}) than incubation at 37°C (10^{-3}). At both temperatures the attained titer remains almost unchanged for 10 days but shows a decrease after 15 days' incubation. The virus is present in the same concentration in the supernatant fluid as in the emulsified whole culture. Infected chorioallantoic membranes maintained in serum ultrafiltrate at room temperature support growth of this virus up to titers varying from 10^{-4} to 10^{-5}.

11530

Search for Microörganisms of the Pleuropneumonia Group in Rheumatic and Non-Rheumatic Children.

ALBERT B. SABIN AND BARBARA JOHNSON

From the Children's Hospital Research Foundation and the Department of Pediatrics, University of Cincinnati College of Medicine.

It has recently been demonstrated[1] that mice of various stocks are carriers of a new group of filtrable microörganisms which biologically can be classed with the causative agent of *pleuropneumonia bovum* but otherwise is quite distinct as regards pathogenicity, affinities for special cell types *in vivo*, and immunological identity. In mice, these microörganisms are usually found in association with the epithelium of the conjunctiva and nasal mucosa without giving rise to any signs of disease. However, when cultures of certain

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

types of these microörganisms are injected intravenously or by certain other routes, experimental diseases are produced in mice which resemble in many respects some of the manifestations of rheumatic fever and rheumatoid arthritis in man.[2] Many attempts have been made, therefore, to isolate similar microörganisms from these human diseases. Failure to obtain such microörganisms from the exudates and tissues of a small number of patients with rheumatoid arthritis or rheumatic fever has already been reported[1-3] and the purpose of the present investigation was (a) to determine whether or not human beings may be carriers of similar or related microörganisms, and (b) to study additional material from patients with rheumatic fever or rheumatoid arthritis.

Cultures were obtained from the nose and throat and in most instances also from the conjunctiva of 100 human beings, 95 of whom were under 15 years of age. Material obtained with sterile cotton swabs was streaked on agar plates containing 30% ascitic fluid. After 4 days' and again after 7 days' incubation at 37°C, all plates were examined with the microscope at a magnification of 100 times and a thorough search was made for colonies which might resemble even remotely those of the pleuropneumonia group. No such colonies, however, were found in any of the cultures. Among the patients that were examined in this manner there were 28 in the active phase of rheumatic fever, 2 in the active stage of Still's disease, 14 with various types of infection of the upper respiratory tract (mostly pharyngitis or tonsillitis associated with otitis media), 3 with pneumonia, and most of the remainder with miscellaneous medical or surgical conditions.

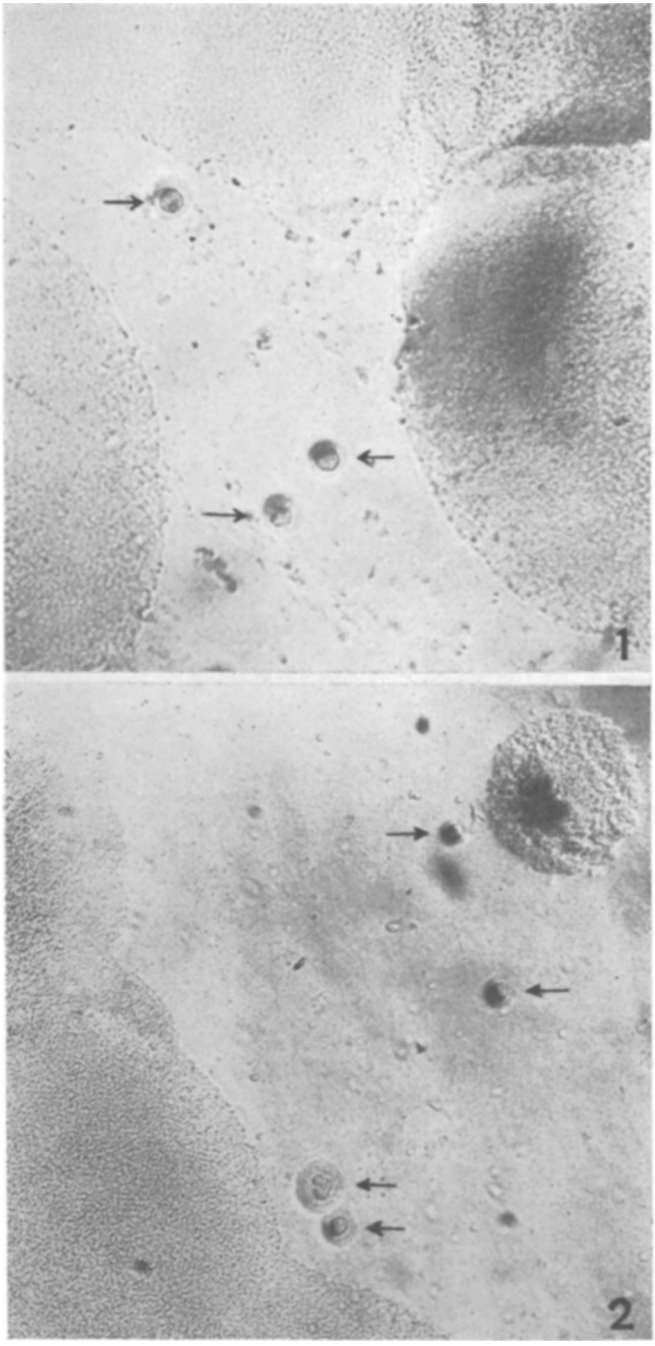
The blood of 9 children with rheumatic fever (acute febrile phase) and of two children during the febrile stage of Still's disease was cultured by adding 5 cc to 25 cc of broth containing 30% ascitic fluid and 0.5% glucose. No growth was obtained despite prolonged incubation and "blind passage." Exudates from the knee-joints of 3 children during their first attack of rheumatic polyarthritis were similarly cultured on fluid and solid media, but without obtaining any growth. Pericardial fluid, the pericardium and myocardium, and vegetations from the mitral valve obtained at necropsy from two children who died with active rheumatic carditis also yielded no growth.

Because experience with the pleuropneumonia group in mice indicated that they may often be intimately associated with the affected

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

[3] Findlay, G. M., Mackenzie, R. D., and MacCallum, F. O., *Brit. J. Exp. Path.*, 1940, **21**, 13.

cells, and since the carrier state in mice was established by streaking the conjunctiva and nasal mucosa rather than exudates from those sites, it was decided to investigate a series of tonsils removed from children for various reasons. A piece of tissue was removed from each tonsil, minced to expose a larger surface, and streaked on a 30% ascitic fluid agar plate. The tonsils of 58 children (116 specimens) were thus examined, and in 3 cases there were colonies, 20 to 40 μ in size, bearing a striking resemblance to those of certain members of the pleuropneumonia group. The appearance of these colonies (to be referred to as "X" colonies) is illustrated in Figs. 1 and 2. They always occurred along the streak either independently of the adjacent bacterial colonies (Figs. 1, 2) or at the border of and in intimate association with a bacterial colony. The plates were examined routinely 4 and 7 days after incubation; in 2 cases the "X" colonies were seen on the 4th day and in the third not until the 7th day, although in a repeat culture from the same tonsil many colonies appeared on the 4th day. Impression films of zones containing these colonies were unsatisfactory because the bacteria from the adjacent colonies obscured the field. Many attempts to passage the "X" colonies in series were without success. When an isolated "X" colony was streaked on 30% ascitic fluid agar or put into broth containing 30% ascitic fluid and 0.5% glucose no growth of any kind occurred. When "X" colonies and adjacent bacterial colonies were passaged together only the bacterial colonies grew out. In 2 of the 3 cases it was possible to obtain "X" colonies several times by repeating cultures from the same tonsils which were kept in the refrigerator, but passage was invariably unsuccessful. There was thus no evidence that the "X" colonies were either a variant or a symbiont of any of the tonsillar bacteria, and their nature remains obscure. It is perhaps significant that they were not observed once among the "swab" cultures from the eyes, nose, and throat of the 100 cases studied by the same method. Swabs from the nose and tonsillar regions of the child, whose tonsils yielded the largest number of "X" colonies, were cultured 7 weeks after tonsillectomy but no "X" colonies were found. The possibility must be investigated that the "X" colonies may represent pleuropneumonia-like microörganisms which are intimately associated with certain cells and have such specific growth requirements that only one generation is possible on the 30% ascitic fluid agar, but it should be stressed that there is still no evidence that there is a human group of pleuropneumonia organisms such as has been shown to exist in cattle, sheep and goats, dogs, rats, and mice.



FIGS. 1 AND 2.

Growth resulting from streaking human tonsils on 30% ascitic fluid agar. Arrows point to "X" colonies. Fig. 1—X112; Fig. 2—X150.

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.

ALBERT B. SABIN AND BARBARA JOHNSON.

From the Children's Hospital Research Foundation and the Department of Pediatrics, University of Cincinnati College of Medicine.

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.