The findings in this series suggest that a negative precipitin test with *S. hemolyticus* nucleoproteins as antigens is unusual in cases of *S. viridans* sub-acute endocarditis, except in the aged. Other sera from patients with *S. viridans* sub-acute endocarditis in whom the previous cardiac damage is due to congenital defects, syphilis, or arteriosclerosis are to be studied for precipitins against the bacterial products used in these experiments.

6161

A Laboratory Method for the Diagnosis of Psittacosis in Man.

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Since the pandemic of psittacosis in 1929 and 1930, instances of disease in human beings associated with parrots and parrakeets have continued to appear, either as isolated occurrences or as small localized epidemics. Frequently the patients have manifested unusual clinical pictures and have run courses not considered to be characteristic of psittacosis. Furthermore, at times the disease has occurred in people associated with birds which have apparently been in good health in this country for considerable periods of time and which until recently would have been considered "safe" in the sense of being free from psittacosis.

A reasonably safe laboratory method for the diagnosis of psittacosis in man is of importance. Our investigations, as well as similar experiences of others have indicated that serological tests are probably not suitable for the detection of psittacosis. Following Krumwiede's observations,[1] our work[2] has shown that mice are highly susceptible to psittacosis and that the experimental disease in them can be easily recognized. We have found, furthermore, that this host can be used in a laboratory test for the diagnosis of psittacosis and when infected can be handled with relative safety and with a minimum of danger of accidental infection.

The method now used by us for the laboratory diagnosis of psittacosis in man is briefly as follows: The patient's sputum to which 20-50 volumes of meat infusion broth, pH 7.8, and a small amount of alundun have been added is thoroughly ground in a

[1] Krumwiede, C., McGrath, M., and Oldenbusch, C., *Science*, 1930, **71**, 262.

[2] Rivers, T. M., and Berry, G. P., *J. Exp. Med.*, 1931, **54**, 105.

mortar. The emulsion is centrifuged for 10 minutes at a speed of 3000 R.P.M. Then the supernatant fluid is filtered through a Berkefeld V candle at a pressure of 15-30 cm. of mercury. Each of 6 mice receives intraperitoneally on 3 successive days 2 cc. of the filtrate. The animals are housed in screened battery jars placed in shallow baths of 5% lysol solution in order to prevent the mechanical spread of the infection by insects. All animals are observed for a period of 30 days.

If a patient dies without a diagnosis having been established and if at the autopsy psittacosis is suspected of being the cause of death, confirmatory evidence frequently can be obtained by the injection of filtrates of lung, liver, and spleen into mice in a manner similar to that just described.

The criteria by which the presence of psittacosis in the inoculated mice is established are: (1) the development in some or all of the animals of illness which is usually fatal within 10-14 days, but occasionally not before 30; (2) the characteristic pathological picture[2] consisting of focal necrotic lesions in the liver and spleen; (3) the absence of ordinary bacterial infections as determined from necropsy cultures; (4) the presence in liver and spleen impression smears of the "minute bodies"[3] of psittacosis; (5) the establishment of serial passages of the virus in mice by means of liver and spleen emulsions from the animals receiving the filtrates; (6) the demonstration that mice which have lived for 30 days following the inoculations of filtrates have developed an active immunity against a potent strain of psittacosis virus. All of these conditions obviously need not be fulfilled in each instance, sometimes one, sometimes another serves to establish the diagnosis.

By procedure similar to that described above material from 24 patients in 17 outbreaks or suspected outbreaks of psittacosis has been examined. Of the 24 patients 16 had psittacosis. Unsuccessful attempts were made to demonstrate the virus in 10 of the 16 patients by inoculation of blood into mice. By means of sputum or sputum filtrates, on the other hand, of 11 patients examined, the virus was obtained with certainty from 8, probably from 1, and was not obtained from 1, while 1 examination is still being conducted. In 5 instances the virus was found in the sputum but was not demonstrated in the blood collected simultaneously or earlier in the course of the disease. In 1 case the virus was obtained from sputum collected 48 and 24 hours before death, yet was not demonstrated in the blood or in the organs—brain, lungs, liver, and spleen

[3] Levinthal, W., *Klin. Woch.*, 1930, **9**, 654.

—removed at autopsy. From 1 patient the sputum was collected on the 24th day of illness, a fact that may account for the very small amount of virus present.

From our investigation it is obvious that the sputum is an excellent material in which to demonstrate by mouse inoculation the presence of psittacosis virus, while the blood is not. Bedson's work[4] indicates that the virus can be obtained from the blood of patients provided such blood is injected into parrots or parrakeets. Our endeavors, however, have been directed towards the development of a satisfactory diagnostic test in the mouse, a quite safe host with which to work. It appears that we have been successful, and for more than a year no parrots or parrakeets have been employed for diagnostic purposes in our laboratory.

6162

Normal and Pathological Permeability of the Lymphatic Capillaries in Human Skin.

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It is possible to test the permeability of the lymphatics by means of the vital dyes which have been used to study the permeability of small blood vessels.[1] Observations on the normal and pathological permeability of the lymphatic capillaries in the mouse ear have been reported in previous papers.[2, 3] The present communication concerns itself with the functioning of the lymphatic capillaries in human skin under various conditions. Dyes of graded diffusibility have been used (pontamine blue, Chicago blue, patent blue V, Neptune blue, and phenol red) in the isotonic vehicles 0.9% sodium chloride solution, Tyrode's solution, and a mixture of Tyrode's solution 3 parts and 1 part homologous serum.

Practically any abrasion of the skin, no matter how slight, involves the passage of material into the skin lymphatics. Material thrust into it by scratch, puncture, injection, or superficial cut enters

[4] Bedson, S. P., Western, G. T., and Simpson, S. L., *Lancet*, 1930, **1**, 235, 345.

[1] Rous, P., and Smith, F., *J. Exp. Med.*, 1931, **53**, 219.

[2] McMaster, P. D., and Hudack, S., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 852.

[3] Hudack, S., and McMaster, P. D., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 853.