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Precipitin Formation in S. Viridans Subacute Endocarditis.

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The sera of nineteen patients* with *S. viridans* sub-acute endocarditis have been studied for precipitins against protein fractions of *S. hemolyticus*,[1] *S. viridans*,[2] *Staph. aureus*,[2] and the group specific carbohydrate of *S. hemolyticus*. The *S. viridans* and *Staph. aureus* fractions were 5 and 7 years old, respectively, whereas the *S. hemolyticus* fractions were freshly prepared. All of the patients had the classical disease picture. Blood cultures were negative in 2 of the cases. These patients are to be studied further. The sera were collected at a time when the disease had been sufficiently advanced to justify the patient's admission to the hospital. On the basis of clinical history and the autopsy findings in some cases, 17 of the patients had rheumatic heart disease, and one of the remaining individuals had a normal heart at post-mortem, except for the fresh bacterial endocarditis.

The precipitin test was performed by adding 0.3 cc. of each serum to 0.3 cc. of the respective antigen and incubating the tubes in a water bath at 37°C. for 2 hours. The material was left in the ice-box over night and the initial reading was made in the morning. The tubes were then centrifuged at 1700 revolutions per minute for 10 minutes and a second reading was made.

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[1] Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1931, **54**, 515. Fraction K represents the portion extracted by 0.2 *N* NaOH from the residue from the *G* fraction.

[2] Lancefield, R. C., *J. Exp. Med.*, 1928, **47**, 481. These nucleoproteins were kindly supplied by Dr. Lancefield.

TABLE I.

Precipitin Formation in *Streptococcus Viridans* Subacute Endocarditis.

Serum No.	*S. hemolyticus* 19 D O.N.	*S. hemolyticus* 19 D C.	*S. hemolyticus* 18 K O.N.	*S. hemolyticus* 18 K C.	*S. hemolyticus* 8 C O.N.	*S. hemolyticus* 8 C C.	*S. viridans* O.N.	*S. viridans* C.	*Staph. aureus* O.N.	*Staph. aureus* C.	Serum and Salt Control O.N.	Serum and Salt Control C.
35	0	+	++	+++	0	++	0	++			0	0
36	++	++	++	+++	0	+++	0	++	+	++	0	++
37	++	++	+	+++	0	++	0	0			0	0
38	+++	+++	++	+++	0	++	0	++	0	+++	0	0
39	+++	+++	+++	+++	0	++	++	+++	0	+++	0	0
40	+++	+++	+++	+++	0	0	++	++	0	++	0	0
41	++	++	++	+++	0	++	++	+++			0	0
42	0	++	++	+++	0	0	0	++	0	+++	0	0
43	++	++	0	++	0	0	0	++	0	+++	0	0
44	++	++	+	++	0	0	0	0	0	++	0	0
45	+	+++	++	+++	+	++	++	++			0	0
80	0	+++	++	+++	0	++	0	+++			0	0
269	++	+++	++	+++	++	++	+++	+++			0	0
270	++	+++	++	+++	0	++	+++	+++			0	0
*271	+++	+++	++	+++	0	++	+++	++			0	0
*272	+++	+++	+	+++	++	++	0	+			0	0
341	0	+	0	+	0	0	0	++			0	0
358	++	++	0	++	0	0	0	+			0	0
375	++	++	0	+	0	0	+	++			0	0

Protein fractions and saline = 0. O.N. = Overnight reading. C = Centrifuged specimen reading.
19 D: Acetic acid precipitable *S. hemolyticus* protein extractable at neutrality; concentration 1:2,000.
18 K: Acetic acid precipitable *S. hemolyticus* protein extractable (after removal of less alkaline extracts) between pH 11 and 13.3; concentration 1:2,000.
8 C: Species specific polysaccharide, concentration 1:200,000.
S. viridans and *Staph. aureus* protein solutions, concentration of soluble protein 1:2,000.
* Cases with negative blood culture.

Table I shows the precipitins in the sera of 16 cases of *S. viridans* endocarditis against the bacterial substances. It is evident that the strongest and most uniform reactions are found in the tests with the 2 protein fractions of *S. hemolyticus,* although there are some reactions with the carbohydrate and with the proteins of the other organ-

isms. The weakest reactions occurred in the serum of the only patient in this series in whom clinical history and autopsy findings failed to disclose evidence of rheumatic fever, and in the sera of two patients aged 56 and 69 years. The latter finding may be correlated with a diminishing antibody response with advancing age.[3] Control tests with the sera and the antigens against physiological saline solution were negative. Preliminary heating of 4 of the sera at 60°C. for 30 minutes diminished but did not prevent the formation of the precipitate. In a number of cases weak positive tests were also obtained with a typhoid "nucleoprotein", with which further studies are now being made.

A control group of 100 sera obtained from patients with a wide variety of diseases *other than* rheumatic fever, rheumatoid arthritis, frank hemolytic streptococcus infection, acute nephritis, pneumonia, or peptic ulcer yielded only an occasional positive precipitin test. These reactions in general were much weaker than those observed in the group of *S. viridans* sub-acute endocarditis. Fourteen sera of other patients with temperatures of 102° or more showed no striking precipitin reactions. Three sera from patients with a past history of rheumatic fever and fever due to extraneous causes showed a negative precipitin test.

The relatively marked precipitin formation against *S. hemolyticus* proteins in the sera of the patients with *S. viridans* sub-acute endocarditis is striking and in agreement with Lancefield's findings that an antigenic relationship exists between the nucleoproteins of the *S. hemolyticus, S. viridans,* and *Staph. aureus* organisms. The reaction is not specific for cases of *S. viridans* endocarditis, since precipitates may be obtained against the same antigens with the sera of patients with acute nephritis, rheumatoid arthritis, peptic ulcer, and frequently in the convalescent stage of pneumococcus pneumonia. In a group of cases of nephritis and peptic ulcer, Derick and Fulton[4] obtained a high percentage of positive skin reactions with comparable protein fractions, although their control group showed a much higher number of positive reactions than did the control group of precipitin tests in our series. Coburn has found precipitins against comparable fractions of the hemolytic streptococcus during the active stages of rheumatic fever.[5] In general, however, the tests were weaker than with the sera of the cases of *S. viridans* sub-acute endocarditis.

[3] Thomsen, O., *Z. Immunf.*, 1917, **26**, 213; Thomsen, O., and Kettel, K., *Ibid.*, 1929, **63**, 67.

[4] Derick, C. L., and Fulton, M. M., *J. Clin. Invest.*, 1931, **10**, 121.

[5] Coburn, Alvin F., in press.

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.

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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.