such cytozoa may contain no trypanosomes. Thus, cultures attempted from twenty-six of such heavily infected birds failed to show any growth. Again, the presence of trypanosomes is not associated with any one form of intracellular parasite. Furthermore, the cultural method shows the existence of several distinct species of trypanosomes, and among these is one which presents at the same time both types described by Schaudinn as stages on the one hand for *halteridium* and on the other hand for the "leucocytozoön" of Danilewsky.

The authors therefore conclude that trypanosomes in birds may be met with as several distinct species wholly unrelated to the intracellular parasites. The greatly diverging conclusions reached by Schaudinn and the authors must be ascribed to the fact that Schaudinn worked with *mixed cultures* as developed in the body of the mosquito, whereas the authors have employed strictly *pure cultures* of these flagellates.

[The authors published the full details of a part of this investigation in the March issue (1905) of the *Journal of Infectious Diseases*. Additional papers will appear in later issues.]

5 (51). **"The gradual decrease in bacteria of the production of agglutinable substance": WILLIAM H. PARK.**

At the last meeting of the Society of American Pathologists and Bacteriologists an informal statement of this fact was made by Dr. Welch for Drs. Marshall and Knox. The experiments of Dr. Collins and the author are reported here because they were undertaken in a slightly different way and also because a certain number of confirmatory observations are of value.

The maltose-fermenting paradysentery bacillus of Flexner was grown for twenty-four hours on each of eleven consecutive days in fresh bouillon solutions of the serum from a horse immunized through oft-repeated injections of the bacillus. The serum strength in the solutions used was 1.5%, 4% and 15%. The serum agglutinated the culture before its growth in the solutions in dilutions up to 1 in 800, and was strongly bactericidal in animals. After eleven transfers the culture grown in the 15% solution ceased to be distinctly agglutinated by the serum in any dilution and ceased to absorb from the serum any appreciable amount of the agglutinins acting upon the original culture. The

cultures grown in the 1.5% and 4% solutions were changed to a less degree and agglutinated in dilutions up to 1 in 100 and 1 in 60 respectively, and continued to absorb agglutinins. The recovery of the capacity to be agglutinated was very slow in the culture grown in the strongest serum solution, when it was from time to time transplanted in fresh nutrient agar. The other cultures recovered this characteristic more rapidly.

The first culture, after growth for sixteen weeks, during which it was transplanted forty-three times, agglutinated in dilutions up to 1 in 200, and after twenty weeks in dilutions up to 1 in 400. The culture grown in 4% solution of serum agglutinated after sixteen weeks in dilutions up to 1 in 500, and one in 1.5% agglutinated in dilutions up to 1 in 800. This diminution and final almost complete lack of development of agglutinable substance in bacteria grown in a serum rich in agglutinin and immune bodies is interesting. It showed not only a rapid variation in bacteria of essential characteristics, but also indicated a possible means of adapting themselves to resist destruction in the living body, since the bacteria which ceased to produce agglutinable substance and probably, also, less substance with affinity for other antibodies, might be considered less vulnerable to these substances.

It is not certain that the agglutinin in the serum causes the change in the bacteria, for solutions may agglutinate and still not produce this effect. The fact has been noted that the horse serum of animals not immunized has much the same effect on the cultures as the immune serum. Although this suggests that the change is not due to antibodies, it does not prove this, since the serum of a horse before injections is rich in antibodies for the typhoid-colon groups, due possibly to the passage of bacteria from the intestines into the circulation.

The author's explanation of the process is that there are substances in the serum which attack certain parts of the bacteria such as the agglutinable substance. In the increase of bacteria in the serum those which produce the least of these substances are least inhibited and therefore develop most rapidly. When cultures are made from serum solution to serum solution daily, a gradual differentiation takes place until finally bacteria producing almost no agglutinable substance develop.