borne out by the fact that the lytic property in normal human serum may exist entirely apart from its agglutinating capacity. The conclusion, therefore, seems justified that the normal meningococcidal power of human and animal blood is wholly unrelated to any previous immunizing experience with the specific antigen. While the possibility cannot be excluded that in those instances in which natural agglutinin and lysin occur together both antibodies reflect the result of common antigenic stimulation, it is more than likely that this relationship is merely fortuitous and that their coexistence should be interpreted as a chance occurrence of 2 different substances, one a specific antibody and the other a nonspecific factor of the blood.

10006

Citrate Solutions for Preservation of Fluid Blood.

JOYCE COTTER AND W. J. MACNEAL.

From the Department of Pathology and Bacteriology, New York Post-Graduate Medical School and Hospital, Columbia University.

Solutions of sodium citrate have been used to prevent coagulation of blood for many years and these solutions have become increasingly important in preserving the fluid blood for transfer from one human being to another. The citrate solutions are also employed in various laboratory procedures, particularly in the preservation of leukocytes for study of phagocytosis. The present study was undertaken because of the suspicion that some untoward reactions observed following citrate transfusions might be related to the citrate.

We obtained hermetically sealed ampules of the citrate solutions of 4 different manufacturers. All of these were perfectly clear. A test of the hydrogen-ion concentration of these 4 specimens revealed the following results:

Specimen	pH
A	8.5
B	8.0
C	8.1
D	8.2

We also prepared some solutions in our own laboratory and found that one solution of sodium citrate, 2.5% in 0.6% sodium chloride, had a pH of 8.7, higher than that observed in any of the 4 commercial samples.

We prepared a solution of citric acid, 2.5% in 0.6% sodium chloride, and found that this solution of citric acid had a pH of 1.17. A number of trial mixtures of citric acid and sodium citrate were then prepared and it was found that a mixture of 5 cc of the citrate with 0.03 cc of the citric acid gave an approximately neutral solution. The various solutions were then tested by mixing 1 cc of freshly drawn human blood with 4 cc of the undiluted citrate solution. These mixtures were incubated at 37°C for 20 hours, then placed in the refrigerator for 24 hours, and then left in the room for a week. The appearance of the preparation was recorded from time to time. At the end of a week there was very marked destruction of the red blood corpuscles with red discoloration of the supernatant liquid in those tubes in which the citrate solution of hydrogen-ion concentration 8.5, 8.1, and 8.2 had been used. In the citrate of pH 8.0 the hemolysis was distinctly less.

Without going into great detail in regard to the many tests which were performed, one may say that the blood, when mixed with citrate solution of pH 7.1 to 7.5, remained for many days, even at room temperature, without evidence of dissolution of the red blood corpuscles. When, however, the citrate solution had a hydrogen-ion concentration greater than 7.6 or less than 6.8, one observed gross evidence of destruction of the blood.

A further problem presented itself in regard to sterilization of these citrate solutions in glass ampules. It is evident that the quality of the glass plays a part in determining the final results. One should, therefore, work with glassware of uniform composition and then will be able, by trial and error, to determine the exact mixture of citric acid and citrate which will, after proper sterilization in the autoclave, retain the desired hydrogen-ion concentration.

With our materials it was found best to add 0.04 cc of 2½% citric acid solution to 5 cc of the 2½% sodium citrate solution, in order to obtain, after autoclave sterilization, a solution with a pH between 7.1 and 7.5. When fresh human blood was mixed with such a solution without it being diluted with saline, the blood corpuscles remained without evidence of hemolysis for at least a week at room temperature.

There is every reason to expect that such a solution diluted further with saline, which is itself neutral in reaction or nearly so, will permit the preservation of blood at low temperatures for many days or weeks. Because of the increasing interest in the use of citrated blood in transfusion, these observations may have some practical value.