dose of penicillin was begun, a direct Coombs test was done for the first time and found to be positive. After 7 days, Benemid was added to the regimen and at that time her hematocrit began to fall. She was transfused but the fall continued. The penicillin and Benemid were discontinued; she was again transfused and thereafter her hematocrit remained stable. The only sample of blood we had an opportunity to study was one drawn on the day of her death. Her direct Coombs test was strongly positive. Her serum agglutinated the red cells of a normal donor only after the cells had been treated with penicillin. The material eluted from the surface of the patient's own thoroughly washed erythrocytes reacted in the same manner as her serum. These observations suggest that the massive doses of penicillin sensitized this patient's red cells in vivo to react with her own serum in a manner entirely analogous to what has been demonstrated in vitro. Whether this sensitization then led to excessive hemolysis and to the fall in the patient's hematocrit remains to be substantiated by more controlled studies.

150

Application of Erythrocyte Powder in Laboratory Practice

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The blood with positive serological reaction for syphilis, as well as erythrocytes of blood stored over 21 days are usually wasted in Blood Banks. The erythrocytes of such blood samples were utilized for the preparation of erythrocyte powder. They were washed with saline and haemolyzed with distilled water. The stromata were then washed 3 times with distilled water cooled to 6°C. Thereafter, the stromata sediment was lyophilized and pulverized in a mortar. The drying could also be performed at 37°C, the stromata being spread in a thin layer; but the lyophilized preparations were more active. About 1500 mg of fine, pinky powder were obtained from 200 ml of packed red cells. The powder could be stored at 4°C for at least two months without any appreciable loss of activity. Before use, the needed amount of the powder was weighed out and washed twice with saline. Attention was given to prepare a suspension without lumps. In this way, the powder was cleared of almost all remaining haemoglobin.

542 Milgrom, Orellana and Layrisse
The powder prepared in this way was successfully applied for the following procedures:

1. Absorption of normal isoagglutinins anti-A and anti-B;
2. Absorption of immune incomplete group antibodies of different specificities;
3. Absorption of anti-human heteroagglutinins from rabbit immune sera;
4. Preparation of pure antibody solutions by elution of the sensitized powder;
5. Complement fixation tests;
6. Experimental immunization procedures.

1. Absorption of antibodies. The erythrocyte powder was used for the absorption of anti-A and anti-B antibodies from test sera of human origin (anti-Rh, anti-Duffy, etc.). Ten to 20 mg of powder were used for the absorption of 1 ml of serum. At first, a small amount of serum was added to the washed powder sediment, and a diffuse suspension was prepared by vigorous stirring with a glass rod; then, the remaining volume of serum was added. The tubes were left for 2-3 hours at room temperature, being frequently shaken. Thereafter, the tubes were centrifuged and the serum withdrawn. The absorption performed in this way was almost always complete, and it had to be repeated only in exceptional cases.

The absorption of anti-A and/or anti-B antibodies from anti-D serum could be performed not only with Rh negative, but also with Rh-positive erythrocyte powder heated after washing (as a moist sediment) for 10 minutes at 56°C the Rh antigen thus being completely destroyed.

Absorption of anti-human hetero-agglutinins from rabbit antiglobulin serum was performed with the same technique, the powder of A erythrocytes being used preferably.

For the absorption of anti-D antibodies, 50 mg of Rh0(+) powder per 1 ml of serum were used. The absorption was performed at 37°C during 2 hours. Repeated absorptions were frequently required for complete exhaustion of antibodies.

2. Preparation of pure antibodies. The elution of blood group antibodies from the red cells has been applied since the experiments of Landsteiner and Miller in 1925, but the methods used did not permit to obtain pure antibodies in a high titer and in a solution free of ballast proteins. Some progresses in these methods were obtained by the application of stromata by Kidd and Weiner.

For the preparation of anti-A and anti-B antibodies, the powder was sensitized with normal sera at room temperature. The sensitized powder was washed 3 times with saline and eluted in saline at 56°C. The eluates obtained were water-clear and contained less than 10 mg% of proteins. The anti-A and anti-B antibodies had a titer up to 1:1000.

For the preparation of anti-Rh eluates, the group O Rh+ powder was sensitized at 37°C with a potent anti-Rh serum. The powder was washed and eluted at 56°C in AB serum or in saline.

The eluate obtained in AB serum had a serum albumin titer at least as high as the original serum, contained no anti-A or anti-B antibodies of the original
serum and could be successfully applied for Rh testing of red cells of all groups as to

Application of Erythrocyte Powder in Laboratory Practice 543

ABO. It could also sensitize the cells for the action of Coombs serum, the careful washing of the sensitized cells was unavoidable in order to obtain positive reaction. In other words, this eluate had all the properties of an incomplete antiserum of group AB.

The eluate obtained in saline was water clear. It strongly sensitized the red cells for the action of Coombs serum. The washing of the cells could be omitted without any influence on the test. The indirect Coombs test could be performed in a very simple manner: 1 drop of eluate + 1 drop of saline suspension of cells; incubation at 37 C or at room temperature and 1 drop of Coombs serum.

These properties of the saline eluate are elucidated in the following way: AntiRh serum (and AB serum eluate) contains, besides the antibodies, normal globulins which have to be washed out because otherwise they would neutralize the antiglobulin antibody before it enters into the reaction with the anti-Rh antibody fixed to the cell. On the other hand, the saline eluate does not contain at all, or contains only traces of ballast proteins and the neutralization of the antiglobulin antibody cannot take place. Therefore the washing of the sensitized cells can be safely omitted.

3. Complement fixation test. Out of 43 normal sera tested, only 5 gave positive reactions due to anti-A and/or anti-B antibodies. Three sera from group O women who gave birth to infants with haemolytic anemia due to ABO incompatibility were tested. Two of these sera, one with high titer of anti-A and the other with anti-B, gave a strongly positive reaction with A and B erythrocyte powder respectively. The third serum, with a high titer of anti-A, gave only a doubtful reaction.

4. Immunization. Daily intravenous injections of a suspension containing 20 mg of washed powder could be performed on rabbits, without producing any morbid symptoms. Very large amounts of powder could be given subcutaneously or intramuscularly without doing any harm. The application of this method for the immunization of animals and human beings should be examined more precisely.