We wish to thank Prof. S. K. Bhattacharyya for his interest and encouragement. Our thanks are also due to Messrs. Sarabhai Chemicals for a gift of *iso*-nicotinic acid.

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A Simple Method of *Rh* Determination

IT has been shown that about 0·5 per cent of human sera possess a thermostable factor agglutinating *O Rh*+ red cells sensitized with anti-*D* antibodies. Only those sera were taken into account which agglutinated sensitized red cells at least in 1 in 8 dilution in saline. Weaker sera gave unreproducible reactions. The strongest serum observed had a titre of 128. According to our experiments[1], the agglutinating factor in the sera observed is an anti-globulin antibody. It may be absorbed from the sera by sensitized red cells and it may be neutralized in the sera by incomplete antibodies regained at 56° C. from cells. This antiglobulin antibody reacts only with immune antibodies after their denaturation in serological reaction, and therefore it is not neutralized by normal serum or by immune serum containing unchanged antibodies (Fig. 1). The discussion on the nature of this antibody and on its eventual role in pathology will be given elsewhere. Here we wish to stress the practical application of our findings.

(1) A suspension of red cells in 3 per cent saline sensitized with incomplete antibody was tested with human antiglobulin sera. Cells sensitized with all anti-*D* sera gave strongly positive reactions. Tests performed with other incomplete antibodies gave various results depending only upon the strength of the sensitization and not upon the specificity of antibody used. Since the human antiglobulin antibody is not neutralized by serum proteins (other than denatured antibody) the washing of sensitized cells can be omitted.

(2) *Slide agglutination test with mixed serum.* All the incomplete sera may be easily changed into saline agglutinating sera simply by mixing with human antiglobulin sera. After preliminary experiments, the following technique was adopted. Undiluted anti-globulin serum is mixed in equal proportions with undiluted incomplete anti-*Rh* serum. To one drop of mixed serum on a slide is added one drop of 3 per cent saline suspension of red cells with corresponding *Rh* antigen. The slides are allowed to stay in a wet chamber at room temperature for 20–30 min., after which clear-cut results are obtained. The agglutination is a very firm one and does not disappear after vigorous shaking.

In this manner fifteen anti-*D*, three anti-*c*, one anti-*C* and one anti-*e* incomplete sera have been tested. In all cases the mixed serum gave the expected reactions. The controls with cells of non-corresponding *Rh* types were always negative. The titre of a mixed serum was 4–16. The mixed serum can be stored frozen for at least seven days. This

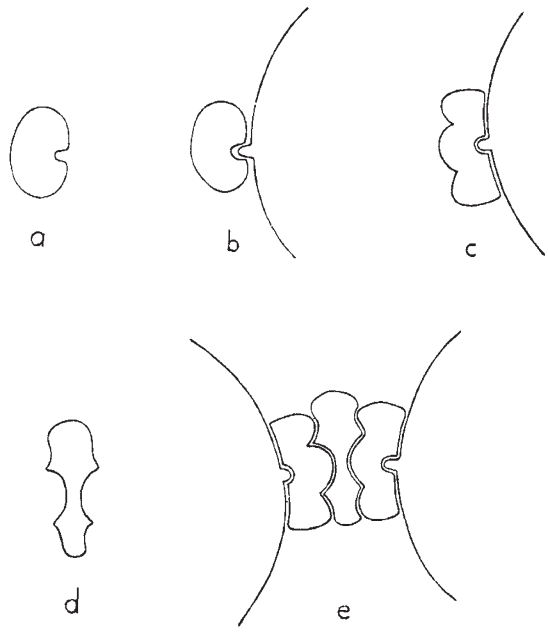

Fig. 1. (*a*) Incomplete antibody; (*b*) serological linkage with red cells; (*c*) denaturation of the antibody molecule in reaction with antigen; (*d*) human antiglobulin antibody; (*e*) agglutination of sensitized red cells

method of *Rh* determination is much simpler than any other used.

(3) *Antiglobulin test with platelets.* The chief difficulty in carrying out the antiglobulin test with platelets (and also leucocytes) is the necessity of saline washing, which causes clumping of these cells. With our human antiglobulin sera, washing can be omitted. Our preliminary experiments are promising.

While one investigation was in progress, we saw the interesting paper in *Nature* of Lewis and Chown[2]. It is now clear that these authors were using a very weak human antiglobulin sera. Therefore they obtained agglutination with mixed sera and could produce this phenomenon only with some anti-*Rh* sera. The negative results with sensitized cells made impossible a correct interpretation of the results.

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Carnosine Phosphate as Phosphate Donor in Muscular Contraction

CARNOSINE phosphate was first synthesized by Severin, Georgievskaya and Ivanov[1] by a method very similar in detail to that for creatine phosphate[2]. They showed that this method yields as a rule a diphosphate, both phosphate bonds being high-energy; but that the one which is not on the imidazole ring is more labile. In the presence of muscle extract the latter is transferred to adenosine diphosphate.