intratypic variant of echovirus type 29 rather than a distinct echovirus serotype.

Summary. An antigenic relationship has been demonstrated between the newly classified echovirus types 29 and 32. The amount of crossing seen in conventional tube neutralization tests was relatively minor, but fairly high reciprocal titers were observed in plaque reduction neutralization tests. In cross hemagglutination-inhibition and complement fixation tests these two viral types were virtually indistinguishable. The question arises as to whether these two new echovirus types should be classified as distinct immunotypes or whether they represent antigenic variants of a single viral type.

1. Panel for Picornaviruses, *Science,* 1963, v141, 153.

2. Schmidt, N. J., Guenther, R. W., Lennette, E. H., *J. Immunol.,* 1961, v87, 623.

3. Schmidt, N. J., Dennis, J., Hagens, S. J., Lennette, E. H., *Am. J. Hyg.,* 1962, v75, 74.

4. Schmidt, N. J., Lennette, E. H., Doleman, J. H., Hagens, S. J., *ibid.,* 1957, v66, 1.

5. Committee on Enteroviruses, *Am. J. Pub. Hlth.,* 1957, v47, 1556.

6. ———, *Virology,* 1962, v16, 501.

7. Kamitsuka, P. S., Soergel, M. E., Wenner, H. A., *Am. J. Hyg.,* 1961, v74, 7.

8. Wenner, H. A., *Ann. N. Y. Acad. Sci.,* 1962, v101, 343.

9. Wenner, H. A., Soergel, M. E., Kamitsuka, P. S., Perine, P., Chin, T. D. Y., Lou, T. Y., *Am. J. Hyg.,* 1963, v78, 247.

Received January 28, 1964. P.S.E.B.M., 1964, v116.

Comparison of Bactericidal and Hemolytic Serum Systems. I. Inhibitory Effects of Normal Human Serum Fractions.* (29165)

L. E. SCHNEIDER, A. MIYAMA, W. BRAUN, O. J. PLESCIA AND B. BJÖRKLUND
Institute of Microbiology, Rutgers University, and Immunological Research Laboratory, Stockholm

Factors responsible for the bactericidal activity of sera *in vitro* have been investigated extensively(1-5) and it has been shown that they involve specific antibody and the known components of the hemolytic complement system. In addition, lysozyme has been implicated as a potentiating factor of the reaction(6). Earlier studies(7) in this laboratory have shown that the bactericidal serum reaction can be enhanced by certain amino acids and carbohydrates and can be inhibited by factors interfering with the normal metabolic activities of the cell. It became of interest, therefore, to determine to what extent possibly unrecognized factors in serum, when present in excess, might modify the bactericidal activity of serum. To this end, each of 32 fractions of a pool of human serum was tested for its ability to modify a basic bactericidal serum reaction. In these tests no enhancing effects but inhibitory effects were detected in the presence of an excess of certain serum fractions. Since the serum fractions responsible for such inhibitions differed from fractions previously shown to inhibit the hemolytic activity of complement (8), the analysis with the 32 serum fractions was extended to include a study of inhibitory effects on immune hemolysis, so that a comparison between effects on bactericidal and hemolytic systems could be made. The results are presented here.

Materials and methods. The bactericidal reactions were carried out by previously detailed methods(7), using pools of so-called "normal" human serum (obtained from Middlesex Hospital, New Brunswick, N. J. or Interstate Blood Bank, Inc., Memphis, Tenn.) as a source of specific antibody, complement and other accessory factors. To the reaction mixture (0.5 ml) containing 0.1 ml of "normal" serum diluted with saline (1/10, 1/20, or 1/40) and 0.2 ml of the fraction to be tested (diluted 1/4, 1/8, or 1/16), was added 0.2 ml of a suspension of *Escherichia coli* 8 in ABA buffer(9) containing 10^3 cells.

* This work was supported in part by grant from U. S. Public Health Service.

The reaction mixtures were incubated in a shaking water bath at 37°C for 60 minutes and then placed in an ice-water bath, after which 0.5 ml aliquots were diluted with 4.5 ml of cold saline. Either 0.5 ml or 0.16 ml of the diluted reaction mixtures was pipetted into individual cups of Disposo trays and 1.8 ml of warm tryptose agar containing tetrazolium was added. Survivors that developed into colonies were counted 24 and 48 hours later. All tests were run in duplicate and plated in quadruplicate. The immune hemolytic reactions were run at 37°C for 45 minutes using 0.1 ml of a sensitized sheep erythrocyte suspension (1×10^9/ml), 0.1 ml of diluted serum fraction, and 0.1 ml of a suitably diluted hemolytic human serum. The extent of hemolysis was measured spectophotometrically at 541 mμ in terms of the hemoglobin liberated into the supernatant.

The 32 fractions used were obtained from a pool of sera from 26 normal, healthy blood donors. This serum pool was fractionated by continuous curtain paper electrophoresis at a current of 35 ma. The sample flow was 1.55 ml/hour, and the material collected was permitted to drip into containers submerged in a cold bath at —12°C. Without thawing, each frozen fraction was then lyophilized. All reconstituted materials were kept at —45°C when not used. A UV extinction curve at 280 mμ for the 32 fractions is shown in Fig. 1.

Results. In the basic bactericidal test system, to which the serum fractions were added, all test organisms were killed when the unsupplemented serum was used at a dilution of 1/10 or 1/20, whereas an average of only 50% or less of the organisms were killed at a serum dilution of 1/40. The serum pool from which the 32 fractions were obtained killed 90% of the *E. coli* 8 at a 1/80 dilution, 20% at 1/160 and none at 1/320. None of the 32 serum fractions was bactericidal by itself. When these fractions were added in final concentrations of 1/2, 1/4, 1/8, or 1/16, inhibitions were observed (Fig. 2) in the presence of fractions 5-10 (roughly γ-globulin) at all concentrations, while fractions 12-27 showed pronounced inhibitory effects only at relatively high concentrations, with a particularly high inhibition caused by fraction 23 (albumin). These inhibitions were similar in different experiments even though the absolute survival in unsupplemented controls varied somewhat.

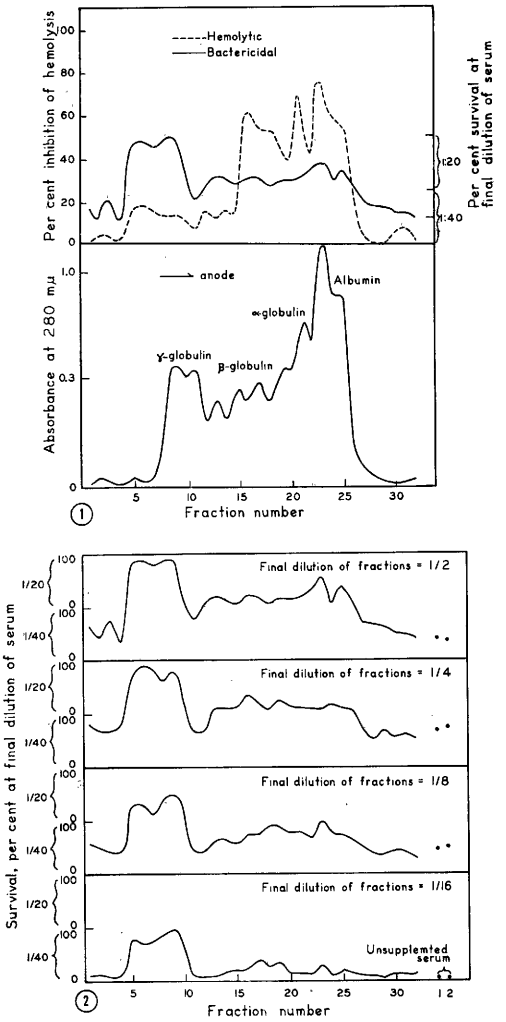

FIG. 1. Upper part: Inhibition of bactericidal and hemolytic reactions by individual serum fractions obtained by curtain electrophoresis from a normal human serum pool. Lower part: Relative content of protein of 32 serum fractions used in the tests shown above. (Points on the curve for bactericidal activity represent the most significant of 2 sets of data obtained at serum dilutions of 1/20 and 1/40. Thus, if survival was 100% at 1/20 and 40% at 1/40, the latter figure was used; if survival was 65% at 1/40 and 0%, or near it, at 1/20, the first figure was used.)

FIG. 2. Inhibition of bactericidal reactions by different concentrations of 32 serum fractions. (See legend to Fig. 1 for explanation of these curves.)

None of the 32 serum fractions was hemolytic by itself in the lysis of sensitized sheep erythrocytes, but the unfractionated human serum pool from which these 32 fractions had been produced was hemolytic at a dilution of 1/20 and not at 1/40. When added to a basic hemolytic serum system, in which 50% of the red cells were lysed (1/100 dilution of a normal human serum pool), several of the fractions caused an inhibition of hemolysis at a final concentration of 1/50 (Fig. 1). The most pronounced inhibitions were obtained with fractions 16-19 (β-globulin), 21 (α-globulin), and 23-25 (albumin).

Discussion. Contrary to initial expectations, none of the 32 different serum fractions caused an enhancement of immune lysis of either bacteria or red cells. Rather, several of the fractions caused an inhibition and it is interesting to note that the relative activities of the responsible fractions were not identical for the hemolytic and bactericidal systems. Thus, at the concentrations employed, which corresponded to their relative concentration in serum, fractions 5-10 which include materials with the electrophoretic mobility of γ-globulins and also highly fluorescent substances,* produced a more pronounced inhibition of the bactericidal reaction than the β-globulin fractions. Conversely, in the hemolytic system the β-globulin fractions were more inhibitory than the γ-globulins. The relative magnitude of these inhibitory effects remained unaltered even when the fractions were added in concentrations calibrated to contain equal amounts of protein.

If the inhibitions produced by an excess of certain serum fractions were directed against serum components required by the two systems, the data would suggest that the relative requirements of the hemolytic and bactericidal systems for the inhibited serum components may differ quantitatively if not qualitatively. Alternately, it was possible that the target of the inhibitory effects was not the serum but rather the cell. As will be reported later, it has become possible to demonstrate that the inhibitory serum fractions acted on serum components rather than on the bacterial cell or the red cell, and it was also determined that the action was selective for the hemolytic components of the complement system. It still remains to be resolved whether the observed differences in requirements for serum components active on bacterial cell and red cells are merely quantitative or are, in fact, qualitative.

Summary. When each of 32 different fractions, obtained from a human serum pool by electrophoresis, was added either to a bactericidal or a hemolytic serum reaction, inhibitory effects were found to be produced by some of the fractions. The bactericidal reaction, employing components present in "normal" human serum, was significantly inhibited by an excess of γ-globulins, and the hemolytic reaction by an excess of β-globulins. Inhibition of both reactions was also produced by an excess of serum fractions having the electrophoretic mobility of albumins. These findings are discussed in relation to quantitative and possibly qualitative differences in the serum components required for the bactericidal and hemolytic activities of serum.

* Fluorescent light of a wave length of 365 mμ when activated with light of 285 mμ.

1. Maaløe, O., *Acta Path. Microbiol. Scand.,* 1948, v25, 414.

2. Muschel, L. H., Treffers, P. H., *J. Immunol.,* 1955, v76, 1.

3. Rowley, D., *Brit. J. Exp. Path.,* 1956, v37, 223.

4. Michael, J. G., Braun, W., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 104.

5. Wardlaw, A. C., *J. Exp. Med.,* 1962, v6, 1231.

6. Ione, K., Tanigawa, Y., Takubo, M., Satani, M., Amano, T., *Biken's J.,* 1959, v2, 1.

7. Michael, J. G., Braun, W., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 486.

8. Cavallo, G., Plescia, O. J., *Lo Sperimentale,* 1958, v108, 186.

9. Wardlaw, A. C., Pillemer, L., *J. Exp. Med.,* 1956, v103, 553.

Received January 28, 1964. P.S.E.B.M., 1964, v116.