A Rough Antigen Frequently Associated with Vi in Some Enterobacteriaceae. (17736)

E. R. KENNEDY* AND C. A. STUART.

From the Biological Laboratories, Brown University, Providence, R.I.

Felix and Pitt(1) first described the Vi antigen as a thermolabile antigen conferring virulence and O-inagglutinability upon organisms possessing this component. Many investigators(2,3,4) have questioned the thermolability of the Vi antigen, others(5,6) its role in virulence and it is well known that an occasional Vi positive strain is completely O-agglutinable in pure O antiserum. Stuart, Wheeler *et al.*(7) found that certain coliform and *Paracolobactrum* cultures gave a characteristic Vi type of agglutination in a presumably pure Vi antiserum. Adsorption of the Vi antiserum with any one of the coliform or *Paracolobactrum* cultures completely removed agglutinins for all these cultures without reducing the titer for Vi positive *Salmonella* strains while adsorption with certain Vi positive *S. typhosa* and *S. ballerup* strains removed all agglutinins. The antigen common to some coliform, *Paracolobactrum* and Vi positive *Salmonella* cultures but differing from the Felix and Pitt antigen was called a Vi-like component. Wheeles and Stuart(8) found the Vi-like antigen in one culture with part of and 2 cultures with the complete somatic structure of Sach's Q-771 strain. Subsequently the same antigen was found in several additional strains of coliform, *Paracolobactrum* and Shigella-like organisms (unpublished data). Brower (9) reported an antigen other than the IX, XII, Vi or d component in a Bhatnagar strain of *S. typhosa* as a "probably rough antigen". Work on the Vi-like substances was revived in this laboratory when a nonmotile variant of the Bhatnagar strain used by Brower and kindly furnished by her was found to be composed almost exclusively of the Vi and Vi-like antigens. The Vi-like antigen will hereinafter be called the Er antigen (a rough antigenic component of many Enterobacteriaceae).

In the present work pure Vi antiserums were prepared in two ways. (1) Antiserums prepared in rabbits with living antigens of strains of *S. typhosa* possessing the Vi but lacking the Er antigen were adsorbed with living antigens of H-901 and 0-901. (2) antiserums of rabbits immunized with alcoholic extracts of *S. ballerup* containing Vi produced for the most part only Vi antibodies. An occasional antiserum prepared from an alcoholic extract of any Vi positive organism, particularly after prolonged immunization, possessed antibodies reacting in low dilutions (20-160) with boiled homologous antigen. Pure Er antiserum was prepared with *S. typhosa* 0-484 (the S-107 strain of Stuart, Wheeler *et al.*(7) containing both the Vi and Er antigens. This antiserum after adsorption with *S. typhosa* possessing Vi but not Er yielded a pure Er antiserum. Another Er antiserum was made from *Paracolobactrum coliforme* 121 rich in Er but lacking the Vi, IX, XII or d antigen. Tests with cultures known to possess the Vi or Er antigen or both indicated that the Er antibodies prepared from the *S. typhosa* and *P. coliforme* strains were identical. The Er antiserum prepared from *P. coliforme* 121 agglutinated *S. typhosa* 0-484 to a titer of 20480 and other *S. typhosa* strains possessing the Er antigen to titers ranging from 80 to 20480. With these antiserums the Er antigen

* Present address, Catholic University, Washington, D.C.

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2. Welch, H., and Mickle, F. L., *Am. J. Publ. Health*, 1937, v27, 351.

3. Smith, E. V. D., *J. Inf. Dis.*, 1938, v63, 21.

4. Luippold, G. F., *Am. J. Publ. Health*, 1946, v36, 15.

5. Kauffmann, F., *Z. Hyg. Infekt.*, 1936, v117, 778.

6. Almon, L., *Bact. Rev.*, 1943, v7, 43.

7. Stuart, C. A., Wheeler, K. M., Rustigian, R., and Zimmerman, A., *J. Bact.*, 1943, v45, 101.

8. Wheeler, K. M., and Stuart, C. A., *J. Bact.*, 1946, v51, 317.

9. Brower, E. H., *Am. J. Hygiene*, 1944, v40, 154.

was found in high concentration in 10 strains of *S. typhosa,* in moderate concentration in 6 strains and in weak concentration in several others from a total of 75 strains studied. It was found in *S. ballerup* strains, in one rough *paratyphi* C strain, in 4 *Paracolobactrum* strains and 2 *Escherichia coli* cultures. These findings would indicate that the Er antigen is relatively widespread in the Enterobacteriaceae. The confusion that can be caused by the presence of Er antibodies in presumably pure Vi antiserum is apparent. The increasing number of reports of Vi antigen in coliform and *Paracolobactum* cultures must be viewed with suspicion unless confirmed by adequate adsorption tests. If for no reason other than to avoid confusion with Vi the general distribution of the Er antigen should be recognized.

In the previous work(7) it was noted that coliform and *Paracolobactrum* cultures with Er antigen exhibited rough tendencies. When antigens of these strains were prepared by adding saline to agar slant cultures and the tubes rotated, the growth came off the agar in flakes. When briskly rotated for 2 or 3 minutes most of the flakes broke up and after allowing the suspension to sediment 10 to 15 minutes perfectly smooth antigens that showed no spontaneous agglutination in 0.85 or 2% saline at 37°C or 55°C were obtained. Upon boiling for 1 or more hours in 0.85% saline varying degrees of spontaneous agglutination did occur with all strains. Young colonies of these cultures on nutrient agar plates showed rough characteristics which disappeared as the age of the colony increased. In broth cultures sediment was noted on the bottom of the tube but smooth, stable antigens could be pipetted from the supernatant fluid. *S. typhosa* strains with Er antigen reacted in a similar manner except that the tendency toward roughness was not usually as marked as with coliform and *Paracolobactrum* cultures. If the Er antigen is a rough component of a culture a comparison of the Vi and Er antigens with respect to distribution and other characteristics is important. The Er antigen has been found in all groups of enteric bacteria possessing Vi: *Salmonella,* coliform and *Paracolobactrum*(10). In the *Salmonella,* including *S. typhosa,* the Er antigen has been limited to those cultures possessing Vi, but not all strains with Vi possess the Er antigen. In the coliform and *Paracolobactrum* the Er antigen alone is usually found. Hirsch(11) found that cultures with Vi were agglutinated by acriflavin. Cultures containing Er antigen showed somewhat stronger agglutination than Vi strains in the same dilution or agglutinated in slightly higher dilutions of the dye than did the Vi strain. Agglutination in acriflavin long has been accepted as one of the better though not infallible criteria of roughness. Both the Vi and Er antigens may be extracted from cultures by alcohol and by saline and heat; the former more readily by alcohol and the latter by saline and heat. These extracts are antigenic in rabbits and will inhibit the agglutination of the Vi antigen or the Er antigen by their respective antibodies (Stuart and Kennedy)(12). White(13) and others have pointed out that treatment of rough *Salmonella* with alcohol may remove the rough character of the culture. When cultures with the Vi or Er antigen are heated to 60°C for one hour most or all of these antigens are freed from the cell and the washed cells are not agglutinated in Vi or Er antiserums respectively. Free in the supernatant fluid the Vi and Er antigens are not completely destroyed for an hour or more at 121°C. The Er antigen is not as readily dissociated from the cell as the Vi and in some strains may resist dissociation for one hour at 60°C. Increasing the time of heating at 60°C or increases in the temperature dissociates the Er antigen but may cause the residual cells to be spontaneously agglutinated. Cultures containing both Vi and Er antigens tested in pure Er antiserum often exhibited Er-inagglutinability comparable to O-inag-

10. Marmion, B. P., *Month. Bull. Min. Health and Emer. Pub. Lab. Serv.,* 1944, v3, 139.

11. Hirsch, W., *J. Path. and Bact.,* 1937, v44, 349.

12. Stuart, C. A., and Kennedy, E. R., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 455.

13. White, P. B., Med. Res. Council, London, supplement to special report No. 91, 1926.

glutinability usually associated with the Vi antigens(14). One difference may be noted between the reaction of Vi and Er antigens in their respective antiserums. With the former the agglutination which occurs after 4 hours at 37°C is frequently completely reversed after several hours at 55°C. The Er antigen which tends to agglutinate weakly at 37°C may agglutinate more strongly in some antiserums at 55°C.

In *S. typhosa* the principal characteristic of rough strains appears to be the complete or almost complete absence of the IX, XII somatic antigens. All rough strains examined in this laboratory to date and meeting this requirement have possessed both the Vi and Er antigens. Agglutination and adsorption tests with an antiserum prepared from "*S. typhi* 6S. rough" showed that the Vi and Er antigens were the only major somatic components of this strain. Saline suspensions of agar slant cultures of this strain while exhibiting no spontaneous agglutination after 2 hours at 37°C and 18 hours at 55°C were completely agglutinated after one hour in a boiling water bath. Saline suspensions of *P. coliforme* 121 which possessed a strong Er but no Vi antigen behaved in the same manner at 37°C, 55°C and 100°C. However, *P. coliforme* 121 completely lost the Er antigen when after numerous platings an extremely rough variant was established.

Finally the presence of the Vi and Er antigens in an organism is definitely associated with the opaque colonial form while their absence is associated with the translucent colony. Arkwright(15) early in his work on the smooth to rough transformation pointed out the tendency of the smooth colony variants to be translucent and the rough to be opaque. With both the Vi and Er antigens the association with colonial form is not absolute and exceptions do occur. Whether the acquisition of the Vi antigen is a preliminary stage in the smooth to rough transformation with the acquisition of the Er antigen a definite step in that direction cannot be answered at this time. In any event the Er antigen is important in itself from a diagnostic viewpoint since strains of many enteric bacteria containing the Er antigen may be reported as Vi positive. As previously pointed out(16) so called "single factor" or "pure typing serums" lend a false sense of security to diagnostic bacteriology and too much reliance is often placed on agglutination tests in such serums. A simple adsorption test usually will differentiate between the Vi and Er antigens but the Er antigen may vary both quantitatively and qualitatively in different cultures and even in the same culture upon different occasions.

Specific designation (Er) is given to the antigen under discussion only because of its frequent association with the Vi antigen in enteric bacteria, particularly the *Salmonella* group.

Conclusions. 1. In some Enterobacteriaceae, particularly *S. typhosa*, a rough antigen (Er) has been found associated with Vi. 2. As with Vi, heat and/or alcohol dissociates the Er component from the cell and the free antigen can be used to produce Er antibodies in rabbits or to inhibit agglutination of cultures possessing this antigen by Er antiserum. 3. Like Vi the presence of the Er antigen is associated with the opaque colonial form while its absence is associated with the translucent form.

14. Kennedy, E. R., Doctorate thesis, 1949, Brown University.

15. Arkwright, J. A., *J. Path. and Bact.*, 1921, v24, 36.

16. Stuart, C. A., and Carpenter, P., *J. Immunol.*, 1949, v61, 161.

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