Requirement of Certain Plasma Factors for Production of Red Band By *Staphylococcus aureus*. (29417)

JULIA T. WELD AND B. H. KEAN
Departments of Public Health and Medicine, Cornell University Medical College, New York City

Two strains of *Staphylococcus aureus* (Guy and Oxford strains) have been shown to produce two different kinds of red band in human blood agar media(1,2). Initial studies showed that plasma was required for development of the red band, that this property was not lost by heating to 60°C for 30 minutes, and that human gamma globulin and human serum albumin could not replace plasma in production of the red bands.

Further studies with the Guy strain were undertaken to determine whether other fractions of human plasma could cause development of the red band. Of the fractions tested* only the alpha globulin IV_1 from one particular fraction lot has been shown to possess this ability.

The present paper reports details of these studies.

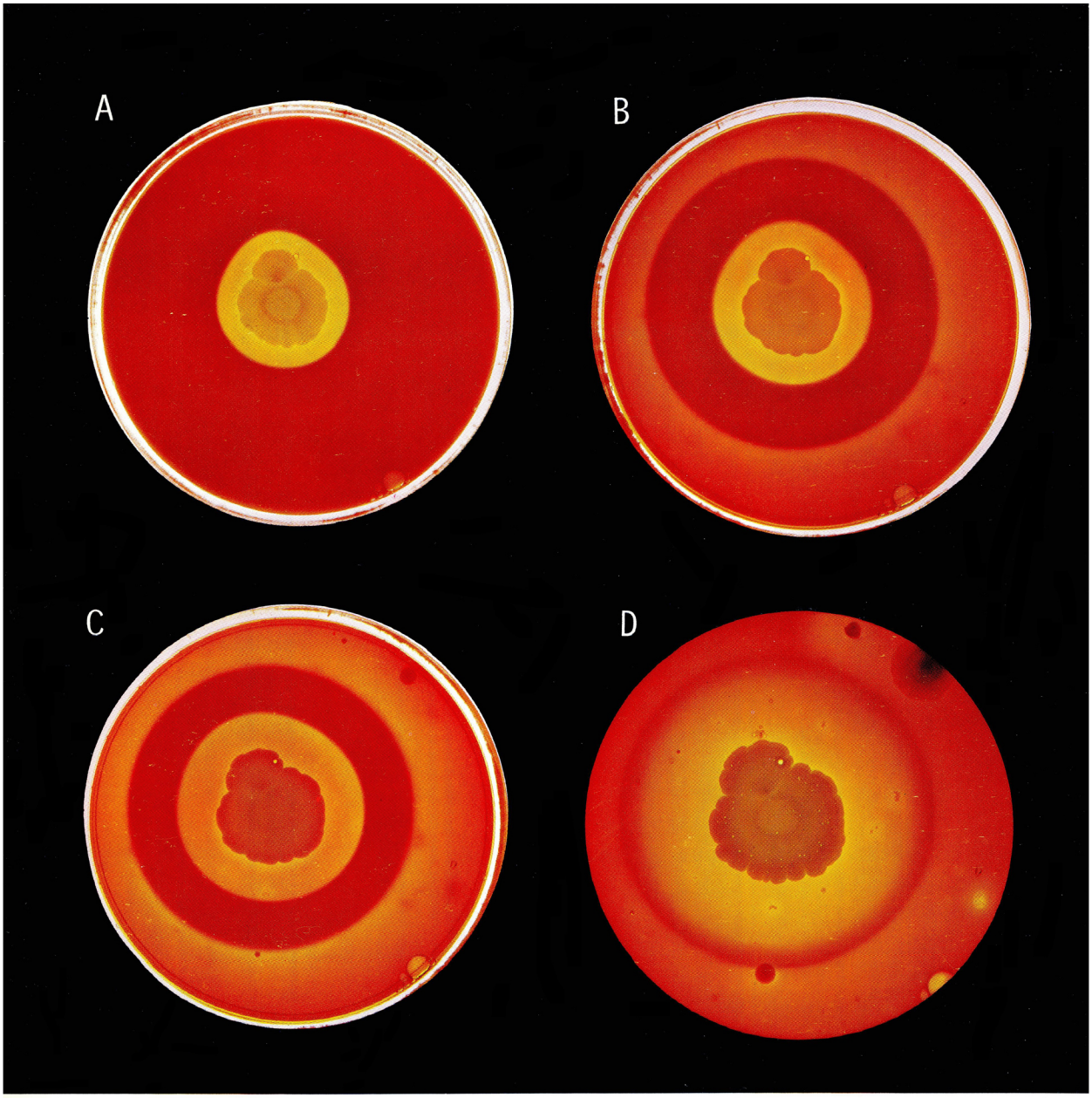
Materials and methods. 1. Preparation of alpha globulin IV_1. Fifty mg of the Pentex, lot 6 fraction IV_1 were added to 20 ml of Difco hemagglutination buffer, to obtain a concentration of 0.25% of the fraction. The material was dissolved by warming at 40°C for one hour or at 45°C for 30 minutes with occasional gentle shaking by hand. A slight amount of low density undissolved material in the preparation was removed by centrifugation. After sterilization by passage through a 0.65 μ Millipore filter, the opalescent filtrate was stored at —20°C until used.

2. Preparation of plates. To a series of 15 ml conical centrifuge tubes, 1 ml of A.C.D. whole human bank blood was added, and the red cells washed twice with 10 ml physiological saline solution. During the course of these studies, 10 different blood samples obtained from the Blood Bank Association of New York were used. The washed sedimented red cells were suspended in 0.5 ml of saline, and 2 ml of the fraction IV_1 filtrate were added. The mixture of washed red cells and fraction IV_1 was added to 18 ml trypticase soy agar at 48°C to 50°C in a small Erlenmeyer flask, well mixed, and poured into two 60 × 15 mm plastic plates, using approximately 10 ml for each plate. Thus the final dilution of fraction IV_1 was 0.025%. Two plates were poured from each of the 2 control media. The first contained the washed red cells from 1 ml of whole blood, 2 ml of buffer and 18 ml of agar. The second contained 1 ml of whole blood, 1.5 ml of buffer and 18 ml of agar. In each experiment the same human blood was used for both the washed red cells and the whole blood agar media. All tests were done in duplicate. Other control plates were prepared containing Fraction IV_1 or human plasma without red blood cells. The *Staphylococcus* strain under study was known to cause development of extensive surface lipid deposits (or plaques) on this medium(3,4).

3. Inoculation of plates. Plates were inoculated centrally with a small loop of broth suspension from a fresh 18 hour slant. In most studies strain Guy was used. Other strains used were #43 (Guy Type), the Oxford strain, the D1245 80/81 (Oxford Type) and the MAM coagulase negative *Staphylococcus* strain previously described.

Results. In plates containing fraction IV_1 (lot 6) with washed red cells, or whole blood, both the Guy strain and strain #43 produced a characteristic red band 1 to 1.2 cm wide surrounding an area of 0.3 to 0.4 cm of specific hemolysis adjacent to the growth of the organisms following incubation for 5 days at 37°C and 1 day at room temperature (see plate). The Oxford and D1245 80/81 strains of *S. aureus* caused development of

* Blood fractions were obtained from Pentex Inc., Kankakee, Ill. These included alpha globulin IV_1, lot 6; alpha globulin IV, lot 7; alpha globulin IV_4, lot 6; gamma globulin Fr. 11, lot 22; beta globulin Fraction 11, lot 8; Mercaptalbumin crystals, lot 3; albumin crystallized, lot 5, albumin 30% solution, lot 12; and azo-albumin, lot 1; Bovine IV_1, lot 7; Rabbit IV_1, lot 2.



Development of the Red Band in Washed Red Cell Agar Medium Containing Alpha Globulin IV_1

One plate of 0.025% (final dilution) alpha globulin IV_1 washed red cell agar medium was inoculated centrally with *S. aureus*, Guy. The colored plate demonstrates the rapidity with which red band develops. Note narrowing of the red band with passing of time.

A 11/18/63 5d at 37°C
B 11/19/63 5d " " , 1d room temp.
C 11/22/63 5d at 37°C, 4d room temp.
D 12/ 5/63 5d " " , 14d " "

red bands 1 cm wide, directly adjacent to the growth of these organisms. The MAM strain did not produce the red band phenomenon.

When the Guy strain was inoculated on control plates containing washed red cells without plasma factors, an area of hemolysis always developed around the colony, but the red band did not develop around the hemolyzed area. When control plates containing whole blood were used, typical red bands developed with 8 of the 10 human bloods employed. Two blood samples did not produce the red band. However, when fraction IV_1, lot 6, agar was added, washed red cells from all 10 bloods showed the characteristic red band.

In contrast to the above, fraction IV_1 was not able to replace plasma in production of lipid plaques by the Guy strain(3,4), despite incubation for 5 days at 37°C and 14 days at room temperature. However, lipid plaques were seen microscopically on the plasma plates in an area of 0.3 cm surrounding the growth of *S. aureus* even after 18 hours at 37°C. No lipid plaques were ever seen on the red band or anywhere else in the hundreds of plates containing fraction IV_1 and washed red cells which were inoculated with *S. aureus,* Guy, whereas numerous large lipid plaques almost always developed, on the whole blood control plates similarly inoculated.

Recently we have found that human alpha globulin fractions IV and IV_4 and bovine fraction IV_1 (but not rabbit fraction IV_1) were also able to replace plasma in the development of the red band. This work is being continued.

Discussion. Fraction IV_1, lot 6, was able to replace plasma in the development of the red bands by both the Guy and Oxford strains of *S. aureus*. Thus it appears likely that the differences in the red bands produced by these two strains may be related to the production of hemolysin by the Guy strain and to absence of hemolysin by the Oxford strain.

The Guy strain did not produce the red band with 2 of the 10 blood samples used in this study but readily caused development of the red band with the washed red cells from all 10 blood samples, when fraction IV_1 was substituted for the original plasma. An unpublished study also showed that all human bloods are not suitable for the production of the red band effect.

The recent finding that typical red bands also appeared in systems containing human alpha globulin fractions IV and IV_4 suggests that some plasma factor present in all 3 of these fraction preparations IV, IV_1, and IV_4, serves as a substrate for the red band production.

The results in this paper emphasize the fact that some human bloods do not contain a factor found in IV plasma protein preparations that is required for the production of the red band by *S. aureus,* Guy.

Summary. 1. The Guy and Oxford strains of *S. aureus* regularly cause development of the red band phenomena when inoculated on agar containing washed red cells and alpha globulin IV_1, lot 6 (Pentex). 2. Fraction IV_1, lot 6, is not able to replace plasma in the development of lipid plaques noted in whole blood containing plates inoculated with the Guy strain. 3. Human alpha globulin fractions IV, IV_4 or bovine fraction IV_1 could also be substituted for whole plasma to produce the red band phenomenon. 4. Further studies are being conducted in efforts to discover what substance in fractions IV, IV_1 and IV_4 serves as a specific substrate for the red band production.

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