mentioned haemagglutinin. Another haemagglutinating component of the acetone and ether preparation was associated with infectivity and was detected at a density of 1·247, suggesting that this component represents "complete virus particles" which have not been destroyed by the acetone and ether extraction procedure.

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[1] Casals, J., and Brown, L. V., *J. Exp. Med.*, **99**, 429 (1954).
[2] Clarke, D. M., and Casals, J., *Amer. J. Trop. Med. Hyg.*, **7**, 561 (1958).
[3] Mussgay, M., and Rott, R., *Virology*, **23**, 573 (1964).

"Normal" Antibodies which react with Heterogenetic Bacillary Antigen

Studies have been reported of a bacteriogenic transfusion reaction[1] in which the recipient's red cells became polyagglutinable, apparently as a result of the presence of a species of *Bacillus* in the donor bottle which was capable of altering erythrocytes *in vitro* in a similar way. It was suggested that modification of the cells by bacterial antigen with subsequent haemolysis by "normal" anti-bacterial antibody might provide the mechanism for such reactions. In further investigations[2], heterogenetic antigens from various genera and species of Gram-positive bacteria were examined, some prepared by the methods of Rantz[3] and Neter[4], and were shown to react with anti-bacterial antibodies found in normal human sera. Crude preparations of at least two such antigens, present in bacilli and in *Staphylococcus aureus*, as well as a third, found only in *Streptococcus*, reacted with antibodies in sera of practically 100 per cent of apparently normal persons. Whether this high frequency of reactivity resulted from the cumulative effect of several antibody specificities or from a single specificity was re-examined with purified antigen.

Antigen was extracted from 72 h cultures of *Bacillus* sp. No. 372–56 by boiling, as described previously[2]. The organisms and debris were removed by centrifugation and the supernate was mixed with ten volumes of acetone, which was decanted after the antigen had been precipitated. Precipitates were dried to remove traces of acetone and redissolved in 0·85 per cent solution of sodium chloride, buffered at *p*H 6·7 with phosphate salts. These antigens were tested[2] for their content of hexose and their ability to modify erythrocytes. Washed human group O red cells were mixed with twenty volumes of an optimal concentration of antigen, incubated at 35° C for 30 min, washed again, and resuspended in buffered saline solution (*p*H 6·7) at a concentration of 2 per cent. Tests were conducted on one hundred consecutive normal blood donor sera by mixing 0·05 ml. of serum with 0·05 ml. of modified cell suspension, incubating the mixtures at 5° C for 30 min, centrifuging lightly, and reading the results microscopically. Duplicate tubes were incubated at 37° C for 1 h, the cells washed, and tested with antiglobulin serum. Each donor serum was also tested against control cells which had been treated with buffered saline instead of antigen solution. Reactive sera of known titre were included in each test protocol to control the sensitivity. Of the one hundred normal donor sera, eighty-six produced agglutination of modified cells in saline and ninety-seven yielded positive results by the antiglobulin test (Table 1). Two sera failed to react by either procedure. An attempt to demonstrate blocking by these two sera failed, although previous work[2] has indicated that blocking may be produced by anti-streptococcal human sera which react with the NSS heterogenetic antigen of Rantz[3], and some of the other twelve non-agglutinating sera appeared to exhibit a slight blocking effect.

Table 1. REACTIONS OF ONE HUNDRED NORMAL SERA WITH ERYTHROCYTES MODIFIED BY PURIFIED BACILLARY ANTIGEN

No. of sera	Haemagglutination	Indirect antiglobulin
85	+	+
12	−	+
1	+	−
2	−	−
Percentage reactive	86	97

Total reactive 98 per cent.

Table 2. ABSORPTION OF NORMAL HUMAN ANTIBODY BY BACILLI (HAEMAGGLUTINATION AND INDIRECT ANTIGLOBULIN (COOMBS) TESTS)

Dilution	Human serum vs. modified cells			Human serum absorbed with bacilli vs. modified cells		
	5° C	37° C	Coombs	5° C	37° C	Coombs
1 : 1	4+	3+	4+	−	1+	4+
1 : 2	4+	2+	4+	−	−	2+
1 : 4	2+	±	4+	−	−	−
1 : 8	−	−	4+	−	−	−
1 : 16	−	−	4+	−	−	−
1 : 32	−	−	4+	−	−	−
1 : 64	−	−	1+	−	−	−
1 : 128	−	−	−	−	−	−

Table 3. EFFECT OF HEATING (56° C) ON NORMAL HUMAN ANTIBODY (HAEMAGGLUTINATION TESTS)

Saline agglutinins	Serum dilutions					
	1/1	1/2	1/4	1/8	1/16	1/32
Unheated	4+	3+	2+	−	−	−
15 min at 56° C	4+	3+	1+	−	−	−
30 min at 56° C	4+	3+	2+	−	−	−
45 min at 56° C	3+	2+	−	−	−	−
60 min at 56° C	3+	1+				
Incomplete antibodies (indirect antiglobulin)						
Unheated	4+	3+	2+	1+	−	−
15 min at 56° C	±	−	−	−	−	−
30 min at 56° C	−	−	−	−	−	−
45 min at 56° C	−	−	−	−	−	−
60 min at 56° C	−	−	−	−	−	−

The reactivity of normal human sera with bacillary carbohydrate antigen was associated with the gamma globulins when sera fractionated by curtain electrophoresis were tested against modified erythrocytes. These antibodies were partially absorbed by bacillary cell suspensions (Table 2). Some of the saline-active antibodies were heat-labile at 56° C (Table 3) if heated for more than 30 min, and the reactivity of sera containing incomplete antibodies was removed even more readily. The instability of these antibodies was confirmed by demonstrating a loss of reactivity after treatment with 2-mercaptoethanol.

Although agglutinins for the purified carbohydrate antigen from bacilli were demonstrated in only 86 per cent of the sera, 98 per cent were reactive in either one or both of the saline and antiglobulin tests. The possibility that this difference was more quantitative than qualitative has not been completely ruled out, although the sensitivity of the testing procedure was at least as great as that of previous tests with crude antigen.

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[1] Chorpenning, F. W., and Dodd, M. C., *Vox Sang.*, **10**, 460 (1965).
[2] Chorpenning, F. W., and Dodd, M. C., *J. Bact.*, **91**, 1440 (1966).
[3] Rantz, L. A., Randall, E., and Zuckerman, A., *J. Infect. Dis.*, **98**, 211 (1956).
[4] Neter, E., Gorzynski, E. A., Drislane, A. M., Harris, A. H., and Rajnovich, E., *Proc. Soc. Exp. Biol. and Med.*, **101**, 484 (1959).