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<td>Fort Detrick/SMUFD ltr dtd 15 Feb 1972</td>
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NEW DATA ON AGGLUTINATION OF ERYTHROCYTES

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FOR DESK USE ONLY. Not for use in Dept. of Army Publication. See AR 310-1, Par 16.
In connection with experiments performed jointly with Friedberger to accelerate hemolysis by precipitating sera (Centralblatt für Bakteriologie und Parasitologie, Vol XLV, No 4), I performed similar experiments on agglutination. It turned out that the erythrocytes loaded with a dose of the corresponding and nonself-agglutinating immune serum clump together very rapidly and tenaciously if one uses as antiprotein serum a precipitating serum for the animal species which the autoceptor has produced.

In my experiments I used rabbit erythrocytes loaded with autoceptor of a goat treated for some time with rabbit erythrocytes. The serum of a rabbit treated with normal goat serum served as antiprotein serum.

Table 1

<table>
<thead>
<tr>
<th>(1) Kaninchenerithrocyten</th>
<th>(2) Autoimmunserum</th>
<th>(3) Kaninchenpräzipitatserum</th>
<th>Agglutination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 eri</td>
<td>0,0001 eri</td>
<td>0,0001 eri</td>
<td>0</td>
</tr>
<tr>
<td>1 eri</td>
<td>0,0005 eri</td>
<td>0,001 eri</td>
<td>(5) Haftig</td>
</tr>
<tr>
<td>1 eri</td>
<td>0,0005 eri</td>
<td>0,01 eri</td>
<td>(7) Stark</td>
</tr>
<tr>
<td>1 eri</td>
<td>0,005 eri</td>
<td>0,1 eri</td>
<td>&quot;</td>
</tr>
<tr>
<td>1 eri</td>
<td>0,05 eri</td>
<td>0,5 eri</td>
<td>(8) dritt</td>
</tr>
<tr>
<td>1 eri</td>
<td>0,5 eri</td>
<td>5 eri</td>
<td>0</td>
</tr>
</tbody>
</table>

1 - Rabbit erythrocytes 5%; 2 - Immune goat serum; 3 - Precipit, rabbit serum; 4 - Agglutination; 5 - moderate; 6 - strong; 7 - very strong; 8 - 2 hours at room temperature. Centrifugation and washing of the erythrocytes with saline solution (0.9%); 9 - 2 hours at room temperature.

It will be noted that neither the antiprotein serum by itself regardless of the dose nor the autoceptor serum in the doses under study and multiples thereof is able to agglutinate rabbit red corpuscles.

*Read on 29 September 1907 at the Berlin Congress for Hygiene and Demography.*
In order to enable this special agglutination to show up clearly, it is absolutely essential to work with thoroughly washed, loaded erythrocytes since the presence of all the amboceptor serum, however small the quantity, interferes with the phenomenon. Agglutination occurs only when immune goat serum is used. It is absent when normal goat serum is used, even tho’ the conditions for precipitation are absolutely the same in both cases (Table II).

Table II

<table>
<thead>
<tr>
<th>(1) Immune serum</th>
<th>(2) Normal serum</th>
<th>(3) Agglutination</th>
<th>(4) Normal serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>(5) mild</td>
<td>(6) middle</td>
<td>(7) strong</td>
<td>(8) Agglutination</td>
</tr>
<tr>
<td>(9) Normal serum</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1-9 - Same as in Table I; 4 - Agglutination with (a) immune serum (b) normal serum

Nevertheless, there is a definite connection. The erythrocytes in contact with the immune serum naturally become loaded with amboceptor, whereas they can draw from the normal serum little or no amboceptor. There still remains the possibility that there is a definitely relationship between the fixed immune amboceptors and the precipitating serum. The following fact indirectly favors this. When heated to 70°, the serum loses both its precipitating ability and its ability to cause the loaded erythrocytes to agglutinate (Table III).

It would be of great value to determine whether precipitation of the precipitin from the antiprotein serum by means of the precipitable substance has removed from the latter the capacity for bringing about agglutination.

According to the experiments performed jointly with Frieden for on acceleration, hemolysis, it can also be seen in the case of agglutination that with contact of the antiprotein serum with the loaded erythrocytes a minimum binding of the substance of importance for agglutination takes place. This substance, however, is difficult to identify and then only after the most careful consideration of the quantitative relations (Table IV).
### Table III

<table>
<thead>
<tr>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
<th>(4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit erythrocytes</td>
<td>Zygammunum-serum</td>
<td>Serum un-treated or heated</td>
<td>Agglutination unheated or heated</td>
</tr>
<tr>
<td>1 cm</td>
<td>0.05 ml cm</td>
<td>0.05 ml cm</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.025 ml</td>
<td>0.025 ml</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.005 ml</td>
<td>0.005 ml</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.0025 ml</td>
<td>0.0025 ml</td>
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<td>0.001 ml</td>
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</tr>
<tr>
<td>1</td>
<td>0.0005 ml</td>
<td>0.0005 ml</td>
<td>0</td>
</tr>
</tbody>
</table>

(2) Precipitated with 5% NaCl; (3) 2 hour undiluted serum; (4) 2 hour heated or unheated serum.

1 - Rabbit erythrocytes; 2 - Immune goat serum; 3 - Precipitated rabbit serum unheated or heated to 70°C; 4 - Agglutination; 5 - Unheated serum; 6 - Heated to 70°C; 7 - Moderate; 8 - Strong; 9 - Very strong; 10 - 2 hours at room temperature. Centrifugation and washing of the erythrocytes with saline solution (0.85%); 11 - 2 hours at room temperature.

### Table IV

<table>
<thead>
<tr>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
<th>(4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit erythrocytes</td>
<td>Zygammunum-serum</td>
<td>Serum agglutinated or not agglutinated</td>
<td>Agglutination agglutinated or not agglutinated</td>
</tr>
<tr>
<td>1 cm</td>
<td>0.05 ml cm</td>
<td>0.05 ml cm</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.025 ml</td>
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<tr>
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<td>0.005 ml</td>
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<td>0.001 ml</td>
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</tr>
<tr>
<td>1</td>
<td>0.0005 ml</td>
<td>0.0005 ml</td>
<td>0</td>
</tr>
</tbody>
</table>

(2) Precipitated with 5% NaCl; (3) 2 hour undiluted serum; (4) 2 hour heated or unheated serum.

1 - Rabbit erythrocytes; 2 - Immune goat serum; 3 - Precipitated rabbit serum precipitated or not precipitated; 4 - Agglutination; 5 - Precipitated serum; 6 - Non-precipitated serum; 7 - Moderate; 8 - Traces; 9 - Strong; 10 - Very strong; 11 - 2 hours at room temperature. Centrifugation and washing of the erythrocytes with saline solution (0.85%); 12 - 0.1 cc of precipitated rabbit serum was precipitated twice with 5% immune goat serum per cc) rabbit erythrocytes in a 5% suspension and each time with the erythrocytes in 10 cc of this suspension.
The facts that I have reported here on rabbit-goat amboceptor, the corresponding erythrocytes and the corresponding rabbit protein serum should also apply to other species of animals.

Likewise the specificity of the reaction should be the same, although further research might disclose whether the phenomenon appears in bacteria the same way that it does in erythrocytes.

It is unjustified, in my opinion, to look upon the data summarized above as a new starting point for explaining or offering theoretical interpretations of the rather delicate processes involved in agglutination.