AGGLUTINATING PROPERTIES OF ESCHERICHIA COLI

AGGLUTINATION OF ERYTHROCYTES, LEUCOCYTES, THROMBOCYTES, SPERMATOZOA, SPORES OF MOLDS, AND POLLEN BY STRAINS OF E. COLI

L. ROSENTHAL

Department of Laboratories, Israel Zion Hospital, Brooklyn, N. Y.

Received for publication December 9, 1942

In examining the biological properties of Escherichia coli, I have found that some strains agglutinated a number of cellular elements of animal and plant origin: erythrocytes, leucocytes, thrombocytes, spermatozoa, yeasts, spores of molds, and pollen.

References to this subject in the literature are few and concern only agglutination by E. coli of red cells (Guyot 1908) and spermatozoa (Rosenthal 1931). Aynaud (1911) in a brief statement includes E. coli among bacteria which agglutinate blood platelets but gives no details and does not specify whether it concerns the whole group of E. coli or selected strains. So far no attempt has been made to connect these separate findings and correlate them as manifestations of an agglutinating factor inherent in strains of E. coli. To the best of our knowledge agglutination of all other elements is described here for the first time.

The purpose of this investigation was: 1) to develop an adequate technique for the performance of the test, 2) to study the various factors which may influence the reaction, 3) to determine how widely the agglutinating property is encountered in the E. coli group and 4) to interpret the mechanism of the reaction by comparing the action of E. coli with that of other known agglutinating factors.

TECHNIQUE

1. Preparation of suspensions for agglutination

a. Erythrocytes. Human blood of any group, washed or unwashed, was added in proportion of 1-2% to physiological salt solution.

b. Leucocytes. Citrated blood (1 ml. of 3.8% solution of sodium citrate and 4 ml. of blood) was allowed to stand until the red cells settled. The supernatant plasma contained leucocytes and thrombocytes. However, the leucocytes frequently showed a tendency to clumping, which, though not as marked as after addition of bacteria, might have interfered with interpretation of the test. In order to stabilize the suspension the following method was found satisfactory. The citrated plasma was mixed with an equal volume of 2% solution of formalin in physiological saline. The mixture was kept overnight in the ice-box, and the following day the supernatant fluid was removed and the sediment suspended in a volume of saline corresponding to the original volume of plasma. This suspension was uniform, did not contain any clumps and could be kept in the ice-box for several days. Controls have shown that formalinized leucocytes were agglutinated only by those strains of E. coli which acted on fresh leucocytes.
c. Thrombocytes. Fresh citrated plasma obtained in the manner previously described was centrifuged for 2 minutes to precipitate the leucocytes. The supernatant layer of plasma was used for the test since the thrombocytes remained uniformly suspended even after an interval of several hours. All attempts to remove the plasma by prolonged centrifuging and to suspend the sediment of thrombocytes in saline proved unsatisfactory since without plasma the thrombocytes showed a marked tendency to autoagglutination.

d. Spermatozoa. Sperm of rats (from epididymis) or of men (condom specimens) was mixed with an equal volume of saline.

e. Yeasts. The strain used in the tests was isolated from Fleishmann’s yeast. The growth of a 24-hour culture on a slanted Sabouraud’s agar was suspended in 2 ml. of saline.

f. Spores of molds. Spores of one-month-old cultures of Aspergillus niger or Penicillium glaucum on a slanted Sabouraud’s agar were suspended in 2 ml. of saline.

g. Pollen. The following pollens were used: 1) Giant Ragweed, 2) Dwarf Ragweed, 3) English Plantain, 4) Red Oak, 5) Sweet vernal grass, 6) Birch, 7) Timothy grass. One gram of each pollen was added to 10 ml. of saline and the mixture vigorously shaken.

2. Performance of the test

The test was performed by placing a drop of the suspension on a slide, introducing into it a loopful of bacterial growth from a culture on agar, and thoroughly mixing. In all positive tests the reaction took place either immediately or within the first 5 minutes. Tilting or rotating of the slide accelerated the reaction. The formation of clumps and flocculi was observed with the naked eye and verified microscopically. In controls without bacteria or with inactive strains the cells remained evenly distributed in the fluid.

FACTORS INFLUENCING THE REACTION

The agglutinating factor was thermodlabile. A suspension of active bacteria, boiled for 2 minutes, lost its agglutinating power. Alcohol had no effect, as was demonstrated by the following experiment. A thick suspension of bacteria was mixed with 10 volumes of alcohol (96°), kept at room temperature for ½ hour and centrifuged. The sediment remained active. Filtrates of broth cultures, young or old, were inactive. Agglutination occurred both in acid or alkaline medium (pH 3–pH12). As an exception, in a medium of pH 10–12 no agglutination of leucocytes by bacteria took place. At pH 4–4.5 leucocytes and thrombocytes showed a tendency to clumping in the absence of bacteria. Treatment of the cellular elements with a 2% formalin solution did not impair their agglutinability. Stromata of red cells (after hemolysis) were also susceptible to agglutination. Addition of serum to the cell suspension did not influence the reaction. The cultures of active strains of E. coli preserved their agglutinating power after numerous transplants.
INCIDENCE OF AGGLUTINATING STRAINS OF E. COLI

Seventy strains of E. coli isolated from the feces of 70 unselected human cases were tested for their agglutinating properties. 46 strains gave negative results. The agglutination of various cells by the remaining 24 strains is shown in table 1.

Thus, 2 strains (N1–N2) agglutinated all cells, 2 strains (N3–N4)—all cells except thrombocytes and pollen, 5 strains (N5–N9)—all cells except erythrocytes and thrombocytes, and so forth. On the other hand erythrocytes were agglutinated by 12 strains, leucocytes—by 13 strains and so forth.

The yeasts and spores of Aspergillus niger and Penicillium glaucum constituted a uniform group agglutinated by the same strains. Equally, all 7 species of pollen used in the test did not differ from one another in their agglutinability.

TABLE 1

Agglutinating properties of 24 strains of E. coli

<table>
<thead>
<tr>
<th>STRAIN NUMBER</th>
<th>ERYTHROCYTES</th>
<th>LEUCOCYTES</th>
<th>THROMBOCYTES</th>
<th>SPERMATOGONIA</th>
<th>YEASTS</th>
<th>SPORES ASPERGILUS</th>
<th>SPORES PENICILLIUM</th>
<th>POLLEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>21</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>22</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>23</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total no. of strains</td>
<td>12</td>
<td>13</td>
<td>11</td>
<td>10</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>8</td>
</tr>
</tbody>
</table>

and thrombocytes, and so forth. On the other hand erythrocytes were agglutinated by 12 strains, leucocytes—by 13 strains and so forth.

The yeasts and spores of Aspergillus niger and Penicillium glaucum constituted a uniform group agglutinated by the same strains. Equally, all 7 species of pollen used in the test did not differ from one another in their agglutinability.

COMPARISON WITH OTHER AGGLUTINATING FACTORS

1. Thomsen's hemagglutinating phenomenon

Friedenreich (1930) isolated several bacterial strains which produce in erythrocytes the following phenomenon described by Thomsen (1927): The red cells
after being in contact for several hours with these bacteria do not show any signs of clumping but are easily agglutinated by subsequent addition of such sera as normally do not affect untreated erythrocytes. One bacterial strain apparently similar to culture “M” of Friedenreich (1930), was isolated by me from a contaminated blood specimen. It was a gram-positive slender mobile rod, which formed greenish-yellow colonies on agar, and slightly liquefied gelatin and Loeffler’s medium. Optimum growth was at 20°C.; at 37°C the development was slow. A comparison between this strain and an hemagglutinating strain of E. coli showed the following differences: 1) Erythrocytes were directly agglutinated by E. coli, even in the absence of any trace of serum or plasma, while the second strain in conformity with the main characteristic of the Thomsen phenomenon did not have any direct agglutinating effect on the red cells, but changed them so that they were agglutinated by subsequent addition of serum. 2) E. coli produced agglutination almost immediately, while the Thomsen phenomenon could be produced only after the red cells were in contact with the bacteria at least for 10-14 hours. 3) Filtrates of E. coli were inactive, while those of Thomsen strain were active. 4) Agglutination by E. coli took place at room temperature and at 37°C., while Thomsen’s phenomenon appeared only at room temperature.

2. Bacterial toxins

Weinberg and Kepinow (1921) found that the addition of bacterial toxin (filtrate of broth cultures of the vibron septique) to a peritoneal leucocytic exudate of a guinea pig previously injected intraperitoneally with Mellins food caused agglutination of the leucocytes after 3 hours incubation of the mixture at 37°C.

Recently Weld and Mitchell (1942) found that staphylococcus toxin agglutinated rabbit leucocytes from pleural exudates which were produced by intrapleural injection of aleuronat and starch suspension. These findings differed from the agglutination of leucocytes by E. coli in 2 respects: 1. E. coli produced the effect almost immediately while in the reaction with the toxins an incubation period of 3 hours (Weinberg and Kepinow) or 50 minutes (Weld and Mitchell) was required. 2. Unlike the experiments with the toxins, filtrates of E. coli broth cultures were inactive.

3. Solutions of dyes

The agglutination of red cells and bacteria by basic dyes has been known for a long time. In my experiments all the cellular elements of animal and plant origin used for the tests with E. coli were equally well agglutinated by these dyes. Thus, the addition of a drop of a solution of crystal violet in saline (1:800) to a drop of a cellular suspension caused prompt agglutination of the cells. However, there is a marked difference between the agglutination by E. coli and that by dyes. As I have shown previously in collaboration with Hornick (1931), serum mixed with the suspension of cells acted as a protective and subsequent addition of a dye solution to this mixture produced no agglutination. Moreover, the addition of serum to the mixture of cells and dye, after the agglutination already took place, was able to restore the uniformity of the original suspension.
In contradistinction to this, the agglutination of cells by *E. coli* was in no way inhibited by addition of serum.

4. Phytoagglutinins

Landsteiner and Raubitschek (1907) and others have shown that various beans and some other plants contain a substance (called originally phasin and later phytoagglutinin) which causes clumping of erythrocytes and leucocytes.

For comparison with *E. coli* I selected Scarlet Runner bean and Jack bean. 5 grams of meal of pulverized beans were mixed with 10 ml. of saline, kept in the ice-box overnight and filtered. The test was performed on a glass slide by mixing a drop of suspension of cells with a drop of filtrate. In conformity with the results obtained by previous investigators the Scarlet Runner extract agglutinated promptly erythrocytes and their stromata, and leucocytes. In addition, as I have found, the extract agglutinated spermatozoa and thrombocytes. The cellular elements of plant origin remained unaffected by this extract. On the other hand, I have observed that the Jack bean extract caused clumping of yeast cells and spores of molds but did not influence cells of animal origin. Phyto-agglutinins acted not only on fresh cells but also on those treated with 2% formalin. The action of phytoagglutinins was not influenced by the pH of the medium, or by addition of serum. Boiling of the extracts destroyed their agglutinating properties. Addition of alcohol to the extract produced a precipitate which, when dissolved in saline, remained active. Landsteiner and Raubitschek believe that the extracts of the beans contain a protein substance which causes the agglutination of the cells. Wienhaus (1909) is of the opinion that the agglutinating property may be due to a special enzyme.

5. Mechanism of the action of *E. coli*

The first 3 agglutinating factors described here, as was pointed out, present features quite distinct from that of *E. coli*. Only the phytoagglutinins in many respects resemble the agglutinins of *E. coli*. In both cases the clumping takes place promptly. The reaction can be performed not only with fresh but also with formalinized cells, both in acid or alkaline medium, the boiling destroys the active principle, alcohol does not affect it. Furthermore, as in the case of strains of *E. coli*, different plant extracts are active against different cells. Therefore it may probably be assumed that the active principle of *E. coli* is of the same nature as that of phytoagglutinins and due to a special protein substance or to an enzyme contained in the bacteria.

**COMMENT**

As is well known present biochemical and serological methods in many cases are insufficient for identification of various strains of *E. coli*. This investigation deals with an additional biological property characteristic of selected strains of *E. coli*—their ability to agglutinate various cells of animal and plant origin. An analogy with the classification of streptococci based on their action on red blood cells would suggest the possibility of using the agglutinating properties of *E. coli* for the same purpose. Our findings constitute merely the first step in the solution of this problem. Further study is necessary to establish whether agglutinating strains form a separate subgroup of *E. coli*. The question of pathogenicity and
virulence of these strains, which in vitro affect the cells so strikingly, also requires elucidation.

The problem presents furthermore some practical aspects. The typing of blood may be obscured by contamination with an hemagglutinating strain of E. coli. According to Lindner (1906) the process of yeast production is at times greatly affected by clumping of yeast cells. In the opinion of Henneberg (1905) bacterial contamination is responsible for this clumping. From what we have seen in our experiments active strains of E. coli can produce such a phenomenon. It may be also of interest to inquire whether the presence in the vaginal flora of spermagglutinating strains of E. coli produces clumping of the inseminated sperm. Similarly one wonders whether pollen-agglutinating strains of E. coli affect the orally ingested pollens which according to Black (1939) and MacQuiddy (1941) pass through the stomach and appear in the intestinal contents.

SUMMARY

Some strains of Escherichia coli have been found to possess agglutinating properties against cellular elements of animal and plant origin: erythrocytes, leucocytes, thrombocytes, spermatozoa, yeasts, spores of molds, and pollen. A technique for the performance of the test and factors influencing the reaction are described. Among 70 studied strains 24 proved active. Comparison with other agglutinating factors (hemagglutinating bacteria of Thomsen, leucagglutinating toxins, basic dyes, phytoagglutinins) shows that agglutinins of E. coli most closely resemble the phytoagglutinins. The importance of the agglutinating properties of E. coli is briefly discussed.

REFERENCES


Black, I. H. 1939 The oral administration of Ragweed Pollen. J. Allergy, 10: 156-158.

Friedenreich, V. 1930 The Thomsen Hemagglutination Phenomenon. Levin and Munkgaard, Copenhagen.


Henneberg, W. 1905 Cited by Lindner.


