THE EFFECT OF SURFACE-ACTIVE AGENTS ON PHAGOCYTOSIS1

L. JOE BERRY, ROBERT W. STARR, III, AND EVELYN C. HALLER

Biological Laboratories, Bryn Mawr College, Bryn Mawr, Pennsylvania

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According to the theoretical formulation of Fenn (1921), based in part on the previous work of Tait (1918) and Rhumbler (1914), a decrease in free surface energy occurs when a particle is ingested by a phagocyte. Mudd and Mudd (1933) offered experimental evidence in support of this concept in their elucidation of the role played by opsonins (and nonimmune serum) in promoting phagocytosis. Bacteria in the presence of serum are more readily ingested because their surface energy is increased, and hence a greater decrease in energy results when they are phagocytosed. An extensive discussion of this relationship is found in the review of Mudd, McCutcheon, and Lucké (1934).

Theoretically, however, phagocytic activity should be subject to variations arising from changes in surface energy, not only of the particles being ingested, but also of the phagocytic cells themselves. Such changes have been suggested as the underlying cause of the increased phagocytosis observed in the granulocytic leucocytes of anemic human beings (Berry, Davis, and Spies, 1945; Berry, Leyendecker, and Spies, 1947) and of rats made anemic by blood loss (Berry and Haller, 1947a). This may also explain the results of Gordon and Katsh (1949), who found that in adrenalectomized rats the splenic macrophages take up less thorotrast (a colloidal solution of thorium dioxide) than those in normal control animals, whereas injections of adrenal cortical extract increase the macrophagic activity over that of controls. Therefore, since phagocytes are known to exhibit a variation in activity under conditions that appear to leave the particles unchanged, it is possible that changes in surface energy are involved.

In order to test the validity of this hypothesis the effect of surface-active agents on the phagocytic function of neutrophilic granulocytes was investigated. The results are described in this report and are offered as additional evidence for the validity of the Fenn theory of phagocytosis.

METHOD

The phagocytic activity of blood neutrophiles was determined by a modification of the in vitro technique of Boerner and Mudd (1935), which has been described in detail in previous publications (Berry, Davis, and Spies, 1945; Berry, Leyendecker, and Spies, 1947). Whole blood is diluted with an equal volume of physiological solution of sodium chloride contained in a paraffined test tube to which a measured volume of standardized bacterial suspension is added. Coagulation is prevented either by the presence of heparin2 in the normal saline or by defibrinating the blood. The phagocytic mixture is agitated at 37 C for a

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fixed period of time; then smears are prepared and stained. The final evaluation of phagocytic function was based, in early tests, on the average number of bacteria ingested per neutrophile in 100 cells. In later experiments, the percentage of neutrophiles showing engulfment of one or more bacteria was found by systematically examining the entire smear. When this was done, the percentage figure was calculated from a total number of cells that varied, from slide to slide, between 100 and 300. While the two methods gave comparable results, the percentage of phagocytes active is theoretically preferable since the size of the clumps of bacteria loses significance (see discussion of this point in Berry and Spies, 1949).

Both human and mouse leucocytes were used in these studies. Human blood was withdrawn from the cubital vein and defibrinated before use. Mouse blood was obtained either by decapitation or by severing the vessels in the axillary region. It was collected in a paraffined watch glass and transferred quickly by tuberculin syringe to the heparinized saline contained in the tubes mentioned above.

Suspensions of 18-hour cultures of Micrococcus candidus served as test particles when the mean number of bacteria per cell was found, and of Staphylococcus aureus when the percentage of cells active was determined. The density of the suspensions was standardized in a Coleman, model 11, spectrophotometer at 650 mμ.

The effect of surface-active agents on phagocytosis was tested in several ways. Sterile solutions containing known concentrations by weight, made up in normal saline, were used either directly as the blood diluent in the phagocytic tube or in preparing the suspensions of bacteria. It was found that an incubation period of 1 hour at 37 C in the presence of the detergent was sufficient to influence phagocytosis. In some mouse experiments, the solutions were injected intraperitoneally, and the mice were bled at intervals following the injections. This blood was used in the phagocytic tests without bringing it again into contact with the detergent. In all cases, the results were compared to those obtained for control samples.

RESULTS

Screening experiments with surface-active agents. The effect of 52 different surface-active agents on the phagocytic activity of human neutrophiles was determined. All solutions were prepared so as to give on final dilution a concentration of 0.0005, 0.00005, and 0.000005 per cent by weight. Table 1 shows the average values of duplicate tests with the most active compounds of each type, arranged in decreasing order of effectiveness. Certain nonionic and anionic detergents more than doubled the mean number of bacteria engulfed per leucocyte, and the best cationic surface-active agent was almost as effective (186 per cent). The probable errors indicate that the validity of the results with these particular substances is good. Of the remaining compounds, not shown in the table, some increased phagocytosis and others depressed it. No effort was made in these preliminary measurements to check the results initially obtained, nor
were additional concentrations tested. The percentage of active ingredients varied from sample to sample, between 20 and 100 per cent, and some were unspecified. Therefore, it is possible that some of the compounds might have been more active than the data indicate had a wider range of concentrations been tested. Nevertheless, the mere fact that surface-active agents were found to

We are indebted to various companies for a generous supply of the compounds tested.

have a profound effect on phagocytosis was sufficient for the purposes of this investigation.

No correlation existed between the pH of the detergent solutions and their influence on phagocytes. The hydrogen ion concentrations were measured with a Leeds and Northrop pH meter, using a glass electrode.¹

In vitro effect of ‘triton” N-100 on phagocytosis. This compound was selected

¹ We wish to thank Miss Barbara Bunce for these determinations.

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**TABLE 1**

**Effect of certain surface-active agents on phagocytic activity of human neutrophiles**

<table>
<thead>
<tr>
<th>DETERGENT</th>
<th>AVERAGE NUMBER BACTERIA INGESTED PER NEUTROPHILE</th>
<th>PER CENT INCREASE OVER CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0001%</td>
<td>0.00005%</td>
</tr>
<tr>
<td>Triton N-100</td>
<td>6.19 ± 0.2</td>
<td>4.75 ± 0.2</td>
</tr>
<tr>
<td>Tween 20</td>
<td>5.64 ± 0.2</td>
<td>4.95 ± 0.2</td>
</tr>
<tr>
<td>Polymethylene glycol 400 monoesterate</td>
<td>6.21 ± 0.2</td>
<td>5.92 ± 0.1</td>
</tr>
<tr>
<td>MPD—1047</td>
<td>3.78 ± 0.2</td>
<td>3.26 ± 0.2</td>
</tr>
</tbody>
</table>

| B. Anionic Detergents             |                                                  |                                |                                |
|----------------------------------|--------------------------------------------------|--------------------------------|
|                                  | 0.0001%  | 0.00005% | 0.000005% | None (control) | 0%   | 5%   | 10%  |
| 1. Decyl benzene Na-monosulfate   | 8.18 ± 0.2 | 6.14 ± 0.2 | 3.82 ± 0.2 | 214  | 160  |
| Carboxymethyl cellulose          | 8.13 ± 0.2 | 6.09 ± 0.3 | 5.06 ± 0.4 | 200  | 150  | 124  |
| Hydroabietyl Na-sulfate           | 4.59 ± 0.2 | 3.74 ± 0.1 | 2.34 ± 0.1 | 196  | 160  |
| Pine-substituted phenol Na-sulfonate | 4.27 ± 0.2 | 3.10 ± 0.2 | 3.03 ± 0.2 | 141  | 168  |
| Dehydrogenated dipolymer Na-sulfonate | 4.30 ± 0.2 | 3.79 ± 0.2 | 2.18 ± 0.3 | 168  | 148  | 124  |

| C. Cationic Detergents            |                                                  |                                |                                |
|----------------------------------|--------------------------------------------------|--------------------------------|
|                                  | 0.0001%  | 0.00005% | 0.000005% | None (control) | 0%   | 5%   | 10%  |
| Triton K-60                      | 3.74 ± 0.2 | 3.02 ± 0.2 | 2.63 ± 0.3 | 2.01 ± 0.2 | 186  | 150  | 131  |
| Surface-active agent S-E         | 2.34 ± 0.2 | 2.45 ± 0.2 | 1.85 ± 0.4 | 1.85 ± 0.3 | 126  | 132  | 100  |
| MPD—1046                         | 5.03 ± 0.1 | 5.39 ± 0.1 | 3.91 ± 0.2 | 128  | 138  |

We wish to thank Miss Barbara Bunce for these determinations.
for additional study since it gave the largest increase in phagocytosis of those tested (table 1). For these experiments, and all those subsequently reported, the percentage of active phagocytes was determined with Staphylococcus aureus as the test organism. Each smear was counted without knowledge of the origin of the slide. This was done to eliminate the possibility of an unconscious bias on the part of the experimenter. Duplicate counts checked within 1 to 2 per cent.

Figure 1 shows the results with human blood, using a series of bacterial densities, as indicated by spectrophotometric readings. The neutrophiles are much more active after previous incubation with "triton" N-100 (0.005 per cent by weight) and the greatest difference between experimental and control blood appears at a density of 45 per cent transmittance. This corresponds to about 29 million bacteria per ml. Figure 2 gives the results of similar experiments carried out with mouse blood. The maximum difference in phagocytosis again falls at a bacterial density of about 29 million organisms per ml.

The effect of intraperitoneal injections of "triton" N-100 on the phagocytic activity of mouse leucocytes, evaluated in vitro. Figure 3 compares the percentage of active phagocytes from animals given 2 mg of triton N-100 in 0.5 ml of saline (equivalent to about 100 mg per kilogram of body weight), by intraperitoneal injection at time zero, with that of uninjected controls. A second injection, indicated by the arrow, maintained the elevated phagocytic activity of the granulocytes for an additional period comparable to that of the initial dose. This amount of detergent increases the ability of the white cells to engulf bacteria for 4 to 5 hours, but at the end of this time there is a significant depression of phagocytosis. When the injections were repeated every 4 hours for 20 hours,
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Figure 2. Same as figure 1 except that mouse granulocytes were used.

Figure 3. Percentage of phagocytes active compared with time following intraperitoneal injections in mice of 2 mg of triton N-100 in 0.5 ml saline. A second injection was given at the arrow. Control values shown by open circles, single injection by solid circles, and second injection by open squares.

Figure 4. Shows the results of a single injection with the dosage level reduced to 0.5 mg of triton N-100 in 0.5 ml saline (about 25 mg per kilogram of body weight). There is an increase
in phagocytosis comparable to that shown in figure 3. Figure 5 establishes the fact that with this amount of detergent the elevation in phagocytosis is maintained over a period of 3 days when injections are given every 4 hours. Similar results were obtained with the dose level reduced to 0.2 mg in 0.2 ml of saline.

No change in phagocytosis was found over a 6-hour period following an oral administration (by stomach tube) of either 0.2 mg or 1 mg of triton N-100.

**Therapeutic test of triton N-100 in mice experimentally infected with Salmonella typhimurium.** Since repeated injections of triton N-100 maintained an elevated phagocytic activity over a 3-day period, it was considered worth while to test the possible therapeutic value of this substance in mouse typhoid. In previous

![Figure 4](http://jb.asm.org/)  

Figure 4. Percentage of phagocytes active compared with time following intraperitoneal injections in mice of 0.5 mg of triton N-100 in 0.5 ml saline. Test blood shown by solid circles and control blood by open circles.

experiments mice were protected against this infection at a time when the blood granulocytes showed improved phagocytosis accompanying blood loss anemia (Berry and Haller, 1947b). Accordingly, 150 mice were infected with a standardized suspension of thoroughly washed *Salmonella typhimurium*. Four hours later half the animals were injected intraperitoneally with 0.2 mg of triton N-100 in 0.2 ml physiological saline, and these injections were repeated every 4 hours for 56 hours. The remaining half of the mice, serving as controls, were injected at the same time with an equal volume of sterile saline. The percentage survival curves for the two groups are given in figure 6. Other than the smaller number of deaths during the first 2 days, no protection was afforded the mice. The total mortality in the two groups was practically identical.
Figure 5. Same as figure 4 except injections were repeated every 4 hours for 68 hours.

Figure 6. Percentage of survival of treated (solid circles) and untreated mice (open circles) following experimental infection at zero hour with *Salmonella typhimurium*. Intraperitoneal injections of 0.2 mg of triton N-100 in 0.2 ml of saline were started 4 hours after infection and were continued for 56 hours. Control animals received only sterile saline injections.
The effect on phagocytosis of treating the test particles (bacteria) with surface-active agents. In the experiments described in previous sections, the phagocytes came into contact with the detergent for at least 1 hour prior to the introduction of bacteria. Phagocytosis increased in every case except those in which excessive quantities were injected in mice over a period of hours. The fact that no protection against the fatal outcome of mouse typhoid infections accompanied this phagocytic change suggested that tests should be carried out when bacteria alone or both bacteria and leucocytes were in contact with the detergent prior to evaluating phagocytosis. The results in table 2 prove that no significant change in phagocytosis occurs when both granulocytes and bacteria are incubated with the detergent prior to completing the phagocytic mixture, but when bacteria alone are incubated phagocytosis is depressed. This holds for the five substances

<table>
<thead>
<tr>
<th>SURFACE-ACTIVE AGENT</th>
<th>No treatment (controls)</th>
<th>Neutrophiles incubated</th>
<th>Bacteria incubated</th>
<th>Neutrophiles and bacteria incubated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triton N-100</td>
<td>66.7</td>
<td>87.5</td>
<td>48.2</td>
<td>70.7</td>
</tr>
<tr>
<td>Triton N-100</td>
<td>72.5</td>
<td>83.7</td>
<td>63.5</td>
<td>70.0</td>
</tr>
<tr>
<td>Tween 20</td>
<td>77.0</td>
<td>87.0</td>
<td>60.5</td>
<td>75.3</td>
</tr>
<tr>
<td>Tween 20</td>
<td>69.5</td>
<td>85.5</td>
<td>59.7</td>
<td>68.2</td>
</tr>
<tr>
<td>Tween 20</td>
<td>64.7</td>
<td>80.5</td>
<td>51.0</td>
<td>60.0</td>
</tr>
<tr>
<td>Na-abietyl sulfate</td>
<td>72.5</td>
<td>84.0</td>
<td>59.5</td>
<td>74.2</td>
</tr>
<tr>
<td>Decyl benzene Na-monosulfate</td>
<td>74.0</td>
<td>91.5</td>
<td>65.2</td>
<td>75.5</td>
</tr>
<tr>
<td>Carboxymethyl cellulose</td>
<td>77.0</td>
<td>85.5</td>
<td>71.0</td>
<td>75.5</td>
</tr>
</tbody>
</table>

(both anionic and nonionic) that gave the largest increases in phagocytosis, as shown in table 1. Results comparable to those of table 2 were also obtained when suspensions of Salmonella typhimurium were used instead of Staphylococcus aureus.

DISCUSSION

According to Ponder (1927), a particle will be ingested by a cell when

$$\frac{\text{S.E. particle} - \text{S.E. cell particle}}{\text{S.E. cell}} = or > \ ( +1 )$$

where S.E. cell represents the free surface energy between the cell and the surrounding medium, S.E. particle that for the particle and medium, and S.E. cell particle that between the interface of cell and particle. Therefore, phagocytosis is favored by a decrease in the surface energy of the cell or cell particle and an increase in surface energy of the particle. Changes in the opposite direction make ingestion less likely. The greater the total decrease in free surface energy that accompanies ingestion, the greater the probability that it occurs. Since
the surface-active agents decrease surface energy, an enhancement of phagocytosis should accompany such a change produced in phagocytic cells by incubation with detergents, and the reverse effect should result when the bacteria are so altered. In addition, only a small change in activity would be predicted when the surface energy of both bacteria and phagocytes is decreased proportionately. These theoretical relationships are fully confirmed by the experiments described in this report.

SUMMARY

Five, out of a total of 52 surface-active agents tested *in vitro*, at least double the mean number of bacteria ingested per human neutrophile, as compared to control values. Similarly, an increase in percentage of neutrophiles active was found when the most effective of these, "triton" N-100, was tested with human and with mouse neutrophiles. This same compound, injected intraperitoneally into mice, elevated the phagocytic activity of the blood granulocytes, evaluated *in vitro*; and with injections repeated every 4 hours for 3 days, a greater phagocytic function was maintained. A similar treatment, however, failed to protect mice against experimentally induced infections with *Salmonella typhimurium*. This fact is explained by the observation that no change in phagocytosis occurred when both blood cells and bacteria were incubated in the presence of the detergent prior to mixing, and a decrease in phagocytosis followed incubation of the bacteria alone. These results are considered in the light of surface energy relationships in phagocytosis.

REFERENCES


Fenn, W. O. 1921 The theoretical responses of living cells to contact with solid bodies. J. Gen. Physiol., 4, 373-385.


