# THE ORGANOTROPIC, BACTERIOTROPIC, AND LEUCOCYTOTROPIC ACTIONS OF CERTAIN ORGANIC CHEMICALS.

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Present knowledge of the manner by which chemicals influence the mechanism of infection and resistance is limited. Investigations in chemotherapy hitherto have been concerned primarily with certain organotropic and bacteriotropic activities,—a search for a chemical of a monotropic character. The influence of definite chemical entities both upon the factors vital to the integrity of the animal against an invading microorganism, and such life processes as are perhaps not essential to actual defense against bacterial disease, has not been thoroughly investigated. The work of Ehrlich, Morgenroth, Browning, and others has demonstrated that it is possible to find chemicals with less of organotropic than of bacteriotropic activity. Frequently, however, when such substances have been used against an experimental infection, the treated animal succumbed as though no drug had been given, or in a shorter time than the controls. In other words, the drug, in doses not fatal under normal conditions, apparently acts upon defensive complexes necessary to the animal in coping with an infection, having a greater influence upon this activity than upon the pathogenic microorganism. Just what the force or combination of forces is that maintains a state of stable resistance in the animal toward microorganisms can only be conjectured. Whatever other elements enter into this biological reaction, obviously the phagocyte has some share. Because of this, as evidenced in the increase of the activity of the leucocyte during the course of many infectious diseases, it was decided to study the influence of certain chemicals, not alone in respect to organotropism and to bacteriotropism, but as to leucocyto-

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tropism as well. The work was undertaken in the hope that information might be gained which would enable a more intelligent choice of chemicals for chemotherapeutic purposes, and might lead to the finding of a delicate indicator by which to judge the relationship between chemical constitution and the activity exhibited by the body against an invading organism.

We wish to report the toxicity for mice (organotropism), the bactericidal action on *Staphylococcus aureus* (bacteriotropism), and the antiphagocytic influence (leucocytotropism), of certain members of seven groups of chemicals—triphenylmethane leuco bases, triphenylmethane dyes, acridines, safranines, phenazines, quinones, and cinchonas.

The work was done while in collaboration with Dr. Jacobs and Dr. Heidelberger in the study of chemotherapy.[1] The manner in which the chemicals here reported influence an experimental pneumococcus infection of mice will appear in a later publication.

The confusion that exists in the literature on phagocytosis seems to be due for the most part to variations in technique. Even in controlled phagocytic studies frequent anomalies appear, unavoidable when dealing with so delicate an indicator as the leucocyte. If, then, instead of considering results from one such experiment or series of experiments, we attempt to compare those from work done under widely differing conditions, definite conclusions are difficult to reach.

The findings of Hamburger,[2] Kolmer,[3] Manwaring and Ruh,[4] Grünspan,[5] and Smith [6] on the influence of quinine on phagocytosis can hardly be compared. Hamburger[7] obtained leucocytes from defibrinated horse blood and suspended them in horse serum. To this suspension he added the drug and incubated the mixture, thus affording opportunity for interaction between quinine and leucocytes

[1] Felton, L. D., and Dougherty, K. M., *J. Exp. Med.*, 1922, xxxv, 761.

[2] Hamburger, H. J., *Centr. Bakt., 1te Abt., Ref.*, 1913, lvii, 105. Hamburger, H. J., and Hekma, E., *Biochem. Z.*, 1908, ix, 512.

[3] Kolmer, J. A., Solis-Cohen, S., and Steinfield, E., *J. Infect. Dis.*, 1917, xx, 333.

[4] Manwaring, W. H., and Ruh, H. O., *J. Exp. Med.*, 1907, ix, 473.

[5] Grünspan, T., *Centr. Bakt., 1te Abt., Orig.*, 1909, xlviii, 444.

[6] Smith, H. L., *Lancet*, 1910, ii, 1342.

[7] Hamburger, H. J., and Hekma, E., *Biochem. Z.*, 1907, iii, 88.

before the charcoal was added. Kolmer,[3] on the other hand, used washed leuco-cytes procured from the peritoneal cavity of rabbits after an injection of aleuronat, and these came into contact with the quinine only after it had been incubated an hour with a heavy suspension of living Type I pneumococci. Thus in the former case, the quinine acted primarily on the leucocyte and secondarily on the sub-stance to be phagocytosed, while in the latter, the primary action was on the pneumococcus. As might be expected, their results contradict each other, Ham-burger[2] claiming an inhibition in phagocytic activity with a dilution of quinine 1:1,000, and Kolmer[3] claiming a stimulation with the same strength of the drug. Manwaring and Ruh,[4] using still another technique, found a 20 per cent stimula-tion from quinine in as low a dilution as 1:200. In this instance whole defibrinated blood was used with a streptococcus which had been suspended in 0.85 per cent NaCl and sterilized at 100° C. Grünspan[5] also places the optimum concentration for this drug at 1:200. Smith[6] gives 1:7,500 as the lowest dilution of quinine stimulating phagocytosis. He used washed human corpuscles, human serum, and living B. coli, sealing them in a Wright tube and incubating in an opsonizer. The fact that the chamber was sealed may have exerted an influence on the process.

*Methods.*

Although present knowledge relating to phagocytosis does not make it possible to employ a method which will give uniformly constant results, by testing the chemicals in groups, each group on the same day, with the same leucocytes, staphylococci, and guinea pig serum, we have endeavored to render the conditions of our ex-periments constant.

The following three methods for determining the degree of phago-cytosis were tried.

1. 0.5 cc. of leucocytic suspension + 0.5 cc. of culture + 0.5 cc. of guinea pig serum 1:10 + 0.5 cc. of chemical were mixed in a 12 mm. tube and incubated for 15 minutes in a water bath at 37.5°C.

2. 0.5 cc. of leucocytic suspension + 0.5 cc. of chemical were mixed and incu-bated as above for 10 minutes. To this were then added 0.5 cc. of culture and 0.5 cc. of guinea pig serum 1:10, the mixture was shaken, and the tube was rein-cubated for 10 minutes in a water bath at 37.5°C.

3. 0.5 cc. of leucocytes suspended in undiluted guinea pig serum + 0.5 cc. of culture + 0.5 cc. of chemical were incubated for 15 minutes in a water bath at 37.5°C.

The last was the method finally adopted, since the simultaneous incubation of all constituents mixed together seemed more nearly to reproduce actual conditions of infection in the animal.

The leucocytes were procured from the peritoneal cavity of a guinea pig 15 hours after an intraperitoneal injection of a solution composed of 3 per cent aleuronat, 6 per cent starch, in 0.85 per cent NaCl, by washing out the exudate with sterile 0.5 per cent citrate in 0.85 per cent NaCl. The cells were washed three times in sterile citrate solution before being used. Opsonin and complement were furnished by fresh guinea pig serum and the organism employed as the indicator of leucocytic activity was an 18 hour broth culture of *Staphylococcus aureus*.

Smears were made after incubation, and the preparations stained by Cross'[8] method. The staphylococci contained in 100 cells were counted and the counts averaged. The result of each dilution of the chemical is shown in Tables I to VIII, the dilution quoted being calculated after the addition of all the components. Uniform dilutions of the different chemicals could not be employed, due to the varying solubilities of the drugs. Control counts were made from tubes containing leucocytes, serum, and organisms, but no chemical.

The toxicity given is the largest, non-fatal intraperitoneal dose in milligrams for 18 gm. mice.

The bactericidal action of the drugs was determined in whole blood with a 2 hour incubation period, by means of a technique described in a previous paper.[1]

## Triphenylmethane Leuco Bases.

As a group, the triphenylmethane leuco bases studied inhibit phagocytosis (Table I), only 33 per cent of the total number of compounds showing a normal count in the highest dilution used. In analyzing this inhibition, consistent correlation with bactericidal potency and toxicity is hard to follow.

For instance, leucomalachite green (D 1) and leucobenzein (D 8) have the same strength as regards bactericidal action, killing *Staphylococcus aureus* at 1:400. But D 1, causing a 60 per cent reduction of the phagocytic index at 1:40,000, permits an approximately normal count at 1:160,000, while D 8 at 1:600,000 shows an 80 per cent inhibition of this activity. The maximum non-lethal dose for D 1

[8] Cross, H. B., *Bull. Johns Hopkins Hosp.*, 1921, xxxii, 350.

is 1.0 mg., for D 8, 2.5 mg., so that D 1, while two and one-half times as toxic and having the same bactericidal action, allows a much greater degree of phagocytosis than D 8.

*p*-Hydroxyleucomalachite green (D 2) and *p*-aminoleucomalachite green (D 3) show divergence in another direction. Both have a maximum non-lethal dose of 1.0 mg. D 3 exerts no bactericidal power at 1:400, while D 2 kills at that dilution. At 1:160,000, D 2 exhibits an approximately normal index, D 3 a 33 per cent reduction. At 1:320,000, D 2 shows a 50 per cent reduction and D 3 only a 25 per cent.

A third variation in effect is seen between *o*-hydroxyleucomalachite green (D 9) and *o*-methoxyleucomalachite green (D 11). The phagocytic indices are reduced 80 per cent by both chemicals at 1:320,000, but neither has any bactericidal power at 1:400, and their maximum non-lethal doses differ widely, D 9 being two and one-half times as toxic as D 11.

## Triphenylmethane Dyes.

In comparing the specific dyes with their leuco bases the same irregularity is found. Neither *o*-hydroxymalachite green (D 7) nor its leuco base (D 9) in 1:400 possesses bactericidal power; D 9, however, is two and one-half times as toxic, and at 1:600,000 reduces the phagocytic index 90 per cent as compared with 25 per cent by D 7 in the same dilution (Table II).

In the case of the *p*-methoxymalachite green (D 12) and its leuco base (D 10), the dye is more toxic and bactericidal than the leuco base, but from 1:15,000 to 1:1,500,000, D 12 shows a normal phagocytic index, while at 1:600,000, D 10 reduces it 66 per cent. It may be noted here that *p*-methoxymalachite green (D 12) and ethylviolet chloride (D 32) are exceptional in allowing normal indices in a bactericidal dilution.

TABLE I.

*Triphenylmethane Leuco Bases.*

| Chemical. | Maximum non-lethal dose intraperitoneally. | | Bactericidal for staphylococci in whole blood in 2 hrs. | Effect on phagocytic index of different dilutions of chemicals. | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mg. | Dilution in mouse. | | 1:40,000 | 1:80,000 | 1:160,000 | 1:320,000 | Control. |
| (D 1) Leucomalachite green. | 1.0 | 1:3,000 | 0 at 1:400 | 3.5 | 3.8 | 8.6 | 11.0 | 9.9 |
| (D 3) *p*-Aminoleucomalachite green. | 1.0 | 1:3,000 | ∞ " 1:400 | 3.2 | 4.1 | 6.0 | 7.1 | 9.9 |
| (D 6) *p*-Methylleucomalachite green. | 2.5 | 1:1,200 | | 4.4 | 4.0 | 7.3 | 4.4 | 9.9 |
| | | | | 1:1,500 | 1:15,000 | 1:150,000 | 1:1,500,000 | Control. |
| (D 13) *p*-Ethoxyleucomalachite green. | 0.5 | 1:6,000 | ∞ at 1:400 | 3.6 | 5.0 | 5.0 | 6.2 | 6.0 |
| | | | | 1:40,000 | 1:80,000 | 1:160,000 | 1:320,000 | Control. |
| (D 2) *p*-Hydroxyleucomalachite green. | 1.0 | 1:3,000 | + at 1:400 | 3.2 | 5.9 | 8.8 | 6.4 | 9.9 |
| | | | | 1:600 | 1:6,000 | 1:60,000 | 1:600,000 | Control. |
| (D 9) *o*-Hydroxyleucomalachite green. | 0.5 | 1:6,000 | ∞ at 1:400 | 2.4 | 2.7 | 2.6 | 2.5 | 12.3 |
| | | | | 1:1,500 | 1:15,000 | 1:150,000 | 1:1,500,000 | Control. |
| (D 14) 2,4-Dihydroxyleucomalachite green. | 1.25 | 1:2,400 | ∞ at 1:400 | 2.8 | 3.9 | 6.6 | 4.3 | 6.4 |
| | | | | 1:600 | 1:6,000 | 1:60,000 | 1:600,000 | Control. |
| (D 11) *o*-Methoxyleucomalachite green. | 1.25 | 1:2,400 | ∞ at 1:400 | 1.2 | 2.3 | 2.1 | 2.5 | 12.3 |

TABLE I—*Concluded.*

| Chemical. | Maximum non-lethal dose intraperitoneally. | | Bactericidal for staphylococci in whole blood in 2 hrs. | Effect on phagocytic index of different dilutions of chemicals. | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mg. | Dilution in mouse. | | 1:40,000 | 1:80,000 | 1:160,000 | 1:320,000 | Control. |
| (D 4) Leucocrystal violet. | 1.0 | 1:3,000 | ∞ at 1:350 | 2.4 | 1.2 | 4.4 | 3.4 | 9.9 |
| | | | | 1:600 | 1:6,000 | 1:60,000 | 1:600,000 | Control. |
| (D 10) p-Methoxyleucomalachite green. | 2.5 | 1:1,200 | 0 at 1:400 | 4.5 | 3.2 | 2.4 | 4.2 | 12.3 |
| (D 8) Leucobenzein. | 2.5 | 1:1,200 | 0 " 1:400 | | 3.2 | 2.6 | 2.0 | 12.3 |

The blanks in the tables indicate that the cells in those dilutions were too disintegrated to count.

In general, the leuco compounds are less bactericidal than the dyes, the difference being quite marked in some cases.

(D 18) Malachite green nitrate......................... 1 : 6,400
(D 1 ) Leucomalachite green.......................... >1 : 400
(D 16) p-ethoxymalachite green chloride................. 1 : 12,800
(D 13) p-ethoxyleucomalachite green chloride........... >1 : 400
(D 15) o-methoxymalachite green nitrate............... 1 : 6,400
(D 11) o-methoxyleucomalachite green nitrate........... >1 : 400

This bactericidal relationship between the dye and its corresponding leuco base is not found in regard to the antiphagocytic action. Without exception both leuco bases and dyes decrease the phagocytic index, and the dilution represented by the largest non-lethal dose in the mouse is more leucocytotropic than bacteriotropic.

*Acridines.*

The acridines represented in Table III yield findings very similar to that with the triphenylmethane dyes, although as a group they are not so bactericidal, nor is perhaps the antiphagocytic action so great. In comparing the chemicals of this group, proflavine (D 26) stands out as possessing almost ideal characteristics, very similarly to p-methoxymalachite green (D 12) and ethyl violet (D 32) of the triphenylmethane dyes; the tropic relationships of the chemicals are such that a dose non-lethal for mice is also bactericidal for staphylo-

## TABLE II.

### *Triphenylmethane Dyes.*

| Chemical. | Maximum non-lethal dose intraperitoneally. | | Bactericidal for staphylococci in whole blood in 2 hrs. | Effect on phagocytic index of different dilutions of chemicals. | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mg. | Dilution in mouse. | | 1:600 | 1:6,000 | 1:60,000 | 1:600,000 | Control. |
| (D 18) Malachite green nitrate. | 0.6 | 1:5,000 | 0 at 1:6,400 | 0.24 | — | 11.5 | 15.0 | 14.1 |
| | | | | 1:1,200 | 1:12,000 | 1:120,000 | 1:1,200,000 | Control. |
| (D 16) *p*-Ethoxymalachite green chloride. | 0.6 | 1:5,000 | 0 at 1:12,800 | — | 1.9 | 2.8 | — | 4.2 |
| (D 19) *p*-Hydroxymala-chite green chloride. | 0.3 | 1:9,000 | ∞ " 1:1,600 | — | — | 1.8 | 2.3 | 4.0 |
| | | | | 1:600 | 1:6,000 | 1:60,000 | 1:600,000 | Control. |
| (D 7) *o*-Hydroxymalachite green. | 1.25 | 1:2,400 | ∞ at 1:400 | 1.8 | 2.1 | 3.0 | 3.0 | 4.1 |
| | | | | 1:1,500 | 1:15,000 | 1:150,000 | 1:1,500,000 | Control. |
| (D 15) *o*-Methoxymala-chite green nitrate. | 0.6 | 1:5,000 | 0 at 1:6,400 | 4.1 | 4.4 | 6.8 | 5.0 | 6.4 |
| | | | | 1:40,000 | 1:80,000 | 1:160,000 | 1:1,320,000 | Control. |
| (D 5) Hexamethylviolet (crystal). | 1.0 | 1:3,000 | 0 at 1:350 24 hrs. 0 at 1:39,400 | 0.0 | 3.0 | 8.8 | 4.4 | 7.4 |
| | | | | 1:900 | 1:9,000 | 1:90,000 | 1:900,000 | Control. |
| (D 20) *p*-Tolylmalachite green chloride. | 0.03 | 1:90,000 | 0 at 1:2,000 | — | — | — | — | 9.1 |

TABLE II—*Concluded.*

| Chemical. | Maximum non-lethal dose intraperitoneally. | | Bactericidal for staphylococci in whole blood in 2 hrs. | Effect on phagocytic index of different dilutions of chemicals. | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mg. | Dilution in mouse. | | 1:1,200 | 1:12,000 | 1:120,000 | 1:1,200,000 | Control. |
| (D 17) o-Chloromalachite green chloride. | 0.6 | 1:5,000 | 0 at 1:1,600 | — | 0.8 | 3.4 | 2.3 | 3.4 |
| | | | | 1:600 | 1:6,000 | 1:60,000 | 1:600,000 | Control. |
| (D 24) p-Chloromalachite green nitrate + 3.5 H₂O. | 0.5 | 1:6,000 | + at 1:2,500 | — | 1.3 | 2.2 | 3.2 | 7.1 |
| (D 21) Brilliant green nitrate + 1 H₂O. | 0.003 | 1:900,000 | 0 " 1:4,000 | 3.1 | 5.0 | 3.3 | 7.9 | 7.8 |
| (D 32) Ethylviolet chloride | 0.125 | 1:24,000 | 0 " 1:8,000 | 4.5 | 13.0 | 12.0 | 13.0 | 12.3 |
| (D 31) 3,4-Methylene-dihydroxymalachite green chloride + 4 H₂O. | 0.06 | 1:50,000 | 0 " 1:8,000 | 6.8 | 11.0 | 7.0 | 12.5 | 12.3 |
| (D 27 a) 2-Ethoxy-4',4"-bisdimethylaminotriphenylcarbinol. | 0.03 | 1:90,000 | 0 " 1:500 | — | 1.0 | — | 9.0 | 14.1 |
| (D 27 b) o-Ethoxymalachite green. | 1.25 | 1:2,400 | 0 " 1:500 | — | — | — | — | 14.1 |
| (D 22) p-Nitromalachite green chloride. | 0.03 | 1:90,000 | + " 1:2,000 | — | 1.3 | 5.5 | 4.8 | 7.1 |
| | | | | 1:1,500 | 1:15,000 | 1:150,000 | 1:1,500,000 | Control. |
| (D 12) p-Methoxymalachite green. | 0.5 | 1:6,000 | 0 at 1:3,200 | 4.5 | 8.0 | 7.5 | 8.5 | 7.6 |

$$\text{Effect on phagocytic index of different dilutions of chemicals.}$$

coccus and in this concentration not antiphagocytic. Since the introduction of acriflavine by Ehrlich[9] members of this group of chemicals have been employed in the treatment of various infections.

Browning and Cohen[10] have recently reported on the antiseptic properties of a rather complete series of acridines, and although not all derivatives were the same

[9] Ehrlich, P., and Benda, L., *Ber. chem. Ges.*, 1913, xlvi, 1931.

[10] Browning, C. H., and Cohen, J. B., *Brit. Med. J.*, 1921, ii, 695.

## TABLE III.

### *Acridines.*

| Chemical. | Maximum non-lethal dose intraperitoneally. | | Bactericidal for staphylococci in whole blood in 2 hrs. | Effect on phagocytic index of different dilutions of chemicals. | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mg. | Dilution in mouse. | | 1:600 | 1:6,000 | 1:60,000 | 1:600,000 | Control |
| (D 54) 3, 6-Dihydroxy-acridine. | 1.25 | 1:2,400 | ∞ at 1:1,000 | 0.5 | 5.2 | 7.9 | 15.0 | 10.3 |
| (D 53) 3, 6-Diamino-9-phenylacridine. | 1.25 | 1:2,400 | ∞ " 1:1,000 | — | 1.3 | 0.5 | 1.6 | 10.3 |
| (D 60) Diamino-*n*-methyl-acridone. | 1.25 | 1:2,400 | ∞ " 1:500 | 2.5 | 4.5 | 11.3 | 7.1 | 15.1 |
| (D 43) 9, 10-Dimethyl-acridinium chloride. | 0.625 | 1:5,000 | ∞ " 1:500 | — | 3.0 | 6.1 | 6.1 | 12.3 |
| | | | | 1:200 | 1:2,000 | 1:20,000 | 1:200,000 | Control |
| (D 34) 3, 6-Diamino-10-methylacridinium chloride + 1 H₂O. | 0.5 | 1:6,000 | 0 at 1:1,000 | 0.8 | 3.9 | 7.2 | 6.2 | 12.3 |
| | | | | 1:600 | 1:6,000 | 1:60,000 | 1:600,000 | Control |
| (D 57) 2-Amino-6-hydroxy-acridine dihydrochloride. | 2.5 | 1:1,200 | ∞ at 1:500 | — | 3.0 | 1.2 | 1.2 | 10.3 |
| (D 26) Diaminoacridine sulfate + 3 H₂O. | 2.5 | 1:1,200 | 0 " 1:500 | 1.7 | 17.8 | 14.0 | 13.0 | 14.1 |
| (D 49) Leucocyanotrypa-flavine dihydrochloride. | 0.312 | 1:9,000 | + " 1:500 | 1.8 | 5.6 | 9.2 | 9.6 | 10.3 |
| (D 42) Cyanotrypaflavine. | 0.078 | 1:38,400 | 0 " 1:500 | 4.0 | 6.4 | 2.6 | 8.7 | 12.3 |
| (D 56) Acridine orange dihydrobromide. | 0.625 | 1:5,000 | ∞ " 1:500 | — | — | 1.9 | 3.2 | 10.3 |
| (D 40) Homoflavine ("acridine yellow"). | 0.6 | 1:5,000 | ∞ " 1:1,000 | — | 4.5 | 5.5 | 7.8 | 12.3 |

as those given here, confirmation was made in respect to bactericidal action on staphylococcus. These authors did not study the influence of the group on the phagocytic index. Gay and Morrison[11] using acriflavine claim negative chemotherapeutic results in experimental streptococcus empyema in rabbits, regardless of

[11] Gay, F. P., and Morrison, L. F., *J. Infect. Dis.*, 1921, xxviii, 1.

the high bactericidal potency of the drug against this organism. They show phagocytosis to be inhibited by strong concentrations, such as have usually been employed, but state that the dye sterilizes considerable quantities of pus in the test-tube in a dose which does not inhibit phagocytosis.

Browning, Gulbransen, Kennaway, and Thornton[12] report that acriflavine kills staphylococcus in a dilution of 1:100,000 in serum and does not inhibit phagocytosis above 1:500. It is difficult to compare our results with theirs, as a different medium was used, the incubation period was shorter in performing the bactericidal test, they did not consider phagocytosis inhibited unless the inhibi-

TABLE IV.

*Safranines.*

| Chemical. | Maximum non-lethal dose intraperitoneally. | | Bactericidal for staphylococci in whole blood in 2 hrs. | Effect on phagocytic index of different dilutions of chemicals. | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mg. | Dilution in mouse. | | 1:600 | 1:6,000 | 1:60,000 | 1:600,000 | Control. |
| (D 63) Methylnaphthophenazonium chloride. | 0.156 | 1:19,200 | + at 1:500 | — | — | 9.7 | 8.6 | 15.1 |
| (D 62) Isorosindulin nitrate. | 2.5 | 1:1,200 | + " 1:500 | — | 2.4 | — | — | 15.1 |
| (D 37) Phenosafranine chloride. | 0.078 | 1:38,400 | + " 1:500 | 1.8 | 8.5 | 7.1 | 9.0 | 12.3 |
| | | | | 1:200 | 1:2,000 | 1:20,000 | 1:200,000 | Control. |
| (D 47) Phenylrosindulin chloride. | 0.625 | 1:4,800 | 0 at 1:500 | 0.8 | 1.3 | 2.8 | 2.1 | 12.3 |

tion exceeded 50 per cent, and they used human materials in their method. Despite the toxic action of proflavine and acriflavine for the phagocyte, they seem to have some local therapeutic action. According to Davis[13] both of these chemicals, following either an intravenous injection or *per os* administration, render the urine bactericidal for both staphylococcus and the colon bacillus. Davis and Harrell[14] also report a therapeutic action of acriflavine in treatment of gonorrheal urethritis.

## *Safranines.*

The small number of safranines studied does not warrant any general deductions as to this group of compounds. But for the staphylo-

[12] Browning, C. H., Gulbransen, R., Kennaway, E. L., and Thornton, L. H. D., *Brit. Med. J.*, 1917, i, 73.

[13] Davis, E. G., and Beck, G. H., *J. Urol.*, 1921, v, 215.

[14] Davis, E. G., and Harrell, B. E., *J. Urol.*, 1918, ii, 257.

coccus, the chemicals shown in Table IV exert a very low bactericidal action. And they are markedly antiphagocytic with the exception of phenosafranine, which produces a 25 per cent reduction in the phagocytic index in a dilution of 1:600,000.

TABLE V.

*Phenazines.*

| Chemical. | Maximum non-lethal dose intraperitoneally. | | Bactericidal for staphylococci in whole blood in 2 hrs. | Effect on phagocytic index of different dilutions of chemicals. | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mg. | Dilution in mouse. | | 1:600 | 1:6,000 | 1:60,000 | 1:600,000 | Control. |
| (D 65) *unsym.*-Diaminophenazine hydrochloride. | 1.25 | 1:2,400 | ∞ at 1:500 | — | 6.3 | 5.6 | 10.6 | 15.5 |
| (D 38) *unsym.*-Diaminosafranine hydrochloride. | 2.5 | 1:1,200 | ∞ " 1:500 | — | 2.8 | 6.5 | 8.9 | 12.3 |
| | | | | 1:200 | 1:2,000 | 1:20,000 | 1:200,000 | Control. |
| (D 46) *sym.*-Diaminophenazine hydrochloride. | 1.25 | 1:2,400 | + at 1:500 | — | 2.8 | 2.9 | 4.0 | 12.3 |
| | | | | 1:600 | 1:6,000 | 1:60,000 | 1:600,000 | Control. |
| (D 36) Toluylene red hydrochloride. | 1.25 | 1:2,400 | ∞ at 1:500 | 3.2 | 6.0 | 3.3 | 8.4 | 12.3 |
| | | | | 1:200 | 1:2,000 | 1:20,000 | 1:200,000 | Control. |
| (D 44) Dimethylnaphthoenrhodine hydrochloride. | 0.6 | 1:5,000 | ∞ at 1:500 | — | 3.5 | 6.5 | 5.0 | 12.3 |

## Phenazines and Quinones.

The phenazines (Table V), and quinones (Table VI) both may be classed as drugs that are not bactericidal for staphylococci, but they are antiphagocytic in dilutions non-toxic for a mouse, with the exception of sodium chloranilate in the quinone group. This compound apparently has no bactericidal action, yet permits of phagocytosis within 25 per cent of normal in a dilution of 1:600.

TABLE VI.

*Quinones.*

| Chemical. | Maximum non-lethal dose intraperitoneally. | | Bactericidal for staphylococci in whole blood in 2 hrs. | Effect on phagocytic index of different dilutions of chemicals. | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mg. | Dilution in mouse. | | 1:600 | 1:6,000 | 1:60,000 | 1:600,000 | Control. |
| (D 58) Quinone. | 0.625 | 1:5,000 | ∞ at 1:1,000 | 4.0 | 2.3 | 1.0 | 4.2 | 10.3 |
| (D 59) *p*-Nitrosophenol. | 0.312 | 1:9,000 | + " 1:50 | 2.3 | 3.2 | 5.6 | 13.0 | 15.5 |
| (D 64) Sodium chloranilate. | 5.0 | 1:600 | ∞ " 1:500 | 11.1 | 14.2 | 14.2 | 2.0 | 15.1 |
| (D 67) Anilino-β-naphtha quinone. | 1.25 | 1:2,400 | ∞ " 1:1,000 | — | 6.0 | 12.0 | 7.7 | 10.3 |

## Cinchonas.

The cinchona compounds in Table VII are the members of a group of alkaloids on which we reported in a previous paper.[1] It was found that the aromatic compounds had the power of killing, rapidly, multiple lethal doses of virulent pneumococci when organisms and drugs were injected simultaneously into the peritoneal cavity of mice. In this respect, all cinchona derivatives were found to be superior to optochin, but the different aromatic substitution products were possessed of varying degrees of bactericidal action *in vitro* and *in vivo*. Intravenous treatment with these drugs lowered the resistance of the mice as did any method of treatment except *per os*, when given at a site other than the one at which the organisms had been injected. Although mice with definitely established pneumococcus infection were not cured, these compounds, under specified conditions, had a measurable amount of protective action, greater perhaps than that of any drug used heretofore against a bacterial infection.

At a glance the compounds in therapeutic doses in Table VII are seen to possess marked antiphagocytic activity, and with the possible exception of C 29, the aromatic compounds are seen to be more leucocytotropic than optochin. The question naturally arises, whether this antiphagocytic property is the cause of the therapeutic failure of the chemicals in an established infection.

TABLE VII.

*Cinchonas.*

| Chemical. | Maximum non-lethal dose intraperitoneally. | | Bactericidal for staphylococci in whole blood in 2 hrs. | Effect on phagocytic index of different dilutions of chemicals. | | | |
|---|---|---|---|---|---|---|---|
| | Mg. | Dilution in mouse. | | 1:1,000 | 1:2,000 | 1:4,000 | Control. |
| (C 29) Hydroquinine chloroacetyl-anilide. | 0.5 | 1:6,000 | | — | 6.0 | 3.7 | 7.7 |
| (C 36) Dihydroquinine *p*-chloro-acetylaminophenol hydrochloride. | 2.5 | 1:1,200 | + at 1:1,000 | — | — | 2.4 | 7.7 |
| (C 40) Dihydroquinine *m*-chloro-acetylaminophenol hydrochloride. | 1.5 | 1:1,900 | + " 1:2,000 | 1.9 | 3.2 | ∞ | 7.7 |
| (C 110) Dihydroquinine 4-chloro-acetylaminopyrocatechol hydrochloride. | 4.0 | 1:750 | + " 1:500 | — | 1.2 | 0.72 | 7.7 |
| (C 11) Optochin (ethylhydrocup-reine). | 3.0 | 1:960 | + " 1:500 | 5.2 | 1.8 | 3.36 | 7.7 |
| (C 9) Hydroquinine. | 1.0 | 1:3,000 | | 4.6 | 6.0 | 4.0 | 7.7 |

Bordet's experiments[15] showing that the resistance of an animal is lowered after it has been partially depleted of leucocytes by means of carmine, and also Lippman's work[16] proving that optochin has no protective action in animals whose leucocyte count has been lowered by thorium, may be cited in support of the postulate that a drug which inhibits the action of the leucocytes will have little value in treatment of a bacterial disease. However, the experiments of neither Bordet[15] nor Lippman[16] are conclusive for the reason that the chemicals used to decrease the number of white cells may have exerted a toxic action on other functions vital to the resistance of the animal. Acton[17] has demonstrated that a number of cinchona derivatives inhibit completely the migration of leucocytes in a dilution of 1:500. The classical work of Binz[18] shows that quinine itself, along with its other general protoplasmic toxicities, inhibits phagocytosis.

[15] Bordet, J., Studies in immunity, translated by Gay, F. P., New York, 1909, 30.

[16] Lippman, *Z. Immunitätsforsch., Orig.*, 1915-16, xxiv, 107.

[17] Acton, H. W., *Lancet*, 1922, i, 124.

[18] Binz, C., Das Chinin. Nach den neuern pharmakologischen Arbeiten dargestellt, Berlin, 1875.

With the four cinchona derivatives, as with optochin, we have been able to show that migration of leucocytes follows almost immediately after the injection of a non-toxic dose of the drug into the peritoneal cavity of a mouse. The experiments reported in Table VIII were carried out by injecting 0.5 mg. of drug in a 1 cc. volume into the peritoneum of mice. At 1, 2, 3, 4, and 24 hours respectively, a small quantity of fluid was removed, diluted with a known amount of Turk's solution, shaken, and counted in the usual manner. Obviously, the drugs have a positive chemotactic influence on leucocytes in mice, since these migrate to the site of injection. Inasmuch as the cinchona compounds, as has been shown above, inhibit the phagocy-

TABLE VIII.

*Chemotactic Influence of Aromatic Cinchona Derivatives.*

| Chemical. | Duration of observation. | | | | |
|---|---|---|---|---|---|
| | 1 hr. | 2 hrs. | 3 hrs. | 4 hrs. | 24 hrs. |
| (C 29) Hydroquinine chloroacetylanilide. | 2,110* | 2,340 | 5,740 | 4,000 | 9,400 |
| (C 36) Dihydroquinine *p*-chloroacetylamino-phenol hydrochloride. | 3,520 | 5,360 | 5,140 | 4,300 | No fluid. |
| (C 40) Dihydroquinine *m*-chloroacetylamino-phenol hydrochloride. | 2,720 | 3,020 | 4,620 | 5,000 | "    " |
| (C 110) Dihydroquinine 4-chloroacetylamino-pyrocatechol hydrochloride. | 3,860 | 4,200 | 6,800 | 9,840 | 63,680 |
| (C 11) Optochin (ethylhydrocupreine). | 740 | 1,660 | 22,300 | 5,360 | No fluid. |

* The numbers refer to cells per c.mm. of fluid.

tosis of staphylococcus and are also proven to have positive chemotactic characteristics, it would seem that the antiphagocytic action is due, to a large extent, to the paralysis of the function of the leucocytes and not to destruction of the cells.

### DISCUSSION AND SUMMARY.

We are dealing, as the results show, with groups of chemicals, all of which, whether bacteriotropic or not, greatly inhibit the engulfing of *Staphylococcus aureus* by leucocytes. Not a sufficiently large number of experiments was performed in attempt to cure experimental staphylococcus infections to warrant any conclusion in regard to possible therapeutic activity against this organism. How-

ever, as will appear in another paper, the only group out of the seven which definitely possessed an *in vivo* bactericidal action against pneumococcus is that of the cinchona derivatives. Certain members of the other chemical groups studied, although bactericidal in a very high dilution,—chemicals in which the concentration of a non-lethal dose was many times greater than that required to kill multiple minimal lethal doses of organisms *in vitro*,—had no certain effect when bacteria and drug were injected simultaneously into the peritoneal cavity of a mouse. In fact, the treated mouse often died before the controls.

If we may assume,—leaving out of consideration the practical significance of *in vivo* chemical destruction and excretion following the injection of the drug into the animal,—that the failure of these chemicals to exhibit a benign influence on a systemic infection in cases in which the drug can be used in a bactericidal dilution, is due to their antiphagocytic property, only one step has been taken in analysis of the factors vital for the defense of the animal against a specific microorganism. Why do these chemicals inhibit leucocytic activity? Is it because of their influence upon complement, opsonin, or the leucocyte itself, or some special one function that determines the ability to ingest bacteria? Only further work can definitely settle this question and also determine whether or not such an analysis would be of practical importance in a rational development of chemotherapy.

The ideal chemotherapeutic agent may be one that has an *in vivo* bactericidal potency and a negligible or stimulatory phagocytic action in doses non-lethal for the experimental animal. However difficult such a drug may be to find, it seems unlikely that the ultimate success in chemotherapy will be so simple. Again, it is conceivable that a secondary action of a drug, although leucocytotropic and not bacteriotropic, may bring about conditions in the animal body that will enable it to throw off the invading organism. Or finally, a drug compatible with the forces necessary to the host's defense and possessing *in vivo* bactericidal action to a greater or less degree may be the chemical sought for, the goal toward which we should strive, to achieve a rational chemotherapy for infectious diseases.

## CONCLUSIONS.

With certain members of the triphenylmethane dyes and leuco bases, safranines, phenazines, quinones, and cinchona groups of chemicals, there exists no consistent parallelism between the bacteriotropic activities and the organotropic and leucocytotropic activities.

All the chemicals tested possess a leucocytotropic action, as measured by the decreased ability of leucocytes to ingest staphylococci. This action against the functional activity of the leucocyte is more pronounced than the organotropism or bacteriotropism (for staphylococcus).

Four aromatic cinchona compounds, hydroquinine chloroacetylanilide hydrochloride (C 29), dihydroquinine $p$-chloroacetylaminophenol hydrochloride (C 36), dihydroquinine $m$-chloroacetylaminophenol hydrochloride (C 40), dihydroquinine 4-chloroacetylaminopyrocatechol hydrochloride (C 110), and optochin (C 11) are markedly antiphagocytic in their therapeutic dose. They possess a positive chemotactic action for leucocytes when injected into the peritoneal cavity of mice.

In the cases of $p$-methoxymalachite green (D 12), ethyl violet chloride (D 32), and diaminoacridine sulfate (D 26) the condition was approached in which the concentration of a non-lethal dose for mice is staphylotropic and not leucocytotropic.