STUDY OF THE ACTION OF FOUR AROMATIC CINCHONA DERIVATIVES ON PNEUMOCOCCUS. A COMPARISON WITH OPTOCHIN.

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Since Morgenroth[1] introduced ethylhydrocupreine (optochin) as a chemotherapeutic agent against the pneumococcus, a number of related compounds has been investigated in the effort to find one more distinctly monotropic. However, up to the present, optochin holds a unique position in that it alone has been proven to influence beneficially an experimental pneumococcus infection in mice, in which animal the bacteriotropism is greater than the coincident organotropism. But this difference in the balance between organotropic and bacteriotropic action of optochin is insufficient to establish a definite therapeutic effect once the pneumococcus infection has become systemic in nature. The use of optochin against pneumococcus infection in man has had a rather comprehensive trial. In pneumonia, the results of investigations of Moore and Chesney,[2] Manliu,[3] Wright,[4] Parkinson,[5] and others have shown its use in this connection to be of little value. This rather discouraging outcome has shown itself to be true, despite the increase in the bactericidal power of the patient's blood (Moore[2] and Wright[4]) and the negligible change in opsonic index (Wright[4]) following its use. Bier[6] has claimed therapeutic effect in treatment of superficial wounds. Uncertain results in pneumococcus meningitis of man were reported by Wolff and Lehmann,[7] Lippman,[8] and Rosenow,[9] while Cordua[10] has found a favorable action in cases of

[1] Morgenroth, J., and Levy, R., *Berl. klin. Woch.*, 1911, xlviii, 1560, 1979. Morgenroth, J., *Naturwissenschaften*, 1913, i, 609. Morgenroth, J., and Bieling, R., *Berl. klin. Woch.*, 1917, liv, 723.

[2] Moore, H. F., and Chesney, A. M., *Arch. Int. Med.*, 1917, xix, 611; 1918, xxi, 659.

[3] Manliu, J., *Berl. klin. Woch.*, 1916, liii, 58.

[4] Wright, A. E., *Lancet*, 1912, ii, 1633, 1701.

[5] Parkinson, C., *Z. Chemotherap., Orig.*, 1913, ii, 1.

[6] Bier, A., *Berl. klin. Woch.*, 1917, liv, 717.

[7] Wolff, S., and Lehmann, W., *Jahrb. Kinderheilk.*, 1914, lxxx, 188.

[8] Lippman, *Berl. klin. Woch.*, 1917, liv, 781.

[9] Rosenow, G., *Deutsch. med. Woch.*, 1920, xlvi, 9.

[10] Cordua, R., *Berl. klin. Woch.*, 1921, xlviii, 1323.

meningococcus meningitis. However, Kolmer and Idzumi[11] were unable to produce a favorable influence on an experimental pneumococcus infection of the meninges of dogs and rabbits.

The realization of the limitations of this compound led us to investigate the cinchona derivatives synthesized and reported by Jacobs and Heidelberger.[12] It seemed to us that regardless of whether or not a substance was found that possessed greater activity than optochin, a biological study of these numerous compounds—so closely related to optochin—might be an aid in further chemical and biological investigations.

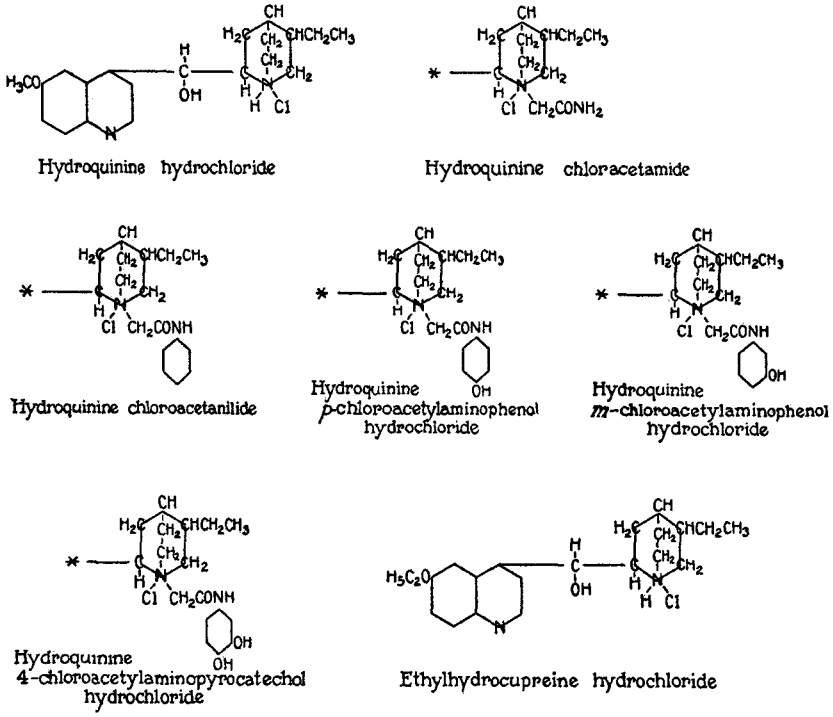
It was found at the outset that certain of these modified cinchona compounds possess a remarkably high bactericidal activity for pneumococci *in vivo*. In this paper we wish to present a study of four active chemicals in comparison with optochin. These four were chosen, as is shown below, for the reason that they represent a uniform series of hydroquinine derivatives, thus affording opportunity for a comparison between the chemical constitution and biological activity of closely related compounds.

To show clearly the relationship between these compounds and the exact chemical differences, structural formulas are given in Text-fig. 1. The first change in hydroquinine is the addition of chloracetamide on the quiunclicine ring, making hydroquinine chloracetamide. In turn, one of the amide hydrogens of the acetamide group is substituted by a benzene nucleus forming hydroquinine chloroacetanilide, the first important member of this series. The remaining drugs are hydroxy substitution products of the latter, in which the OH group is substituted in the para or the meta, or in both para and meta positions in the benzene nucleus, yielding respectively hydroquinine *p*-chloroacetylaminophenol hydrochloride, hydroquinine *m*-chloroacetylaminophenol hydrochloride, and hydroquinine 4-chloroacetylaminopyrocatechol hydrochloride. To avoid repetition of these names in this paper, the laboratory number will be used, as follows: the chloroacetylanilide, C 29; the *p*-chloroacetylaminophenol, C 36; the *m*-chloroacetylaminophenol, C 40; and the 4-chloroacetylaminopyro-

[11] Kolmer, J. A., and Idzumi, G., *J. Infect. Dis.*, 1920, xxvi, 355.

[12] Jacobs, W. A., and Heidelberger, M., *J. Am. Chem. Soc.*, 1919, xli, 2090.

catechol, C 110. As can be seen from the chart, these substances are derived from the hydroquinine and not from the ethylhydrocupreine nucleus, the only difference in the two compounds being that one is a methoxy and the other an ethoxy substitution on the quiniline nucleus. There is in these chemicals a combination of two bactericidal compounds—the quinines and benzenes. Since Lister introduced



TEXT-FIG. 1. Structural formulas of the cinchona alkaloids studied.

phenol into antiseptic surgery practically all the hydroxybenzene compounds have been employed for the same or similar purposes, but abandoned for one reason or another as impracticable. These new organic chemicals can therefore be thought of either as additive compounds of the benzene derivatives to the hydroquinine nucleus or the hydroquinine to the benzene nucleus by means of a common linkage CH_2ONH.

General Procedure.

Since we undertook to make a comparative study of the different chemicals, work was planned so that each experiment, in so far as was practical, embraced all the drugs under the same conditions. Young mice of a healthy stock, weighing from 15 to 18 gm., were employed throughout. Animals of this weight were used because it was early found that large, and especially old, female mice reacted irregularly. Doses of the chemical were approximated to 18 gm. mice. Each mouse was autopsied at death and one loop of heart's blood smeared on a blood agar plate, presence or absence of growth being noted at the end of 24 hours. Animals that survived were kept for 30 days and then killed. Cultures were made from a few animals living the 30 day period, but inasmuch as no pneumococci could be found, this procedure was abandoned. Unless otherwise stated in the tables pneumococci were present at death in the blood stream of all mice recorded. Since we found considerable variation in numbers of organisms in both the treated animals and the controls, little stress was laid on this factor. For each experiment, controls were run in duplicate with what had been found from previous experience to be 1, 10, and 20 M.L.D. This minimum lethal dose was calculated each time for the given experiment after the death of the controls, and was taken to be the smallest number of organisms, or the highest dilution of the culture (made in physiological salt solution), causing the death of duplicate mice in 48 hours.

The chemicals were dissolved in boiling sterile distilled water. The pneumococcus used was a Type I (Neufeld).[18] Its virulence was such that generally two to four organisms (fluctuations occurring from time to time from two to twenty organisms) of a 6 hour culture, as determined by plating, injected into the peritoneal cavity of a mouse, resulted in the death of the animal within 48 hours. The intravenous virulence of this organism was rather low, requiring approximately 200,000 diplococci to cause death, or 10,000 times as many as by the intraperitoneal route.

Solubilities.

In Table I are given the solubilities of the chemicals in water, physiological salt solution, and serum. They have a practical bearing as can be seen below, in view of the bactericidal results obtained *in vivo* and *in vitro*. In making the determinations with water and physiological salt solution, each respectively was added slowly in measured amounts on 100 mg. of substance until solution was obtained. The solution in serum represents the concentration of the drug that does not cause precipitation in the serum, and can hardly be termed solubility. Both the aromatic compounds and optochin

[18] The strain of pneumococcus was kindly furnished by Dr. O. T. Avery.

are protein precipitants. C 29 is the least and optochin the most soluble, while C 36, the para-hydroxybenzene derivative, is more soluble than C 40, with the OH group in the meta position. C 110, having the OH group both in the para and meta positions, holds an intermediate position between these two compounds.

TABLE I.

Solubilities.

Chemical.	Percentage of chemical in solvent.		
	Water.	Physiological salt solution.	Horse serum.
C 29	0.3	0.12	0.06
C 36	10.0	10.0	0.12
C 40	2.0	2.0	0.05
C 110	4.0	4.0	0.25
Optochin.	10.0	10.0	0.4

Bactericidal Action in Vitro.

Bactericidal action has been measured in two ways, (*a*) by a constant dose of organisms inoculated into varying concentrations of the chemical and (*b*) by a constant concentration of drug to which were added varying numbers of organisms. Since Chesney[14] has shown

TABLE II.

*Bactericidal Action in Whole Blood in Vitro.**

Chemical	C 29	C 36	C 40	C 110	Optochin.
Dilution sterile in 2 hrs	1:1,500	1:750	1:1,000	1:500	1:8,000

* Neufeld pneumococcus.

that a young culture is more resistant to bactericidal agents than an old one, a 6 hour culture of the Neufeld pneumococcus was employed in these tests, in order that conditions might resemble as nearly as practicable those *in vivo*. In the first method whole defibrinated blood was used as medium.

[14] Chesney, A. M., *J. Exp. Med.*, 1916, xxiv, 387.

To 2 cc. of the blood was added the chemical in water solution with concentrations adjusted to give a constant volume in making the following series of dilutions: 1:500, 1:750, 1:1,000, 1:1,500, 1:2,000, 1:4,000, 1:8,000, and 1:12,000. This series was inoculated with 0.2 cc. of a broth culture per tube and incubated for 2 hours in a water bath at 37°C. One loopful from each tube was then distributed over one-fourth of a blood agar plate. These plates were incubated for 96 hours and read. From Table II it can be seen that under these conditions the aromatic derivatives are less active than optochin, the latter being about eight times as potent as C 29, the best of the others.

In the second method, bactericidal activity was determined by exposing the organisms in physiological salt solution, 50 per cent serum, and 50 per cent defibrinated rabbit blood, to the action of the chemicals for 5 minutes.

The different dilutions of pneumococci were made in 0.4 cc. of physiological salt solution, undiluted serum, and whole blood, respectively, by adding 0.1 cc. of the appropriate dilution of the culture to make the final concentration the one desired. The chemicals were dissolved in water, so that 0.5 cc. contained 1 mg. of substance. In the final test (Table III) there was a volume of 1 cc. with 1 mg. of chemical and a series of dilutions of a 6 hour culture of 1:10, 1:100, 1:1,000, 1:10,000, and 1:100,000. The controls in each case were made at the end of the experiment, 0.5 cc. of physiological salt solution being added to make the volume up to 1 cc. To each tube containing 0.5 cc. of culture was added 0.5 cc. of the chemical. The mixture was put in the water bath at 37°C. for 5 minutes, and then 0.05 cc. was pipetted into 100 cc. of meat infusion broth (pH 7.4), thus bringing the chemical dilution above its bactericidal potential (1:2,000,000). The flasks were under observation for 4 days and results recorded each 24 hour period except for the test on blood, the first observation in this case being made 12 hours after inoculation. Controls all grew out in 24 hours, while in this length of time no visible growth occurred in the flasks containing organisms subjected to the chemicals. The order of the rapidity of bactericidal action in decreasing ratio is as follows: C 29, C 40, C 36, optochin, and C 110. Neither serum nor blood diminished the activity of C 29 or optochin. The unchanged benzene nucleus derivative possesses the most rapid action; the one with the OH group in the meta position is more active than the one with the OH in the para position; while the compound with the OH group in both meta and para positions is still less active. Optochin in this experiment proves to have a slow rate of activity, as had been found by Solis-Cohen, Kolmer, and Heist.[15]

In addition, the dilution of the drugs that inhibited bacterial growth, with resultant death, was determined on meat infusion broth (pH 7.8).

[15] Solis-Cohen, S., Kolmer, J. A., and Heist, G. D., *J. Infect. Dis.*, 1917, xx, 313.

TABLE III.

Bactericidal Action with a 5 Minute Contact Period. 1 Mg. of Chemical Used in Physiological Salt Solution, Serum, and Whole Blood.

Chemical.	Dilution of pneumococcus (Neufeld).	Physiological salt solution.						50 per cent horse serum.						50 per cent defibrinated rabbit blood.					
		Chemical.				Control.		Chemical.				Control.		Chemical.				Control.	
		24 hrs.	48 hrs.	72 hrs.	96 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.	24 hrs.	48 hrs.	12 hrs.	24 hrs.	48 hrs.	72 hrs.	12 hrs.	24 hrs.
C 29	1:10	0	+			+		0	+			+							
	1:100	0	+			+		0	+			+		0	0	+		+	
	1:1,000	0	0	0	0	+		0	+			+		0	0	0	0	+	
	1:10,000	0	0	0	0	+		0	0	0	0	+		0	0	0	0	+	
	1:100,000	0	0	0	0	±	+	0	0	0	0	±	+	0	0	0	0	±	+
C 36	1:10	0	+			+		0	+			+							
	1:100	0	+			+		0	+			+		+				+	
	1:1,000	0	+			+		0	+			+		+				+	
	1:10,000	0	0	0	0	+		0	+	+	+	+		0	+			+	
	1:100,000	0	0	0	0	±	+	0	0	0	0	±	+	0	+			±	+
C 40	1:10	0	+			+		0	+			+							
	1:100	0	+			+		0	+			+		+				+	
	1:1,000	0	0	0	0	+		0	+			+		+				+	
	1:10,000	0	0	0	0	+		0	0	0	0	+		0	+			+	
	1:100,000	0	0	0	0	±	+	0	0	0	0	±	+	0	+			±	+
C 110	1:10	0	+			+		0	+			+							
	1:100	0	+			+		0	+			+		+				+	
	1:1,000	0	+			+		0	+			+		+				+	
	1:10,000	0	0	+		+		0	0	+		+		+				+	
	1:100,000	0	0	0	0	±	+	0	0	0	0	±	+	0		+		±	+
Optochin.	1:10	0	+			+		0	+			+							
	1:100	0	+			+		0	+			+		+					
	1:1,000	0	+			+		0	+			+		0	+			+	
	1:10,000	0	0	+		+		0	+			+		0	0	0	+	+	
	1:100,000	0	0	0	0	+	+	0	0	0	0	±	+	0	0	0	+	±	+

0.1 cc. of a 6 hour culture was pipetted into each tube (10 cc. volume) of broth containing the series of dilutions represented in Table IV. The inoculated broth was incubated for 4 days, and then 1 cc. from the last tube showing no visible growth was plated for confirmation. Hydroquinine (C 9) and hydroquinine chloracetamide (C 20) were added to the experiment for comparison.

It is rather interesting to note that the substitution of the chloracetamide on the hydroquinine nucleus decreases its bactericidal activity. With the exception of optochin, the same relationship persists between this group of chemicals as in the preceding experiment. C 29 is as active as optochin under these conditions.

TABLE IV.

Bactericidal Action in the Presence of Broth (pH 7.8).

Chemical.	Dilution in broth.														
	1:2,500	1:5,000	1:10,000	1:15,000	1:20,000	1:30,000	1:40,000	1:60,000	1:80,000	1:120,000	1:160,000	1:240,000	1:320,000	1:480,000	1:640,000
C 9								+	++	++	++	++	++	++	++
C 20		++	++	++	++	++	++	++	++	++	++	++	++	++	++
C 29											+	++	++	++	++
C 36								++	++	++	++	++	++	++	++
C 40										++	++	++	++	++	++
C 110				+	++	++	++	++	++	++	++	++	++	++	++
Optochin.											++	++	++	++	++

Toxicity.

Instead of the toxic dose the largest tolerant dose is given, as determined by averaging several titrations (Table V). The injections *per os* were made by means of a small silver stomach tube attached to a syringe by flexible rubber tubing, the volume injected always being 0.5 cc. From the standpoint of the largest tolerant dose, the same relative positions of the aromatic compounds exist organotropically as were shown to exist parasitotropically; that is, with these four aromatic compounds toxicity runs parallel with bactericidal power—the most toxic, C 29, is the most bactericidal. If rapidity of bactericidal action is used as the criterion, this is also true of optochin. The intravenous toxicity is perhaps largely a physical phenomenon due to protein precipitation.

In the balance between the organotropism and parasitotropism lies the hope of chemotherapy. It is perhaps unreasonable to suppose that a drug will ever be discovered that is strictly monotropic in its action. Manifestly, all the drugs used in clinical medicine show distinctly a therapeutic dose beyond which they set up complex phenomena causing aggravation rather than amelioration. Just as a drug is active often because of its affinity for a certain monocellular tissue in the animal body, so must we look for a drug that has a greater toxicity for monocellular organisms—bacteria—than for any cells vital to the defensive mechanism of the animal. It is perhaps impossible, at this stage of our knowledge, to make this comparison. However, if the bactericidal action of the largest tolerant dose of these

TABLE V.

Toxicity.

Chemical.	Largest tolerant dose.			
	Intravenous.	Intraperitoneal.	Subcutaneous.	*Per os.*
	mg.	*mg.*	*mg.*	*mg.*
C 29	0.2	0.5	2.0	5.0
C 36	0.5	2.5	5.0	10.0
C 40	0.2	1.5	3.0	8.0
C 110	0.5	4.0	6.0	12.0
Optochin.	0.6	3.0	4.0	10.0

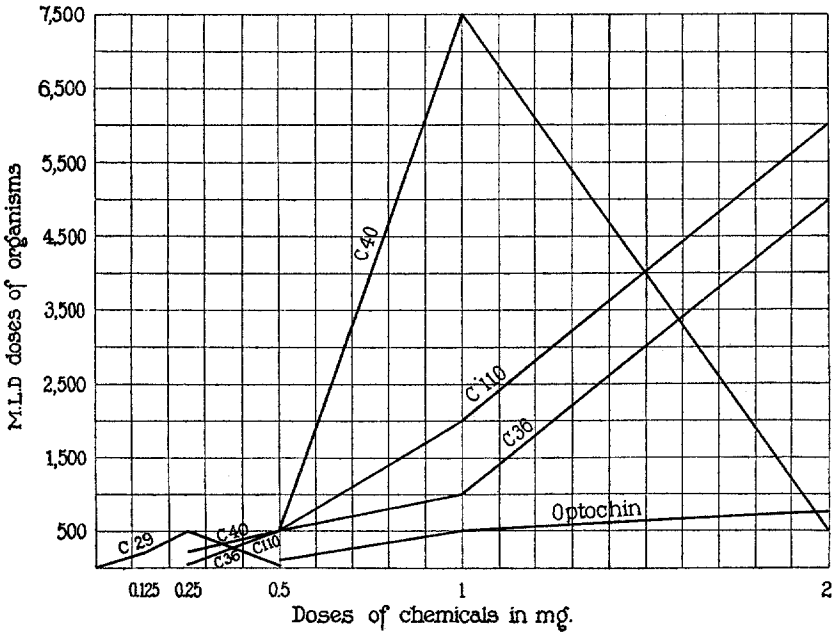
chemicals (Table V) for the experimental animal—the mouse—is compared with the number of virulent pneumococci that are killed *in vitro* (Table III) in as short a time as 5 minutes, the first step of this comparison is taken; that is, a non-toxic dose of the drug (for mice) is capable of killing *in vitro* many multiple lethal doses of virulent organisms. The following experiments were designed to show whether these relationships exist *in vivo*.

We wish to present the results of these *in vivo* experiments from a twofold standpoint. First, will the drugs kill multiple lethal doses of virulent pneumococci when chemical and organism are injected simultaneously into a walled off body cavity, such as the peritoneal? Second, will the drugs diffuse, and influence the experimental infection when injected by routes other than the one by which the animal was

infected? Briefly, is there either a local or a systemic bactericidal action *in vivo*, or both?

Simultaneous Injection of Multiple Lethal Doses of Pneumococci with Varying Amounts of Drugs.

The simplest possible procedure, perhaps, by which to estimate *in vivo* bactericidal activity resulting in an animal following the injection of a given drug is a simultaneous injection of organism and



TEXT-FIG. 2. Representation of the number of minimum lethal doses of organisms influenced by different doses of drugs—a therapeutic zone phenomenon.

chemical into the peritoneal cavity. By using as an indicator a microorganism of known virulence, it is possible to obtain within limits of biological accuracy an estimation of the number of organisms killed. The curves depicted in Text-fig. 2 are only approximate, owing to the wide range in dosage of organisms. These curves represent a titration of the number of minimum lethal doses of pneumococci killed by each of four doses of drugs, decreasing by halves from the

largest non-lethal dose. The number of minimum lethal doses of organisms, namely 1, 10, 100, and 1,000, in the preliminary titrations, was the same for all the doses of each chemical. Subsequently dilutions representing minimum lethal doses intermediate between the above doses were injected to furnish data given in Text-fig. 2. All titrations were made in duplicate.

The work with the aromatic compounds gave consistent results throughout. Optochin, on the other hand, varies in its effectiveness, especially in the 2 mg. dose. With this dose, the 740 M.L.D. from which the mice were protected is the average of five titrations: 400, 100, 200, and 1,000 to 2,000 M.L.D. The last two figures were obtained when the pneumococcus was at its greatest virulence, two to four organisms causing the death of the control in 48 hours. At the time of the other three experiments, 20 to 40 organisms were necessary to cause death of the controls in this interval. The fact that approximately ten times as many organisms were present in the unit injected at one time as at the other possibly accounts for the variable results. As the aromatic compounds are more rapid in their action, this may account for the fact that the results are more constant than with the more slowly acting optochin.

Hydroquinine has been shown by Morgenroth, Kolmer,[15] and Tugendreich and Russo[16] to have slight but distinct therapeutic value, though it is not so active as ethylhydrocupreine. By using the same technique as employed with these aromatic derivatives, it was found that hydroquinine protected against 100 M.L.D. at a time when this was represented by approximately 200 pneumococci. Hydroquinine chloracetamide, in the same experiment, protected against only 10 M.L.D. The results with these two chemicals show a parallelism between *in vitro* and *in vivo* bactericidal action (Table IV).

The very striking zonal reaction noted with C 29 and C 40 shows that there is an optimum concentration of the chemicals, below their toxic dose, at which virulent pneumococci are killed *in vivo*. If a therapeutic amount larger or smaller than this is injected, the mice die of septicemia. Manifestly, whatever the mode of action in this case may be, the normal defensive mechanism of the animal

[16] Tugendreich, J., and Russo, C., *Z. Immunitätsforsch., Orig.*, 1913, xix, 156.

is weakened, the relation between mono- and polytropism being reversed. This same phenomenon occurs with the other chemicals, as will be shown later in this paper.

Comparison of Single and Repeated Injections of the Same Amount of Drug.

To make a still more vigorous test on this local effect, experiments were carried out on single and repeated injections of drugs, 1 and 2 hours after the animal was experimentally infected.

It has been shown by many workers that it is more difficult to cure an infection if time elapses between inoculation of the animal and application of remedial measures. That this is true with these chemicals is shown in Table VI. The experiment thus represented was carried out to show the relative effectiveness between single doses, and the same amount divided into fifths. These two methods of treatment were applied to mice 1 hour after injection of organisms, those receiving a single dose of drug being injected with 10 M.L.D., while those having the dose divided were injected with 10, 20, and 50 M.L.D.

The single dose of the drug proved ineffective, except with optochin, in which case one mouse with 10 M.L.D. survived. The repeated divided dose presented a different picture, as many as 50 M.L.D. of the organisms being killed with the aromatic compounds. Optochin in these small doses was unable to cure an animal infected with more than 10 M.L.D. The drop in the effectiveness of the chemicals when injected 1 hour after the organism is remarkable. The simultaneous procedure with C 40, for instance, proved to be lethal for 8,000 M.L.D. of organisms; injections deferred for 1 hour had no influence on 10 M.L.D. The power of the drug was thus decreased approximately 8,000 times.

Inasmuch as optochin, under these conditions, did not hold up as well as the other derivatives, the possibility was suggested that dosage was at fault. To establish this point and to determine the optimum concentration for five hourly injections, the experiment represented in Table VII was carried out.

2 hours after the injection of the pneumococci, a series of five injections of each doses of the chemicals, at intervals of 1 hour, was begun. The repeated 1 mg. doses of C 36 and C 40 were toxic for the mice. However, at death the C 36 animals had pneumococci in their blood streams, while with C 40, the animal with

TABLE VI.

Comparison of Single and Repeated Divided Doses of Drug, Given 1 Hour after Injection of the Organism.

Chemical.	Length of life.					
	After single dose of chemical.			After repeated doses of chemical, five times at hr. intervals.		
	Dose.*	No. of mice.	No. of M. L. D.*	Dose.*	No. of mice.	No. of M. L. D.*
			10			10 / 20 / 50
	mg.			*mg.*		
C 29	0.5	4	24 hrs.; 9 days; 16 days; 24 days.	0.1	2	L.; L.† / 144 hrs.; L. / L.; L.
C 36	1.0	4	60 " 72 hrs.; 36 hrs.; 120 hrs.	0.2	2	" " / L.; L. / " "
C 40	1.0	4	24 " 72 " 48 " 60 "	0.2	2	" " / " " / " "
C 110	2.0	4	72 " 240 " 96 " 168 "	0.4	2	" " / " " / " "
Optochin.	2.0	4	72 " 120 " 140 " L.	0.4	2	" " / 48 hrs.; L. / 48 hrs.; 48 hrs.
Controls.	0	6	0.00000001 cc., 48 hrs. = 1 M. L. D.	0	6	0.00000001 cc., 48 hrs. = 1 M. L. D.

* Both drug and organisms were injected intraperitoneally.

† In the tables L. indicates lived.

TABLE VII.

Intraperitoneal Varying Doses of Drug Repeated Hourly for 5 Hours, Beginning 2 Hours after Injection of the Organism. Titration of Chemicals.

Chemical.	No. of M. L. D.	No. of mice.	Length of life after varying doses.			
			1.0 mg.	0.5 mg.	0.25 mg.	0.125 mg.
C 29	10	2		36 hrs.; 24 hrs.	36 hrs.; 36 hrs.	10 days; L.
	100	2		24 " 24 "	36 " 36 "	36 hrs.; 36 hrs.
C 36	10	2	24 hrs.; 24 hrs.	36 " L.	L.; L.	
	100	2	24 " 36 "	60 " "	36 hrs.; 96 hrs.	
C 40	10	2	24 " 24 "	36 " 60 hrs.	L.; L.	
	100	2	36 " 24 "	36 " 36 "	60 hrs.; L.	
C 110	10	2	L.; L.	L.; L.	L.; L.	
	100	2	36 hrs.; L.	60 hrs.; L.	36 hrs.; 36 hrs.	
Optochin.	10	2	L.; L.	7 days; L.	48 " 48 "	
	100	2	60 hrs.; L.	36 hrs.; 60 hrs.	36 " 36 "	
Controls.	0	4	Succumbed to 0.0000001 cc. = 1 M. L. D.			

TABLE VIII.

Intraperitoneal Doses of Drug Repeated Hourly for 5 Hours, Beginning 2 Hours after Injection of Varying Doses of the Organism.

Titration of Organisms.

Chemical.	Dose.	No. of mice.	Length of life.		
			No. of M. L. D.		
			10	20	40
	mg.				
C 29	1.25	4	L.; L.; L.; 96 hrs.	36 hrs.; 36 hrs.; 36 hrs.; 36 hrs.	36 hrs.; 36 hrs.; 36 hrs.; 36 hrs.
C 36	0.25	4	96 hrs.; 96 hrs.; 96 hrs.; 24 hrs.	L.; L.; L.; L.	36 " 36 " 36 " L.
C 40	0.25	4	L.; L.; L.; L.	96 hrs.; 36 hrs.; 36 hrs.; 36 hrs.	36 " 36 " 36 " 36 hrs.
C 110	0.5	4	36 hrs.; L.; L.; L.	36 " L.; L.; L.	36 " L.; 36 hrs.; L.
Optochin.	1.0	4	36 " " " "	L.; L.; L.; L.	36 " 36 hrs.; 96 hrs.; 96 hrs.

the 10 M.L.D. gave a sterile heart's blood culture, that of the 100 M.L.D. mouse being positive. All other mice of this experiment proved to have a septicemia at death. It can be seen with repeated, as with single injections, of C 29 and C 40, that there is a definite optimum concentration at which these drugs act; in addition, C 110, C 36, and optochin show this zonal phenomenon with repeated doses, C 110 and optochin having a broader zone than the other chemicals. Optochin, however, as in the preceding experiment, did not influence more than 10 M.L.D. when administered in small (0.25 mg.) repeated doses.

In the experiment represented in Table VIII, we estimated the number of minimum lethal doses of pneumococci killed by the optimum concentration of the drug. The results parallel those represented in Table VII, and in addition show that C 36 and C 110 and optochin influence as many as 20 M.L.D. The irregularity seen with C 36 has occurred in other experiments. Often with a small number of organisms, the infection proceeded as in the control mice, while a larger number of organisms was killed.

Diffusion of Drugs.

Thus far our work indicates that within certain definite limits these cinchona compounds have marked *in vivo* bactericidal activity. Drugs to be effective in an infectious disease should have more than this local action. They must be able to penetrate or diffuse throughout the animal body and perhaps in the last analysis maintain a definite concentration in the blood stream. This is especially true with the experimental infection of pneumococci in mice, which is a typical septicemia and in no way comparable to a lobar pneumonia. With this idea in view, the following experiments were carried out.

Subcutaneous Administration of Drugs Following Intraperitoneal and Intravenous Injection of Pneumococci.—This is the method which Morgenroth and Levy[1] and Moore[17] used in their investigations with optochin on mice. Their results show that optochin does diffuse into the circulation, inasmuch as an experimental infection was controlled in its incipiency.

Table IX is a record of three experiments in which the drug was administered subcutaneously, and the organism injected either intraperitoneally or intravenously. In the first experiment duplicate mice were given 1 and 10 M.L.D.

[17] Moore, H. F., *J. Exp. Med.*, 1915, xxii, 269, 389, 551.

TABLE IX.

Subcutaneous Injection of Drug.

Chemical.	Organism injected intraperitoneally.			Organism injected intraperitoneally.			Organism injected intravenously.		
	Dose.	No. of M. L. D.	Length of life.	Dose.	No. of M. L. D.	Length of life.	Dose.	No. of M. L. D.	Length of life.
	mg.			*mg.*			*mg.*		
C 29	0.5	1	L.; L.	2.0	1	48 hrs.; 48 hrs.	0.5	$\frac{1}{5}$	60 hrs.; 48 hrs.
		10	48 hrs.; 36 hrs.		10	48 " 48 "		1	48 " 48 "
C 36	1.0	1	L.; L.	4.0	1	36 " 48 "	1.0	$\frac{1}{5}$	48 " 96 "
		10	36 hrs.; 36 hrs.		10	36 " 48 "		1	48 " 48 "
C 40	1.0	1	L.; L.	2.0	1	L.; 48 hrs.	1.0	$\frac{1}{5}$	L.; L.
		10	36 hrs.; 48 hrs.		10	48 hrs.; 48 hrs.		1	60 hrs.; 60 hrs.
C 110	2.0	1	36 " 48 "	4.0	1	48 " 48 "	2.0	$\frac{1}{5}$	60 " 48 "
		10	36 " 36 "		10	48 " 48 "		1	60 " 60 "
Optochin.	2.0	1	L.; L.	4.0	1	L.; L.	2.0	$\frac{1}{5}$	L.; 60 hrs.
		10	48 hrs.; L.		10	" 96 hrs.		1	60 hrs.; 48 hrs.

of pneumococci intraperitoneally, followed immediately by the drug. There seem to be indications that the drugs, save C 110, do have a systemic distribution in sufficient quantity to influence the progress of the attack of virulent organisms. This action is slight, however, optochin having greater effectiveness than the other compounds. In the second experiment a large non-toxic dose was given to duplicate mice with 1 and 10 M.L.D. The infection was not influenced, except in the case of optochin. The same zonal phenomenon persisted that occurred with C 29 and C 40 and in fact all the aromatic compounds (Text-fig. 1); that is, a large non-toxic dose of the drug did not favorably influence the course of the infection, the mice, if anything, being more sick than those given smaller doses. The third experiment represents an attempt to influence the course of an experimental septicemia. Again in duplicate, mice were given $\frac{1}{5}$ and 1 M.L.D. (1:1,000 and 1:5,000) of a 6 hour culture of Neufeld pneumococci intravenously, followed immediately by the drug subcutaneously. It is seen that not only did the mice injected with 1 M.L.D. of the drug die, but all save those with C 40 and optochin of the $\frac{1}{5}$ M.L.D. series. Seemingly with this dose, at least, the natural defensive mechanism is interfered with, so that the animal receiving one-fifth of the number of pneumococci (approximately 40,000 organisms) necessary to cause the death of a mouse, died in the same time as the controls.

Intravenous Injection of Drug and Organism; Intravenous Injection of Organism and Intraperitoneal Injection of Drug, and Vice Versa.—If the subcutaneous injection of the drug decreased the ability of the animal to cope with virulent organisms, one might expect that injection of the drug intravenously would also destroy a part of this defensive process.

That this is the case is shown in the first experiment in Table X. Although optochin was not so toxic as the aromatic compounds, a single dose of the drug did not influence the progress of the infection with 1 M.L.D. of organisms. In the next experiment with the intravenous organism and intraperitoneal drug, the outcome was practically the same as with the intravenous injection of the organism and subcutaneous injection of the drug. Again not only does the intraperitoneal injection of the chemical not affect favorably an experimental septicemia in mice, but causes the animal to succumb to a number of organisms below the minimum lethal dose.

Diffusion of Drugs through the Gastrointestinal Tract.—Inasmuch as there has been found to be a definite therapeutic dose for these chemicals when given by the intraperitoneal and intravenous routes, varying amounts were employed to test their diffusibility from the gastrointestinal tract.

TABLE X.

Simultaneous Injection of Drug and Organism.

Chemical.	Both drug and organism injected intravenously.			Organism injected intravenously, drug intraperitoneally.			Organism injected intraperitoneally, drug intravenously.		
	Dose.	No. of M.L.D.		Dose.	No. of M.L.D.		Dose.	No. of M.L.D.	
		½	1		½	1		½	1
	mg.			*mg.*			*mg.*		
C 29	0.1	72 hrs.; 144 hrs.	60 hrs.; 11 days.	0.5	36 hrs.; 36 hrs.	36 hrs.; 36 hrs.	0.1	L.; L.	36 hrs.; 36 hrs.
C 36	0.2	48 " 120 "	36 " 36 hrs.	1.0	120 " 48 "	36 " 36 "	0.1	" 36 hrs.	36 " 36 "
C 40	0.1	36 " L.	36 " 7 days.	1.0	48 " 60 "	36 " 48 "	0.1	" L.	36 " 36 "
C 110	0.3	L.; L.	60 " 72 hrs.	2.0	L.; L.	60 " 72 "	0.3	" 36 hrs.	36 " 36 "
Optochin.	0.3	" "	120 " 48 "	2.0	" "	120 " 48 "	0.3	" 36 "	36 " 36 "

A series of four doses was used—both single and repeated—each being one-half the amount of the preceding (Table XI). Mice were injected intraperitoneally with 2 to 4 M.L.D. of organisms immediately followed by the drug *per os*. With the single dose there was demonstrated a diffusibility sufficient to influence this small number of pneumococci. As with the intraperitoneal injection of the drug, there is in this case also a concentration of chemicals above which the organisms are not killed, and the animal dies of septicemia. Although C 29 and C 110 delayed death, there were no actual survivals. C 36, C 40, and optochin did prevent the development of an infection, optochin, as before, being active in a large dose. On the other hand, repeated doses of all the drugs exerted a destructive influence upon the organism.

From this experiment it would be very difficult to say which one of the chemicals was the best. To determine this fact, the optimum concentration of the drug was used in another experiment with multiple lethal doses of pneumococci (Table XII).

Three doses of the chemical were given at 2 hour intervals, beginning immediately after the injection of the organisms. Although there was very little variation in effect of the different chemicals, results prove that inasmuch as there were some survivals, some action other than purely local was exerted. In the same table are presented the results concerning the effectiveness of drugs after intravenous injection of the organism. It happens that by this method of administration the same destructive action of the defensive mechanism of the animal does not occur with C 40, C 110, and optochin, as occurred by intraperitoneal route. In fact, with these compounds, there was a survival of the mice injected with 1 M.L.D. This experiment with intravenous injection of the organism was repeated, with multiple minimum lethal dose, but no more than 1 M.L.D. was influenced with C 40, C 110, and optochin, while with C 29 and C 36, death of the mice ensued on less than 1 M.L.D.

The rapidity with which the bactericidal activity of these compounds is neutralized within the animal body is shown in Table XIII.

A therapeutic dose of each drug was injected into the peritoneal cavity of mice, in triplicate, and 5 minutes later, 1, 10, and 20 M.L.D. of pneumococci were injected by the same route. An amount of chemical which in simultaneous injections kills 500 to 8,000 M.L.D. is fixed, changed, exhausted, or somehow destroyed in 5 minutes, so that all the mice injected with 10 M.L.D. died, and none receiving 20 M.L.D. survived.

TABLE XI.

Per Os Therapy after Intraperitoneal Injection of 2 M.L.D. *of the Organism.*

Chemical.	Single doses immediately after injection of organism.			Three doses, 2 hrs. apart; first immediately after injection of organism.		
	Dose.	No. of mice.	Length of life.	Dose.	No. of mice.	Length of life.
	mg.			*mg.*		
C 29	2.5	4	48 hrs.; 72 hrs.; 72 hrs.; 120 hrs.	2.5	3	24 hrs.; 24 hrs.; 48 hrs.
	1.25	3	48 " 48 " 96 "	1.25	3	24 " 24 " L.
	0.625	3	72 " 48 " 48 "	0.625	3	48 " 72 " "
				0.25	3	48 " L.; L.
C 36	5.0	4	48 hrs.; L.; L.; L.	5.0	3	24 " 24 hrs.; 36 hrs.
	2.5	3	72 " " "	2.5	3	24 " 48 " L.
	1.25	3	72 " " "	1.25	3	48 " L.; L.
				0.625	3	54 " " "
C 40	5.0	4	48 hrs.; 48 hrs.; 48 hrs.; 54 hrs.	5.0	3	24 " 48 hrs. 48 hrs.
	2.5	3	L.; L.; L.	2.5	3	24 " 48 " 48 "
	1.25	3	72 hrs.; L.; L.	1.25	3	48 " 48 " L.
				0.625	3	L.; L.; L.
C 110	5.0	4	48 hrs. 60 hrs.; 72 hrs.; 72 hrs.	5.0	3	24 hrs.; 24 hrs.; 48 hrs.
	2.5	3	48 " 96 " 72 "	2.5	3	24 " 48 " 60 "
	1.25	3	48 " 72 " 72 "	1.25	3	48 " L.; L.
				0.625	3	72 " " "
Optochin.	5.0	4	72 hrs.; L.; L.; L.	5.0	3	48 hrs.; 48 hrs.; 48 hrs.
	2.5	3	72 " 72 hrs.; 72 hrs.	2.5	3	48 " 72 " L.
	1.25	3	48 " 72 " 72 "	1.25	3	48 " L.; L.
				0.625	3	48 " 48 hrs.; L.

TABLE XII.

Per. Os Therapy. Three Doses Repeated at 2 Hour Intervals; First Dose Simultaneous with Injection of the Organism.

Chemical.	Dose.	No. of mice.	Length of life. No. of M. L. D. injected intravenously.		Dose.	No. of mice.	Length of life. No. of M. L. D. injected intraperitoneally.		
			½	1			2	20	40
	mg.				*mg.*				
C 29	0.25	4	24 hrs.; 24 hrs.; 48 " 6 days.	24 hrs.; 48 hrs.; 48 " 72 "	0.25	4	48 hrs.; 48 hrs.; L.; L.	96 hrs.; L.; L.; L.	48 hrs.; 72 hrs.; 72 " 72 "
C 36	0.5	4	36 " 36 hrs.; 4 days; 6 days.	36 " 48 " 96 " 6 days.	0.5	4	48 hrs.; 72 hrs.; L.; L.	48 hrs.; 48 hrs.; L.; L.	48 " 72 " 72 " L.
C 40	0.5	4	L.; L.; L.; L.	36 " 48 hrs. L.; L.	0.5	4	L.; L.; L.; L.	72 hrs.; L.; L.; L.	48 " 72 hrs.; 6 days; L.
C 110	1.0	4	" " " "	L.; L.; L.; L.	1.0	4	48 hrs.; L.; L.; L.	48 hrs.; L.; L.; L.	48 hrs.; 48 hrs.; L.; L.
Optochin.	1.0	4	" " " "	" " " "	1.0	4	48 hrs.; L.; L.; L.	48 hrs.; L.; L.; L.	48 hrs.; 48 hrs.; 48 " L.
Controls.	0	4	0.0005 cc. 6 days; L. L.; L.	0.0001 cc. 36 hrs.; 48 hrs.; 96 hrs.; 120 hrs.	0	4	48 hrs.; 48 hrs.; 48 " 48 "	36 hrs.; 36 hrs.; 36 " 36 "	36 hrs.; 36 hrs.; 36 " 36 "

TABLE XIII.

Protection Experiment. Drug Injected 5 Minutes before the Organism.

Chemical.	Dose.	No. of mice.	Length of life. No. of M.L.D. 1	10	20
	mg.				
C 29	0.5	3	36 hrs.; 36 hrs.; 36 hrs.	36 hrs.; 36 hrs.; 36 hrs.	36 hrs.; 36 hrs.; 36 hrs.
C 36	1.0	3	12 " 72 " 36 "	36 " 36 " 36 "	60 " 36 " 36 "
C 40	1.0	3	36 " L.; L.	36 " 48 " 240 "	36 " 36 " 36 "
C 110	2.0	3	36 " " "	36 " 72 " 120 "	36 " 72 " 72 "
Optochin.	2.0	3	48 " " "	36 " 72 " 72 "	36 " 60 " 72 "
Controls.	0	3	36 " 36 hrs.; 36 hrs.	36 " 36 " 36 "	36 " 36 " 36 "

Our work as so far reported has been restricted to a Type I pneumococcus (Neufeld). Moore[2] showed that there is a difference in the bactericidal action of optochin with pneumococci of the different types, but it is not necessarily specific for types. Using the same procedure of simultaneous intraperitoneal injection of organism and drug, described above in connection with the Neufeld pneumococcus, we have titrated an additional Type I and two each of Types II and III (Table XIV). It would appear from these results that the chemicals are most active against Type I, less so against Type II, and least against Type III. That this specificity perhaps is not as

TABLE XIV.

Comparison of the Relative Protection Afforded by Different Drugs against Multiple Lethal Doses of Type I, II, and III Pneumococci. Simultaneous Injection Intraperitoneally of Drug and Organism.

Chemical.	No. of M. L. D.					
	Type I.		Type II.		Type III.	
	Neufeld.	No. 15.	No. 28.	No. 32.	No. 14.	No. 27.
C 29	500	100	400	20	0	1
C 36	5,000	1,000	400	50	0	0
C 40	8,000	1,000	500	100	0	1
C 110	6,000	1,000	400	50	10	1
Optochin.	700	400	400	50	10	1
No of organisms per M.L.D.	4	18	9	8	700,000	5,000,000

marked as it would seem is borne out, even in the case of these six strains, by two considerations. First, the virulence of the Type III strains was not as great as that of either of the Type I or of the Type II, the Type III having a minimum lethal dose in one strain of 700,000 and in the other of 5,000,000 organisms. It is possible that we are dealing not with type specificity but with organisms more or less resistant to chemicals and of varying numerical degrees of virulence. This is emphasized in the experiment shown in Table XV. Then, during another investigation, the virulence of the Type I (Neufeld) was reduced, so that 1 M.L.D. contained 7,000 instead of two to four organisms. This organism was used in multiple lethal doses, 1, 10, 100, and

TABLE XV.

Effect of Drugs on a Neufeld Type I Pneumococcus of Lowered Virulence (Decreased Approximately 4,000 Times).

Chemical.	Dose.	No. of mice.	Length of life.			
			No. of M.L.D.			
			1,000	100	10	1
			No. of organisms.			
			6,500,000	650,000	65,000	7,000
	mg.					
C 29	0.5	2	48 hrs.; 48 hrs.	48 hrs.; 48 hrs.	96 hrs.; 112 hrs.	L.; L.
C 36	2.0	2	48 " 48 "	48 " 48 "	48 " 96 "	" "
C 40	1.0	2	48 " 48 "	48 " 48 "	L.; L.	" "
C 110	2.0	2	48 " 48 "	48 " 48 "	48 hrs.; L.	" "
Optochin.	2.0	2	48 " 48 "	48 " 48 "	48 " 48 hrs.	48 hrs.; 96 hrs.
Controls.	0	2	48 " 48 "	48 " 48 "	48 " 48 "	48 " 48 "

1,000, and followed immediately by the chemical (simultaneous method). The results with organism in this stage of activity were very similar to those with Type III above, the chemicals, except C 40, being active against not more than 1 M.L.D.

Rabbit Experiment.

Two experiments were carried out with rabbits (Table XVI). Organisms and drugs were injected simultaneously. Regardless of toxicity, 25 mg. per kilo of each chemical were used. The pneumococcus (Neufeld) in a preliminary titration demonstrated a virulence such that 0.000001 cc. of a 6 hour serum broth culture

TABLE XVI.

Rabbit Experiment.

Chemical.	Pneumococci 0.00001 cc.					Pneumococci 0.000001 cc.				
	Dose per kilo.	Rabbit No.	Weight.	Life.	Culture.	Dose per kilo.	Rabbit No.	Weight.	Life.	Culture.
	mg.		*gm.*	*days*		*mg.*		*gm.*	*days*	
C 29	25	1	2,300	3	++	25	9	2,100	4	++
C 36	25	2	1,900	11	++	25	10	1,775	L.	
C 40	25	3	1,540	L.		25	11	2,000	"	
C 110	25	4	1,850	7	++	25	12	2,020	"	
Optochin.	25	5	1,950	2	++	25	13	1,675	3	++
	25	6	2,000	3	++	25	14	2,200	3	++
Controls.	0	7	1,800	3	++	0	15	1,950	3	++
	0	8	2,100	3	++	0	16	2,100	3	++

killed a 2 kilo rabbit in 3 days. In the first experiment with 0.0001 cc., or 100 M.L.D., the rabbit treated with C 40 remained alive (30 days), those treated with C 36 and C 110 showed a slight delay, while the controls, the C 29, and the optochin animals succumbed in 2 or 3 days. In the second experiment the same procedure was carried out, except that 1 cc. of 0.00001 dilution of organisms was employed for injection. The C 29, optochin, and control animals died in 4, 3, and 3 days respectively, those of C 36, C 40, and C 110 survived.

These results show that the bactericidal activity of the aromatic compounds is not restricted to mice. Optochin seemingly does not act with sufficient rapidity in rabbits to kill this number of organisms *in vivo*. The results of this experiment confirm the work with optochin of Moore,[17] Scott,[18] and Lewis.[19]

[18] Scott, W. M., *J. Path. and Bact.*, 1914–15, xix, 130.

[19] Lewis, J. H., *Arch. Int. Med.*, 1918, xxii, 593.

DISCUSSION.

The aromatic compounds described are a series of chemicals possessing rapid bactericidal action both *in vitro* and *in vivo*. There is, however, a pronounced variation in their action from the bacteriotropic and organotropic standpoint. C 29 ranks first in rapidity of action *in vitro*, but because of its polytropic characteristics is the poorest *in vivo*. The para-hydroxy substitution product (C 36) is an improvement in that the decrease in bactericidal action is more than compensated for by the lessening of its toxicity for mice. The bactericidal activity *in vitro* of the meta-hydroxy compound (C 40) is almost as great as the unsubstituted benzene derivative (C 29), and its toxicity is reduced 50 per cent. The dihydroxybenzene compound (C 110), judged by its ability to kill multiple lethal doses of pneumococci *in vivo*, holds an intermediate position between the para and meta derivatives. Its *in vitro* bacteriotropic activity, however, is much less than that of both the other aromatic chemicals and optochin. This substance is an example of a drug which, when injected simultaneously with multiple lethal doses of a virulent pneumococcus into the peritoneal cavity of mice, causes the death of a greater number of organisms than a corresponding amount *in vitro*. Apparently in a therapeutic dose its activity is auxiliary or perhaps stimulatory to the other natural forces of an animal which combat an infection. From the above it may be seen that there is a relationship between chemical constitution and chemotherapeutic activity.

The ultimate test of a chemotherapeutic agent is whether its use results in the cure of an established infection. Mice are unsatisfactory animals with which to make this test. The incubation period for the pneumococcus is so short, as well as the length of life following a fatal dose of the organism, that any remedial measure, if treatment be delayed till the infection is systemic in nature, may give misleading results.

Our method of investigation has shown that when pneumococci and drugs are injected simultaneously into the peritoneal cavity the death of multiple lethal doses of the organisms results. Any delay in the administration of the drugs neutralizes their effectiveness. Although an experimental infection was arrested when treatment was

postponed 2 hours after the injection of multiple lethal doses of organism (with chemicals and pneumococci injected intraperitoneally), at no time have we been able to cure a mouse having a systemic infection. The treatment involving the longest delay was administered after an 8 hour interval. In this experiment, not reported above, mice were injected with 1 M.L.D. and treatment was begun 8 hours later. 60 per cent of the mice treated with the aromatic compounds, with the exception of C 29, survived. Inasmuch, however, as pneumococci did not appear in the blood stream of the controls until 12 hours had elapsed, this study merely proves the chemicals to have a local therapeutic action, though of a high degree of efficiency. However, that there is some therapeutic activity resulting from diffusion of the drugs, as tested by injecting chemicals and organisms through different routes, is shown by the favorable results obtained in *per os* therapy.

Binz,[20] in an extensive series of experiments, has shown that, along with other physiological and pharmacological attributes of certain of the cinchona alkaloids, (1) they are general protoplasmic poisons, (2) stimulating in small amounts the activity of ameba, Infusoria, and white blood cells, in larger amounts paralyzing, and (3) decreasing the oxidizing and reducing power of the blood and weakening the enzymotic action. The summary of his work indicates that quinine in small amounts stimulates normal functional activity, in large amounts causing paralysis of this same mechanism. We are unable to say that these aromatic derivatives produce the same effect, but it has been shown that in experiments with pneumococcus infection of mice their action is zonal; that is, within certain dosage the bacteriotropic action of the chemicals is greater than the deleterious influence on the defensive mechanism of the animal. Amounts above this therapeutic dose, although non-lethal, paralyze processes vital for the animal's defense, permitting the invading organism to develop without restraint. It is difficult to understand why this large, non-lethal dose does not kill the bacteria, when seemingly more than a sufficient quantity to do so is injected. There is an apparent reversal of relationship from bacteriotropism with small doses to organotropism with the large. In other words, there is a greater affinity for the animal

[20] Binz, C., *Virchows Arch. path. Anat.* 1869, xlvi, 67; Das Chinin. Nach den neuern pharmakologischen Arbeiten dargestellt, Berlin, 1875.

tissue than for the pneumococci, when large amounts of chemical are injected. This zonal phenomenon was found to occur in intraperitoneal, subcutaneous, and *per os* administration of all the drugs, optochin having a less pronounced zonal effect than the aromatic compounds.

The question arises as to whether it would be advisable to use drugs for chemotherapeutic purpose that present this zonal phenomenon, which at first glance seems so discouraging. That this may not be an insuperable barrier is suggested by the fact that it is present in some degree in all drugs used in clinical medicine. Drugs in general have a therapeutic zone beyond which treatment cannot be carried with safety to the patient. The practical value of any therapeutic agent lies in its ability to produce desired effects in relatively nontoxic quantities.

The question naturally arises whether the aromatic cinchona compounds which are reported in this paper may have therapeutic applications in man. This cannot be answered until further and wider studies have been made. Our experiments have been confined almost entirely to the one animal species; but that other larger animals can be used may be inferred from the experiments successfully carried out on rabbits. That the list of synthesized pharmacological agents effective against a bacterial infection has been enlarged is obvious. But we feel that the time for the employment of any of the drugs here described in man has not yet arrived. Further investigations along the lines followed may not impossibly lead to the goal being sought.

CONCLUSION.

It has been shown with one strain of pneumococcus (Type I, Neufeld), that hydroquinine chloroacetanilide (C 29), hydroquinine *p*-chloroacetylaminophenol hydrochloride (C 36), hydroquinine *m*-chloroacetylaminophenol hydrochloride (C 40), and hyroquinine 4-chloroacetylaminopyrocathecol hydrochloride (C 110) have a rapid pneumococcidal activity both *in vitro* and in the peritoneal cavity of mice, and to a lesser extent in rabbits. In comparison, optochin is slower in action, but its power is not so easily destroyed either *in vitro* or *in vivo*.

The introduction of the hydroxy group of the benzene nucleus of hydroquinine chloroacetanilide changes the relationship between

organotropism and bacteriotropism. In comparing the rapidity of *in vitro* bactericidal action and intraperitoneal toxicity, C 29 exhibits the most rapid pneumococcidal action and is the most toxic for mice. C 36, the para-hydroxy derivative, is one-fifth as toxic as C 29 and only one-tenth less active bactericidally. C 40 is one-half as toxic and has approximately the same bactericidal power, while C 110 is one-eigth as toxic and one-fifth as pneumococcidal; and optochin is one-sixth as toxic and has one-fifth the bactericidal action. Arranged in the order of their ability to kill pneumococci when injected simultaneously with them into the peritoneal cavity, the drugs are: C 40, C 110, C 36, optochin, and C 29.

The chemotherapeutic action of the aromatic compounds is essentially local in character. But by *per os* therapy there is demonstrated a certain amount of diffusion of this activity, not shown by any other method of administration, C 40 and C 110 having about the same value as optochin.

Intravenous injection of the drugs in small doses destroyed to a greater or less extent the natural defenses of the animal, optochin being perhaps less injurious than the aromatic compounds. This same destruction of natural resistance followed intraperitoneal and subcutaneous injections of the chemicals as measured by intravenous injections of the organisms.

The maximum tolerant dose in a single injection (intraperitoneal) is not so efficacious as the same dose divided in fifths and injected at hour intervals. Optochin under these conditions is not so active as the aromatic compounds. In general, repeated doses are more curative than single.

There is a zone between the therapeutic and toxic doses, both single and repeated, for all these chemicals alike, where the natural resistance of the animal to an infection is reduced. This effect is noted especially with C 29, C 36, and C 40. In the case of optochin the therapeutic dose is nearer the toxic than with C 110, C 36, and C 40. Apparently these chemicals exhibit a variability in *in vivo* bactericidal activity according to different strains of pneumococci and numerical virulence.

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