THE ACTION OF ETHYLHYDROCUPREIN (OPTOCHIN) ON TYPE STRAINS OF PNEUMOCOCCI IN VITRO AND IN VIVO, AND ON SOME OTHER MICRO-ORGANISMS IN VITRO.

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In 1911 Morgenroth and Levy (1) introduced the drug ethylhydrocuprein (optochin), a derivative of hydroquinine, in the treatment of experimental pneumococcic infection. Subsequent tests have, as a rule, shown the compound to possess a parasiticidal effect on the pneumococci. In the experiments so far reported, different strains of pneumococci, chosen more or less at hazard, were studied; no attempt, however, has been made to investigate the action of the drug on type strains of the various groups of pneumococci. It seemed of interest to investigate the action of the drug from this viewpoint.

Neufeld (2) showed that strains of pneumococci differed among themselves in respect to their immunity reactions (protective bodies). The investigations of Cole (3) and of Dochez and Gillespie (4) have resulted in a serological classification of the pneumococci. It has been shown by these authors that the pneumococci can be divided into at least four groups. The organisms of Groups I and II are specific in their immunity reactions; that is, an immune serum produced against any member of Group I has a specific protective action against, and a specific agglutinative action on, any member of Group I, but has no effect on any members of Groups II, III, or IV. Similarly, a serum immune to any member of Group II will react in the same way with all members of that group, but not with any members of Groups I, III, or IV. In Group III are included all members of the *Pneumococcus mucosus* type. To Group IV belong all pneumococci not belonging to Groups I, II, or III. A serum immune to any member of Group IV exhibits protective and agglutinative reactions with the strain used for its production, but in no instance with any other member of Group IV or

with any member of the other three groups. Lister (5) has arrived at a somewhat similar classification from opsonic studies.

EXPERIMENTAL.

The pneumococci used in the present study were representative of these four serological groups and were obtained from the Hospital of The Rockefeller Institute, with the exception of the microorganism designated as South Africa (9), which was obtained from the South African Institute for Medical Research; this organism has been placed in a fifth group by Lister (5) as a result of his opsonic studies. All strains of pneumococci used in the present study were isolated from cases of human pneumonia, except those called I Les and II White, which were obtained from cases of pneumococcal meningitis by lumbar puncture.

The Action of Ethylhydrocuprein in Vitro.

Wright (6) has shown that the action of ethylhydrocuprein hydrochloride on pneumococci *in vitro* is only slightly lessened by the presence of serum, while with antiseptics such as lysol and cresol, under similar conditions, the contrary is the case. Tugendreich and Russo (7) have shown that the hydrochloride of the drug in a concentration of 1 in 16,000 kills the pneumococcus *in vitro* in three hours at room temperature, and that it is much more potent in this respect than the hydrochlorides of the homologous compounds: isopropyl, isobutyl, and isoamylhydrocuprein; this superiority is even more marked over quinine and hydroquinine. In the present study the action of ethylhydrocuprein hydrochloride on type strains of the different groups of pneumococci was worked out and compared with that on other microorganisms under similar conditions.

Technique.—A solution of the drug was made in broth and sterilized by boiling or filtration through a Berkefeld filter. Various dilutions of this stock solution were made; to 2 cc. of each dilution in a test-tube 0.1 cc. of a 24 hour broth culture was added and the tubes were incubated for 18 hours at 37° C. At the end of this period the tubes were examined, and the results, as regards growth or inhibition of growth, noted; a large loopful of the two

TABLE I.

The Action in Vitro of Ethylhydrocuprein Hydrochloride on Some Representatives of the Four Serological Groups of Pneumococci.

Serological group of pneumococcus.	I.			II.			III.			IV.		
Designation of strain of pneumococcus....	106[5]	(B42)1[4]	Les	(B21)1[10]	38[3]	White	(A66)14[2]	(E22)4[3]	(B48)1[4]	(M)4[3]	(A67)11[3]	South Africa (9)
Highest dilution causing inhibition of growth.....	500,000	1,000,000	500,000	1,000,000	1,000,000	1,000,000	500,000	10,000,000	1,000,000	500,000	1,000,000	1,000,000
Highest dilution causing death.....	20,000	1,000,000	100,000	1,000,000	50,000	1,000,000	100,000	10,000,000	100,000	50,000	1,000,000	1,000,000

lowest dilutions showing growth macroscopically, and of all tubes showing no growth, was in each case plated in about 12 cc. of blood agar and incubated. The concentration of the drug was thus enormously diluted. The plates were examined at the end of 24 hours and again at the end of 48 hours. Thus, one could differentiate between an inhibitory effect of the drug on the growth of the microorganism and actual death. Some typical results are given in Table I.

Explanation of Tables.—In the top row the Roman numeral stands for the serological group to which the strain of pneumococcus belongs; a letter and number in brackets, if present, denote the particular strain; the Arabic number represents the number of animal passages of the strain and the exponent the number of cultivations on artificial medium since the last animal passage. In the remaining two rows each number represents the greatest dilution in which the growth of the corresponding microorganism was inhibited or killed, as the case may be. In the case of the pneumococci the range of dilutions of the drug examined was as follows: 1 in 10,000; 1 in 20,000; 1 in 50,000; 1 in 100,000; 1 in 500,000; 1 in 1,000,000; and 1 in 10,000,000.

From the results given in Table I it is seen that ethylhydrocuprein hydrochloride causes in very high dilutions, *in vitro,* an inhibition of growth, and death of the pneumococcus, the latter occurring, generally speaking, in somewhat lower dilutions; and that no constant or considerable differences are seen in these effects on typical representatives of the four groups of pneumococci.

It was thought of interest to compare the action on pneumococci with that on other organisms, more especially streptococci. The results are set forth in Table II.

Description of the Microorganisms Mentioned in Table II.

Streptococcus 1 was a non-hemolytic streptococcus cultivated by Dr. Beattie from a case of rheumatic fever. The culture used was obtained from the Bacteriological Department of the Museum of Natural History, New York.

Streptococci 5 and 7 were two different strains of non-hemolytic streptococci obtained from the Pathological Department of Mt. Sinai Hospital, New York. They were cultivated from the blood of patients suffering from sabacute endocarditis.

TABLE II.

The Action in Vitro of Ethylhydrocuprein Hydrochloride on Microorganisms Other than Pneumococci.

Micro-organism.	*Micrococcus catarrhalis.*	*Streptococcus mucosus.*	Streptococcus 1.	Streptococcus 5.	Streptococcus 7.	Streptococcus 8.	Streptococcus 59.	Streptococcus R.	Streptococcus C.	*B. coli communis.*	*B. typhosus.*	*B. paratyphosus* B.	*Staphylococcus albus.*	B. Friedländer.
Highest dilution causing inhibition of growth	10,000	20,000	Not inhibited in 10,000	20,000	10,000	10,000	10,000	20,000	10,000	Not inhibited in 10,000	Not inhibited in 10,000	Not inhibited in 10,000	Not inhibited in 10,000	10,000
Highest dilution causing death	Not killed in 10,000	Not killed in 10,000	Not killed in 10,000	20,000	Not killed in 10,000	Not killed in 10,000	Not killed in 10,000	Not killed in 10,000	Not killed in 10,000	—	—	—	—	Not killed in 10,000

TABLE III.*

The Action in Vitro of Quinine Hydrochloride.

Microorganism.	I105[6].	II38[5].	IIIA66.12[4].	IVM.4[3].	*Streptococcus mucosus.*	*Micrococcus catarrhalis.*	Streptococcus I.	*B. coli communis.*	*Staphylococcus albus.*
Highest dilution causing inhibition of growth	50,000	50,000	50,000	50,000	5,000	5,000	5,000	1,000	1,000
Highest dilution causing death	5,000	10,000	5,000	10,000	1,000	5,000	2,000	1,000	1,000

* The first four organisms are representatives of the four groups of pneumococci.

Streptococcus 8 was a laboratory strain of hemolytic streptococcus.

Streptococcus 59 was a green-producing, non-hemolytic streptococcus cultivated by Dr. H. F. Swift from the blood of a patient suffering from rheumatic pericarditis.

None of these strains had a capsule, fermented inulin, or was bile-soluble.

The *Streptococcus mucosus* was a diplococcus growing in short chains; it showed, with His's stain, a well defined capsule in the body fluids of an infected animal; it was not bile-soluble; it was pathogenic for mice, causing a sticky exudate in the peritoneal cavity; it did not ferment inulin; on cultivation for a few passages on blood agar the growth, which was previously moist on solid media, became dry in character.

The streptococcus strains called R and C were isolated from normal sputum and were mucous in type; that is, they gave a mucoid growth on blood agar and possessed a capsule; these qualities were, however, lost after a few passages on artificial media. Neither was bile-soluble, and neither fermented inulin.

From Table II it is seen that the action, if any, of ethylhydrocuprein, in the dilutions examined, on the microorganism mentioned therein was considerably less than that on the pneumococci.

The action *in vitro* of the hydrochloride of quinine (from which ethylhydrocuprein is derived) on pneumococci and on some other microorganisms is shown in Table III. It is seen therefrom that, while the action of quinine hydrochloride is greater on the pneumococci than on the other bacteria, this action is far less in the former case than that of optochin.

The Action of Ethylhydrocuprein (Optochin Base) on Representatives of the Four Groups of Pneumococci in Vivo.

Owing to the fact that certain biochemical relationships (such as bile solubility) exist between trypanosomes (8) and spirilla (9), on the one hand, and the pneumococci (10) alone among the *Cocaceæ*, on the other, Morgenroth (1) investigated the action on the pneumococci of that group of compounds which has given results in the therapy of trypanosomal and spirillar infections; namely, quinine and its derivatives.

Morgenroth and Levy showed that ethylhydrocuprein (a derivative of hydroquinine) exerted a considerable protective action, and a certain degree of curative action (1) on experimental pneumococcal infection in the mouse. Later Gutmann (11) and Morgenroth and Kaufmann (12) reported experiments in which this protective action on several strains of pneumococci was shown. Levy (13) studied the curative effect of the drug on pneumococcal infection of the mouse by a strain of *Pneumococcus mucosus*, an interval of from two to six hours having been allowed to elapse between the intraperitoneal infection and the first

administration of the drug. The dosage of the infection does not seem to have been accurately measured in Levy's experiments, nor do the results seem to have been checked by autopsy; an effect of the drug, however, on the infection can be seen; of 16 mice, 10 survived up to the 8th day, when the observation was discontinued. This work is summarized by Rosenthal (14).

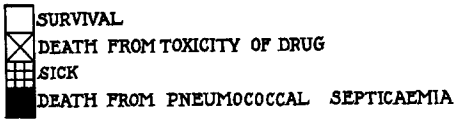
So far, the strains studied in this connection were chosen more or less at haphazard, no attempt having been made to study strains of pneumococci in relation to classification. In the following experiments typical representatives of the four groups of pneumococci were used.

Technique.—A 2 per cent solution of the free base ethylhydrocuprein (optochin base) was made in sterile olive oil by rubbing up in a mortar and standing the product so obtained in the incubator at 37° C. over night. Mice of 18 grams' weight and upwards were used. The infection was given in a constant volume of 1 cc. of broth intraperitoneally. The treatment consisted of 0.5 cc. of the 2 per cent optochin base solution in oil (calculated in every case) per 20 grams of mouse given under the skin of the back immediately after the infection; this was followed by an equal dose on the second day of the experiment, and by 0.4 cc. per 20 grams of mouse on the third and fourth days. The mice were observed for a period of from 10 to 14 days. An autopsy was performed on every mouse that succumbed (whether a treated animal or a control), and the heart's blood, and peritoneal fluid if necessary, were examined in a smear preparation, Gram's stain and His's capsule stain being used. If no Gram-positive and capsule-bearing bacteria were found in the smear by these methods, cultures were made on defibrinated rabbit blood agar with abundant inoculation, incubated for 24 hours at 37° C., and then examined.

In tabulating the results the graphic method recommended by Morgenroth is employed. In the text-figures each square denotes one experimental animal; a black square indicates death from pneumococcal septicemia; a white square, survival; an oblique cross denotes that the animal died, but that the examination of the heart's blood was negative, in which instance death was apparently caused by the toxicity of the drug, the infecting pneumococci having been first killed off by its action;[1] and, finally, a square subdivided into

[1] Morgenroth pointed out that the toxic dose for mice is not far removed from the curative dose.

six smaller ones denotes that the animal was sick at the time of observation (Text-fig. 1).

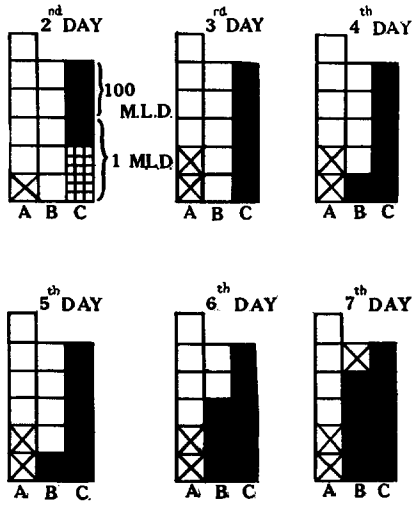


TEXT-FIG. 1. Explanation of the method used in the text-figures.

The condition of the animals is first given in the diagram on the second day of the experiment; that is, 24 hours after the infection and first treatment; consequently, the first diagram in each experiment is marked 2d day.

The minimum lethal dose of the infecting microorganism was in each case determined for forty-eight hours unless otherwise stated; in all cases that dose was taken as the m. l. d. which was with certainty fatal within the time limit stated for several mice. In case of doubt reestimations were made. With this statement it is unnecessary to give the estimations in detail.

Throughout the observations the mice were kept at about 75° F.[2]

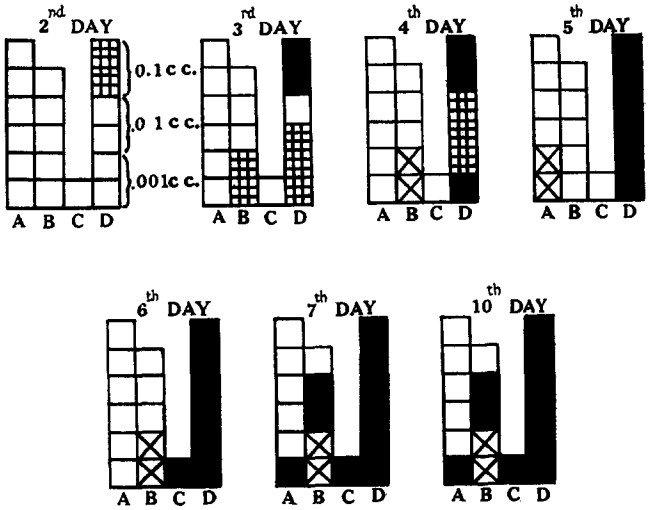


TEXT-FIG. 2. Experiment 1. Strain I 106.[4] The m.l.d. was 0.000,000,001 cc. (48 hrs.). The infecting doses used: 10 m.l.d. (Column A); 1,000 m.l.d. (Column B). Column C, controls (untreated).

[2] The toxicity of the drug is said to be greater when the animals are kept exposed to cold.

Comment on Experiment 1.—(Text-fig. 2.) Of the 6 treated animals infected with 10 times the m. l. d., 2 died of toxicity, the heart's blood at autopsy being sterile; while the remaining 4 animals remained permanently well; of those infected with 1,000 times the m. l. d. and treated, 1 died of toxicity (showing sterile heart's blood at autopsy), and the remaining 4 of pneumococcal septicemia; the last mentioned 4 animals, however, survived much longer than the untreated controls.

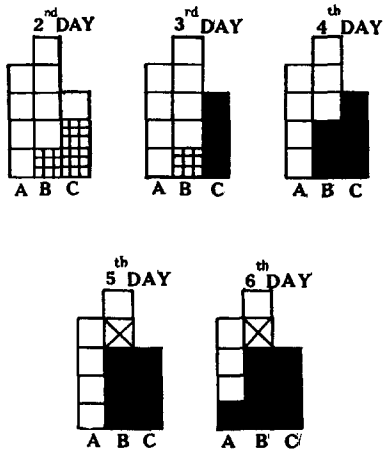
This microorganism was of extraordinarily high virulence for mice, it having had 106 animal passages. The m. l. d. above stated was regularly fatal and showed 6 to 14 colonies when plated.



TEXT-FIG. 3. Experiment 2. Strain I (B42)I^{6}. The m.l.d. was 0.001 cc. The infecting doses used: 10 m.l.d. (Column A); 100 m.l.d. (Column B); 500 m.l.d. (Column C). Column D, controls (untreated).

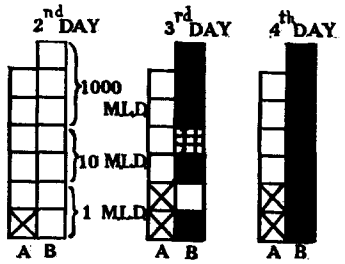
Comment on Experiment 2.—(Text-fig. 3.) This strain was relatively much less virulent than the previous one. A dose of 0.001 cc. or 0.01 cc. was always fatal within 4 to 5 days, but sometimes the animals infected with the larger of these doses survived those infected with the smaller by a day. In view of the protracted course of the infection in this case the time limit for the m. l. d. was judged as 5 days. Of those animals injected with 10 times the m. l. d. only 1 died of pneumococcal septicemia, all the others

surviving. Of those injected with 100 times the m. l. d. 1 survived; 2 died of toxicity; and 2 of pneumococcal septicemia, but later than the controls.



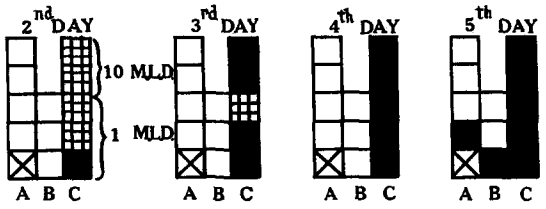
TEXT-FIG. 4. Experiment 3. Strain II 34^{19}. The m.l.d. was 0.000,000,001 cc. (48 hrs.). The infecting doses: 10 m.l.d. (Column A); 100 m.l.d. (Column B). Column C, controls (untreated).

Comment on Experiment 3.—(Text-fig. 4.) Of the 4 animals infected with 10 times the m. l. d. and treated, 1 died on the 6th day of pneumococcal septicemia and 3 survived. Of the 5 infected with 100 times the m. l. d. and treated, 3 died of pneumococcal septicemia and 1 survived; the remaining animal died of toxicity. On plating the m. l. d., 6 to 12 colonies resulted. This strain was one of very high virulence, notwithstanding the fact that it had undergone nineteen artificial cultivations since the last animal passage.



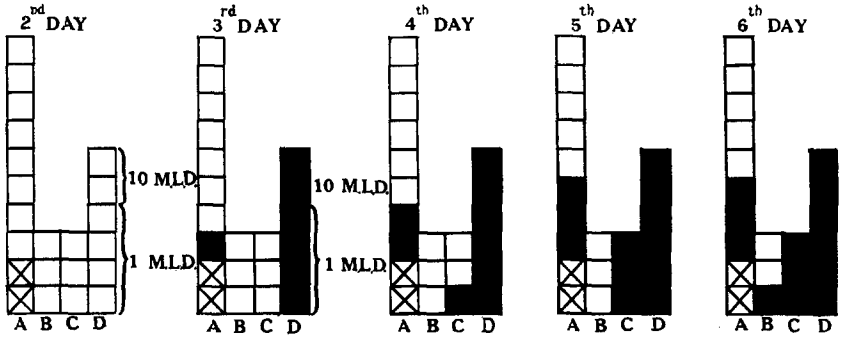
TEXT-FIG. 5. Experiment 4. Strain II (B21) 3^{2}. The m.l.d. was 0.0,000,001 cc. (fatal in 4 days). Dose used: 1,000 m.l.d. (Column A). Column B, controls (untreated).

Comment on Experiment 4.—(Text-fig. 5.) If the m. l. d. is judged to kill in 4 days, 4 mice out of 6 infected with 1,000 times the m. l. d. survived the controls permanently. Of the 2 given in Text-fig. 5 as dying of toxicity the heart's blood of 1 was sterile, and a Gram-negative bacillus was recovered from that of the other, but no pneumococci.



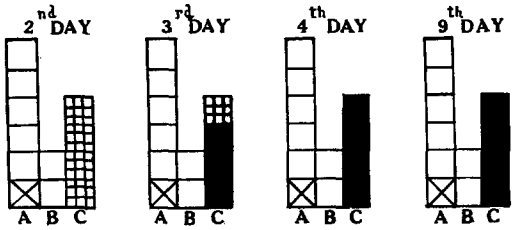
TEXT-FIG. 6. Experiment 4 a. Strain II (B21) 2^{14}. The m.l.d. was 0.01 cc. (48 hrs.). Doses used: 1 m.l.d. (Column A); 50 m.l.d. (Column B). Column C, controls (untreated).

Comment on Experiment 4 a.—(Text-fig. 6.) In this experiment 1 animal represented in Column A (1 m. l. d.) and 1 in Column B (50 times the m. l. d.) died on the 8th and 10th days, respectively, the heart's blood showing on examination a Gram-negative bacillus but no pneumococci. As we were troubled with mouse typhoid at this time, this was probably the cause of death. 5 animals survived, 2 died of pneumococcal septicemia, and 1 of toxicity.



TEXT-FIG. 7. Experiment 5. Strain III (A66) 12^{8}. The m.l.d. was 0.000,000,001 cc. (48 hrs.). Doses used: 10 m.l.d. (Column A); 100 m.l.d. (Column B); 1,000 m.l.d. (Column C). Column D, controls (untreated).

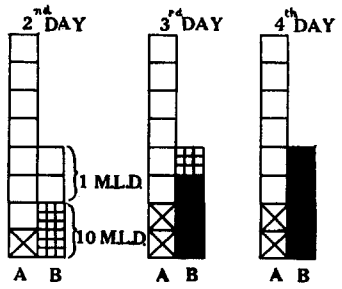
Comment on Experiment 5.—(Text-fig. 7.) The Strain III (A 66)12[6] was a very virulent one. The m. l. d., 0.000,000,001 cc. of a 24 hour broth culture, when plated on blood agar, showed on incubation at least 15 typical colonies. The animal represented by the upper segment of Column C was found almost completely eaten by its fellows on the 6th day; no autopsy could be done, consequently the cause of death is somewhat doubtful. After the 6th day no further deaths occurred in this series. 5 out of 10 animals injected with 10 times the m. l. d. survived; 3 died of pneumococcal infection, and 2 of toxicity (giving a sterile heart's blood). Of 3 injected with 100 times the m. l. d. 2 survived, and the remaining animal succumbed to the infecting pneumococci. Of those injected with 1,000 times the m. l. d. none survived.



TEXT-FIG. 8. Experiment 6. Strain III (E22)12[6]. The m.l.d. (0.000,001 cc.) was fatal in 48 hrs. 0.00,000,001 cc. of a broth culture was observed to be regularly fatal within four days; the m.l.d. was not, however, at the time of this experiment determined for a longer period than 48 hrs.; consequently the dose of 0.000,001 cc. was probably considerably greater than the m.l.d. for a mouse. Column A, 1 m.l.d. (0.000,001 cc. (48 hrs.)). Column B, 10 m.l.d. (0.00,001 cc. (48 hrs.)). Column C, controls, 1 m.l.d. (untreated).

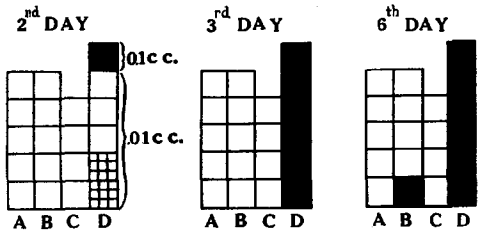
Comment on Experiment 6.—(Text-fig. 8.) Among the treated animals no death occurred up to the 10th day when observation was discontinued. 1 animal of the series died of toxicity, and none of pneumococcal septicemia.[3]

[3] Further experience with this strain has shown it to be more susceptible to the action of ethylhydrocuprein *in vivo* than any other strain of pneumococcus examined by us. We have been able with the drug to protect against 10,000 times the m.l.d.



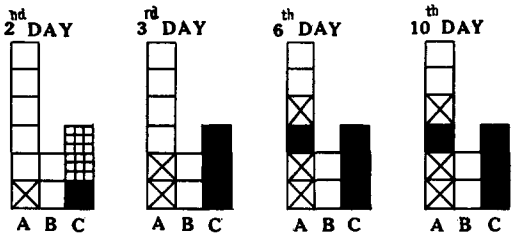
TEXT-FIG. 9. Experiment 7. Strain IV M.4[3]. The m.l.d. was 0.00,000,001 cc. of a 24 hr. broth culture. Dose used: 10 m.l.d. (Column A). Column B, controls (untreated).

Comment on Experiment 7.—(Text-fig. 9.) No further deaths occurred after the 4th day. Of 8 animals injected with 10 times the m. l. d. and treated, 2 died of toxicity and 6 survived. This was an unusually virulent strain of Group IV. The virulence, however, rapidly fell off on artificial cultivation.



TEXT-FIG. 10. Experiment 8. Strain South Africa (9).5[2]. The m.l.d. was 0.01 cc. Doses used: 10 m.l.d. (Column A); 20 m.l.d. (Column B); 50 m.l.d. (Column C). Column D, controls (untreated).

Comment on Experiment 8.—(Text-fig. 10.) Of the mice infected with 10 times the m. l. d. and treated all survived; of those infected with 20 times the m. l. d. and treated only 1 died of pneumococcal septicemia, all the others surviving; all those infected with 50 times the m. l. d. and treated survived. The controls infected with 1 m. l. d. all died within 48 hours.



TEXT-FIG. 11. Experiment 9. Strain IV (S 10)3^{19}. The m.l.d. was 0.01 cc. (48 hrs.). Dose used: 10 m.l.d. (Column A); 50 m.l.d. (Column B). Column C, controls, 1 m.l.d. (untreated).

Comment on Experiment 9.—(Text-fig. 11.) Of the 5 animals infected with 1 m. l. d. and treated 2 survived, and 3 died of toxicity. From the heart's blood of one of these latter a Gram-negative bacillus was recovered. One died of pneumococcal septicemia. Both the animals infected with 50 times the m. l. d. and treated recovered.

DISCUSSION.

The results of the present study agree, in the main, with those of the other workers mentioned. We have not had in our experiments such a large percentage of cures as is claimed by Morgenroth, namely 90 to 100 per cent; nor have we seen any definite protective action of optochin *in vivo* with an amount of infection greater than 1,000 times the m. l. d. of a highly virulent strain.[4] Moreover, the greater the virulence of the strain (by passage through mice) the greater was the difficulty in protecting against increasing multiples of the m. l. d. Constant results are difficult to obtain in the mouse, owing to the repeated injections apparently causing the animals a considerable degree of traumatism and consequently rendering them liable to intercurrent troubles. This was true of an epidemic of mouse typhoid which tended to obscure the results. The relative toxicity of the drug for mice is another factor which tends to render results not quite clear. Olive oil, the vehicle in which the drug is given, does not undergo absorption for a week or more, and the disturbance to the tissues caused by the repeated injections of this inert body seems to afford

[4] See page 281, footnote 3.

a suitable nidus for the growth of bacteria which may kill the animal before the observation is concluded. It seems likely that the detrimental factors, toxicity of the drug, the traumatism inflicted by the injections, and the effect of the infection itself, summate in their effects and thus render the probability of the animal surviving less likely, although its body may have been completely sterilized from pneumococcal infection. The effect of ethylhydrocuprein *in vitro* and *in vivo* on the pneumococcus is considerable and specific and is seen on type strains of all four groups of pneumococci.

From the text-figures it is seen that, of 85 mice infected with 100 times the m. l. d. or less and treated, 15 mice, or 17.6 per cent, died of pneumococcal septicemia; 13 mice, or 15.2 per cent, died of toxicity of the drug or some obscure cause, the heart's blood being sterile at autopsy (the corresponding controls invariably died of pneumococcal septicemia); 56 mice, or 66.8 per cent, survived. Of these 85 treated mice, 69, or 81 per cent, either recovered or died of causes other than pneumococcal septicemia; such, for example, as the toxicity of the drug.

CONCLUSIONS.

1. Ethylhydrocuprein hydrochloride in very high dilution inhibits the growth of, and in 18 hours kills, representatives of all four groups of pneumococci *in vitro*. The killing effect is generally seen in somewhat lower dilutions than the inhibiting effect. No constant or considerable difference is seen in these actions on representatives of the four groups of the pneumococci. The action of ethylhydrocuprein hydrochloride on the pneumococci *in vitro* is so strongly specific that it may possibly be used as a test for a true pneumococcus.

2. The inhibitory or killing effects of ethylhydrocuprein hydrochloride *in vitro* on bacteria other than pneumococci are slight or absent. The effects are greater on streptococci than on any other organisms examined, but are still much less than on the pneumococci. This action distinguishes between the streptococcus group, including *Streptococcus mucosus* sometimes found in normal

mouths, on the one hand, and the true pneumococcus (including *Pneumococcus mucosus*), on the other.

3. Quinine hydrochloride inhibits the growth of, and kills the pneumococcus *in vitro;* much stronger concentrations, however, are necessary than in the case of ethylhydrocuprein. This effect of quinine hydrochloride is also seen on other organisms, but in a less degree.

4. Ethylhydrocuprein (optochin base) has a well marked protective action against experimental pneumococcal infection in mice in the case of type strains of all four groups of pneumococci; this protective action may be efficient against many multiples of the minimum lethal dose.

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