A FURTHER STUDY OF THE BACTERICIDAL ACTION OF ETHYLHYDROCUPREIN ON PNEUMOCOCCI.

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In a previous communication[1] we stated that ethylhydrocuprein inhibits the growth of, and kills, pneumococci *in vitro* in very considerable dilutions of the drug, and that it exerts a considerable protective action in experimental pneumococcal infections in mice. The present study was undertaken with the object of gaining some information as to the rate of absorption of the drug into the circulation in the animal body, as to how long the resulting bactericidal effect, if any, of the serum on pneumococci lasted, and as to the mode of action of the drug on these microorganisms.

Ethylhydrocuprein, a derivative of hydroquinine, was introduced by Morgenroth[2] in 1911 in the treatment of experimental pneumococcal infection in mice. It has since been subjected to study by many observers, who, generally speaking, agree that it has a bactericidal action on pneumococci, *in vitro* and *in vivo*. Wright[3] showed that the blood serum of mice previously treated with the drug killed pneumococci in the test-tube.

In the present study we considered it advisable to make use of rabbits as our experimental animals, and, having determined the tolerated and toxic doses of the hydrochloride of the drug (optochin hydrochloride) and of the free base (optochin base) for these animals, we proceeded to study the action of each of these preparations when given by different routes as set forth below. The hydrochloride was given to the rabbits subcutaneously, dissolved in 5 cc. of

[1] Moore, H. F., *Jour. Exper. Med.*, 1915, xxii, 269.

[2] Morgenroth, J., and Levy, R., *Berl. klin. Wchnschr.*, 1911, xlviii, 1560, 1979.

[3] Wright, A. E., Morgan, W. P., Colebrook, L., and Dodgson, R. W., *Lancet*, 1912, ii, 1633, 1701.

distilled water; intravenously, dissolved in 10 cc. of physiological salt solution; and by mouth, dissolved in 25 cc. of distilled water. The free base was given subcutaneously and intramuscularly dissolved in from 5 to 6 cc. of sterile olive oil.

EXPERIMENTAL.

Our experiments show that normal rabbits of approximately 2,000 grams tolerate a single dose of 0.1 gram of the hydrochloride given subcutaneously and 0.125 gram of the base in oil given in the same manner, per kilo of body weight. It seems, however, that the tolerance of normal rabbits of greater weight—3,000 grams and upwards—is less than this. The tolerance of normal rabbits of about 2,000 grams' weight for a single dose of the base in oil when this solution is given intramuscularly (into the erector spinal mass) is still lower—0.075 gram per kilo of body weight. Finally, the animals are able to bear, without showing signs of toxicity, only a small dose of the hydrochloride dissolved in normal salt solution given slowly intravenously; namely, a dose lying between 0.02 and 0.05 of a gram per kilo of body weight. In this case the drug must be given well diluted and very slowly; for, if given in any considerable concentration, a reaction between it and the blood plasma takes place with a resulting heavy precipitate which causes speedy death.

We have observed that the tolerance of rabbits previously infected with pneumococci seems to be somewhat less than that of normal animals.

The drug in a toxic dosage gives rise to certain characteristic symptoms in rabbits, the variety and intensity of which depend in part on the dose, in part on the route by which it is given, and in part on the location of the injection. In order of severity, in the case of subcutaneous or intramuscular injection, the symptoms are as follows: quietness of the animal and disinclination to eat; halting movement of the legs nearest the side of injection; spastic and incoordinated movements of the same; and complete paralysis of the extremities nearest the injection site. Finally, the paralysis may spread to the other limbs and the animal may lie on the floor collapsed, and die. The respirations are at first hurried and, with a larger dose, later diminish in rate, and symptoms of dyspnea may

appear. When a toxic dose is given intravenously, the animal shows convulsive movements, more or less severe according to the size of the dose, and may finally die with convulsions and exophthalmos. The bactericidal action of the drug described below is seen in the serum of animals showing severe toxic signs as well as in animals which received a dose well below the toxic limit.

Technique.—Normal rabbits weighing about 2,000 grams were used. Each rabbit was bled from the marginal ear vein, the ear having previously been thoroughly cleansed with bichloride of mercury and alcohol and wiped dry with a sterile sponge. The drug was then administered in the manner and amount mentioned in each protocol below. The animals were bled either as before from the marginal ear vein, or directly from the heart—which latter may be done with safety many times—at stated intervals after the administration of the drug. The blood in each case received in centrifuge tubes was, after the final bleeding, placed in the ice chest until the following morning, when the clot was loosened and centrifugalized. 3 cc. of serum from each centrifuge tube were pipetted off the clot into a test-tube, a separate pipette being used in each case, and inactivated for one-half hour at 56° C. in a water bath. (In a few special cases the active serum was used with the object of comparing the bactericidal power of such serum with the same serum inactivated, as described above.) The 3 cc. of serum in each test-tube were inoculated with 0.5 cc. of a dilution of an 18 hour broth culture of a pneumococcus of from 1 in 100,000 to 1 in 1,000,000. Stock strains of pneumococci of Groups I and II were used, generally the latter. The pneumococci having been thoroughly mixed with the serum, 0.5 cc. of the mixture was plated in about 10 cc. of 1 per cent glucose agar, which had been previously melted and cooled to 40° C. By this means the drug, even if present in the serum in sufficient concentration to prevent the growth, or cause death, of the pneumococci, was so diluted in the plate as to leave the pneumococci free to grow unhindered. The Petri dishes used were 10 cm. in diameter. The plates were incubated for from 20 to 24 hours, at 37° C., and at the end of this period the number of colonies in each was counted. The tubes containing the inoculated serum were also incubated at 37° C. for definite periods, as stated in the

tables, at the end of each of which periods 0.5 cc. of the contents of each tube was plated as before, and these plates were incubated in the same way. In this way we were enabled to gain information on the bactericidal action on pneumococci of the serum of animals treated with ethylhydrocuprein in relation to the points already stated.

Explanation of the Protocols.—The numerals in the protocols represent the number of colonies resulting from plating 0.5 cc. of the inoculated serum from the corresponding test-tube either immediately after the tubes were inoculated, or after a definite period of incubation; the figures in the vertical columns correspond to the intervals between the administration of the drug and the various bleedings of the animal; the figures in each horizontal row represent the number of colonies per 0.5 cc. of serum from each particular bleeding after a definite period of incubation.

Experiments Illustrating the Effects on Pneumococci of the Serum of Animals Treated with Ethylhydrocuprein Hydrochloride (Optochin Hydrochloride) Dissolved in Water, Given Subcutaneously (under the Skin of the Back).

Experiment 1.—(Table I.) Rabbit 98 E; weight 1,600 gm. Received 0.1 gm. of ethylhydrocuprein hydrochloride per kilo of body weight, in 5 cc. of distilled water, subcutaneously. No toxic symptoms. Pneumococcus: stock strain of Group II.

TABLE I.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies when plated after 7 hrs.' incubation.	No. of colonies when plated after 22½ hrs.' incubation.
Before giving drug.....	119	Almost infinity	Infinity.
1¼ hrs. after.....	105	36	0
4 " "	126	Almost infinity	Infinity.
5 " "	116	" "	"

Experiment 2.—(Table II.) Rabbit 115 D; weight 1,900 gm. Received 0.1 gm. of ethylhydrocuprein hydrochloride per kilo of body weight, in 5 cc. of distilled water, subcutaneously. No toxic symptoms. Pneumococcus: stock strain of Group II.

TABLE II.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies when plated after 7½ hrs.' incubation.	No. of colonies when plated after 20 hrs.' incubation.
Before giving drug.....	410	Infinity	Infinity.
1⅞ hrs. after.....	445	29	3
2½ " "	492	176	Infinity.

Experiment 3.—(Table III.) Rabbit 95 E; weight 2,200 gm. Received 0.1 gm. of ethylhydrocuprein hydrochloride per kilo of body weight, in 5 cc. of distilled water, subcutaneously. No toxic symptoms. Pneumococcus: stock strain of Group II.

TABLE III.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies when plated after 6⅛ hrs.' incubation.	No. of colonies when plated after 21 hrs.' incubation.
Before giving drug.....	329	Almost infinity	Infinity.
1 hr. after.....	268	46	18
2 " "	273	135	146
4⅚ " "	316	Several thousand	Infinity.

Experiment 4.—(Table IV.) Rabbit 141 D; weight 2,130 gm. Received 0.1 gm. of ethylhydrocuprein hydrochloride per kilo of body weight, in 5 cc. of distilled water, subcutaneously. No toxic symptoms. Pneumococcus: stock strain of Group I rendered highly virulent for rabbits by passage.

TABLE IV.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies when plated after 6 hrs.' incubation.	No. of colonies when plated after 22 hrs.' incubation.
Before giving drug.....	281	Almost infinity	Infinity.
1⅚ hrs. after.....	351	62	0
2⅔ " "	285	134	Several thousand.
3⅔ " "	282	188	Infinity.
4⅔ " "	297	506	"
5⅚ " "	306	Almost infinity	——

Experiment 5.—(Table V.) Rabbit 2; weight 2,300 gm. Received 0.2 gm. of ethylhydrocuprein hydrochloride per kilo of body weight, in 5 cc. of distilled water, subcutaneously. Severe toxic appearances; died immediately after last bleeding. Pneumococcus: stock strain of Group II.

TABLE V.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 2½ hrs.' incubation.	No. of colonies after 4 hrs.' incubation.	No. of colonies after 6¼ hrs.' incubation.	No. of colonies after 10¼ hrs.' incubation.
Before giving drug.....	456	478	Almost infinity	Infinity	—
2½ hrs. after.....	460	382	245	35	1
7⅓ " "	478	456	390	379	127

Experiment 6.—(Table VI.) Rabbit 3; weight 1,700 gm. Received 0.15 gm. of ethylhydrocuprein hydrochloride per kilo of body weight, in 5 cc. of distilled water, subcutaneously. Severe toxic symptoms; died immediately after last bleeding. Pneumococcus: stock strain of Group II.

TABLE VI.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 2 hrs.' incubation.	No. of colonies after 5 hrs.' incubation.	No. of colonies after 11 hrs.' incubation.	No. of colonies after 24 hrs.' incubation.
Before giving drug.....	187	188	Almost infinity	Infinity	Infinity.
1¼ hrs. after.....	194	127	64	0	0
2½ " "	187	175	123	56	46
4¾ " "	192	181	212	Several thousand	Infinity.

Comment on Experiments 1 to 6, Inclusive.—A study of the figures shown in the protocols of these experiments reveals certain facts. The serum of animals given a suitable dose (*e. g.,* 0.1 gram per kilo of body weight) of ethylhydrocuprein hydrochloride subcutaneously has a strongly bactericidal action *in vitro* on the pneumococci, these microorganisms growing freely in normal rabbit serum. This property is possessed by serum obtained as early as one hour after the administration of the drug—in fact, it is at its maximum about this time, or a little later; having attained a maximum potency the bacteriolytic effect gradually falls off, and, in the case of a dose of 0.1 gram per kilo of body weight (a dose well tolerated), it passes into an inhibitory effect on the growth of the pneumococci within from 2½ to 3 hours after the animal received the drug; the inhibitory effect seems to restrain the free growth of the microorganisms for some hours, after which this action seems to be overcome; this inhibitory effect on growth, in its turn, disappears about 5 hours after the drug has been given. Further, the killing off of the pneumococci in the serum of animals treated with the drug is a gradual process, lasting several hours, according to the dosage of the drug and the amount of inoculation, etc. The same effects are to be seen in the serum of animals which have received a toxic dose of the drug, except that the duration of these actions is longer, and, perhaps, more powerful.

Experiments Illustrating the Effects on Pneumococci of the Serum of Animals Treated with the Free Base Ethylhydrocuprein (Optochin Base) Dissolved in Sterile Olive Oil, Given Subcutaneously (under the Skin of the Back).

Experiment 7.—(Table VII.) Rabbit 142 D; weight 2,200 gm. Received 0.075 gm. of ethylhydrocuprein base per kilo of body weight, in 6 cc. of olive oil, subcutaneously. No toxic symptoms. Pneumococcus: stock strain of Group I rendered highly virulent for rabbits by passage.

TABLE VII.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 4 hrs.' incubation.	No. of colonies after 22 hrs.' incubation.
Before giving drug.....	301	Almost infinity	Infinity.
1½ hrs. after.....	305	116	0
2½ " "	290	227	Almost infinity.
3½ " "	297	189	" "
4½ " "	351	408	Infinity.
5 " "	358	Several thousand	——

Experiment 8.—(Table VIII.) Rabbit 91 E; weight 2,200 gm. Received 0.1 gm. of ethylhydrocuprein base per kilo of body weight, in 6 cc. of olive oil, subcutaneously. No toxic symptoms. Pneumococcus: stock strain of Group II.

TABLE VIII.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.		No. of colonies after 4 hrs.' incubation.		No. of colonies after 22 hrs.' incubation.	
	Serum inactivated.	Serum not inactivated.	Serum inactivated.	Serum not inactivated.	Serum inactivated.	Serum not inactivated.
Before giving drug.....	321	—	Almost infinity	—	Infinity	—
1 hr. after.....	342	292	94	74	1	0
2½ " "	382	350	158	162	98	37
4¾ " "	305	323	207	257	Several thousand	Almost infinity.

Experiment 9.—(Table IX.) Rabbit 105 D; weight 1,750 gm. Received 0.1 gm. of ethylhydrocuprein base per kilo of body weight, in 6 cc. of olive oil, subcutaneously. No toxic symptoms. Pneumococcus: stock strain of Group I.

TABLE IX.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 3 hrs.' incubation.	No. of colonies after 7½ hrs.' incubation.	No. of colonies after 20 hrs.' incubation.
Before giving drug...	573	Several thousand	Infinity	—
2 hrs. after.....	349	200	41	0
3 " "	402	224	76	3
4⅙ " "	400	270	123	17
5⅙ " "	410	238	138	8

Experiment 10.—(Table X.) Rabbit 106 D; weight 1,700 gm. Received 0.1 gm. of ethylhydrocuprein base per kilo of body weight, in 6 cc. of olive oil, subcutaneously. No toxic symptoms. Pneumococcus: stock strain of Group I.

TABLE X.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 3 hrs.' incubation.	No. of colonies after 7½ hrs.' incubation.	No. of colonies after 20 hrs.' incubation.
Before giving drug...	397	Several thousand	Infinity	—
2 hrs. after.....	330	197	59	0
3 " "	338	229	125	14
4⅙ " "	358	298	190	552*
5⅙ " "	374	460	—	—

* Macroscopically there appeared to be no growth in the corresponding tube.

Experiment 11.—(Table XI.) Rabbit 104 D; weight 1,650 gm. Received 0.125 gm. of ethylhydrocuprein base per kilo of body weight, in 6 cc. of olive oil, subcutaneously. Animal showed a tendency to lie quiet for some hours after the injection. Pneumococcus: stock strain of Group I.

TABLE XI.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 3 hrs.' incubation.	No. of colonies after 7½ hrs.' incubation.	No. of colonies after 20 hrs.' incubation.
Before giving drug...	594	Several thousand	Infinity	Infinity.
2 hrs. after.....	520	149	51	0
3½ " "	419	159	107	0
4½ " "	368	256	1,200	Infinity.

Experiment 12.—(Table XII.) Rabbit 101 D; weight 1,720 gm. Received 0.15 gm. of ethylhydrocuprein base per kilo of body weight, in 6 cc. of olive oil, subcutaneously. Animal apparently well up to 6 hours after injection; died during the last bleeding. Pneumococcus: stock strain of Group II.

TABLE XII.

Serum obtained.	No. of colonies per 0.5 c. when plated immediately after inoculation.	No. of colonies after 5½ hrs.' incubation.	No. of colonies after 8½ hrs.' incubation.	No. of colonies after 25 hrs.' incubation.
Before giving drug...	976	Almost infinity	Infinity	Infinity.
2 hrs. after.....	1,100	180	57	0
3½ " "	1,328	386	292	0
5 " "	1,152	403	283	Almost infinity.
6 " "	1,284	Several thousand	Infinity	Infinity.

Experiment 13.—(Table XIII.) Rabbit 102 D; weight 1,770 gm. Received 0.15 gm. of ethylhydrocuprein base per kilo of body weight, in 6 cc. of olive oil, subcutaneously. No toxic symptoms. Pneumococcus: stock strain of Group II.

TABLE XIII.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 5½ hrs.' incubation.	No. of colonies after 8½ hrs.' incubation.	No. of colonies after 25 hrs.' incubation.
Before giving drug...	1,176	Infinity	Infinity	Infinity.
2 hrs. after.....	1,232	452	224	10
3½ " "	958	339	169	Infinity.
5 " "	1,152	500	441	Almost infinity.

Experiment 14.—(Table XIV.) Rabbit 121 F; weight 2,050 gm. Received 0.15 gm. of ethylhydrocuprein base per kilo of body weight, in 6 cc. of olive oil, subcutaneously. No toxic symptoms. Pneumococcus: stock strain of Group II.

TABLE XIV.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 5½ hrs.' incubation.	No. of colonies after 23 hrs.' incubation.
Before giving drug.....	68	Almost infinity	Infinity.
2 hrs. after.....	58	0	0
3 " "	64	0	0
4 " "	59	7	0
5 " "	43	18	0

Comment on Experiments 7 to 14, Inclusive.—These protocols, like those of Experiments 1 to 6, show that the serum of animals given subcutaneously a suitable dose of ethylhydrocuprein base (optochin base) dissolved in olive oil has a strongly bactericidal action *in vitro* on the pneumococci, that this property is possessed by serum obtained as early as 1 hour after the administration of the drug, and

that having attained a maximum potency the bacteriologic effect gradually falls off. In the case of a dose of 0.1 gram per kilo of body weight (a dose well tolerated), the bactericidal action passes into an inhibitory effect on the growth of the pneumococci in about four hours after the animal received the drug; here again, the inhibitory effect seems to restrain the free growth of the microorganism for some hours, after which this action seems to be overcome. The inhibitory effect, in its turn, ultimately disappears. As before, the protocols show that the killing off of the pneumococci in the serum of the treated animals is a gradual process. The effects are more lasting than when the hydrochloride in water is given by the same route.

Experiments Showing the Effects on Pneumococci of the Serum of Animals Treated with Ethylhydrocuprein Hydrochloride Dissolved in 10 Cc. of Normal Saline Solution and Given Slowly Intravenously.

Experiment 15.—(Table XV.) Rabbit 9; weight 2,000 gm. Received 0.02 gm. of ethylhydrocuprein hydrochloride per kilo of body weight, in 10 cc. of normal saline, slowly, intravenously.

Slight convulsions toward end of injection; immediately after injection the animal lay stretched out on floor and apparently could not rise. Complete recovery in a few minutes. Pneumococcus: stock strain of Group II.

TABLE XV.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 7 hrs.' incubation.	No. of colonies after 18 hrs.' incubation.
Before giving drug.....	405	Infinity	Infinity.
5 min. after.....	426	533	About 2,000.
1½ hrs. "	360	Infinity	Infinity.

Experiment 16.—(Table XVI.) Rabbit 10; weight 2,700 gm. Received 0.078 gm. of ethylhydrocuprein hydrochloride per kilo of body weight, in 10 cc. of normal saline, slowly, intravenously.

Convulsions and exophthalmos toward the end of the injection; immediately after injection the animal lay on floor and seemed collapsed; twitching of legs. Recovered in a few minutes. Pneumococcus: stock strain of Group II.

TABLE XVI.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 3½ hrs.' incubation.	No. of colonies after 7 hrs.' incubation.	No. of colonies after 20 hrs.' incubation.
Before giving drug...	304	249	Infinity	Infinity.
5 min. after.....	329	235	87	10
1½ hrs. "	340	315	Infinity	—
4½ " "	350	292	"	—

The serum of rabbits which had been given intravenously doses of the hydrochloride smaller than those given to Rabbits 9 and 10 showed no bactericidal or inhibitory effect on the pneumococci. An animal given 0.05 gm. per kilo of body weight intravenously died immediately after the injection with convulsions.

Comment on Experiments 15 and 16.—The serum of rabbits given a non-fatal dose of hydrochloride of the drug intravenously does not show such a strong or prolonged effect on pneumococci as when the drug is given subcutaneously. When given intravenously, toxic signs are more easily obtained than when other routes are used.

Experiments Showing the Effects on Pneumococci of the Serum of Animals Treated with Ethylhydrocuprein Base (Optochin Base), Dissolved in Olive Oil, and Given Intramuscularly.

Experiment 17.—(Table XVII.) Rabbit 120 D; weight 1,620 gm. Received 0.075 gm. of ethylhydrocuprein base per kilo of body weight, in 6 cc. of olive oil, intramuscularly. No toxic symptoms. Pneumococcus: stock strain of Group II.

TABLE XVII.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 10 hrs.' incubation.	No. of colonies after 22 hrs. incubation.
Before giving drug.....	61	Infinity	Infinity.
1½ hrs. after.....	60	142	"
3⅙ " "	71	Almost infinity	"
4⅙ " "	77	Infinity	"
4⅚ " "	73	"	"

Experiment 18.—(Table XVIII.) Rabbit 121 D; weight 1,850 gm. Received 0.075 gm. of ethylhydrocuprein base per kilo of body weight, in 6 cc. of olive oil, intramuscularly. No toxic symptoms. Pneumococcus: stock strain of Group II.

TABLE XVIII.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 10 hrs.' incubation.	No. of colonies after 22 hrs.' incubation.
Before giving drug.....	74	Infinity	Infinity.
1½ hrs. after.....	64	15	2
3½ " "	91	Several thousand	Infinity.
4¼ " "	91	Almost infinity	"
5 " "	89	Infinity	"

Experiment 19.—(Table XIX.) Rabbit 169 D; weight 2,000 gm. Received 0.1 gm. of ethylhydrocuprein base per kilo of body weight, in 6 cc. of olive oil, intramuscularly. Distinct toxic symptoms within 2 hours after administration of drug. Recovered next day. Pneumococcus: stock strain of Group II.

TABLE XIX.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 4½ hrs.' incubation.	No. of colonies after 21 hrs.' incubation.
Before giving drug.....	1,012	Almost infinity	Infinity.
1½ hrs. after.....	984	124	1
2½ " "	952	413	Infinity.
4½ " "	1,010	Several thousand	"

Comment on Experiments 17 to 19, Inclusive.—These experiments, illustrating the results of giving the base of the drug dissolved in oil intramuscularly, show that the drug is more toxic when given by this route than when given subcutaneously in the same vehicle and that the bactericidal effect on the pneumococci is not so prolonged, and probably not so intense, as when the subcutaneous route is used.

In addition to the experiments mentioned above, we have studied the bactericidal action of the serum of rabbits into the stomachs of which the hydrochloride of the drug, dissolved in 25 cc. of distilled water, was introduced by means of a stomach tube; the tolerance is greatest by this route, but the bactericidal effects are slight or absent; even by giving doses as large as 0.3 to 0.4 gram per kilo of body weight to rabbits of 2,000 grams' weight, and otherwise using the same technique as described above, we have not been able to demonstrate any bactericidal action of the serum of these animals on pneumococci within a period of from 2½ to 6 hours after administration of the drug, and only a slight inhibitory effect on their growth.

In order to gain some idea of the bactericidal action on pneumococci of the serum of man treated with the drug the experiments immediately to be described were carried out. The technique was the same as that described above.

Experiment 20.—(Table XX.) Normal man, M.; weight 50.8 kilos. Received, *per os,* in capsules, 0.5 gm. of ethylhydrocuprein hydrochloride. No toxic signs or symptoms. Pneumococcus: stock strain of Group II.

TABLE XX.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 2½ hrs.' incubation.	No. of colonies after 8 hrs.' incubation.	No. of colonies after 12 hrs.' incubation.	No. of colonies after 21 hrs.' incubation.
Before giving drug..	395	3,000 (approximately)	Infinity	Infinity	—
1 hr. after.....	458	382	391	1,500	Several thousand.
2 " "	560	311	337	3,000 (approximately)	Several thousand.
5 " "	374	400	Several thousand	Almost infinity	Almost infinity.

Experiment 21.—(Table XXI.) Normal man, A; weight 68.96 kilos. Received *per os,* in capsules, 0.5 gm. of ethylhydrocuprein hydrochloride. No toxic signs or symptoms. Pneumococcus: stock strain of Group II.

TABLE XXI.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 4⅔ hrs.' incubation.	No. of colonies after 9⅔ hrs.' incubation.	No. of colonies after 21 hrs.' incubation.
Before giving drug...	517	Almost infinity	Infinity	Infinity.
1½ hrs. after.....	560	540	Almost infinity	"
2½ " "	521	230	225	Almost infinity.
5⅔ " "	518	483	Several thousand	Infinity.

Experiment 22.—(Table XXII.) Man, B.; weight 52 kilos. Received 0.5 gm. of ethylhydrocuprein hydrochloride dissolved in 5.0 cc. of sterile redistilled water, subcutaneously, in left flank. Next day complained of pains in legs, had a slight rise of temperature, and the area around the injection site was hyperemic and showed a slight superficial edema. Pneumococcus: stock strain of Group II.

TABLE XXII.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.		No. of colonies after 3 hrs.' incubation.		No. of colonies after 8¾ hrs.' incubation.		No. of colonies after 21 hrs.' incubation.	
	Serum inactivated.	Serum not inactivated.	Serum inactivated.	Serum not inactivated.	Serum inactivated.	Serum not inactivated.	Serum inactivated.	Serum not inactivated.
Before giving drug....	76	112	1,300	2,000	Almost infinity	Infinity	Infinity	Infinity.
1 hr. after.....	68	54	25	40	34	19	Almost infinity	Infinity.
2 " "	65	52	29	27	22	140	Infinity	Infinity.
3 " "	61	68	38	37	153	467	"	Infinity.
4½ " "	56	59	24	31	713	431	"	Infinity.

Experiment 23.—(Table XXIII.) Woman, S.; weight 51 kilos. Received 0.5 gm. of ethylhydrocuprein hydrochloride dissolved in 5 cc. of sterile redistilled water, subcutaneously, in abdominal wall. Next day the patient complained of general malaise and headache, had a slight rise of temperature, and had a painful hyperemic area of infiltration and edema about 3 inches in diameter around the injection site which disappeared in a few days. Pneumococcus: stock strain of Group II.

TABLE XXIII.

Serum obtained.	No. of colonies per 0.5 cc. when plated immediately after inoculation.	No. of colonies after 3 hrs.' incubation.	No. of colonies after 8¾ hrs.' incubation.	No. of colonies after 21 hrs.' incubation.
Before giving drug.....	60	51	Almost infinity	Infinity.
1 hr. after.....	56	35	1,320	"
2½ " "	74	34	439	"

Comment on Experiments 20 to 23, Inclusive.—The figures show that the serum of a man to whom had been given a single dose of 0.5 gram of the hydrochloride of the drug either by the mouth or subcutaneously, has a decided inhibitory effect on the growth of the pneumococcus lasting for several hours, after which this action seems to be overcome, and has a bactericidal action which is much less strong than that shown in the protocols of the animal experiments described above. Similar effects are seen whether the drug be administered subcutaneously or by mouth. The employment of the

former method, however, subjects the patient to considerable discomfort.

The serum of a normal man to whom 1.8 grams of the hydrochloride of the drug were given by the mouth in 24 hours, that is, in four doses of 0.45 gram each given at regular intervals, showed a cumulative effect; namely, the bactericidal action was much stronger 2¾ hours after the last, than at a similar period after the first, dose.

DISCUSSION OF RESULTS.

In serum, the pneumococci show a tendency to form short chains composed of from two to four diploid forms. The figures in the foregoing protocols consequently must be regarded, not as giving the total number of pneumococci per 0.5 cc. of the inoculated serum, but as an index to that number. The figures, in other words, are relative, not absolute, but the conclusions drawn with regard to the bactericidal action of ethylhydrocuprein are perfectly valid, for the growth in normal serum serves as a control. Moreover, we have cumulative evidence of this bactericidal action of the drug in the fact that the progressive diminution in the number of pneumococci is seen in a considerable number of different experiments. Again, this progressive diminution frequently goes to the point of complete disappearance of living pneumococci in a few hours.

The protocols, therefore, set forth above show that the serum of rabbits treated with ethylhydrocuprein exerts a bactericidal action on the pneumococci in the test-tube, and later inhibits their growth. The intensity and the time of appearance and disappearance of these actions depend on the dosage, upon the route by which the drug is given, and on the method of administration. With the dosage used in the experiments above described, these effects last longest when the base is given in oil subcutaneously; they are not quite so lasting when the hydrochloride is given in water, subcutaneously; and are still less lasting when the base is given in oil, intramuscularly. To obtain bactericidal effects in rabbits by giving the hydrochloride dissolved in physiological saline intravenously, one must give toxic doses dangerous to the life of the animal. The giving of the hydrochloride directly into the stomach of rabbits does not seem to be efficient from the point of view of a resulting bactericidal action of the serum on the pneumococci.

It was thought that a possible explanation of the progressive decrease in the number of colonies in those tubes in which this was demonstrated might be an agglutinative effect of the drug on the pneumococci. We have frequently examined the contents of the tubes microscopically, macroscopically, and by cultural methods for sterility (the latter in the cases in which the plates showed no colonies), and we have never found any evidences of agglutination.

It will be noticed in the protocols of several experiments that, in the case of serum obtained when, apparently, the optochin concentration in the blood is diminishing, the number of colonies in the incubated tubes at first shows a progressive decrease (the number in the control normal serum, obtained before giving the drug, showing, at the same time, a progressive increase); but, that, later, after a few hours' incubation, the pneumococci progressively increase, until they become too numerous to count in the plates. This phenomenon is more apparent in tubes lightly inoculated than in tubes more heavily inoculated with pneumococci. Evidently, if the optochin concentration in the serum falls below a certain point in relation to the number of pneumococci present, the pneumococci which survive the bactericidal action may, after a few hours in contact with the drug in the incubator, acquire the property of overcoming this action of the drug and, therefore, grow freely. It would be interesting to investigate this property, so rapidly acquired, from the point of view of fastness. It is not due to a destruction of the bactericidal action of the serum caused by simple incubation, because serum incubated for 24 hours, and then inoculated and studied as above described, shows the same intensity of bactericidal action on pneumococci as samples of the same serum not previously subjected to incubation before inoculation. This overcoming of the action of the drug seems to be an entirely new, and, relatively, quickly acquired property of the pneumococci themselves.

It does not seem that there is any considerable difference in the bacteriolytic or inhibitory effects of serum obtained after administration of the drug whether such serum be used after having been inactivated for ½ hour at 56° C., or active.

CONCLUSIONS.

1. The serum of rabbits which have been previously treated with a single dose of ethylhydrocuprein (optochin) exerts a bactericidal action on, and, later, inhibits the growth of pneumococci in the test-tube.

2. These actions are most evident in the serum of rabbits when the base (optochin base) is given in oil subcutaneously; somewhat less when the hydrochloride of the drug is given in water subcutaneously; slight when the base is given in oil intramuscularly; and least evident, or absent, when the hydrochloride in water is introduced directly into the stomach. To get these effects by the intravenous route, toxic doses must be given, and, even with toxic non-fatal doses, the effects do not last long.

3. In the case of the base given in oil subcutaneously to rabbits in a dosage of 0.1 gram per kilo of body weight, the bactericidal action of the serum is at its maximum about one hour after administration, and it passes into an inhibitory effect about four hours after the drug has been given.

4. In man the same inhibitory and bactericidal actions of the serum are present when a single dose of 0.5 gram of the hydrochloride of the drug is given by the mouth or subcutaneously, but the bactericidal action is not so marked as in rabbits.

5. When the optochin concentration in the serum has, apparently, diminished to a certain point in relation to the number of pneumococci present, the pneumococci which have survived the bactericidal action for a few hours acquire the power of growing freely.