A C T A A D R I A T I C A
INSTITUT ZA OCEANOGRAFIJU I RIBARSTVO — SPLIT
FNR JUGOSLAVIJA

Vol. V. No. 7.

THE BACTERICIDAL AND BACTERIOSTATICAL ACTION OF ANTIBIOTICS ON MARINE BACTERIA

V. Cvilć

SPLIT 1953

THE BACTERICIDAL AND BACTERIOSTATICAL ACTION OF ANTIBIOTICS ON MARINE BACTERIA

I. Penicillin and Streptomycin

(With 5 Graphs)

by

Vlaho Cviić

Institute of Oceanography and Fisheries, Split

The ascertainment of the bactericidal and bacteriostatical action of antibiotics on marine bacteria is important for several problems of biological oceanography. It is particularly important for the question of investigating the physiology of marine invertebrates nourishment, — respectively of the role that bacteria have in their feeding.

During the investigation we must needs sterilize the organism, i. e. remove all the bacteria brought into the experiment and preventing us to obtain precise results. Although to the present day there are a lot of methods to sterilize the organism, no one of them proves to be satisfactory, namely no one allows of an exact ascertainment of the quantitative participation of marine bacteria in the nourishment of some sea organisms. Of all the present methods for the sterilization of Protozoa, the most satisfactory has proved to be by renewed rinsing of the organism with sterilized water (Luck et al. 1931). Yet neither this method can fully satisfy, since it does not eliminate the periphytic and epiphytic bacteria from the organism and they prove to be a hindrance to the experiment. The other methods of sterilizing Protozoa are still more unsuitable, such as the cataphoretic method of electrical fields (Amster 1922), the method of negative geotacsy (Luck et al. 1931) or the method of sterilization of the cyst by means of chemical substances (Frosch 1897, Severtzoff 1924, Walter 1908, Oehler 1924). — Still greater troubles appeared in experiments when examining the food material of higher invertebrates as Copepods, Nematods, Tubificids, Chirononuds, some Lamelibranchiata, Ascidia, Crustacea from mud and sand, Rotifers as well as larvae of different marine organisms. Here the difficulties are increased by the fact that owing to the bigger external and especially internal surface of the organism, there is a greater possibility of attachment and development of the epiphytic and periphytic bacteria.

The second question is the sterilization of the phitoplanktonic organisms during the physiological studies (i. e. assimilation) which requires clean cultures of phitoplanktonic organisms. Here too, up to now, the method of renewed rinsing of the organisms with sterilized water proved to be the most successful (ZoBell and Allen 1933) but nevertheless some bacteria still did remain on the organism. The other methods, as the use of mineral basis for the cultivation of Diatoms (Allen 1913), Schreiber 1927) the use of cataphoretic methods of electrical fields (Chapman 1929), the use of bactericidal substances and aniline dyes (ZoBell and Long 1938), the use of higher temperature, irradiation or different chemical substances, proved also to be unsuitable, since they do not eliminate completely the bacteria or they have an equally letal action both on the bacteria as on the phitoplankton organisms.

Besides these two main problems several others exist too, such as the cleansing of the sea-water aquarium infected by the bacteria, the sterilization of sea fish and finally the intricated problem of the fouling of ships' keels, the destruction of submarine constructions, nets, ropes etc. by marine bacteria. Some of these problems have an eminently practical character and therefore are important from this point of view too.

It is true that owing to the present conditions in the production of antibiotics they cannot be used to solve these problems in practice, but by means of a simplification of the production or by the invention of synthetically produced antibiotics, there is no doubt that this question will be current too. Leontjev (1950) used antibiotics as a means for the sterilization of organisms when experimenting with hemoflagellates Leishmania donovani and Tripanozoma crusi and to the basis he added penicillin (1000 units/ccm) and streptomycin (1000 units/ccm). It has been ascertained that these quantities of antibiotics do not provoke any troubles to these flagellates either with regard to their growth or to their morphology or physiology, and they fully prevent the growth of bacteria. King (1950) in an aquarium where he kept Axolotle which suddenly began to be destroyed, added 20 units/ccm of penicillin and thus caused the death to be lessenelto its normal. Tarr, Santhcott and Bissett (1950) used different antibiotics as a means against the destruction of fish caused by bacteria and they ascertained that the most suitable action was afforded by aureomycin and that penicillin and streptomycin have a weak effect. However the authors themselves remark that the results obtained ought to be considered as preliminary. Fish (1950) has

ascertained that washing off Enteromorphae in a solution of penicillin conc. 5000 units/ccm and then cultivating them in a broth to which penicillin in conc. of 500 units/ccm has been added, prevents the growth of bacterial population for seven days. The same author has verified that even conc. of penicillin of 1000 units/ccm has no negative effect on the nimbleness of a green flagellata. Spencer (1952) has found in his experiments with Nitzschia closterium forma minutissima, that conc. of penicillin of 100, 500 and 1000 units/ccm fully prevents the growth of bacterial population for twenty-one days. He has ascertained that streptomycin has a weaker bactericidal action than penicillin. Conc. streptomycin, the author says, of 1000 units/ccm has no inhibitory effect on the bacterial population in the cultivation of Nitzschia clostridium; the bacterial population develops in such a conc. after eleven days, while conc. of 500 units/ccm exerts an inhibitory effect on the growth of Nitzschias. The same author has found that a combination of penicillin and streptomycin in conc. of 500—500 units/ccm has a fully bactericidal action on the bacterial population.

Although we have a pretty large number of methods for sterilizing organisms, which is to prove that the problems to which they are to be appllied are of the uttermost importance, — we may however say that up to the present day, we have no general method which might completely satisfy. — Some of the existing methods, it is true, may satisfy to a certain extent, but they have been tried only for one or some groups of organisms only, and in most cases they are rather unreliable methods. In our work, owing to the importance of the problem itself we tried to study it in detail examining firstly the effects of antibiotics on marine bacteria both on some particular species and on the bacterial population in natural sea-water. It was our aim to find out the lowest concentrations of particular antibiotics which have a decisive bactericidal action on marine bacteria. — In the second phase of our work we shall examine the effect of the most suitable concentrations of antibiotics obtained with regard to the different species of marine invertebrates and marine phitoplanktons.

In this work the laboratory-technicians Ojdana Marović and Peter Bilić have afforded to the author a precious technical help and he would fain offer them his heartest thanks.

MATERIAL AND METHODS

Forty one species of marine bacteria have been used in the experiments, Of these, sixteen species are from different localities of the Pacific ocean, which we got from the Scripps Institution of Oceanography — University of California, thanks to the kindness of Dr. ZoBell, chief of the Microbiological laboratory of that Institute. The remaining twenty five species were taken from different localities of the Adriatic Sea, and have not as yet been fully determined and they are therefore mentioned with numbers, which are used as a temporary mark. We give a table with short characteristics of the species used in our experiments. (Table No. 1).

The sea-water employed in the experiments has been taken from the gulf of Kaštela. — In order to avoid as far as possible the influence of land and the eventual intrusion of soil bacteria in the sea-water, the water has been taken 1.000—1.500 m. from the coast at a depth of 0—1 m. Some quantities of sea-water for the single experiments were put into sterilized Erlenmeyers or small flasks (250—500 ccm in each) under sterilized conditions.

The antibiotics used were: Penicillin G. (crystal) supplied by »Galenika« (Zemun, F. P. R. Y.) packages of 200.000 in vials and Dihydrostreptomycin sulfate (crystal) supplied by Pfizer (Brooklyn, USA) packages of equiv 5 gr. in vials. The antibiotics are besides all the precautions of sterilization also diluted with sterilized water and added in a solutional condition to the broth or sea-water in the ordained quantity. — In the experiments the penicillin is dosed according the customary Oxford International units (O. E.) and from 1600 to 1650 units are contained in 1 mgr. The Streptomycin is dosed in equiv. milligrams.

To cultivate the species we have empleyed a broth composed as follows: Beef extract 2 gr. Bacto-pepton 2 gr., yeast extract 1 gr. Fe PO_4 0,05 gr. 750 ccm of rested sea-water, 250 ccm of distilled water, pH. 7,6. All the species in this broth have grown perfectly well. The broth to which was added a determined concentration of antibiotics, was then subdivided in test tubes (of 6 ccm each) and these were then inoculated to the species from cultures 48 h. old. — The cultures were incubated in the dark at a room temperature and then examined. Where it has not been possible to ascertain with certitude a growth in the broth, the agar-culture was inoculated. To each series of test tubes was added one controlling non-inoculated test tube.

Tab. 1.

No. *Br.*	Mark of the strain *Oznaka soja*	Name of the strain *Ime soja*	Shape *Oblik*	Gram-stain *Gram-bojanje*
1	502	Pseudomonas enalia *(ZoBell-Upham)*	rods *štapići*	gram —
2	510	Micrococcus euryhalis *(ZoBell-Upham)*	coccus *koki*	gram +
3	511	Serratia marinorubra *(ZoBell-Upham)*	short rods *kratki štapići*	gram —
4	513	Actinomyces marinolimosus *(ZoBell-Upham)*	filamentous micelium *nitasti micelium*	gram —
5	514	Vibrio marinopraesens *(ZoBell-Upham)*	twisted rods *savijeni štapići*	gram —
6	517	Achromobacter stenohalis *(ZoBell-Upham)*	rods *štapići*	gram —
7	518	Vibrio algosus *(ZoBell-Upham)*	twisted rods *savijeni štapići*	gram —
8	546	Vibrio marinovulgaris *(ZoBell-Upham)*	twisted rods *savijeni štapići*	gram —
9	552	Micrococcus infimus *(ZoBell-Upham)*	coccus *koki*	gram +
10	557	Micrococcus maripuniceus *(ZoBell-Upham)*	coccus *koki*	gram +
11	562	Bacillus imomarinus *(ZoBel-Upham)*	rods *štapići*	gram +
12	565	Pseudomonas oceanica *(ZoBell-Upham)*	rods *štapići*	gram —
13	581	Pseudomonas aestumarina *(ZoBell-Upham)*	rods *štapići*	gram —
14	585	Bacillus filicolonicus *(ZoBell-Upham)*	rods *štapići*	gram +
15	618	Actinomyces halotrichis *(ZoBell-Upham)*	granulous micelium *granati micelium*	gram —
16	642	Sarcina pelagis *(ZoBell-Upham)*	coccus *koki*	gram +
17	186	From Adriatic Sea *Iz Jadranskog mora*	short rods *kratki štapići*	gram —
18	202	— „ —	coccus *koki*	gram +

No. *Br.*	Mark of the strain *Oznaka soja*	Name of the strain *Ime soja*	Shape *Oblik*	Gram-stain *Fram-bojanje*
19	204	From Adriatic Sea *Iz Jadranskog mora*	short rods *kratki štapići*	gram —
20	205	— „ —	short rods *kratki štapići*	gram —
21	209	— „ —	rods *štapići*	gram +
22	210	— „ —	coccus *koki*	gram +
23	213	— „ —	rods *štapići*	gram —
24	215	— „ —	rods *štapići*	gram +
25	216	— „ —	rods *štapići*	gram +
26	217	— „ —	coccus *koki*	gram +
27	218	— „ —	rods *štapići*	gram —
28	219	— „ —	rods *štapići*	gram +
29	220	— „ —	rods *štapići*	gram —
30	221	— „ —	coccus *koki*	gram +
31	223	— „ —	rods *štapići*	gram —
32	224	— „ —	rods *štapići*	gram —
33	225	— „ —	rods *štapići*	gram +
34	226	— „ —	short rods *kratki štapići*	gram —
35	235	— „ —	rods *štapići*	gram +
36	236	— „ —	rods *štapići*	gram —
37	237	— „ —	rods *štapići*	gram +

No. *Br.*	Mark of the strain *Oznaka soja*	Name of the strain *Ime soja*	Shape *Oblik*	Gram-stain *Gram-bojanje*
38	238	From Adriatic Sea *Iz Jadranskog mora*	rods *štapići*	gram —
39	239	— „ —	coccus *koki*	gram +
40	240	— „ —	granulous micelium *granati micelium*	gram —
41	262	— „ —	rods *štapići*	gram —

The calculation of bacteria during the experiment with sea-water was performed by means of the plate on agar nutrient of the following composition: 8 gr. nutrient-broth, 1 gr. glucose, 2 gr. pepton, 0,1 Fe. Po4, 15 gr. agar-agar, 750 ccm of sea-water, 250 ccm of distilled water pH-7,6. — The inoculated plates were incubated in the dark at a room temperature (22—24° C) during 5—6 days and then reckonede. — In case of doubtful results the tests were repeated over again for three times, whereas each test was made twice. The same can be said for the tests with the single species.

EXPERIMENTS

1: Bacteriostatical action of penicillin and streptomycin on the single species of marine bacteria

Up to now we have no particulars in literature about the bacteriostatical or impeding action of penicillin and streptomycin on marine bacterial species. In order to get a picture as far as possible complete both of the resistance or of the susceptibility of marine bacteria towards these antibiotics, we have taken in our experiments twelve, respectively nine different concentrations of penicillin and streptomycin. — The results obtained (fig. 1 and table 2) show that the differences in the resistance of marine bacteria against penicillin are very big. The concentration of penicillin of 1 unit/ccm has an inhibiting action on 20 species (50%) out of 40, while middlestrong concentration up to 600 units/ccm have an

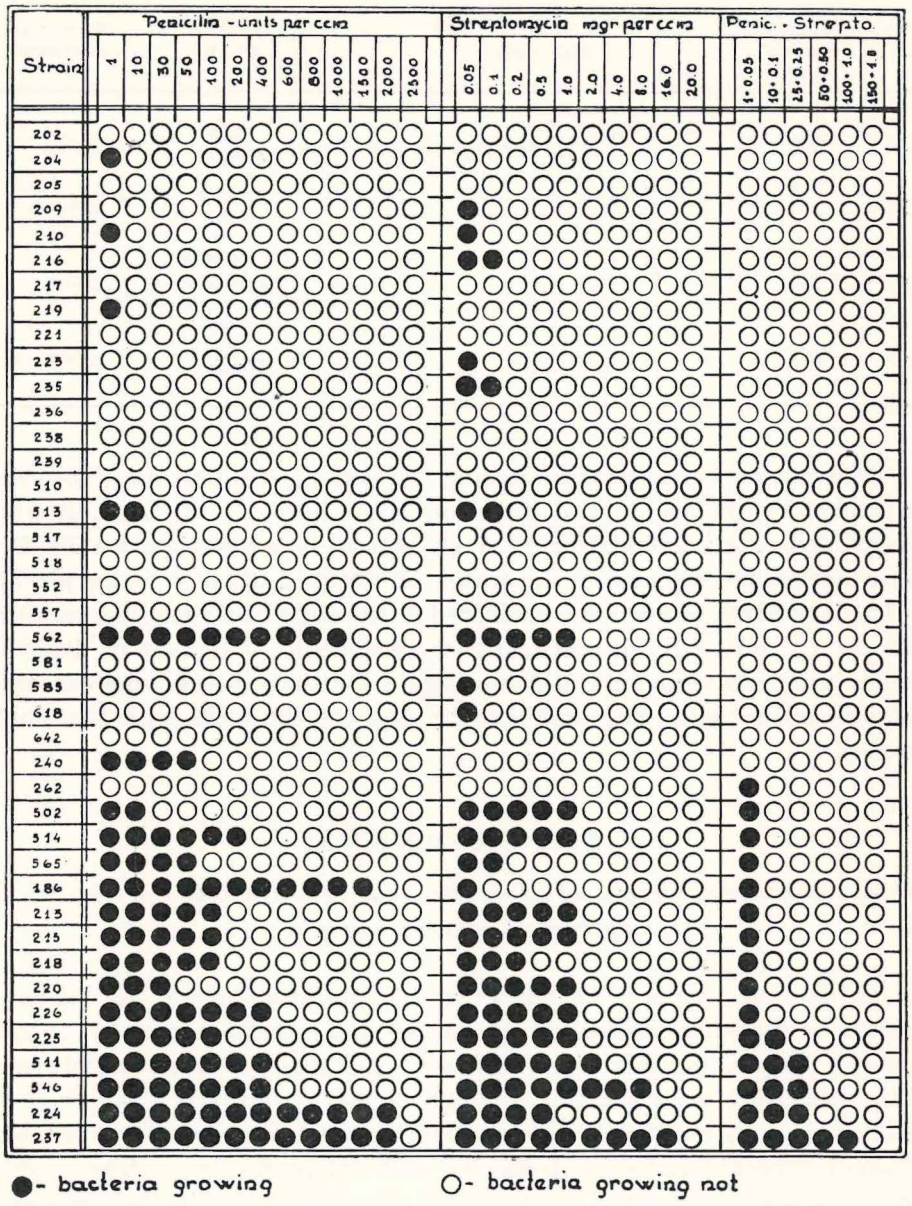

Fig. 1. Bacteriostatical action of penicillin and streptomycin on 41 strains of marine bacteria

inhibiting result on 36 species (90%), the remaining species (10%) resist also against concentrations of penicillin of 1000, and respectively of 2000 units/ccm. — A concentration of penicillin of 2500 u/ccm has a bacteriostatical effect on all the 40 species. On table No. 2 we show the detailed reaction of the species towards the different concentrations of penicillin. In the experiment there were altogether 40 species, of which 9 coccus (all 9 gram-positive) and 31 rod-like (of which 9 gram-positive and 22 gram-negative). As it appears from this table all the coccus are susceptible (9 species, those rodlike and one species with granulous micelium — No 618). Out of a total of 23 species susceptible to concentrations of penicillin up to 30 units/ccm, fourteen species (nine of which coccus) are gram-positive, and nine species are gram-negative. The middle-resistant species, i. e. species which withstand concentrations of penicillin from 30 to 600 units/ccm are thirteen in number and all of them rod-like, — and

Tab. 2. Bacteriostatical action of Penicilin and Streptomycin on 41 strains of marine bacteria.

Bakteriostatičko djelovanje penicilina i streptomycina na 41 sojeva morskih bakterija.

Penicillin			Streptomycin			Penicillin+Streptomycin		
Concetr. Units per ccm *koncetr. jed. ccm*	Strain destroyed *ugiba sojeva*	% of strains destroyed *% ugiba sojeva*	Concetr. mgr. ccm *koncentr. mgr. ccm*	Strains destroyed *ugiba sojeva*	% of strains destroyed *% uginulih sojeva*	Koncetr. Units+mgr. per ccm *koncetr. jed.+ mgr. na ccm*	Strains destroyed *ugiba sojeva*	% of strains destroyed *% uginulih sojeva*
1	20	50 %	0,05	17	42,5%	1+0,05	25	62 %
10	23	57,5%	0,1	23	57,5%	10+0,1	35	87,5%
25	25	62,5%	0,25	27	67,5%	25+0,25	36	90 %
50	26	65 %	0,50	28	70 %	50+0,5	39	97,5%
100	28	70 %	1,0	29	75 %	100+1,0	39	97,5%
200	32	80 %	2,0	37	92,5%	200+2	40	100 %
400	33	82 %	4,0	38	95 %	—	—	—
600	36	90 %	8,0	38	95 %	—	—	—
800	36	90 %	16,0	39	97,5%	—	—	—
1 000	36	90 %	20,0	40	100 %	—	—	—
1.500	37	92,5%	—	—	—	—	—	—
2.500	40	100 %	—	—	—	—	—	—

of them eleven species are gram-negative and 2 species gram-positive. — The most resistant species are No. 562, 186, 224 and 237. All these four species are rod-like, of which two are gram-positive (562 and 237), and two are gram-negative (186 and 224). — However it is quite clear from these particulars, that coccus are the most susceptible sea bacteria towards penicillin, and that there are no sensible general differences of susceptibility with the rod-shaped, respectively of resistance between gram-positive and gram-negative species.

The bacteriostatical action of streptomycin is like that of the penicillin's. Out of a total of 40 species, a koncentration of 0,05 mgr/ccm streptomycin has an inhibiting action on seventeen species (42.5%). Middle strong concentrations of streptomycin (till 8 mgr/ccm) have an inhibiting action on a total of 38 kinds (95,0%) and only two kinds (No 546 and 237) resist even to a concentration of streptomycin of 16 mgr/ccm. A concentration of streptomycin of 20,0 mgr/ccm has a bacteriostatical effect on all the 40 species. From the results shown in fig. 1, it appears that all coccus (9 species) and 12 species of rod-like shapes as well as two species with granulous micelium (240 and 618), are susceptible to streptomycin. Out of a total of 23 species susceptible towards a concentration of streptomycin till 0,1 mgr/ccm twelve species are gram-positive (of which 9 are coccus), and eleven species are gram-negative. There are 15 middle resistant species towards streptomycin, i. e. species which can withstand concentrations of streptomycin till 2 mgr/ccm; all of them are rods of which five are gram-positive and 10 are gram-negative. The most resistant against streptomycin are the species No. 546 (gram-regative rods) and 237 (gram-negative rods). It appears therefore from the aforesaid results that coccus shaped marine bacteria are susceptible both to penicillin and streptomycin, while in the reaction of gram-positive and gram-negative rod-like shapes against streptomycin there is no regularity.

Twenty-three species are susceptible towards penicillin and the same species, — two excepted (No. 216 and 235) are also susceptible to streptomycin. On the other hand out of 23 species susceptible to streptomycin, — two excepted (No. 240 and 186), all are susceptible to penicillin as well. Of the middle resistant species (13 against penicillin and 15 against streptomycin) ten of the same species are middle-resistant against both the antibiotics. A somewhat bigger difference in the resistance against penicillin and streptomycin is shown by the species No. 186 and 224 (resistant against penicillin but susceptible towards streptomycin) and the species No. 562 which is rather susceptible towards streptomycin. Highly

resistant against penicillin and streptomycin is the species No. 237 (gram-positive rod-shaped Clostridium sp). — We can therefore conclude that there is some parallelism between the action of penicillin and streptomycin on the species of marine bacteria, respectively in the resistance afforded by the marine bacteria against these antibiotics.

Since the action of penicillin and streptomycin on the single species has shown a certain parallelism, some experiments have been made about the action of both combined antibiotics on the single species. Six different concentrations have been taken for each antibiotic, and attention has been paid to the fact that such concentrations should correspond to those of the previous experiments with the single antibiotic. — The results of these experiments are given in the fig. No. 1 and table No. 2 as well as on the Fig. No. 2. — The combination of penicillin-streptomycin has a keener destructive action on the single species than each antibiotic separately. — Of the 40 chosen species, thirty-five species (87.5%) are susceptible to the

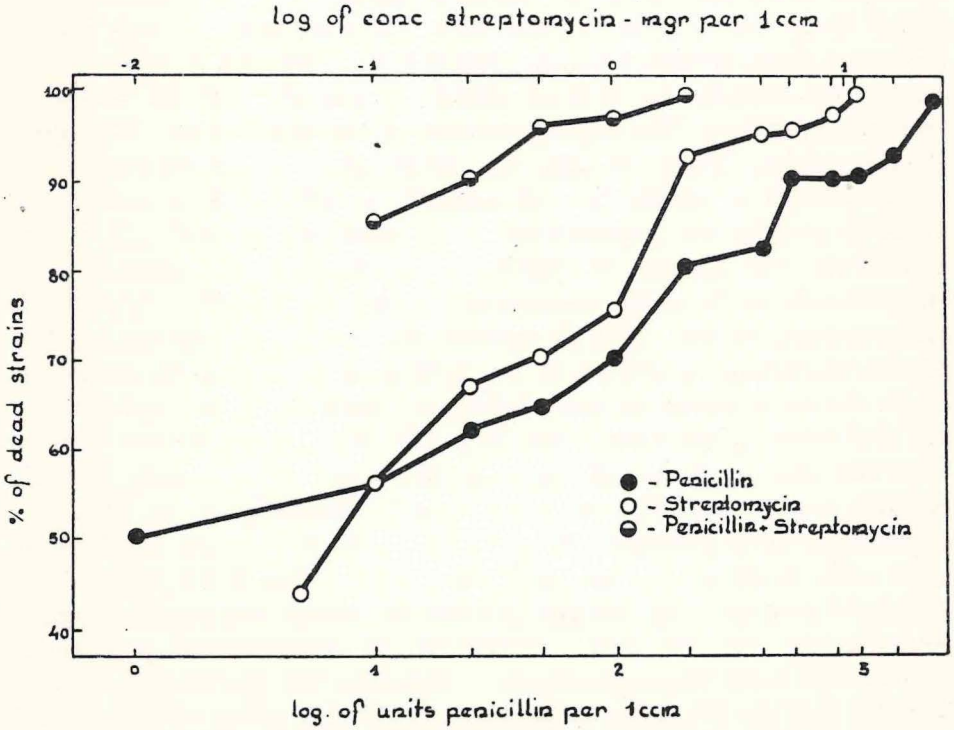

Fig. 2. Resistence of the strains of marine bacteria against different concentrations of penicillin and streptomycin

combined 10 units/ccm penicillin + 0,1 mgr/ccm streptomycin, while the antibiotics tal en separately in these concentrations had an inhibiting action od 23 species (penicillin) and respectively on 23 species (streptomycin), i. e. 57.5% of the species. There are 39 species (i. e. 97,5%) of middle-resistant species, viz. species which can withstand a concentration of 50 units/cm penicillin + 0,50 mgr/ccm streptomycin, — while the single penicillin in that concentration has an inhibiting result on 26 species (65%) and the single streptomycin on 28 species (70%). The concentration of 100 units/ccm penicillin + 1,0 mgr/ccm streptomycin is supported by only one species (No. 237), and a concentration of 150 units/ccm penicillin + + 1,5 mgr/ccm streptomycin proved to have a bacteriostatical action on all the 40 species. The effect of the single penicillin in such a concentration (100 units/ccm) has been tolerated by 12 species and 11 species can tolerate the concentration of streptomycin alone (1,0 mgr/ccm).

Out of 26 species susceptible to the combination penicillin-streptomycin, there are 9 species coccus (all gram-positive), 24 rod-shaped species and 3 species with micellium (of which 22 gram-negative and 5 gram-positive). Three species (No. 224, 546 and 511) are of a middleresistance against the combination of both antibiotics and all three are rod-shaped and gram-negative. The most resistant is the species No. 237 (gram-positive rods). These results too show that all coccus-shaped are susceptible to a combination of penicillin-streptomycin as well as the greatest part of the gram-positive (one excepted No. 237). — When comparing the species susceptible to penicillin or respectively to streptomycin with those susceptible to the combination of penicillin-streptomycin, we note that 21 species (52,5%) are susceptible to either of the antibiotics as well as to the combination of both the antibiotics. In all the three cases the most resistant species is No. 237 (Clostridium sp) and after it, the species No. 224, 546 and 511. — We can conclude therefore that in all the thre cases the effect of the antibiotics on marine bacteria has a parallelism as far as their susceptibility is concerned, respectively their resistance, since a total of 25 species out of 40 behave quite alike in all the three cases. A certain remarkable difference is observeld only with the species 185 and 562 which are resistant against penicillin but are far more susceptible to streptomycin and to the combination penicillin-streptomycin. — Likewise the species 213, 215, 218, 220, 226 and 514 which are more susceptible to the action of the combined penicillin-streptomycin than to the action of these two antibiotics given separately.

II: The bactericidal action of antibiotics on bacteria in sea water

Three series of experiments have been performed: the action of penicillin, the action of streptomycin and the action of the combination of penicillin-streptomycin on the bacterial population in natural sea-water under laboratorial conditions. In all the three series of experiments 7—8 different concentrations of antibiotics have been taken, endeavouring to determineas exactly as possible the critical concentration, in order to get the lowest concentration having a bactericidical action on all bacteria present ih the sea-water. — Besides each experiment the reaction of the bacterial population was followed in a controlling vessel wihout the addition of antibiotics. The tests were made in a room temperature and the vessels were kept in the dark.

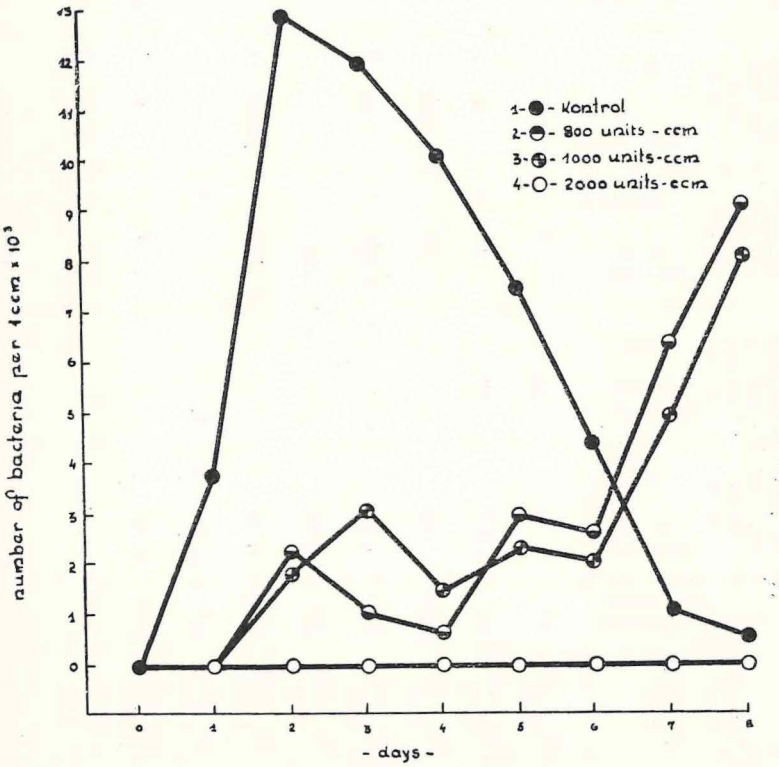

Fig. 3. Reaction of the bacterial population in natural sea water against different concentrations of penicillin

Tab. 3. Bactericidal action of Penicillin on marine bacteria.
Baktericidno djelovanje penicilina na morske bakterije.

No. *Br.*	Date of test *Datum probe*	Temper of room *Temper. sobe*	100 units per ccm *100 jed. na ccm*		300 units per ccm *300 jed. na ccm*		500 units per ccm *500 jed, na ccm*		800 units per ccm *800 jed. na ccm*		1.000 units per cm *1.000 jed. na ccm*		1.500 units per ccm *1 500 jed. na ccm*		2.000 units per ccm. *2.000 jed. na ccm*		Kontrol *Kontrolna*	
			bact. ccm *bakt. ccm*	strains *sojeva*	bact. ccm *bakt. ccm*	strains *sojeva*	bact. ccm *bakt. ccm*	strains *sojeva*	bact. ccm *bakt. ccm*	strains *sojeva*	bact. ccm *bakt. ccm*	strains *sojeva*	bact. ccm *bakt. ccm*	strains *sojeva*	bact. cm. *bakt. ccm*	strains *sojeva*	bact. ccm *bakt. ccm*	strains *sojeva*
1*	22. VIII 1952	25°C	32	8	46	7	43	7	37	8	47	8	52	7	49	6	39	8
2	23. VIII 1952	26°C	9.870	5	12.100	3	17	5	7	3	2	2	1	1	—	—	3.710	5
3	24. VIII 1952	28°C	5.190	3	8.740	2	1.523	3	1.978	2	2.075	2	—	—	—	—	12.940	5
4	25.VIII 1952	28°C	9.180	3	19.170	2	8 364	3	3.028	2	1.010	2	2	2	—	—	11.900	5
5	26. VIII 1952	28°C	18.300	2	8.320	2	1.949	4	1.543	3	689	2	1	1	—	—	10.010	6
6	27. VIII 1952	27°C	8.780	3	2.560	2	12.060	2	2.425	2	2.760	2	—	—	—	—	7.450	5
7	28. VIII 1952	26°C	15.200	2	4.620	3	10.739	2	2.686	2	2.085	2	—	—	—	—	4.300	5
8	29. VIII 1952	27°C	9.750	3	5.280	3	3.805	3	6.490	3	5.185	2	—	—	—	—	1.110	4
9	1 .IX 1952	28°C	—	—	—	—	10.606	2	9.148	3	8.259	2	—	—	—	—	617	4
	Average: *Prosjek:*		10.883	—	8.684	—	6.135	—	3.413	—	2.752	—	1	—	—	—	6.504	—

1* Number of bacteria in 1 ccm before adding the antibiotic.
Broj bakterija na 1 ccm prije dodavanja antibiotika.

The action of the different concentrations of penicillin in natural sea-water under laboratorial conditions and the reaction of the bacterial populations against the antibiotics are shown on Table No. 3 and fig. 3. — We can see from the results that low concentrations of penicillin of 100 and 300 units/ccm do not provoke any diminution of the population, but on the contrary in relation with the population ih the controlling vessel it develops far better and after 4—5 days it attains a maximum of 19—18000 bacteria per 1 ccm, against 10000 bacteria per ccm in the controlling vessel. Likewise a concentration of penicillin of 800 units/ccm has a remarkable bactericidical effect and the development of the population is weaker than in the controlling vessel. A general bactericidical action is obtained with a concentration of 2000 units penicillin per ccm. In sea water into which penicillin in different concentrations has been added the number of bacterial species rapidly decreases and after 24—48 hours from a total of 7—8 species it falls to 3—2 bacterial species which withstand against penicillin and appear till the end of the experiment. In the controlling vessel the picture is different, for in it from 8 species existing at the beginning of the experiment the number of species during the maximum development of the population, iz. after 3 days falls to 5 bacterial species. The fact that the bactericidal action of penicillin on the development of the bacterial population in sea water is actually increased with the rising of the concentration of this antibiotic in the sense of a delaying in the multiplication of resistant species, is to be noticed from the average number of bacteria for 1 ccm in the single experiments. Such an verage in a concentration of penicillin of 100 units/ccm amounts to 10883 bacteria per ccm and decreases with the rising of the concentration of penicilin, so that in a concentration of 1000 units/ccm it amounts to 2762 bacteria per ccm. (Table No. 3). In the controlling vessel the average number of bacteria per ccm was of 6504, i. e. an average as in the concentration of penicillin of 500 units per ccm. — Otherwise the bacterial population in the controlling vessel developed in a normal way, attained its maximum after 3 days and to the end of the experiment it was in a gradual dectreasing.

The reaction of the bacterial population in sea-water under laboratorial conditions against the different concentrations of streptomycin is shown in the table No. 4 and fig. 4. — We can notice from the results obtained that the bactericidal effect of streptomycin is weaker than the effect of penicillin. In the lower concentrations of streptomycin (1,2 to 12,0 mgr/ccm) the bacterial population develops much better than in the controlling vessel,

and its decreasing with the rising of the concentration in relation with the one observed in the controlling vessel, is much slower. Only at a concentration of 20,0 mgr/ccm of streptomycin the bactericidal effect is visible and the total bactericidal action appears already at 26,0 mgr/ccm. As for the

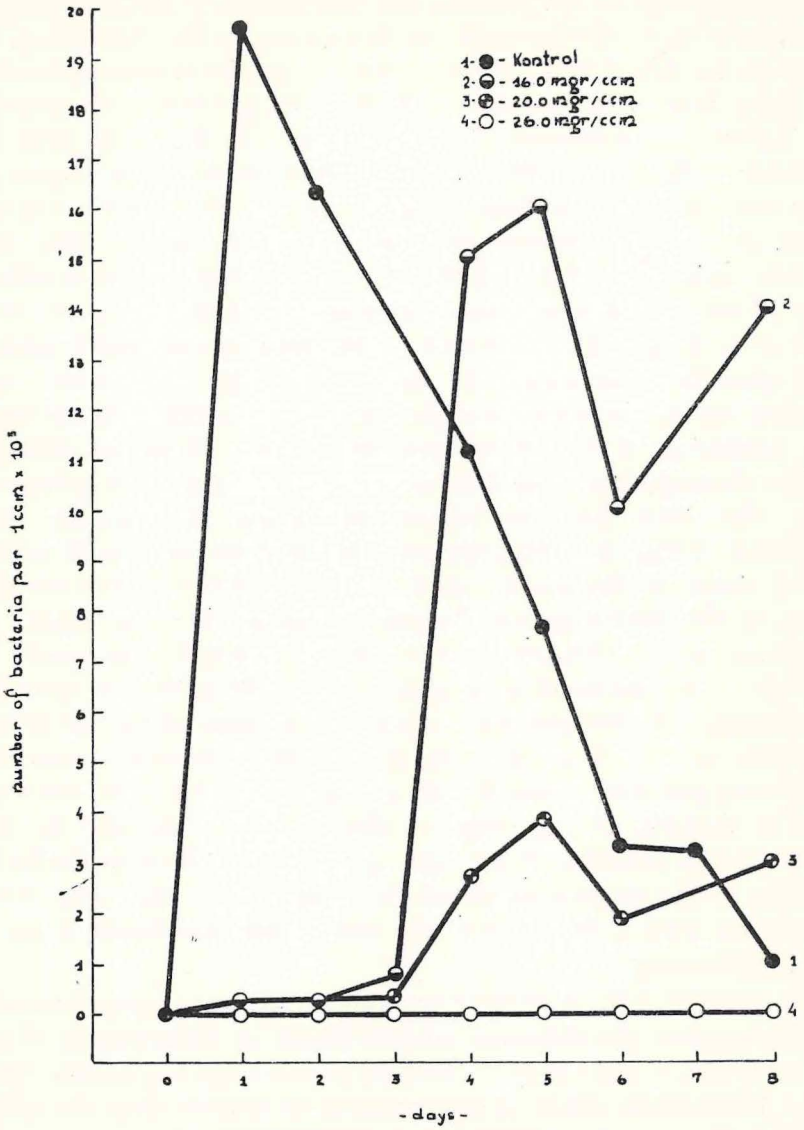

Fig. 4. Reaction of the bacterial populaton in natural sea water against different concentrations of streptomycin

Tab. 4. Bactericidal Action of Streptomycin on marine bacteria.
Baktericidno djelovanje streptomicina na morske bakterije.

No. *Br.*	Date of test *Datum probe*	Temper. of room *Temper. sobe*	1,20 mgr ccm		3,60 mgr ccm		6,0 mgr ccm		10,0 mgr ccm		12,0 mgr ccm		16,0 mgr ccm		20,0 mgr ccm		26,0 mgr ccm		Kontrol	
			bact. ccm *bakt. ccm*	strains *sojeva*	bact. ccm *bakt. ccm*	strains *sojeva*	bact. ccm *bakt. ccm*	strains *sojeva*	bact. ccm *bakt. ccm*	strains *sojeva*	bact. ccm *bakt. ccm*	strains *sojeva*	bact. ccm *bact. ccm*	strains *sojeva*	bact. ccm *bact. ccm*	strains *sojeva*	bact. ccm *bakt. ccm*	strains *sojeva*	bact. ccm *bact. ccm*	strains *sojeva*
1*	4. IX 1952	27°C	49	6	54	7	55	7	48	6	53	6	48	7	61	8	51	7	53	7
2	5. IX 1952	27°C	11	2	4	2	6	2	2	2	2	1	—	—	1	1	—	—	19.680	4
3	6. IX 1952	27°C	11.030	2	4.950	3	6.950	3	3.130	2	21	2	9	2	5	2	—	—	16.380	5
4	7. IX 1952	25°C	25.900	2	19 520	2	20.320	3	12.050	2	1.150	2	620	2	32	2	—	—	12,560	5
5	8. IX 1952	26°C	over-*preko* 40.000		16 200	2	18.100	2	18.310	2	25.500	2	15.100	2	2.700	2	—	—	11.210	6
6	9. IX 1952	25°C	„		13.750	2	21.040	2	13.020	2	17,220	2	16.000	2	3.900	2	—	—	7.655	4
7	10. IX 1952	24°C	„		20.100	2	24.150	2	18.410	2	19.100	2	10.300	2	1.850	2	—	—	3.450	4
8	11. IX 1952	24°C	„		26.300	3	25.470	2	19.810	2	19.300	2	—	—	—	—	—	—	3.375	3
9	12. IX 1952	25°C	„		29.430	2	—	—	—	—	—	—	14.100	—	1.648	—	—	—	9.410	—
	Average: *Prosjek:*		—	—	16.280	—	16.570	—	12.100	-	11.760	—	8.018	—	1.449	—	—	—	10.465	—

1* Number of bacteria in 1 ccm before adding the antibiotic.
Broj bakterija na 1 ccm u morskoj vodi prije dodavanja antibiotika .

selection of the resistant bacterial species, Streptomycin has a stronger effect than penicillin and already at the lowest concentration of 1,20 mgr/ccm the number of bacterial species is lowered after 24 hours from 6 to 2 species. By increasing its concentration streptomycin too has an action in delaying the multiplication of the resistant species of bacteria, which may be noted by the average number of bacteria per ccm for the experiments with the single concentrations. While the average number of bacteria per ccm is of 16280 in a concentration of 3,60 mgr/ccm, it decreases to 1648 bacteria per ccm with the rising of the concentration to 20,0 mgr/ccm of streptomycin. In the controlling vessel the average number of bacteria per ccm was 9410, which corresponds to the average number with a concentration of 12,0 to 16,0 mgr streptomycin per ccm and the development of the bacterial population itself in the controlling vessel ran in a normal way.

On table No. 5 and fig. 5 is shown the reaction of the bacterial population in sea water under laboratorial conditions against the destructive activity of a combination of penicillin and streptomycin. From the results obtained it is visible that both antibiotics taken together have a much more destructive action than if administered indipendently. This corresponds

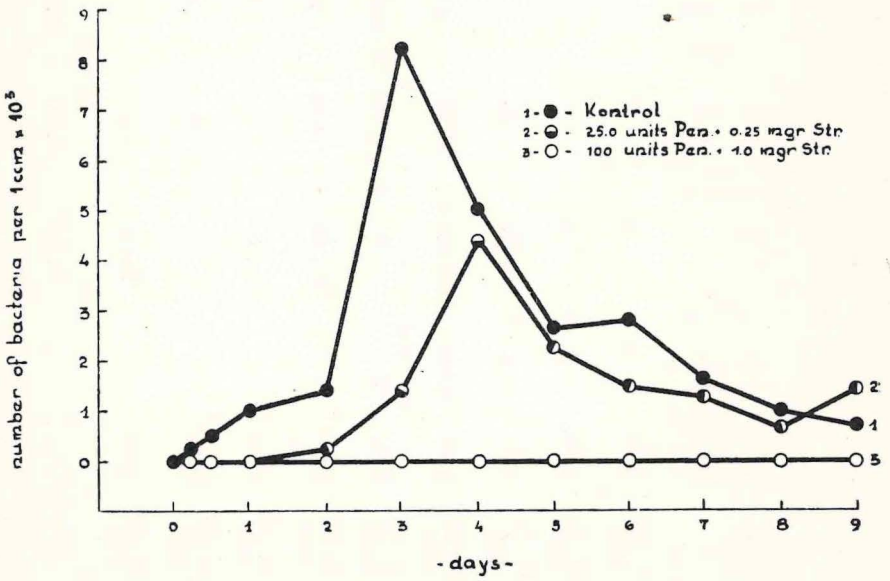

Fig. 5. Reaction of the bacterial population in natural sea water against different concentrations of penicillin and streptomycim

Tab. 5. Bactericidal action of the combination Penicillin and Streptomycin on marine bacteria
Baktericidno djelovanje kombinacije penicilina i streptomicina na bakterije u morskoj vodi.

No. *Br.*	Date of test *Datum probe*	Temper of room *Temper. sobe*	Hour *Sat*	1,0 unit ccm Pen+0,01 mgr ccm Str. *1,0 jed. ccm Pen+0,01 mgr ccm Str.*		5,0 units ccm Pen.+0,05 mgr ccm Str. *5,0 jed. ccm Pen.+0,05 mgr ccm Str.*		10.0 units ccm Pen.+0,1 mgr ccm Str. *10,0 jed. ccm Pen.+0,1mgr ccm Str.*		25,0 units ccm Pen.+0,25 mgr ccm Str. *25,0 jed. ccm Pen.+0,25 mgr ccm Str.*		50,0 units ccm Pen.+0,50 mgr ccm Str. *50,0 jed. ccm Pen.+0,50 mgr ccm. Str.*		100,0 units ccm Pen.+1, 0mgr. ccm Str. *100,0 jed. ccm Pen.+1,0 mgr ccm Str.*		Kontrol *Kontrolna*	
				bact. ccm *bakt. ccm*	strains *sojeva*	bact. ccm *bakt. ccm*	strains *sojeva*	ba t. ccm *bakt. ccm*	strains *sojeva*	bact. ccm *bakt. ccm*	strains *sojeva*	bact. ccm *bakt. ccm*	strains *sojeva*	bact. ccm *bakt· ccm*	strains *sojeva*	bact. ccm *bakt. ccm*	strains *sojeva*
1 *	29. IX 1952	22oC	8	158	8	143	8	156	7	164	7	139	8	1 2	7	145	9
2	29. IX 1952	23oC	11	78	3	31	2	18	3	10	3	2	2	—	—	227	8
3	29. IX 1952	23oC	20	111	2	19	3	7	2	2	2	1	1	—	—	442	8
4	30. IX 1952	23oC	8	2.980	2	30	2	9	2	5	2	—	—	—	—	943	6
5	1. X 1952	23oC	8	15.200	2	981	2	271	1	142	1	3	—	—	—	1.323	5
6	2. X 1952	22oC	8	2.520	3	4.880	2	813	2	1.360	2	—	—	—	—	8.160	4
7	3. X 1952	21oC	8	1.030	2	5.290	1	4.430	2	4.350	2	—	—	—	—	4.900	5
8	4. X 1952	21oC	8	3.620	2	1.310	2	5.900	2	2.170	2	—	—	—	—	2.610	6
9	5. X 1952	21oC	8	1.830	2	628	2	2 380	2	1.520	1	—	—	—	—	2.780	5
10	6. X 1952	22oC	8	798	2	535	3	892	1	1.350	2	—	—	—	—	1.530	4
11	7. X 1952	22oC	8	627	2	802	2	1.328	2	940	2	—	—	—	—	920	5
12	8. X 1952	22oC	8	2.900	2	2.670	2	2.110	2	1.230	2	—	—	—	—	780	6
		Average : *Prosjek :*		2.967	—	1.652	—	1.650	—	1.279	—	—	1	—	—	2.2 3 7	—

1* Number of bacteria in 1 ccm before adding the antibiotics.
Broj bakterija na 1 ccm prije dodavanja antibiotika.

to the results obtained by Jawetz and collaborators (1952) and other authors (Spencer 1952) who ascertained that the sinergism of penicillin and streptomycin »in vitro« is manifested in a strengthened destructive activity, which is greater than the one of the single antibiotics. A combination of low concentrations of these antibiotics has an effect on the development of the bacterial population retarding the multiplication of the resistant species of bacteria, and a concentration of 5 units penicillin per ccm and of 0,05 mgr streptomycin per ccm has a visible destructive effect already after 3 till 8 hours of activity and increases with the rising of the concentration of antibiotics (Table 5).

While with the lowest concentration the number of bacteria per ccm of sea water falls from 158 to 78 bacteria/ccm, already after 3 hours of activity and begins to rise after 8 hours (111 bacteria per ccm), with a concentration of 25 units penicillin + 0,25 mgr streptomycin per ccm it falls after 3 hours from 164 to 10 bacteria/ccm and begins to rise only after 48 hours (142 bacteria/ccm). A complete destructive action is perceived already with a concentration of 100 units penicillin per ccm and 1,0 mgr streptomycin per ccm. — The selection of the resistant species

Tab. 6. The average number of bacteria in 1 ccm of sea water during the experiments of 8 days with different concentrations of Penicillin and Streptomycin.

Prosječni broj bakterija na ccm morske vode u toku eksperimenata od 8 dana pri raznim koncentracijama penicilina i streptomycina.

Penillin		Steptomycin		Penic.+Streptomycin	
Conc. units per ccm *konc. jed. na ccm*	Average number of bacteria ccm *prosječni broj bakterija ccm*	Conc. mgr. per ccm *konc. mgr. na ccm*	Average number of bacteria ccm *prosječni broj bakterija ccm*	Conc. per ccm units mgr *konc. ccm jedinica mgr*	Average number of bacteria ccm *prosječni broj bakterija cc*
100	10.883	1,20	—	1+0.1	2.967
300	8.684	3,60	16.280	5+0,05	1.625
500	6.135	6,0	16.570	10+0,10	1.650
800	3.413	10,0	12.100	25+0,25	1.279
1.000	2.762	12,0	11.760	50+0,50	1
1.500	1	16,0	8.018	100+1,0	0
2.000	0	20,0	1.648	—	—
—	—	26,0	0	—	—

is somewhat faster and already with low concentrations of both the antibiotics it falls from 8 to 2—3 bacterial species and with higher concentrations even to 1 bacterial species. The average number of bacteria per ccm in the experiments for the single concentrations of both antibiotics slightly surpasses the average number of bacteria per ccm in the controlling vessel (2237 bacteria per ccm) already with the lowest concentration of 1 unit/ccm penicillin + 0,01 mgr/ccm streptomycin.

In comparing the total destructive activily of the combination penicillin and streptomycin with the single effects of these antibiotics on the bacterial population in sea water (Table 6.) we get the following result: penicillin alone has a total destructive effect on bacteria in sea water in a concentration of 2000 units/ccm and streptomycin alone has a complete destructive effect in a concentration of 26,0 mgr per ccm. — The combination of both antibiotics has a general destructive effect on bacteria in sea water in a concentration of 100 units penicillin per ccm + + 1,0 mgr streptomycin per ccm. — Consequently the combination of these antibiotics has a total bactericidal effect in a concentration of about 20 times lower than they would have if administered independently.

DISCUSSION

In comparing the results obtained in testing the effect of antibiotics on marine bacterial species with the results in testing the effect of antibiotics on the bacterial population in sea water, it appears that in both cases in general the number of the resistant as well as of the susceptible species towards the antibiotics, corresponds. Four species are resistant against Penicillin, three species against streptomycin and one species against the combination of both the antibiotics (fig. 1). In the experiments with sea water the number of bacterial species in bigger concentrations of antibiotics falls to 2—3 species, and in the combined antibiotics to only one species. The most resistant species against penicillin (No. 224 and 237) are destroyed in a concentration of 2500 units/ccm and a concentration of 2000 units/ccm in sea water has a total destructive effect. The most resistant species against streptomycin (No. 237) is destroyed in a concentration of 20 mgr/ccm and a concentration of 26,0 mgr/ccm

has in sea water a total destructive power. The most resistant species (No. 237) against the combined penicillin and streptomycin is destroyed in a concentration of 150 units/ccm penicillin + 1,5 mgr/ccm streptomyicin, and the total bactericidal concentration of both the antibiotics in sea water is of 100 units/ccm penicillin + 1,0 mgr streptomycin. It appears therefore that penicillin and a combination of penicillin + streptomycin have a stronger destructive effect in sea water than in broth, while matters turn to the contrary in the action of streptomycin. W a k s m a n (1948 p. 186) on the basis of the reaction of pathogenic bacteria against penicillin says that the effect of penicillin on bacterial cultures in a rich surroundings (broth or serum) is stronger than in a poor surroundings (water or salted solution).

It is a matter of further research to ascertain whether in the case of sea bacteria there is a definitive exception to this phenomenon with regard to penicillin.

According to the results of S p e n c e r (1952) it appears that streptomycin is not so bacteriostatic on bacterial flora coupled with cultures of Diatomea, as penicillin proves to be. Our results about the effects of penicillin and streptomycin on sea bacterial species (fig. 2) and with our results agree also those of the destructive action of these antibiotics on bacteria in sea water (Tables 3 and 4), show that at the beginning of the experiment the effect of penicillin is stronger and in the course of the experiment streptomycin performs a quicker selection on susceptible and respectively resistant species than penicillin and that streptomycin has a more stimulating effect on the multiplication of the remaining resistant species. In the fig. 2 at the beginning the curve of the effect of penicillin is steeper (50%) than that of streptomycin (42%), but they soon cross each other (57,5%) and later the curve of streptomycin is steeper than of penicillin. From the tables 3 and 4 it is visible that the number of species, particularly in lower concentrations, falls faster in streptomycin than in penicillin, and the average number of bacteria in experiments with different concentrations of streptomycin is bigger (16280 to 12100 bacteria/ccm) than that in penicillin (10883 to 6135 bacteria/ccm).

J a v e t z and collaborators (1952) have found that for a total bactericidal action of a combination of penicillin and streptomycin on 5 species pathogenic bacteria, it is necessary that at least either of the antibiotics should be in such a concentration in which it has alone a destructive action. In our experiments it has been proved that in synergetic

result of both antibiotics, in concentrations in which they have a total destructive power on the bacterial population in sea water or on single species of sea bacteria, it is not necessary that either of the antibiotics should be in a concentration in which it has an indipendent total destructive power. In our case the bacteristatic combination of bouth antibiotics for the population of bacteria in sea water is at a concentration of 100 units/ccm penicillin + 1,0 mgr/ccm of streptomycin, while penicillin alone has a bacteriostatic action in a concentration of 2000 units/ccm while streptomycin alone in a concentration of 26,0 mgr/ccm. — The total bactericidal effect of the combination penicillin and streptomycin on bacterial species is at a concentration of 200 units/ccm + 2,0 mgr/ccm and penicillin alone is completely bactericidal in a concentration of 2500 units/ccm, while streptomycin alone is so in a concentration of 20,0 mgr/ccm (fig. 2).

In the experiments with the action of antibiotics on the bacterial population in sea water, it has been ascertained that low concentrations of penicillin and especially of streptomycin, not only have inhibiting action, but they have a stimulating effect on the develoment of the bacterial population. While the maximum development of the population in the controlling vessel (Table 3) was of 12.940 bacteria per 1 ccm in a concentration of penicillin of 100 units and 300 units/ccm, it was of 18300 or respectively of 19170 bacteria per 1 ccm. — In a concentration of streptomycin of 1,0, 2,0, 3,6 and 6,0 mgr/ccm the number of bacteria in the population was of over 40.000, 29.430 respectively 25.470 bacteria per 1 ccm and in the controlling vessel it was of 19.680 bacteria per 1 ccm. Miller and Bohuhoff (1947) as well as Kushrock and others (1947) have ascertained such a phenomenon ih the action of streptomycin on some species of pathogenic bacteria and Waksman (1948 p. 195) relates that low concentrations of antibiotics, lower than those that have an inhibiting effect, do stimulate the delelopment of organisms or processes of metabolism in the organism. — Very likely both this phenomenon as well as the phenomenon of adaptability of the organism to antibiotics, which for different species requires different periods of time, — and with the reciprocal antigonistic effect of enzyme of different bacterial species in the population, — are the cause of the quasiregular decreasing and increasing of the population in the course the experiment. So in the experiment on the action of penicillin, we have in the course of 9 days in all concentrations, after the first increase of the bacterial population its decrease, and then again its increase, then once more its

decrease and increase. We have similarly two decsreases and three incereases of the population in the experiments of streptomycin and the combination of both antibiotics almost in all concentrations.

It has been shown that in the susceptibility or respectively in the resistance between the single bacterial species against penicillin and streptomycin there are big differences, and that the resistance of a particular species against the action of a determined concentration of antibiotics is characteristic and permanent. Such a phenomenon for pathogenic bacteria has already been observed by many authors (Waksman 1941, Fleming 1942, Graddock 1942, Rose et al. 1945, Stokinger et al 1943), and was used by them for the selection of the single bacterial species and for the distinction of some bacteria from others. — According to the results obtained in our experiments with the single species of sea bacteria, it is possible to introduce, in determining the species, among others, also the characteristic: »reaction against antibiotics«. For this purpose it will be necessary to examine separately the susceptibility of the single species but also of each species and the reaction against the different antibiotics.

SUMMARY

Experiments have been made about the bacteriostatical and bactericidal action of penicillin and streptomycin on single species of sea bacteria (altogether 41 species), as well as on sea bacteria in natural sea water under laboratorial conditions.

Most of the sea bacterial species are susceptible towards penicillin (70%), towards streptomycin (75%) and towards combined penicillin and streptomycin (97%). — The coccus (gram-positive) are all susceptible to very low concentrations of antibiotics, in all three cases and for the rods gram-positive and gram-negative no regularity could be ascertained in the reaction against these antibiotics. There is a certain parallelism in the reaction of the species against penicillin, streptomycin and their combinations, so 52,5% of the species are suceptible in all the three cases and 10% of the species are resistant in all the three cases. The action of the combined penicillin and streptomycin on the species is about 20 times more effective than the action of either antibiotics indipendently.

The results of the effects of penicillin and streptomycin on bacteria in sea water, in general agree with those of the action of these antibiotics on the single species of sea bacteria. The same concentration of antibiotics and their combinations have a somewhat more effective destructive action in the natural sea water than in the culture of the single species in broth. The lowest concentration of penicillin, streptomycin and their combinations with a total destructive effect on bacteria in sea water have been established: penicillin 2000 units/ccm, streptomycin 26,0 mgr/ccm, penicillin and streptomycin 100 units/ccm + 1,0 mgr/ccm. The sinergetic action of penicillin streptomycin is about 20 times more effective than the action of either antibiotic separately.

Some phenomena of the sinergetic action of penicillin and streptomycin have been discussed as well as of the stimulating effect of these antibiotics on the development of bacterial population, some regularities in the mutability of the bacterial population in the course of the experiments, and the possibility of using the reaction of the species against penicillin and streptomycin for the purpose of determining the bacterial species.

BIBLIOGRAPHY:

Chu S. P. (1946): Note on the tehnique of making bacteria-free cultures of marine diatoms. Jour. of the Mar. Biol. Ass. Vol. XXVI. No. 3.

Czekalowski W. J. (1950): Penicilin inactivators of bacterial origin. The Jour. of Pathol. and Bact. Vol. XII. No. 1. — 1950.

Durišić M. (1948): Inhibitorna i baktericidna moć streptomycina. — Srpski arhiv za celokupno lekarstvo, sv. 3197 — 1948.

Fish G. R. (1950): A method of obtaining bacteria-free cultures of a marine flagellate and Enteromorpha intestinalis using penicilin. Maddelanden fram Getebergs botaniska Tradgard, Bol. 18, 28—9.

Jawetz E., Gunmison B. J., Bruff B. J. and Coleman R. V. (1952): Studies on Antibiotic Symergism and Antagonism. — Jour. Bact. Vol. 64, No. 1. — 1952.

King H. K. (1950): Penicilin in the Aquarium. — Nature, Vol 166, No. 4233.

Leontjev I. F. (1950): Primenjenije antibiotikov dlja očistki kuljtur gemoflagelatov ot mikrobov. — Priroda (Moskva) No. 7 — 1950.

Luck J. M., Sheets G. and Thomas O. J. (1931): The role of Bacteria in the nutrition of Protozoa. — The Quarterly Review of Biology, Vol. VI., No. 1. 1931.

Spencer P. C. (1952): On the use of Antibiotics for isolating Bacteria — free cultures of Marine Phytoplankton organisms. Jour. of the Marine Biolog. Accos. Vol. XXXI., No. 1, 97—106.

Tarr H. L. A. Southeott B. A., and Bissett H. M. (1950): Eeffect of several Antibiotics and Food preservatives in retarding Bacterial spoilage of Fich. — Progress Rep., Fich. Research Board of Canada, No. 83 — 1950.

Waksman A. S. (1948): Antagonismes microbiens et Substances antibiotique (Paris — 1948.).

ZoBell E. C. (1946): Marine Microbiology, Chronica Botanica, Waltham 1946. USA.

ZoBell E. C. and Feltham B. C. (1938): Bacteria as food for certain marine invertebrates. — Sears Jour of Mar. Research, Vol. I. No. 4. — 1938.

ZoBell E. C. and Long J. H. (1938): Studies on the isolation of bacteria — free cultures of marine phytoplancton. Jour. of Mar. research, Vol. I, No. 4 — 1938.

Zshucke J. (1951): Die ausnützung der elektiven Wirkung des Streptomycins zur Verbesserung der kulturellen Typhus-und Paratyphus diagnose in stuhl und Urin. — Zentr. f. Bakt. Parasith. Infektions. und Hyg. — Originale, Band 178. Heft 1—2, 1951.

Received for publication November 26, 1952.

BAKTERICIDNO I BAKTERIOSTATIČKO DJELOVANJE ANTIBIOTIKA NA MORSKE BAKTERIJE

I. Penicilin i streptomycin

(sa 5 grafikona u tekstu)

Vlaho Cviić

Institut za oceanografiju i ribarstvo, Split

Kratak sadržaj

Izvršeni su eksperimenti sa bakteriostatičkim i baktericidnim djelovanjem penicilina i streptomycina na pojedine sojeve morskih bakterija (ukupno 41 soj) i na morske bakterije u prirodnoj morskoj vodi pod laboratoriskim uslovima.

Većina sojeva morskih bakterija je senzibilna prema penicilinu (70%), prema streptomycinu (75%), te prema kombinaciji penicilin-streptomycin (97%). Koki-oblici (gram-pozitivni) su svi senzibilni na veoma niske koncentracije antibiotika u sva tri slučaja, a za štapićaste gram-pozitivne i gram-negativne oblike nije utvrđena pravilnost u odnosu na ove antibiotike. Postoji izvjestan paralelizam u odnosu sojeva prema penicilinu, streptomycinu, te njihovoj kombinaciji, tako su 52,5% soja senzibilna u sva tri slučaja, a 10% sojeva je rezistentno u sva tri slučaja. Djelovanje kombinacije penicillin-streptomycin na sojeve je oko 20 puta efikasnije, nego djelovanje svakoga antibiotika posebno.

Rezultati djelovanja penicilina i streptomycina na bakterije u morskoj vodi podudaraju se uglavnom sa rezultatima djelovanja ovih antibiotika na pojedine sojeve morskih bakterije. Iste koncentracije antibiotika i njihove kombinacije djeluju nešto jače baktericidno u prirodnoj morskoj vodi, nego na kulture pojedinih sojeva u bujonu. Utvrđene su najniže koncentracije penicilina, streptomycina i njihove kombinacije, koje djeluju totalno baktericidno na bakterije u morskoj vodi: penicilin 2.000 jed./ccm. streptomycin 26,0 mgr/ccm, penicillin-streptomycin 100 jed./ccm. Sinergetsko djelovanje penicilina-streptomycina za oko 20 puta je efikasnije nego djelovanje svakoga antibiotika posebno.

Diskutirane su neke pojave sinergerskog djelovanja penicilina i streptomycina, stimulatornog djelovanja ovih antibiotika na razvoj bakterijske populacije, neke pravilnosti kolebanja bakterijske populacije u toku eksperimenata, te mogućnost upotrebe odnosa sojeva prema penicilinu i streptomycinu u svrhe determinacije bakterijskih sojeva mora.

Tiskanje završeno 25. V. 1953.

Tisak: Novinsko - izdavačko poduzeće „Slobodna Dalmacija“ - Split