# SELECTIVE ANTIBIOTIC ACTION OF VARIOUS SUBSTANCES OF MICROBIAL ORIGIN[1]

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Received for publication, January 9, 1942

## INTRODUCTORY

Since Pasteur first demonstrated that certain microörganisms are able to exert antagonistic or antibiotic effects upon other organisms, an extensive literature has accumulated (Waksman, 1941). The presence of living organisms is often necessary for the phenomenon of antagonism to take place. In many cases, the antagonist was found to produce an active substance responsible for this action. The active agent has been isolated, purified, and crystallized only in very few instances. Pyocyanase was the first antagonistic substance to have thus been obtained (Emmerich and Low, 1899). Several others have been isolated recently.

Certain facts have now become recognized concerning the nature of the phenomenon produced by antagonistic microörganisms: 1. The various active substances isolated from the different organisms vary considerably in their chemical nature; 2. these substances are selective in their action upon various organisms, showing variation even as regards specific types or strains of the different groups of bacteria acted upon; 3. the substances vary in the mechanism of their action, some being primarily bacteriostatic, and others bactericidal but not bacteriolytic, whereas still others are both bactericidal and bacteriolytic; 4. the antagonistic capacity is widely distributed among microörganisms and is not limited to any one group of bacteria or fungi.

Active antibiotic agents have now been obtained from representative types of spore-forming bacteria, non-spore-forming bacteria, actinomycetes and fungi. Some of these agents have not yet been isolated in a pure state; however, they are well recognized, both chemically and biologically, and can be characterized by their specific properties. The following substances or preparations have received the greatest consideration: 1. pyocyanase, 2. pyocyanin, 3. gramicidin, 4. tyrocidine, 5. penicillin, 6. gliotoxin, 7. actinomycin, and 8. streptothricin. The first four are of bacterial origin, the next two are produced by fungi, and the last two by actinomycetes. To these may be added several other preparations, of which the exact chemical nature or mode of action is less known, namely, actinomycetin, prodigiosin, fluorescin, microbial-lysozyme, active substances obtained from species of *Aspergillus* and from various other fungi and bacteria. Among the antagonistic phenomena which are now well recognized but for which no active substance has as yet been demonstrated, one may men-

[1] Journal Series paper, New Jersey Agricultural Experiment Station, Rutgers University, Department of Soil Microbiology.

tion the action of certain strains of *Escherichia coli* upon other bacteria, of various yeasts and of many fungi (Nakhimovskaia, 1938, 1939; Waksman, 1941).

The origin, chemical nature and activities of the more important antagonistic substances of microbial origin thus far recognized are summarized in table 1. Because different methods have been used for testing the action of these substances upon microörganisms and because different test organisms, known to vary greatly in the degree of sensitivity, have been employed, it is difficult to compare the results obtained by the different investigators, who have first

TABLE 1

*Summary of known chemical properties of antibiotic agents of microörganisms*

| PREPARATION | ORGANISMS | CHEMICAL NATURE | ORGANISMS ACTED UPON | HEAT STABILITY |
|---|---|---|---|---|
| Pyocyanase | *P. aeruginosa* | Lipoid | Gram-positive and gram-negative bacteria | Thermostable |
| Pyocyanin | *P. aeruginosa* | Pigment | Largely gram-positive bacteria | Thermostable |
| Tyrocidine | *B. brevis* (*Tyrothrix* species) | Polypeptide | Largely gram-positive bacteria | Thermostable |
| Gramicidin | *B. brevis* (*Tyrothrix* species) | Polypeptide | Largely gram-positive bacteria | Thermostable |
| Penicillin | *P. notatum* | Non-nitrogenous body | Various aerobic and anaerobic bacteria | Thermolabile |
| Gliotoxin | *Trichoderma*, *Gliocladium* | Sulfur—containing ring compound | Fungi and bacteria | Thermostable |
| Actinomycin | *A. antibioticus* | Polycyclic nitrogen compound | All bacteria and fungi | Thermostable |
| Actinomyces lysozyme | Various actinomycetes (*A. violaceus*) | Protein | Stated to be similar to lysozyme | Thermostable |
| Streptothricin | *A. lavendulae* | Organic base | Various gram-negative and gram-positive bacteria | Thermostable |

isolated or tested these preparations. Further, the action of these substances has only seldom been compared with that of known chemical compounds having similar properties or producing similar action.

## EXPERIMENTAL

This study was undertaken for the purpose of throwing light upon the relative activity of antibiotic substances of microbiological origin and of well defined chemical compounds, by the use of the same technique and the same test organisms. In the study of the bacteriostatic action of these preparations, two

gram-negative bacteria, two spore-forming and two non-spore-forming gram-positive bacteria, and two actinomycetes were used as test agents. These organisms were selected because of previously established variation in the degree of their sensitivity to the active substances. The two actinomycetes varied but little in their response; hence, only the results obtained with one of these are reported. In many cases, other test organisms, in addition to the above were also employed.

In view of the marked effect of the composition of the medium upon the activity of the substance, three media were used, namely, nutrient agar, nutrient broth, and brain-heart-infusion agar. The cultures were streaked over the solidified agar plate or inoculated into the tubes containing the broth. Incubation took place at 28° or 37°C., depending on the optimum conditions for the growth of the particular test organism, for 1 to 3 days. The active substances were dissolved in water, or, when required, in alcohol and diluted with water. Each active substance was added, in ten different concentrations, to 10 ml. portions of the test media.

In order to avoid complication that would result from the reporting of all the details thus obtained, only those concentrations are reported at which bacterial growth became inhibited. Complete inhibition of growth of the test organism by a given concentration of the bacteriostatic agent is emphasized by the use of a minus (−) sign. When no inhibition was obtained even with the highest concentration employed, a plus (+) sign is used. To illustrate this: 3 = partial inhibition of growth of the particular test organism by the addition of 3 milligrams of the substance to 10 ml. of agar or broth; 3− = complete inhibition of growth by 3 milligrams of the substance; 3+ = no inhibition at all with 3 milligrams of the substance, no higher concentration being used for making the particular test.

The following 14 preparations and chemical compounds were employed in these studies, nine of these being of microbiological and one of biological, although not microbiological, origin; four were well defined chemical compounds of non-biological origin.

1. *Actinomycin*, isolated from *Actinomyces antibioticus*, a crystalline substance (Waksman and Woodruff, 1941). The results reported in table 2 were calculated on a dry basis of purified actinomycin A.

2. *Streptothricin*, isolated from a strain of *Actinomyces lavendulae*. (Waksman and Woodruff, 1942). This was a highly purified preparation, although not in a crystalline state. A concentrated solution was used, containing, per 1 ml., 4 mg. of dry matter. The results are reported on the basis of dry material.

3. *Gramicidin*, first isolated from *Bacillus brevis* by Dubos (Dubos, 1939; Dubos and Hotchkiss, 1941).

4. *Tyrocidine* was also isolated by Dubos from *B. brevis* (Dubos, 1939; Dubos and Hotchkiss, 1941).

5. *Tyrothricin*, a mixture of substances 3 and 4, or the total active material obtained from *B. brevis*.

6. *Pyocyanase*, a crude preparation isolated from an active culture of *Pseudomonas aeruginosa*, by extraction with ether and concentration.

7. *Pyocyanin*, which is also produced by *P. aeruginosa*; however, for these studies, a synthetic preparation was used.

8. *Penicillin*, a highly purified preparation obtained from a culture of *Penicillium notatum*.

9. *Gliotoxin*, isolated from *Gliocladium fimbricatum* in a crystalline form.

10. *Lysozyme* or fresh egg-white. The results are reported on the basis of ml. of egg-white. The inclusion of this material is justified, because of the claims of Russian investigators that certain actinomycetes produce an active substance which is lysozyme-like in nature, and also because various bacteria are believed to be able to produce lysozyme.

11. *Tolu-p-quinone*, included in this study for two reasons: (a) actinomycin was found to contain a quinone group; (b) a comparison of the bacteriostatic action of several quinones has shown that tolu-p-quinone is a highly active bacteriostatic agent.

12. *Phenol*, the ordinary commercial product.

13. *Lauryl sulfate* (Duponal M. E.) was included because of its marked detergent properties.

14. *Sulfanilamide*, included in this study because of its selective action against various bacteria, comparable to substances of microbial origin.

The results presented in table 2 show that the medium chosen for measuring bacteriostatic action of different preparations is of considerable importance. From 3 to 10 times as much actinomycin and streptothricin is necessary to inhibit the growth of the various test bacteria on brain-heart-infusion agar as on nutrient agar. This may be due to the presence in the brain-heart agar of specific inhibitors for these substances. Blood serum was found, in other experiments not reported here, to have no effect on the action of these substances; however, it is known to interfere with the action of tyrocidine. Nutrient broth proved much more favorable than agar media for demonstrating bacteriostasis of the products of *B. brevis*. This may be due to the low solubility of these substances in water and to their poor diffusion in agar.

The two gram-negative test bacteria were inhibited by streptothricin, pyocyanase, pyocyanin, gliotoxin, tolu-p-quinone, and high concentrations of phenol and sulfanilamide. The two aerobic spore-forming bacteria were inhibited by a number of the preparations, particularly by actinomycin and gliotoxin. It is of special interest to note that *Bacillus mycoides* (at least the particular strain employed in this test) was highly resistant, whereas *Bacillus subtilis* was sensitive to streptothricin, lysozyme, and sulfanilamide. Both organisms were resistant to gramicidin. *Micrococcus lysodeikticus* and *Sarcina lutea* were highly sensitive to actinomycin, gramicidin, tyrocidine, penicillin, gliotoxin and lysozyme. The *Actinomyces* sp. was sensitive to most of the substances when used in not too high dilutions.

Because of the claims that peptone interferes with the activity of the active substances, a synthetic medium was also used, namely Czapek's agar. The

## TABLE 2

*Bacteriostatic effects of agents of biological origin compared with pure chemical substances*

Concentration of active substance, in milligrams, per 10 ml. agar or broth at which growth of the test organisms becomes inhibited; complete inhibition is designated by − sign; no inhibition with the given concentration is designated by + sign.

| ACTIVE SUBSTANCE | TEST ORGANISM | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *A. aerogenes* | | | *E. coli* | | | *B. mycoides* | | | *B. subtilis* | | | *M. lysodeikticus* | | | *S. lutea* | | *Actinomyces* sp. | |
| | TEST MEDIUM | | | | | | | | | | | | | | | | | | |
| | NA* | NB | BHI | NA | NB | BHI | NA | NB | BHI | NA | NB | BHI | NA | NB | BHI | NA | BHI | NA | BHI |
| Actinomycin | 3+ | 3 | 3+ | 3+ | 3− | 3+ | .001− | .001− | .01 | .0003 | .0003− | .01− | .0003 | .0003− | .003− | .0003− | .003− | .03 | .1− |
| Streptothricin† | .1 | .3− | 1 | .03− | .03− | .3− | 3+ | 3+ | 3+ | .003 | .01 | .03 | .03 | .03− | .3 | .03− | .3− | .03− | 1− |
| Tyrothricin | 30+ | 3+ | 3+ | 30+ | 3+ | 3+ | .3− | .1 | .3− | .3− | .03− | .3− | .003 | .001− | .003− | .1 | .1− | .3+ | .3− |
| Gramicidin | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | .01 | .0003− | .01− | .03 | .1 | 3 | 3− |
| Tyrocidine | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | .1− | .03− | .1− | .1− | .01− | .1− | .03 | .01− | .03 | .03 | .03 | .1− | .1 |
| Pyocyanase | 1− | 3+ | 3 | 1 | 3+ | 3 | .3− | .3− | 1− | .3− | .3− | 1− | .1 | .3− | 1− | .3− | .3 | 1− | 1− |
| Pyocyanin | 1 | 3+ | 3 | .3− | .3 | 1− | .1− | .1− | .3− | .03 | .03 | .3− | .1− | .03 | .1− | .03− | .1− | .3− | .3− |
| Penicillin | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | 3+ | .01 | .01 | .01 | .03− | .03− | − | .01 | .01 | 1+ | 1+ |
| Gliotoxin | 1 | 3− | 3− | 1 | 1 | 1 | .1 | .03 | .1− | .003 | .03− | .01 | .0001 | .0003 | .001 | .001− | .0001− | .1− | .1− |
| Lysozyme‡ | 1+ | 1+ | 1+ | 1+ | 1+ | 1+ | 1+ | 1+ | 1+ | .03 | .03− | 1− | .003− | .001− | .0003 | .001 | .0003 | 1− | .3 |
| Tolu-p-quinone | 3− | 3− | 3 | 3− | 3− | 3− | .3 | 1− | 3− | .3 | .3− | 3− | .3 | .1 | 1 | .1 | 3− | 1 | 3− |
| Phenol | 30− | 10 | 30− | 30− | 10 | 30− | 30− | 30− | 30− | 10− | 10− | 30− | 10− | 10− | 10− | 10 | 10− | 10 | 10 |
| Lauryl sulfate§ | 100+ | 10+ | 100+ | 100+ | 10+ | 100+ | 1− | 1− | 3− | .3 | .03− | .3 | 1− | .3 | 3− | 1− | 3− | 1− | 3− |
| Sulfanilamide | 10 | 10 | 10+ | 10 | 10 | 10+ | 10+ | 10+ | 10+ | 1 | 1 | 10 | 10 | 10+ | 10 | 10 | 1 | 10 | 10 |

* NA = nutrient agar; NB = nutrient broth; BHI = brain heart infusion agar.
† Crude preparation.
‡ Fresh egg white, in ml.
§ Duponal M.E.

## TABLE 3

*Bacteriostatic action of various agents on a synthetic medium**

| ACTIVE SUBSTANCE | TEST ORGANISM | | | | | |
|---|---|---|---|---|---|---|
| | *A. aerogenes* | *E. coli* | *B. mycoides* | *B. subtilis* | *S. lutea* | *Actinomyces* sp. |
| Actinomycin | 2+ | 2+ | .001 | .0003 | 0.0003 | .1 |
| Tyrocidine | 10+ | 10+ | .03 | .03 | 0.03 | .03− |
| Gramicidin | | | 10 | 10+ | 0.1 | 3+ |
| Phenol | 30− | 10 | 10 | 3 | 10 | 30− |
| Sulfanilamide | 10 | 10 | 10 | .1− | 1− | 10 |

* Czapek's agar used; for *S. lutea*, 0.3 per cent asparagine replaced 0.2 per cent $NaNO_3$.

results obtained (table 3) tend to show that, for most organisms, the activity of sulfanilamide is greater on the synthetic medium than on the peptone-containing media. This is somewhat true also of tyrocidine and of gramicidin. Actinomycin and phenol, however, did not show any marked differences on the two types of media.

Considerable variation was found in the degree of sensitivity of the different test bacteria to the various bacteriostatic agents employed. One may definitely conclude from these results that no one organism can be used as a single test agent for comparing the bacteriostatic properties of different substances. If phenol is used as a standard, the ratio between the inhibiting concentration of the particular substance against a certain organism and that of phenol gives the relative bacteriostatic activity of the substance for the specific organism. One may thus calculate that the "bacteriostatic phenol coefficient" of actinomycin for *B. subtilis* is 33,000 units; streptothricin, 3,330 units; tyrothricin, 33; tyrocidine, 100; pyocyanase, 33; pyocyanin, 330; penicillin, 1,000; lysozyme, 330; gliotoxin, 3,300; toluquinone, 33; and sulfanilamide, 10. Using *S. lutea* as the test organism, the corresponding coefficient figures for the above substances become 33,000, 330, 100, 330, 33, 330, 1,000, 10,000, 10,000, 100, and 1.

In order to compare these results with those obtained by other investigators, it is sufficient to take sulfanilamide. In these tests, sulfanilamide gave a bacteriostatic action against the two cocci and *B. subtilis* of 1 to 10 mg. per 10 ml. agar, or in dilutions of 1:1,000 to 1:10,000. MacKay (1941) reported recently that sulfanilamide permitted the growth of *Staphylococcus aureus* and *B. subtilis*, after 24 hours, in concentration of 1:5,000, on various serum media.

When a crude preparation of penicillin was employed, in the form of the original culture medium, it was found to be also effective against gram-negative bacteria as well as certain gram-positive organisms not affected by the purified preparation. The concentrations of medium required to inhibit *E. coli* and *Aerobacter aerogenes* varied from 0.1 to 0.3 ml. per 10 ml. agar. *Brucella abortus* was inhibited by even lower concentrations. The action of the purified material is in conformity with the results obtained by the British investigators (Abraham, *et al.*, 1941). The importance of the composition of the medium, method of extraction, nature of test organism and of strain of antagonist employed, which have been found to be of great importance in establishing the activity of the active substance of antagonistic organisms (Waksman, 1941) are thus shown to hold particularly true of the active agents of *Penicillium notatum*.

The bacteriostatic action of several substances of microbial origin has been determined for several other gram-negative bacteria (table 4). Marked differences were again brought out in the degree of sensitivity of the same organism to different preparations; different species of the same genus show different degrees of sensitivity against the same active substance. A comparative examination of the effect of some of the other substances against *B. abortus* gave the following results, in milligrams per 10 ml. of nutrient agar: actinomycin, 0.1, streptothricin, 0.05, gramicidin, 1, pyocyanase, 0.1− and gliotoxin, 0.01−.

A detailed study of the bactericidal action of two substances of microbial origin, namely actinomycin and streptothricin, brought out that they behaved differently by this method as well. Actinomycin affected *E. coli* to a limited extent, unless used in relatively high concentrations, but it acted vigorously upon *B. subtilis* and *M. lysodeikticus*. Streptothricin, on the other hand, had a marked bactericidal action upon both gram-negative and gram-positive bacteria. One further difference in the selective bactericidal action of these two substances was observed, namely, actinomycin brought about the death of the cells without accompanying lysis, whereas streptothricin exerted a bactericidal action often accompanied by lysis, especially in case of micrococci.

Actinomycin has a slow bactericidal effect against bacteria. This is evident even with gram-negative bacteria which are ordinarily highly resistant to this substance. The results of an experiment using *E. coli* cells as the test organism may be reported here. *E. coli* was grown on nutrient agar. The cells were removed, washed, and then suspended in sterile tap water. One ml. of the cell

TABLE 4

*Bacteriostatic action of actinomycin upon different species of Azotobacter*

| ORGANISM | CONCENTRATION OF ACTINOMYCIN, MG. PER 10 ML. AGAR | | |
|---|---|---|---|
| | 1.0 | 0.2 | 0.05 |
| *A. vinelandii* | 0 | 2 | 3 |
| *A. agile* | 1 | 3 | 3 |
| *A. agile* | 2 | 3 | 3 |
| *A. indicum* | 0 | 1 | 3 |
| *A. chroococcum* | 0 | 2 | 3 |
| *A. beijerinckii* | 1 | 3 | 3 |
| Fresh soil culture | 2 | 3 | 3 |

0 = no growth; 1–3 = growth.

suspension and varying amounts of an aqueous suspension of actinomycin were added to sterile test tubes, and the volume made to 10 ml. with sterile tap water. The tubes were incubated at 28°C. After 3, 7, 24, and 48 hours incubation, the numbers of surviving cells were determined by plating. The results were found (Waksman and Woodruff, 1940) to represent a monomolecular reaction similar to the action of chemical disinfectants. The results can be presented graphically by plotting the logarithms of the numbers of viable cells against the time of incubation. The first plating was made after 3 hours incubation. Since the cells in the control did not change in number during the experiment, as evidenced by the straight line on the graph, this line was extended to the 0 hour ordinate, and the extension indicated by a broken line. With the highest amount of actinomycin, all cells were dead after 48 hours incubation. Since all cells died at some time between 24 and 48 hours incubation, the graph line was extended in a smooth curve to meet the abscissa, the last portion of this line being broken to indicate the extension.

An examination of the graphs (fig. 1) reveals that 0.004 mg. of actinomycin per 10 ml. suspension of resting cells of *E. coli* was insufficient to bring about a significant reduction in cell numbers. Ten times that concentration, or 0.04 mg., proved slowly bactericidal and 0.4 mg. per 10 ml. proved markedly bactericidal. Both of these values are less than that required to inhibit *E. coli* growth (1–2 mg. per 10 ml. nutrient agar). This would seem to indicate that nutrient agar may contain some substance inhibitory to the action of actinomycin, or that the rapid multiplication of *E. coli* on nutrient agar is sufficient to overcome the slow bactericidal action of actinomycin, this action becoming evident only when cell multiplication is prevented.

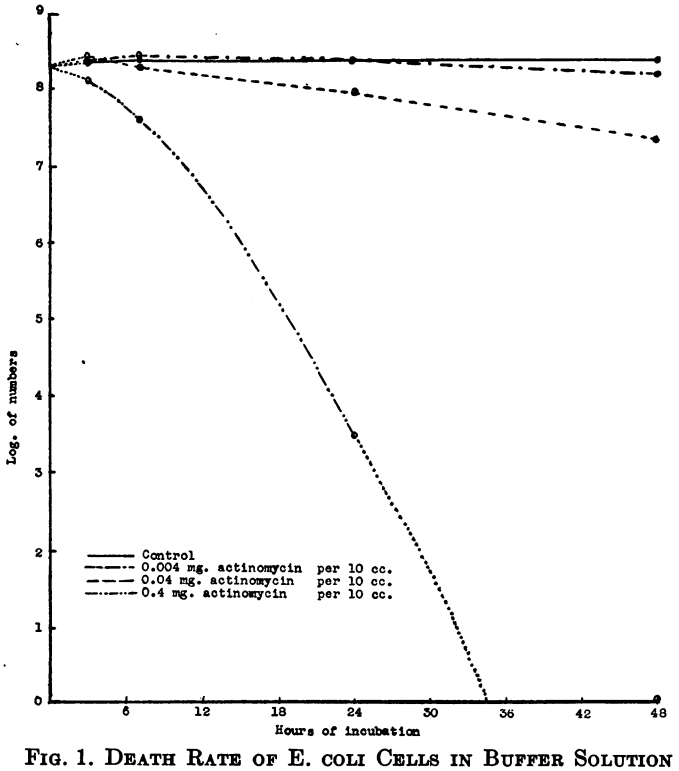


FIG. 1. DEATH RATE OF E. COLI CELLS IN BUFFER SOLUTION

Further comparative studies of the bactericidal action of different substances of microbial origin tended to emphasize their selective nature even more markedly. For example, gliotoxin, which exerted a fairly strong inhibiting effect upon *E. coli*, had only a limited bactericidal action, even in concentrations of 2 mg. per 10 ml. of broth culture. On another gram-negative organism, *Brucella abortus*, gliotoxin also exerted a lower bactericidal effect than actinomycin, crude penicillin, streptothricin and pyocyanase; the last two were by far most active in destroying this gram-negative pathogen. On the other hand, gliotoxin appeared to be more active bacteriostatically against certain bacteria than some of the other substances and preparations.

## DISCUSSION

The recent interest in the antagonistic interrelationships of microörganisms has led to the preparation of several substances of microbial origin which have marked bacteriostatic and bactericidal properties. Some of these have been isolated in the form of pure chemical compounds, whereas others have so far been demonstrated only in the form of crude preparations containing the active principle. The methods employed by various investigators for measuring bacteriostasis vary considerably, both as to medium employed and as to test organisms used. No adequate comparison can, therefore, be made of the activity of the various antibiotic substances produced by different antagonistic microörganisms.

In this investigation, nine products of microbial origin, one other agent of biological origin and four synthetic chemical compounds have been tested by means of standard procedures for measuring bacteriostasis; seven test organisms and three different media were employed. Two of the substances, actinomycin and streptothricin, were of actinomyces (*A. antibioticus*, *A. lavendulae*) origin; three, tyrothricin, tyrocidine and gramicidin, were obtained from a spore-forming bacterium (*B. brevis*); two, pyocyanase and pyocyanin, from a non-spore forming gram-negative bacterium (*P. aeruginosa*); two, gliotoxin and penicillin, were of fungus origin (*Gliocladium fimbricatum* and *Penicillium notatum*). Egg white was used as the source of lysozyme, because an antagonistic agent obtained by certain investigators from different microörganisms was described as resembling lysozyme. For comparative purposes, phenol, tolu-p-quinone, lauryl sulfate, and sulfanilamide were also included.

The differences in the degree of sensitivity of the particular test bacteria to the various substances were most striking. Gramicidin was found to be most specific in its action, being limited to the cocci (*S. lutea* and *M. lysodeikticus*) and acting to a slight extent upon actinomycetes. Purified penicillin was next to it in specific action. Actinomycin, tyrothricin, tyrocidine and gliotoxin acted primarily upon the gram-positive organisms and actinomycetes, much less upon gram-negative bacteria. This is in contrast to the generalized, even if more limited action of phenol and tolu-p-quinone, which behave alike in regard to both gram-positive and gram-negative organisms. Pyocyanase, pyocyanin and the crude penicillin (*P. notatum* culture filtrate) were similar to the chemical compounds in their action, since they were found to be generally bacteriostatic over the wide range of the test organisms employed, no sharp division being obtained upon the basis of the gram stain. Streptothricin was unique in its action: the gram-positive spore-former *B. subtilis* was most sensitive but the other spore-former, *B. mycoides*, was not affected at all by this substance; the gram-negative *E. coli* was more sensitive to streptothricin than either *M. lysodeikticus* or *S. lutea*. Sulfanilamide proved to be only slightly bacteriostatic; however, it had a definite retarding effect upon the growth of the various organisms used in this study.

As a whole, the substances of microbial origin were found to be stronger bac-

teriostatic agents than the chemicals tested. This is based upon a comparison of the actual weights of the active materials. However, if the molecular weights had been used as a basis, the differences would have become even more magnified since the microbial agents are of considerably higher molecular weight than the chemical disinfectants.

It was found further that a high bacteriostatic action is not necessarily accompanied by a high bactericidal action. Gliotoxin, for example, was probably the most active bacteriostatic substance. However, its bactericidal properties were much lower than those of other preparations. Streptothricin, on the other hand, was highly active bacteriostatically and bactericidally against certain gram-negative bacteria. Certain gram-positive organisms, such as *B. mycoides*, usually very sensitive to the action of other compounds such as actinomycin, was highly resistant to streptothricin.

These results tend to disprove certain claims (Dubos and Hotchkiss, 1941) in regard to specific morphological differences in the behavior of bacteria in accordance with the gram stain. It is true that as a rule the gram-positive bacteria are more sensitive to most of the antibiotic compounds than the gram-negative organisms. But there are substances which act quite differently, showing marked variations within each group. One might just as well separate all spore-forming bacteria on the basis of the action of streptothricin, or separate the spore-forming gram-positive bacteria from the gram-positive cocci on the basis of their sensitivity of gramicidin.

It may also be noted here that some of the antibiotic substances have marked fungistatic and fungicidal properties. This is true especially of gliotoxin and actinomycin. These substances further vary in the degree of their toxicity to animals and in their action *in vivo*. The latter depends upon the solubility of the substances in water, their interaction with blood serum, their rate of excretion, absorption by special organs, etc.

In general, the following eight criteria must be carefully watched in comparing the selective action of antibiotic substances of microbial origin:

1. The test organism employed for measuring antagonistic action, namely, fungi vs. bacteria, gram-positive vs. gram-negative bacteria, one species vs. another species, one strain of the same species vs. another strain, etc.
2. Method of testing, including the composition of the medium, solid vs. liquid media, diffusion of active substance vs. suspension in medium.
3. Bacteriostatic vs. bactericidal action.
4. Mechanism of inhibition of bacterial growth by active substance.
5. Degree of purity of active substance.
6. Solubility of active substance, namely aqueous vs. alcoholic solution.
7. Stability of substance, as influenced by temperature, aeration, reaction.
8. Action *in vitro* vs. action *in vivo*.

## SUMMARY

A comparative study has been made of the bacteriostatic and bactericidal properties of various known substances of microbial origin. Their action was

compared with that of egg-white lysozyme and different chemical agents commonly used as disinfectants. Considerable variation was obtained by the use of different test organisms and different media for testing. A number of different organisms and several media were employed for measuring the comparative bacteriostatic action of the different preparations. From three to ten times as much actinomycin or streptothricin was necessary to cause the same inhibition in brain-heart-infusion agar as in nutrient agar. Inhibition by the slightly soluble products of *Bacillus brevis* was more marked in nutrient broth than in the agar media.

Striking differences were found to exist in the selective action of these substances upon various bacteria. Gramicidin was most specific, acting primarily upon gram-positive micrococci. Actinomycin, tyrothricin, tyrocidine, purified penicillin, gliotoxin and the chemical detergent (lauryl sulfate) acted in low concentrations upon gram-positive bacteria and only to a limited extent upon gram-negative organisms. Pyocyanase, pyocyanin and crude penicillin were similar in their action over the whole range of the test organisms used. Streptothricin was unique in its action, being highly active against certain gram-negative bacteria and having no action against certain gram-positive organisms. Upon a weight basis, the substances of microbial origin were found to be much stronger bacteriostatic agents than the chemical antiseptics tested.

Marked differences were also obtained in the selective bactericidal action of the different preparations. Certain substances possessing high bacteriostatic properties were not necessarily also highly bactericidal.[2]

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[2] The authors are indebted to Merck & Co. for supplying samples of tyrothricin, gramicidin, tyrocidine, pyocyanin and sulfanilamide preparations, to Dr. W. F. Bruce of Cornell University, for the gliotoxin, and to Drs. H. W. Florey and N. G. Heatley, of Oxford University, for the purified penicillin.

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