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THE EFFECTS OF VARIOUS METALS

ON THE

GROWTH OF CERTAIN BACTERIA.

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BY

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THE EFFECTS OF VARIOUS METALS ON THE GROWTH OF CERTAIN BACTERIA.[1]

NÄGELI[2] found that certain metals, even in minute traces, inhibit the growth of certain diatoms. Copper he found to be specially active. Pure gold he found to be inactive; but gold coins placed in water of a neutral reaction inhibited the growth of the diatoms. Certain bodies, such as sulphur, carbon, and the like, were found to deprive the metals of their inhibitory power: if these substances were placed along with the metals in the water containing the diatoms, the latter were unaffected by the metals.

Miller,[3] of Berlin, tested the effect of various metals used in dentistry upon the growth of bacteria. Miller's method consists in inoculating a tube of melted jelly with a large number of organisms and pouring the contents of the tube out on a sterilized glass plate. Bits of the metal to be tested are laid on the jelly while this is still soft. Where the metals have an inhibitory effect upon the growth of the micro-organisms there is a clear zone left around the metal after the colonies grow out on the other parts of the plate.

The various commercial preparations of gold tested in this way gave the following results:

1. Velvet gold has no antiseptic properties.
2. Wolrab's cylinders have very little.
3. Pack's pellets inhibited the growth for about five millimetres all round.
4. Quarter century gold foil and Abbey's non-cohesive foil about the same as Pack's pellets.
5. Rolled gold and sponge gold had no effect.
6. Tin gold had much less effect than gold alone.
7. Tin and platinum had no effect.
8. All gold loses its antiseptic power on being glowed.

[1] Work from the Pathological Laboratory, Johns Hopkins University.

[2] Nägeli, C. von, Ueber oligodynamische Erscheinungen in lebenden Zellen. Denkschrift d. schweiz. naturforsch. Gesell., 1893, Zürich, Bd. xxxiii. 1. See also review in Botan. Centralbl., Bd. lv. 93.

[3] Miller, Demonstration einer Methode zur Bestimm. d. antisep. Eigenschaften v. Zahnfüllungsmitteln, Verhand. d. deutsch. odontolog. Gesell., 1889, Bd. i., 1, p. 34.

Behring[1] tested in the same manner the action of several metals upon the growth of the anthrax bacillus, diphtheria bacillus, bacillus pyocyaneus, cholera bacillus, glanders bacillus, and typhoid bacillus, with the following results:

Abbey's cylinders (gold) gave a clear zone of inhibition with anthrax bacilli (1.5 centimetres), with diphtheria bacilli (three to five centimetres), with cholera bacilli (0.4 centimetre), with bacillus pyocyaneus (one centimetre); but had no effect upon typhoid bacilli or glanders bacilli.

Silver leaf and metallic mercury, and to a small extent copper, nickel, and zinc, gave inhibitory zones. Tin, lead, and iron were negative.

Behring also tested certain insoluble salts of mercury, zinc, and lead. Calomel gave about the same results as metallic mercury, the oxide of mercury being somewhat more active. Cinnabar was entirely indifferent. Mercury and its compounds had just the same effect on all the organisms examined. Gold, silver, and copper coins, and to a limited extent nickel coin, had some effect. Gold coin had no effect on the typhoid or on glanders bacilli. The clear zones tested by inoculation into bouillon showed the micro-organisms to be absent. Behring removed the metal and re-inoculated the clear zones. In these re-inoculations the growth was much retarded, showing that some of the metal must have been in solution in the medium. Behring observed a peculiar discoloration of the silver and lead with the typhoid and cholera bacilli, whereas with the anthrax bacilli and the Finkler-Prior's and Deneke's spirilla there was no discoloration. Gold acts more powerfully upon the anthrax and pyocyaneus bacilli than silver does. Behring draws the conclusion that the metals are dissolved by the products of some of the bacteria more readily than by the products of others.

Uffelmann[2] tested the effect of metals on cholera bacilli by smearing the surface of coins with liquefied jelly cultures of cholera bacilli. The bacilli were dead in seventeen minutes after they were smeared on a copper coin. Cholera bacilli in a cholera stool smeared on silver coins in the same way were dead in twenty-five minutes after they were put on. On a brass coin the cholera bacilli, in a liquefied culture, were alive after thirty hours, but dead after sixty hours. On platinum foil there were living cholera bacilli after one hour, but not after five hours.

In the experiments given below I have made use of Miller's method already described. For the most part agar plates were used, and the metals were put on as soon as the plates were made. In some cases the metals were absolutely pure, in some cases they were commercial, but marked chemically pure, in one set brass foil was used, and a few preliminary experiments were made with impure metals. I am indebted to Professor Morse, of the chemical laboratory of the Johns Hopkins University, for

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[1] Zeitschr. f. Hyg., etc., 1890, Bd. ix. p. 482.

[2] Uffelmann, Beiträge zur Biologie des Cholerabacillus. Berlin. klin. Wochenschr., 1892, No. 48, p. 1212.

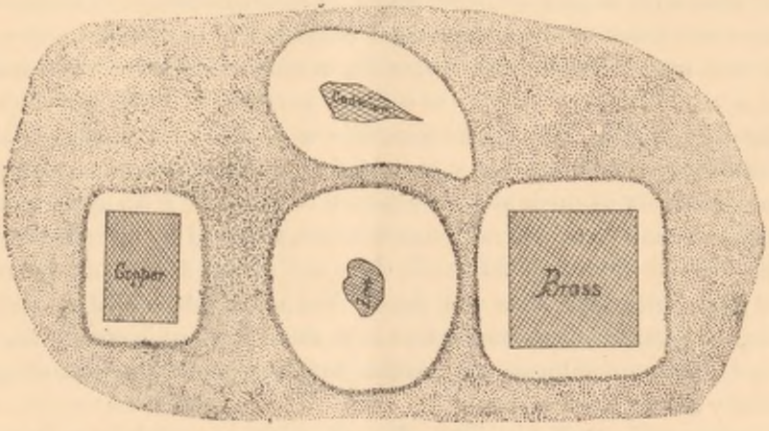
absolutely pure zinc and cadmium. Professor Chittenden, of Yale, kindly sent a sample of pure silicon. The copper, silver, and gold I purified myself, with valuable aid from Dr. G. de Chalmot and Dr. H. M. Parks, both skilful chemists, formerly connected with the chemical laboratory of Johns Hopkins University. The foils mentioned in the description of the experiments were kindly furnished me by Professor Halsted, who used them in his experiments on the dressing of wounds. For the other metals besides those referred to I am indebted to Dr. Berkely, Pathological Laboratory, Johns Hopkins University.

There was no special selection of cultures. These were taken of various ages, and were usually oblique agar cultures. In order to get the reactions described below it is necessary to inoculate the melted medium with a very large amount of the culture, four or five loops to a tube; where smaller amounts were used it was often impossible to make out anything but a clear zone, and the peculiar figures described below were not obtained. If the layer of the medium is too thick the reaction is also interfered with.

Experiments with Copper.—A few preliminary tests were made with various micro-organisms, with ordinary copper wire, and with copper coins. The wire and coins were merely polished with ammonia and sand and then rinsed with water. The rest of the experiments were made with pure copper and with copper foil.

There is no marked difference in the behavior of the micro-organisms towards the copper in these different forms. In all cases there is a clear zone, in some cases narrower, in others wider, and then a narrow zone

FIG. 1.



Action of cadmium, copper, zinc, and brass on the staphylococcus pyogenes aureus.

where there is increased growth. This intensified zone does not have as sharply marked borders as with certain other metals. Both the clear zone and the intensified zone vary appreciably in width, even with the same micro-organism. With staphylococcus pyogenes aureus (Fig. 1) the clear

zone measures in some cases as much as five millimetres in width, and in some cases only two millimetres. The average width of the clear zones in fifteen of the experiments with copper foil is 3.1 millimetres. With the micro-organisms of anthrax, cholera, typhoid, and the colon bacillus the clear zone varies from one millimetre to five millimetres in width with the same organisms at different times. In nearly all cases the medium around the copper becomes greenish and the foil becomes dissolved away, sometimes disappearing entirely.

Experiments with Brass.—The tests with brass were made exclusively with brass foil. The zones obtained with the different micro-organisms were similar to those obtained with copper. The organisms tested with this alloy were staphylococcus pyogenes aureus (Fig 1), typhoid bacillus, bacillus coli communis, bacillus anthracis, bacillus choleræ Asiaticæ, bacillus prodigiosus, bacillus pyocyaneus. The average width of the clear zones in fifteen of the experiments with staphylococcus pyogenes aureus is 2.5 millimetres.

Experiments with Silver.—The results with silver were somewhat less uniform than with copper and brass. A number of preliminary tests were made with polished silver coins, but no record of these was kept. There seemed, however, to be no striking difference between the results with the coins and the results subsequently obtained with carefully purified silver.

The zones obtained around the silver with staphylococcus pyogenes aureus are first a clear zone, one to two millimetres, sometimes even less than one millimetre, followed by a very narrow intensified zone. The intensified zone is sharply marked off from the clear zone and not so sharply marked off on the outside. The intensified zone is better marked with silver than with copper or brass, but it is also narrower. With anthrax cultures the action of the silver varied considerably in different cases. In some cases no zone at all was observable, in some cases there was a zone of about seven millimetres where the colonies were not as thick as on the rest of the plate, and in still others there was a perfectly clear zone of about one millimetre, followed by a zone of five or six millimetres where the colonies were not as thick as on the rest of the plate. With cholera bacilli there was a clear zone, five millimetres broad, followed by an indistinct intensified zone. With typhoid bacilli there was a clear zone about one millimetre; the intensified zone was faint. With the colon bacillus and the bacillus pyocyaneus there was a clear zone about five millimetres wide, then an intensified zone quite sharply marked towards the metal, but shading off gradually on the outside.

Experiments with Gold.—The effect of gold was tested in a few cases with gold foil, and a great many experiments were made with purified gold. No inhibition was observed with staphylococcus pyogenes aureus, colon bacillus, typhoid bacillus, or cholera bacillus. In some cases there was inhibition of the growths of anthrax cultures, and this difference of action is probably to be accounted for by the fact pointed out by Miller, that gold

when freshly glowed has no inhibitory power. In those cases where inhibition was noticed the gold had not been glowed for several weeks. The clear zones when obtained were sharply marked and about one millimetre broad, and there was also some intensification outside.

Experiments with Magnesium.—The magnesium used was the ordinary magnesium ribbon. Tests were made only on staphylococcus pyogenes aureus and the cholera bacillus. With both of these organisms there was a clear inhibitory zone, followed by a zone of increased growth, sharply marked off from the clear zone and gradually fading out on the outside. With the staphylococcus aureus the clear zones average about one millimetre, with cholera bacillus the clear zones average about two millimetres. In every case the bit of metal was raised up above the medium by an accumulation of gas bubbles.

Experiments with Zinc.—A good many experiments were made with ordinary scrap zinc, cast in a sheet, but no note was kept of these. There was a clear zone, however, in every case, and there was probably not much difference between the action of this and of the pure zinc.

With pure zinc all the organisms tested give a broader or narrower clear zone, surrounded by an intensified zone. There is also always a milky discoloration of the medium all around the metal, showing that the metal has been acted upon. This discoloration disappears on the addition of an acid or an alkali.

With staphylococcus pyogenes aureus (Fig. 1) the clear zone measures from five to eight millimetres in breadth, the average being seven millimetres. The intensified zone is very evident, but not sharply marked enough to measure. With cholera there is a wide, clear zone about 1.5 centimetres, and the effect of the metal is seen as far as three centimetres away from the metal. With other organisms the clear zone is usually five millimetres or more broad, followed by a broad, intensified zone that is not sharply marked.

Experiments with Cadmium.—All experiments with cadmium were made with the pure metal. With this metal the reactions obtained with various micro-organisms differ quite strikingly, as a rule. With staphylococcus pyogenes aureus (Fig. 2) there is a clear zone from five to nine millimetres wide, varying in different plates, but no markedly intensified zone. With the colon bacillus the clear zone is about four millimetres wide, surrounded by a very much intensified zone of four millimetres. With typhoid bacillus (Fig. 3) there is a clear zone, five millimetres, then a faintly intensified zone two millimetres broad.

With the cholera bacillus there is a clear zone from five to nine millimetres, not sharply marked on the outside. The most peculiar zone of any with any micro-organism is that obtained with the micro-organism of anthrax and the metal cadmium. In this case (Fig. 4) there is a perfectly clear zone five millimetres wide, then an intensified zone of two millimetres' breadth, and a second inhibitory zone one millimetre wide. In some

cases this second inhibitory zone is not entirely free from colonies, but it can always be made out very distinctly.

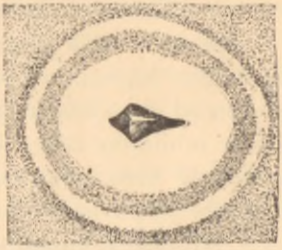
FIG. 2.

Staphylococcus pyogenes aureus. (Cadmium.)

FIG. 3.

Bacillus typhi abdominalis. (Cadmium.)

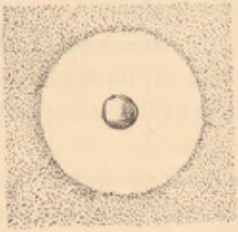
FIG. 4.



Bacillus anthracis. (Cadmium.)

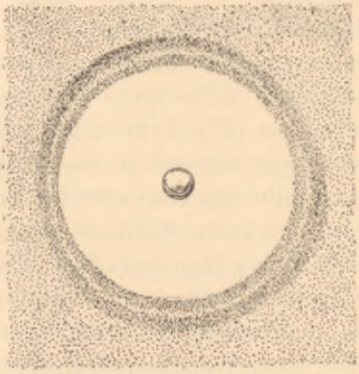
Experiments with Mercury.—There is considerable difference in the behavior of different micro-organisms towards mercury. The zones obtained with staphylococcus pyogenes aureus are first a clear zone about seven millimetres around the metal, followed by a slightly intensified zone which varies in different cases from one to three millimetres in width. With the cholera bacillus there is a clear zone two millimetres around the metal, then a very narrow, intensified zone that is well marked. With bacillus pyocyaneus there is a clear zone four millimetres broad around the metal and outside an intensified zone, sharply marked towards the clear zone and shading off gradually on the outside. With the micro-organism of anthrax there is a broad, clear zone nine millimetres around the metal, surrounded by a very slightly intensified zone that is not sharply marked. The colon bacillus obtained from normal fæces of different individuals and the typhoid bacillus also obtained from different cases show each a characteristic behavior towards the mercury. With the colon bacillus (Fig. 5) there is a clear zone

FIG. 5.



Bacillus coli communis. (Mercury.)

FIG. 6.

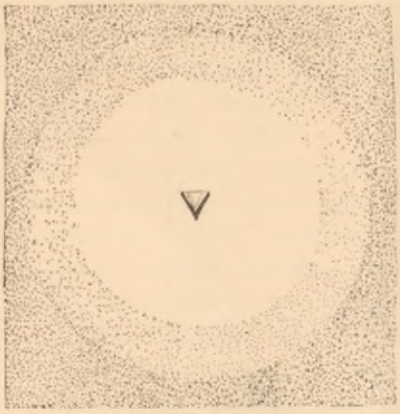


Bacillus typhi abdominalis. (Mercury.)

often seven millimetres broad, sharply marked on the inside, then an intensified zone gradually shading off on the outside. With the typhoid

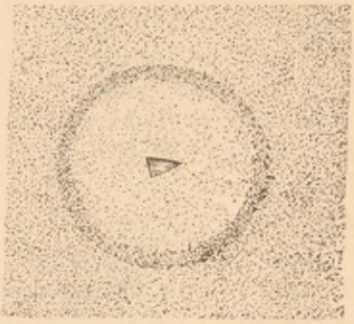
bacillus (Fig. 6) the clear zone is much broader, often one centimetre across, but the peculiarity is the character of the intensified zone. This

FIG. 7.



Staphylococcus pyogenes aureus. (Antimony.)

FIG. 8.



Bacillus coli communis. (Antimony.)

is about two millimetres across, more intense on the outside away from the metal, and in different cases is more or less double,—*i.e.*, there is a narrow, almost clear zone running all around which divides the intensified zone into two zones. Although the number of experiments with both the colon bacillus and with the typhoid was not inconsiderable, it would hardly be safe to regard the reactions as characteristic until a greater number of tests have been made.

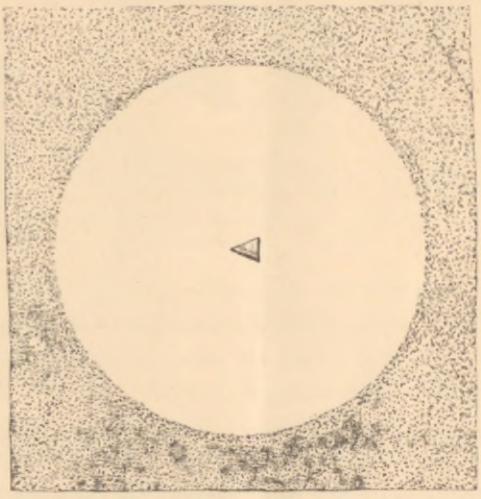
Experiments with Aluminium Foil, with Charcoal, Silicon, Niobium.—A number of tests with charcoal gave no reaction. Aluminium, silicon, and niobium also failed to show any reaction.

Experiments with Antimony.—Antimony with staphylococcus pyogenes aureus (Fig. 7) gives a clear, sharp zone, about one centimetre, then a zone about five millimetres wide where there is diminished growth. In one of the plates with aureus there was only a very narrow, clear zone. The reason for this difference was not apparent. With the colon bacillus (Fig. 8) there is an inhibitory zone, eight millimetres, where the growth of colonies is somewhat thinner than on the rest of the plate, but no clear zone. The intensified zone is quite distinct and about one millimetre broad. With the typhoid bacillus (Fig. 10) there is an almost clear zone, one centimetre, then an intensified zone, two millimetres broad. With the anthrax bacillus (Fig. 9) there is a perfectly clear zone, 1.8 centimetres, then an indistinct, intensified zone. With the cholera bacillus (Fig. 11) there is no sharply marked clear zone, but diminished growth can be made out as far as 1.5 to two centimetres around the metal.

Experiments with Bismuth.—Staphylococcus pyogenes aureus with bismuth gives a clear zone about two millimetres wide, and an indistinct, nar-

row, intensified zone. With anthrax cultures there is a clear zone one millimetre wide. Pyocyaneus, cholera, typhoid, and colon bacilli gave no reaction with bismuth.

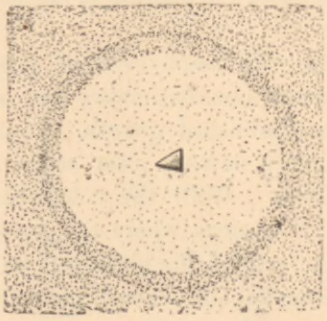
FIG. 9.



Bacillus anthracis. (Antimony.)

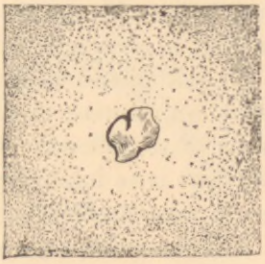
Experiments with Iron.—A bright polished wire nail gave a clear zone about seven to ten millimetres with the typhoid bacillus and with the colon bacillus. There was also discoloration of the medium around the nail. Other organisms were not tested.

FIG. 10.



Bacillus typhi abdominalis. (Antimony.)

FIG. 11.



Cholera. (Antimony.)

Experiments with Nickel.—Pure nickel failed to give any reaction with most of the micro-organisms tested. There was a clear zone with an anthrax culture in the only test that was made with this organism. A nickel five-cent coin gave a clear zone with typhoid bacilli and with colon, but it was possible to detect traces of copper in the medium around the coin.

Experiments with Platinum.—Platinum wire and platinum black failed to give any reaction with any of the micro-organisms tested.

From the above results it is evident that certain metals have at least no marked effect upon the growth of the bacteria tested, and it is notable that it is precisely those metals that are resistant towards chemical reagents in general which fail to show any reaction or do so only to a limited extent. On the other hand, metals that are readily attacked by chemical reagents all exhibit a marked inhibitory action upon the growth of the bacteria. The effect is, therefore, probably due to a solution of the metal in the medium, and putting bits of metal on the cultures is really equivalent to the addition of a small amount of that salt of the metal formed by the action of the nutrient medium. Traces of the metal may, moreover, be detected by chemical reagents in the nutrient medium surrounding the metal. This was done in many cases by simply adding the chemical reagent in small drops to the medium. In some cases the medium was cut out, incinerated on platinum foil, and tested by Behrens's methods.[1] But in many cases a chemical test was not necessary, for the discoloration of the medium alone sufficed to show that the metal had gone into solution, and with the copper and brass foils there were visible holes dissolved out of the metal. Behrens states that the solution of the metal is more or less affected by the products of the growth of the different bacteria. However this may be, some metals are dissolved out in sterile media and may be detected by chemical means.

The explanation of the clear zones is thus quite evident, but the explanation of the intensified zones and of the second inhibitory zone, sometimes seen, is not very apparent. The ideas suggested themselves that the bacteria are driven off from the neighborhood of the metal and collected in the intensified zone, or that the presence of a very small amount of the metal in the medium attracts micro-organisms from all sides. Neither of these explanations seems probable in a solid medium, particularly as many of the organisms tested have no independent motion, and, moreover, the results of experiments made to test this point were negative. These experiments were made as follows: Petri dish cultures of various bacteria, particularly of the motile organisms, were made in very dilute agar, so that the organisms could move about freely and at the same time not be much affected by jarring. After these cultures had grown out, bits of metal were put on the plates and then studied with the microscope. Drop cultures made with diluted agar and having a very small bit of metal on them were also tried. The organisms did not seem to move in any one direction more than in any other.

Another explanation, however, seems to me to explain satisfactorily the

[1] Behrens, Beiträge zur mikro-chemischen analyse. Zeitsch. f. anal. Chem., Jahr. 30, Wiesbaden, 1891. (Detailed in full in Ann. de l'Ecole polyt. de Delft, 1891.)

clear zone immediately around the metals and the intensified zone,—namely, that the dissolved oxides or salts of the metals are in too great concentration in the clear zone, and the trace that is present in the intensified zone may stimulate growth. This does not explain the second inhibited zone.

The length of time it is necessary to leave the metals in contact with the agar, in order to develop inhibitory action, was tried with brass, copper, cadmium, and zinc. Plates of staphylococcus pyogenes aureus were made in the usual way and the metals put on and removed at various intervals. With cadmium there was a clear space where the metal had lain and for one millimetre around where the metal had been left on for a minute. Where the metal had been left on for three or four minutes or more, the clear space usually extended over three millimetres around where the metal had lain. With zinc the results are similar as regard length of time, but the edges of the clear zone are not well defined and there is an intensified zone that is not apparent with cadmium. With brass there was no effect produced by leaving the metal on for thirty-six minutes; after this there was more and more marked inhibition up to fifty minutes, but no clear space except where the metal was left on for a longer time than this. With copper no visible effect was produced in less than thirty-six minutes. After this time there was more and more marked inhibition, but only where the metal had been allowed to lie on for fifty minutes was there a clear space.

Just how far the reactions described above are constant and characteristic will have to be determined by future experiment. It seems probable that they would be constant under the same conditions, since the number of experiments is quite large and the results were gratifyingly uniform, especially in those cases where the figures produced are peculiarly striking. These particularly were repeated a great many times.

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BY

HENRY W. CATTELL, A.M., M.D.

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