

ened the writer's confidence in the hypothesis and led to further investigations. One of these which was of special interest related to acids. For a number of reasons it was supposed that acid would not cause a decrease of permeability. But investigation showed that such a decrease actually occurred in the presence of HCl and it was then a simple matter to predict that antagonism would be found between NaCl and HCl. This turned out to be the case, the amount of antagonism corresponding to the amount of decrease of permeability.⁶

The hypothesis was further tested by investigations on other salts, the most interesting of which are those which (in contrast to those just mentioned) are more effective than CaCl₂ in decreasing permeability, such as La₂(NO₃)₆, Ce₂(NO₃)₆, etc. Here also it was found that the degree of antagonistic action could be foretold by observing the amount of decrease of permeability produced by the pure salts. The results of these investigations afford strong support to the hypothesis.

It seems to the writer that the hypothesis offers a rational explanation of antagonism by showing that salts antagonize each other because they produce *opposite effects on the protoplasm* and by stating definitely what these effects are (it should be noted that they have been measured with considerable accuracy).

The soundness of this point of view is indicated not only by the fact that we are able to predict both qualitatively and (to a considerable extent) quantitatively the effect of combinations of salts⁷ but also by the very signif-

icant fact that we are able to extend this conception to organic compounds and to show that non-electrolytes which decrease permeability can also antagonize such substances as NaCl. These facts indicate that the hypothesis may be applied in a general manner so as to include both electrolytes and non-electrolytes.

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ISOLATION OF *BACILLUS RADICICOLA* FROM SOIL

EVER since the epoch-making achievement of Hellriegel and Wilfarth, reported in 1887, which established the symbiotic relationship between bacteria and legumes in the fixation of atmospheric nitrogen, the legume bacteria, named in 1901, *Bacillus radiculicola* by Beijerinck, have been the object of numerous investigations in all parts of the world. These investigations have assumed a variety of forms and were planned from both the economic and pure science points of view. There has ever remained, nevertheless, the unsolved problem of the direct isolation of *Bacillus radiculicola* from the soil. Sporadic attempts, rather few in number, have been made to attain that end, but,

what there is may perhaps be correlated with the fact that all substances which decrease permeability do not act alike, some producing a much greater decrease than others. Moreover these substances will, if the exposure be sufficiently prolonged, alter their action and increased permeability. The rapidity of this change varies with different substances and this may be related to the fact that some of these substances antagonize each other to some degree. This will be more fully discussed in a subsequent paper.

Experiments on some plants (in which the criterion of antagonism is not electrical resistance but growth) show a fairly strong antagonism between magnesium and calcium. It is possible that for these plants magnesium belongs in the first class.

It will be noted that the hypothesis, as here set forth, says nothing about the mutual relations of substances belonging to the same class but merely states that substances of one class will antagonize those of the other. In this form the hypothesis is completely justified by all the experiments, including those on organic substances.

⁶ *The Journal of Biochemistry*, 19, 1914.

⁷ It should be noted that mixing solutions of two salts which belong to different classes does not produce an effect which is merely intermediate between the two. For example, tissue may be killed by an exposure of 24 hours in NaCl or in CaCl₂ but remain normal in a mixture of these in the proper proportions. Cf. Pringsheim's *Jahrb. f. wiss. Bot.*, 54, 645, 1914.

The writer has found cases in which two substances which can decrease permeability are able to antagonize each other. So far as the writer's experiments with *Laminaria* have gone there is no great amount of antagonism in such cases and

to quote Russell,¹ "none of these organisms (*B. radiculicola*), however, could be found in the soil, nor indeed has any one yet succeeded in finding them there, although their existence can not be doubted." In the literature available to us we have found but one instance in which a claim is made of the direct isolation of *B. radiculicola* from soil not artificially inoculated. That one is the investigation of Gage² who has himself rendered questionable the value of his work by an unfortunately confused use of terminology which has only served to make more difficult than otherwise a comprehension of the present status of the subject. Kellerman and Leonard³ in studying Greig-Smith's claim to having discovered a specific medium for *B. radiculicola* could not find experimental evidence to confirm it. Incidentally the last-named investigators tried to obtain *B. radiculicola* from different soils some of which grew legumes but were unsuccessful in the attempt except in the case of one soil into which pure cultures of *B. radiculicola* had been introduced after its isolation from alfalfa nodules.

While not deeming the matter one of great moment in any sense, since there can be no doubt, as Russell remarks, that *B. radiculicola* is present in any soil in which nodules are found on legumes, the writers decided to attempt the isolation of that organism, and, as a matter of record, submit this brief paper in evidence of the success of their attempt. One of us had for three or four years used as a source of *B. radiculicola* for student work in the laboratory the nodules of a large specimen of *Vicia sicula* growing in the Botanic Gardens on the campus of the University of California, and we therefore decided to attempt the isolation of *B. radiculicola* from the soil in which that plant had grown. The plant had been removed a year or more prior to our initiation of the experiment and the soil had remained bare and unused during that time. Seeds from the plant in question were scattered all over the

surface of the ground, and we gathered them for the later tests which are described below. The soil, so far as we can ascertain, had never been artificially inoculated with cultures of *B. radiculicola*.

Some of the soil just described was taken from below the surface at a depth of about six to eight inches, placed in a sterile container and removed to the laboratory. About 30 grams of the soil were there placed in a sterile bottle, 150 c.c. of sterile water added, and the whole shaken, after being stoppered, for fifteen minutes. The necessary dilutions were then made for purposes of pouring plates. The agar employed at first was of two kinds. The first was similar to that employed by Fred and was constituted as follows:

1,000 grams water
10 grams maltose
1 gram K_2HPO_4 (separately neutralized)
1 gram $MgSO_4$
2 or 3 drops each of 10 per cent. solutions of NaCl,
 $FeCl_3$, $MnSO_4$ and $CaCl_2$
15 grams agar-agar.

The second was a soil extract agar prepared by dissolving 15 grams of agar and 10 grams of maltose in an aqueous extract from the soil above described. The aqueous extract was obtained by boiling one part of soil with three parts of water for one hour and filtering.

In the preliminary tests the soil extract agar gave by far the better results with both the soil to be studied and with commercial cultures of *B. radiculicola* which were employed as controls. By better results we mean that a larger number of colonies developed on the plates poured with the soil extract agar than on those prepared with Fred's *radiculicola* agar. In the later work therefore the soil extract agar was employed exclusively.

From plates of the proper dilution prepared as above described transfers were made to soil extract maltose agar slants by means of a platinum needle from all colonies which appeared to be characteristic of *B. radiculicola* and in fact of any others which appeared to be different from one another. Transfers were thus made from forty-four colonies. After three or four days of growth on the slants, slides were pre-

¹ "Soil Conditions and Plant Growth," D. Van Nostrand Co., 1912, p. 95.

² *Cent. für Bakt.*, 2^{te} Abt., Vol. 27, p. 7.

³ *SCIENCE*, N. S., Vol. 38, p. 95.

pared from all of these organisms and microscopic examinations after several transfers and platings showed the forty-four cultures to be pure. The form of the organisms as viewed under the high power of the microscope varied from short to long rods to oval forms. The detailed results of these examinations, however, can not be given in this brief paper.

The next step in the investigation was to test the powers of inoculation of the forty-four organisms obtained as above described. Our procedure was as follows: A large quantity of fertile sandy soil from Anaheim, Cal., was sterilized in the autoclave for four hours at about $1\frac{1}{2}$ atmospheres of pressure. When it had cooled it was distributed in quantities making a thickness of three inches in quart-size glass fruit jars. The latter were then securely stoppered with cotton and sterilized in the autoclave, thus giving the soil a double sterilization. The jars were then put away for three days to allow the soil to become normally aerated again, and several samples were carefully withdrawn for testing as to sterility. No colonies developed on the agar plates even after many days. The soil thus being shown to be sterile, we proceeded with the *Vicia* seeds as follows. The seeds were placed in a 1 to 1,000 HgCl_2 solution and kept there for ten minutes. They were then thoroughly rinsed with distilled water and treated with concentrated H_2SO_4 for 20 minutes to aid germination. They were then again thoroughly rinsed in sterile distilled water and removed to a sterilized moist chamber containing several layers of water-saturated filter paper. The seeds which thus gave perfect germination in 3 or 4 days as against very poor germination for similar seed untreated with H_2SO_4 were then transferred to the jars with sterile forceps and pressed into the soil by means of a sterilized glass rod without removing the stoppers from the jars. It may be added here that every jar received fifty c.c. of a .5 per cent. dextrose solution to furnish optimum moisture conditions and a proper source of energy for *B. radiculicola*. Five seeds were planted in every jar and the inoculation was accomplished by

the addition, in every case, of a 5 c.c. suspension of the agar slant culture with sterile distilled water. The jars were removed to the greenhouse and remained there for fifty-four days, sterile distilled water being carefully added when necessary. All the plants in all the jars appeared to grow equally well and attained a height of about eight inches. Evidently there was an ample supply of nitrogen in the ammonia or closely related forms to supply even the plants in the five control jars which received no inoculation. Besides the control jars and the forty-four others above described, there were five jars inoculated with commercial cultures as follows: (1) Farmogerm, (2) Nitrogen-gathering Bacteria, (3) Ferguson's Nitrogen Fixing Bacteria, (4) Mulford's Nitro-Germ (weak culture), (5) Mulford's Nitro-Germ (strong culture). After the period mentioned the plants were carefully removed from the soil in every jar and the roots examined, with the following results.

1. No nodules were found on the roots of any of the plants in the control jars.

2. Twenty-one of the forty-four inoculations with bacteria isolated from the soil above described gave positive results and nodules were found on the roots of some or all of the plants in those jars.

3. The balance or twenty-three inoculations gave negative results and none of the plants in those jars showed the presence of nodules on the roots.

4. All the commercial culture inoculations produced nodules except the weak culture obtained from one of the Mulford transfers.

These results would seem therefore to record the first isolation, so far as we know, of *B. radiculicola* directly from the soil; to show that that organism so obtained at least in some forms and places can be readily made to grow on agar plates in large numbers; and to make desirable the use of soil extract-maltose agar for such purposes.

The writers will welcome criticisms of their work which may occur to their colleagues, and to be corrected if, in error, as to priority (excepting Gage's investigation) so far as the recorded isolation of *B. radiculicola* is con-

cerned. Many other facts of interest besides those above discussed have come to light in our investigation, but the limited space of this paper will not permit of their discussion, nor of the submission here of the detailed data which furnish the basis for the discussion above given.

C. B. LIPMAN,
L. W. FOWLER

THE AMERICAN PHYSICAL SOCIETY

THE seventy-fifth meeting of the Physical Society was held in Randall-Morgan Laboratory of the University of Pennsylvania, December 29, 1914, to January 1, 1915. It was a joint meeting with Section B of the American Association for the Advancement of Science. Morning and afternoon sessions were held on Tuesday, Wednesday and Thursday. Vice-president Anthony Zeleny, of Section B, presided on Tuesday and Wednesday afternoons, and President Merritt at the other four sessions.

On Tuesday afternoon the program consisted of the Vice-presidential Address before Section B on "Recent Evidence for the Existence of the Nucleus Atom," by A. D. Cole, and the presidential address of the American Physical Society on "Luminescence," by Ernest Merritt. On Wednesday afternoon there was a symposium on the Use of Dimensional Equations, led by E. Buckingham, who was followed by A. C. Lunn, A. G. Webster, W. S. Franklin and others.

The following program of papers was presented:

"An A. C. Bridge for the Measurement of the Dielectric Loss and Dielectric Constant at High Voltages and Low Frequencies," by Chester A. Butman.

"Influence of the Concentration of Electrolyte upon Electrode Potentials," by Arthur W. Ewell.

"A New Method of Obtaining a Hysteresis Loop," by W. N. Fenninger.

"On Rotation and Magnetization," by S. J. Barnett.

"Note on Thermo E.M.F.'s in which the Resultant Peltier Effect is Zero," by H. C. Barker.

"Linear Resistance Change with Temperature of Certain Molten Metals," by E. F. Northrup.

"The Effect of Temperature on the Dielectric Strength, the Dielectric Loss and the Dielectric Constant of Paraffine Oil," by Chester A. Butman.

"A Preliminary Note on the Variation of Stray Power Losses in a Dynamo," by W. N. Fenninger.

"Relation Between the Energy of the Cathode Rays and the Frequency of the X-Rays Produced by Them," by William Duane.

"Thermionic Currents from a Wehnelt Cathode," by W. Wilson.

"Mobility of Ions at Different Temperature and Constant Gas Density," by Henry A. Erikson.

"The Radioactive Content of Certain Minnesota Soils," by James C. Sanderson. (Read by H. A. Erikson.)

"Conducting Gas Layer at a Metallic Surface," by G. W. Stewart.

"X-Rays From the Electrical Discharge," by Elizabeth R. Laird.

"X-Rays Produced by Slow-moving Cathode Rays," by Elizabeth R. Laird.

"Light Due to Recombination of Ions," by C. D. Child.

"Electric Furnace Evidence on the Relation of Spectrum Lines Having Constant Differences in Wave-Number" (by title), by Arthur S. King.

"The Mechanical Equivalent of Light," by H. E. Ives, W. W. Coblentz and E. F. Kingsbury.

"Fluorescence of the Uranyl Salts under X-Ray Excitation," by Frances G. Wick.

"The Efficiency of Energy Transformation in the Corona Method of Precipitating Fumes," by W. W. Strong.

"Leakage of Gas Through Quartz Tubes" (by title), by E. C. Mayer.

"A New Method for Measuring Gravity at Sea, with Some Trans-Pacific Observations," by Lyman J. Briggs.

"The Oxidation of Nitrogen," by W. W. Strong.

"The Alleged Dissymmetrical Broadening of the D Lines of Sodium," by E. A. Eckhardt.

"Exhibit of Mechanical Models Illustrating (a) Subdivision of Alternating Current Between Two Branches in Parallel, (b) The Alternating Current Transformer, (c) Coupled Circuits in Wireless Telegraphy," by W. S. Franklin.

"Some Causes of Variation in the Sensitivity of Moving Coil Galvanometers," by Paul E. Klopsteg. (Presented by A. Zeleny.)

"A New Standard Phone and Phonometer for any Pitch," by A. G. Webster.

"A New Form of Radiation Pyrometer" (by title), by S. Leroy Brown.

"The Doppler Effect in X-Ray Spectra and Application to the Kinetic Theory of Solids," by L. Gilchrist and D. A. Keys.

"On Acoustic Impedence, and an Approximate Theory of Conical Horns," by A. G. Webster.

"Vapors with Positive Specific Heat in Energy Conversion" (by title), by J. E. Siebel.

"Progress of B-Particles through Matter," by A. F. Kovarik and L. W. McKeethan.

"A Thirty-two Element Harmonic Synthesizer," by Dayton C. Miller.

"The Result of Plotting the Separation of Homologous Pairs against Atomic Numbers instead of Atomic Weights," by Herbert E. Ives and Otto Stuhlmann.

"Beaded Lightning," by W. J. Humphreys.

"A Practical Measurement of Colors," by H. E. Wetherill.

"Preliminary Note on a Mercury-vapor Tube