ABSTRACTS*

GENERAL BACTERIOLOGY


An apparatus has been developed which permits contact of disinfectant and bacteria for as short a period of time as 0.2 second. It consists essentially of a pipette mounted over a platinum loop which in turn is placed a suitable distance above a revolving Petri dish containing water or other neutralizing agent. A known quantity of suspension is placed in the loop and a drop of disinfectant is allowed to fall from the pipette through the loop into the dish. The advantage of the method lies in the fact that it permits the determination of k values with such disinfectants as tincture of iodine and 5 per cent phenol without dilution. With Staphylococcus aureus at 35°C., the former has a value of 325 and the latter of 103. These figures mean that the former is capable of reducing $1 \times 10^{163}$ power organisms to 1 survivor in the space of 0.5 minute, about the minimum contact period with ordinary methods used at present. The significance of this number may be partially appreciated when it is considered that it is over a quintillion quintillion times the number of electrons which could be packed side by side in a space the size of the universe. With the time of exposure used in the apparatus it is possible to have survivors even with the use of as few as 10,000 organisms per cubic centimeter.


Before undertaking the present experiments, the agar cup plate method was standardized. Using the standardized technique, four mercury antiseptics (mercury bichloride, mercurochrome, metaphen and merthiolate) were studied (1) in plain agar and (2) in horse blood.

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agar mixtures. The blood content of the test media varied from 1 to 50 per cent. A stock culture of *Staphylococcus aureus*, having the same resistance to phenol as the F. D. A. strain, was used.

All the mercury antiseptics showed a decreased zone of inhibition of bacterial growth as the blood concentration was increased. The results obtained were expressed by the formula

\[ C = Ke^{-mz} \]

(C, concentration of blood; K, a constant for each antiseptic having the value of C when the zone is zero; e, base Napierian log; m, coefficient of inactivation of the antiseptic; z, zone in millimeters.)

Parallel studies in blood broth and blood agar mixtures showed (1) that the agar, *per se*, had no appreciable effect on the zone size and (2) that in general a correlation could be established between the ordinary antiseptic dilution procedure and the agar cup plate method.

GS. Some Factors Affecting the Germicidal Efficiency of Hypochlorite Solutions. A. S. RUDOLPH AND MAX LEVINE, Department of Bacteriology, Iowa State College, Ames, Iowa.

Studies were made on the comparison of the germicidal efficiency of hypochlorite solutions containing 25, 100, and 500 p.p.m. available chlorine at constant temperature and initial reaction (pH). The effects of reaction and temperature were studied by using hypochlorite solutions containing 25 p.p.m. available chlorine and varying either the pH or temperature, keeping all other factors constant.

To 100-cc. portions of various hypochlorite solutions, contained in two-necked flasks which were held in a water bath at the desired temperature, was added 1 cc. of a freshly prepared suspension of the spores of *Bacillus metiens*. At appropriate intervals an amount of 5 cc. of the spore-hypochlorite mixture was transferred to 45 cc. of sterile distilled water, containing slightly more than enough sodium thiosulphate to neutralize the chlorine carried over. Dilutions were made and plated on standard nutrient agar. Counts were made after incubation for 24 hours at 30°C.

The results show that increasing the concentration of the available chlorine four-fold reduces the killing time to about one-half. Changes in pH affect the germicidal power of hypochlorite solutions to a much greater extent than do changes in the concentration of available chlorine, the germicidal power decreasing with decreasing hydrogen-ion concentration. Germicidal efficiency increased with rising temperatures. The killing time was reduced from 50 to 75 per cent for a rise of 10°C.

Suspensions of spores of *Bacillus metiens* were prepared in Butterfield's dilution "C" water by scraping and washing the growth from standard nutrient agar cultures incubated for 20 days at 30°C. The suspensions were filtered through sterile filter paper to remove clumps and heated at 80°C for 10 minutes to kill vegetative cells. These suspensions showed no appreciable change in resistance to a commercial calcium hypochlorite solution containing 1000 p.p.m. available chlorine during eight months storage in the refrigerator at 10°C (50°F.), nor did the resistance differ significantly from that of dried spores suspended in powdered lactose.


When non-spore-forming bacteria were exposed on agar plates at a distance of approximately 6 cm. to the emanations of a single Sterilamp, most species of pathogenic and non-pathogenic bacteria were killed in less than 2 minutes. The action of the emanations is a direct one and is not due to changes produced in the medium on which the organisms are placed. Mold spores showed practically no susceptibility to the light, but bacterial spores were very susceptible. The bactericidal powers of the Sterilamp do not appear to be any greater than other sources of ultra-violet light previously known.


It is a well known fact that ultra-violet radiations lower the coagulation temperature of pure proteins. Quite as well established is the fact that death in microorganisms is coincident with, or rapidly succeeded by, coagulation of the cell protoplasm. Prompted by these considerations, we have studied the sporocidal action of various combinations of heat and light.

This work shows that bacterial spores may be sensitized to heat by preliminary exposure to ultra-violet light. Spore suspensions, exposed
to suitable ultra-violet radiation followed by heat at 98°C., undergo greater destruction than similar suspensions subjected to the same lethal factors applied in the reverse order. The increased mortality attributable to sensitization varies with the species and the extent of the killing influence. The sensitization mortality represents only a very small part of the total mortality, but in terms of the total number of survivors the comparative differences are significant.

The resistant spores in a culture are more susceptible to heat sensitization than are those which are easily killed by adverse influences. With both heat and ultra-violet radiation, a second application of the same treatment is more sporocidal than combinations of heat and light having the same killing influence. The heat sensitization of spores by ultra-violet radiation is observed when the spores are exposed in a dry or wet condition. Very short ultra-violet rays (1250–2000 Å) are found to be more effective in sensitizing spores to heat than are those which are transmitted by quartz.

G7. Studies on the Biological Effects of Supersonic Vibrations. PAUL J. BEARD and W. F. GANTVOORT, Stanford University, Stanford University, Calif.

Apparatus for the generation of supersonic vibrations is described. It consists of an oscillator with two 500-watt tubes which can be used singly or in parallel. It follows in principle the apparatus described by Wood and Loomis, but with certain modifications. The wave length can be varied between 300 and 1000 kilocycles and the power can be adjusted by means of a variable resistance in the primary circuit. The energy is transmitted to a piezo-active quartz crystal submersed in a dielectric. The latter flows through a cooling system at a rate of 5 gallons per minute and the temperature can be held at any desired level.

On applying energy to the crystal the dielectric becomes so violently agitated that it builds up a fountain about 5 cm. in height. Objects suspended in this fountain are subjected to the effects of the vibrations. Double-distilled water containing triple-distilled mercury developed, with 600 kilocycles, a turbidity lasting over several hours. Exposure for 4 minutes was lethal for mice. Exposure for 15 minutes reduced a culture of Escherichia coli from 70,000,000 to 20,000 cells per cubic centimeter.

G8. An Apparatus for Desiccating Stock Cultures. ALDEN F. ROE, Department of Bacteriology, Hygiene and Preventive Medi-
An apparatus suitable for use in the laboratory with average facilities is described. It consists of a shielded Pyrex glass desiccator with an internal, cooled unit to maintain cultures in the frozen state during desiccation.

The cultures are first concentrated, then frozen with dry ice. After being prepared thus, they are placed in the cooling unit of the desiccator and the latter is evacuated and stored in a low temperature refrigerator for from 12 to 18 hours. Water vapor is absorbed by an efficient dehydrating agent, such as magnesium perchlorate. The water transfer is small, being approximately 10 cc. in the desiccation of 20 cultures.

The individual ampoules containing the desiccated suspensions (from 6 to 12 ampoules per culture) are evacuated in groups, sealed off, and are ready for storage. But a single ampoule need be opened when a culture is desired.

Data are presented on (a) the relative resistance of dried cultures to certain physical agents as well as (b) the value of different suspending media prior to drying.


Until recently there were no filters available for bacteriological work which could be chemically cleaned as well as sterilized before use and which were adaptable to the filtration of small amounts of material. Sintered glass filters of No. 5 porosity presented these possibilities, if they could be relied upon to withhold bacteria. Recently the authors have described tests performed to ascertain the reliability of these filters in withholding bacteria. Since this report the filters have been employed in research and in teaching work for the filtration of solutions of soluble bacterial antigens, carbohydrates, etc., and have been found to be reliable in preventing the passage of bacteria. These filters can be used with very little loss for the filtration of small amounts of material.

Attention was devoted chiefly to preparations or substances which, when supplied in minute amounts, cause development of some of the more exacting bacteria. The test substances were added in varying quantities to synthetic media. *Staphylococcus albus*, *Streptococcus hemolyticus* (scarlet fever), *Corynebacterium diphtheriae*, *Brucella abortus*, *Shigella dysenteriae* and, at times, *Saccharomyces cerevisiae* were used. These organisms failed to develop in synthetic media composed of amino acids, glucose and inorganic salts.

Certain fractions of extracts prepared by us from spleen, liver and yeast caused growth of 5 of the 6 organisms. Amounts of 0.3 microgram (gamma) per cubic centimeter of culture medium were at times sufficient to produce visible growth after several days. Larger amounts resulted in more rapid and luxuriant growth. The factor for propionic acid bacteria of Tatum, Wood and Peterson gave results similar to our preparations.

The following materials showed no growth-promoting effect when used singly under the conditions of our tests: the “sporogenes vitamine” of Knight, three plant auxins, phenyl-acetic acid, glutathione, d l-methionine, threonine, d-lysine, ascorbic acid, lactoflavin, adenine, uracil, allantoin, pimelic acid.

Nicotinic acid and vitamin B, together, but not separately, permitted growth of a staphylococcus, confirming Knight’s work. Beta-alanine and nicotinic acid produced the same effect. Beta-alanine in a restricted synthetic medium permitted growth of a strain of diphtheria bacillus.

The growth-promoting effect could not be reproduced by adding small amounts of metals to the synthetic medium. Also, alterations in the physical character of the medium, particularly those designed to lower the oxidation-reduction potential, did not produce the growth-promoting effect.


Earlier work has shown that a considerable portion of the growth-inducing effect of meat extract for various strains of the diphtheria bacillus is due to the presence of nicotinic acid and β-alanine (or of carnosine) and of salts of potassium, magnesium and calcium. With certain strains, minute traces of pimelic acid, also presumably present in tissue extractives, show a definite growth-enhancing effect. With optimal quantities of these substances, however, only about two-thirds
of the amount of growth produced by whole meat extract can be obtained.

Further investigation has shown that by increasing the quantity of cystine added to the control medium and by supplying small amounts of salts of iron and manganese, even heavier growth is obtained than was formerly believed to be maximal. Thus, when estimating growth by the total nitrogen of the washed bacteria, about 2.25 mgm. of nitrogen were obtainable in the earlier work as against a supposed maximum of approximately 3.5 mgm. With the same strain of organism, and using the same basic medium (casein hydrolysate), 6.0 mgm. of nitrogen are now readily obtainable. It is probable, therefore, that no other organic constituent of meat infusion will be found to be essential for the strains used. That perhaps one or more additional inorganic substances will play a part is indicated by the slightly better growth obtained when a small amount of hay ash is included in the medium.


The recent studies of Mueller on the growth requirements of the diphtheria bacillus and our own on toxin production have led to the formulation of a simple medium for high-titer toxin production on a large scale. By using a complete acid hydrolysate of gelatin as a base supplemented by methionine, cystine, tryptophane, minute amounts of pimelic acid, β-alanine and nicotinic acid, the necessary salts, suitable concentrations of lactic acid, glucose and maltose and by regulation of the minute traces of iron, toxin can be produced routinely having an \( L_f \) value between 45 and 60 \( L_f \) per cubic centimeter. Over 600 liters of toxin averaging better than 30 \( L_f \) per cubic centimeter have been produced on a medium closely related to the above. The many advantages of this medium over previous formulas are as follows: (1) relatively inexpensive; (2) made rapidly and easily; (3) reproducibility excellent (no lot has fallen below 24 \( L_f \) per cubic centimeter); (4) quite specific for Corynebacterium diphtheriae, thus reducing the risk of contamination; (5) constant low nitrogen content (1.7 mgm. of nitrogen per cubic centimeter), thus toxoid formation, with only 0.2 per cent formalin, occurs in 4–5 weeks at 35°C. with no significant variation in the time necessary for complete detoxication; (6) absence of any significant amount of substance in the medium with higher molecular
weight than the simple amino acids, which makes it possible to obtain toxin that is about 70 per cent pure by means of a single ammonium sulfate fractionation and dialysis.


It is probable that oxygen tension is important in the localization and invasion of pathogenic microorganisms. In test tube cultures of pathogenic fungi it is a factor determining depth of growth and invasion of the medium.

Certain pathogenic fungi, grown on Sabouraud’s proof medium (1 per cent peptone, 4 per cent glucose, 1.5 per cent agar, pH 5.6) under atmospheric oxygen tension, show predominantly a surface growth; under 99.8 per cent oxygen a subsurface growth; and under 1 per cent oxygen, a sparser, limited, superficial, sometimes dry film-like growth. Removal of the latter type growth to atmospheric conditions often fails to provoke further growth. The organisms have apparently “asphyxiated” themselves. Similar fungi grown on a medium (0.25 per cent d-glutamic acid, 4 per cent glucose, 1.5 per cent agar, pH 5.6) producing a predominantly subsurface growth under atmospheric oxygen tension, grow more deeply in the medium under 99.8 per cent oxygen and predominantly on the surface under 1 per cent oxygen.

This work demonstrates that invasion of a medium is mutually dependent on constituents of the medium and oxygen tension. Invasion in vivo may likewise be dependent on constituents of the tissue and oxygen tension. Certain organisms invade the medium and grow on the surface using peptone (made from muscle) as the source of nitrogen, grow 0.4 cm. subsurface on substituting a hydrolysate of skin and almost 1 cm. below the surface on substituting a hydrolysate of hair. Variation in certain types of lesion in high and low altitudes substantiate further the importance of oxygen tension.


Further studies on the recently proposed taxonomic key for sub-grouping the mesophilic, aerobic spore-bearing bacilli have led to an investigation of the carbohydrate fermentations of a large number of
cultures. These cultures were obtained in part from soil, and in part from various type culture collections, the latter supplying nearly a hundred differently named species. A synthetic agar medium, with monobasic ammonium phosphate as the source of nitrogen, and incubation periods of two weeks have been employed.

It is observed that those species which have been placed in the same sub-group because of other characteristics attack the same carbon compounds with but a few exceptions, and that the variations which do occur are usually limited to a few sugars. It is also observed that a single species frequently varies in its utilization of some one carbohydrate. Certain strains of Bacillus megatherium readily attacked mannose, while others did not; yet in subsequent tests with the mannose-negative strains, mannose-positive daughter colonies were obtained. Subcultures from these colonies attacked mannose as readily as the originally positive strains. Similar "slow" fermentations of certain carbohydrates are observed for other species.

From the data accumulated, it seems that the fermentation of carbohydrates under the above conditions is useful in the characterization of the various sub-groups of the mesophilic-aerobic bacilli, but that owing to the variations observed within the sub-group, it can not be used to establish the validity of a particular species.

G15. The Carbon Metabolism of Bacterium radiobacter. ALVIN W. HOFER, New York State Agricultural Experiment Station, Geneva, N. Y.

A study has been made of glucose utilization by four strains of Bacterium radiobacter in a mineral salts solution containing a low concentration (0.15 per cent) of sugar. After two weeks of incubation in Eldredge tubes at 25°C., the carbon dioxide was measured by titration of the barium hydroxide in the opposite arm of the tube. The amount of cellular material was estimated by centrifuging, drying and direct weighing of the cells. Glucose was determined by the Schaffer-Hartmann method. An attempt was made to measure the gum, but this was unsuccessful. It was found that 48-58 per cent of the glucose utilized was converted to carbon dioxide, 12-15 per cent to cells, and 30-38 per cent remained unaccounted for, probably because of conversion into gum. When incubated for 7 weeks, the amount of carbon dioxide produced was 65 per cent.

Figures for sucrose were very similar to those for glucose. Eighteen other carbon sources were tested simply by determining the percentage
of original carbon converted to carbon dioxide. This figure checked closely with the figures for glucose and sucrose, suggesting that the carbon metabolism of the organism is identical for the various carbon sources.

G16. The Bound Water Content of Vegetative and Spore Forms of Bacteria. C. A. Friedman and B. S. Henry, Department of Bacteriology, University of Washington, Seattle, Wash.

The authors have previously shown that the total water content of the vegetative cells and the spores of Bacillus subtilis, Bacillus megatherium and Bacillus mycoides is essentially the same. This finding confirmed the work of Virtanen and Pulkki which indicated that the observed heat-resistance of spores is not due to a low water content. The present paper shows that the water existing in a free or unbound state, as determined by the cryoscopic method, is greater in the vegetative cells than in the spores of the three species of bacteria mentioned above. The theory is advanced that the bound water in the spores is not involved in the coagulation of the protein due to heat and that, while the total moisture content of the two types of cells is the same, the difference in free water might account for the observed difference in the heat-resistance of the cells.


It was shown by the author in 1936 that violacein, the violet pigment of Bacillus violaceus (Chromobacterium violaceum), contains one or more pyrrolic groups in its molecule. Work on the culturing of the organism and extraction of the pigment has been suspended, but an examination of the small quantity of violacein remaining on hand indicates that the pyrrolic group (or groups) occurs in a form similar to that found in indigo. Decomposition of the pigment by heating for several hours in a solution of sodium hydroxide in the presence of air gave a grape-like smell similar to that of anthranilic acid or its esters. Acidification with hydrochloric acid and filtration to remove amorphous brown material, gave a solution which, after careful diazotization with nitrous acid, produced the color reactions typical of anthranilic acid when treated with alpha-naphthol or dimethylaniline under the proper conditions. Due to the small amounts of pigment remaining, it was not possible to isolate anthranilic acid as such. Accordingly, the results must be
regarded only as a strong indication of the production of the acid or of some related compound. Since the pyrrolic product (previously obtained by strong reduction of violacein) is apparently not indol but a compound of higher molecular weight, and since the results of other workers indicate that the molecular weight of violacein is considerably greater than that of indigo, it seems highly probable that violacein may consist of an indigo nucleus with additional groups of considerable size attached.

G18. Classification and Pathogenicity of Microorganisms. III. Toxins and Toxic Products. ERNEST A. PRIBRAM, Loyola University Medical School, Chicago, Ill.

The "toxic products" of microorganisms attacking animal tissue are irregularly distributed among organisms of the same genus. A deeper study of their nature, however, reveals that the "toxic products" produced by species of related botanical units show common characteristics. In this abstract the term "toxic products" includes toxins with antigenic characteristics as well as substances which do not produce antibodies, but which are not simple chemical compounds.

The "toxic products" of microorganisms may be classified, as follows:

I. Hematotrope: a, erythrocytotoxins; b, granulocytotoxins; c, fibrinolytic toxins;

II. Lymphotrope: a, for lymph follicles of intestinal tract; b, for lymph nodes;

III. Histiotrope: a, for endothelium of vessels; b, for connective tissue and muscle fibers;

IV. Neurotrope: a, nerve cells; b, adrenals.

These "toxic products" have a distribution, as follows: Micrococcus Ia, b; Streptococcus Ia, b, c; Vibrio Ia; Pseudomonas (lipoids) Ia; Escherichia Ia, IIa; Brucella IIa; Salmonella IIa; Eberthella IIa; Shigella IIa, IVa; Pasteurella IIb, IIa; Hemophilus IIIa; Bacillus Ia, IIIb; Clostridium Ia, IIIb, IVa, b; Corynebacterium IIIb, IVa, b; Fungi: Eumycetes (Aspergillus spores) IVa; Basidiomycetes Ia, IVa.


The tables to be presented reduce procedures, calculations, and possibilities for error in the standardization of concentrated staphylococcus antitoxins to a minimum. The dilutions of the reagents, the kind of
animal employed in the hemolytic, dermonecrotic and lethal tests, and
the methods and quantities of injection into the animals are embodied
in the tables. Through the introduction of a dilution factor, \( B = C_h/20 \),
the procedures for the dermonecrotic and lethal tests have been fixed
for staphylococcus antitoxins of any concentration.

The calculation of results is prescribed by the following formulas:
\[
C_h = N_h \times V_h, \text{ for the hemolytic test;}
\]
\[
C_r = N_r \times B \times V_r, \text{ for the dermonecrotic test; and}
\]
\[
C_l = N_l \times B \times V_l, \text{ for the lethal test.}
\]
\( C_h, C_r, \) and \( C_l \) are the corrected and \( N_h, (N_r \times B), (N_l \times B) \) = the
non-corrected numbers of antihemolytic, antidermonecrotic and anti-
lethal units per cc. of the antitoxin being standardized.

\( B = \) a dilution of the antitoxin such that 1 cc. contains 20 standard
antihemolytic units. As stated above, \( B = C_h/20. \)

\( V_h, V_r, \) and \( V_l \) = factors of correction. These factors are functions
of the volume of the U. S. Standard Staphylococcus Antitoxin which is
indicated by the end-point of the control titrations.

Predetermined numerical values of \( N_r \) and \( N_l \) and of \( V_r \) and \( V_l \) in
the above equations are entered into the tables to correspond to the
end-points of the titrations, so that at the completion of the tests, the
worker need only substitute such values for the symbols in the formulas
and perform the task of multiplication.

G20. The Production of Staphylococcus Toxin in Fluid Media. E. P.
Casman, Abington Memorial Hospital, Abington, Pa.

The addition of agar to the medium and cultivation under a gaseous
mixture of carbon dioxide and oxygen have been two important pro-
cedures in the production of staphylococcus toxin. Small quantities
of semi-solid medium are distributed in suitable containers to obtain a
shallow layer and, after inoculation and incubation, the cultures are
filtered to remove the agar. In order to facilitate the study of the factors
involved in toxin production and for obvious practical considera-
tions, elimination of the use of agar in the medium is desirable. It was
found that by slowly bubbling the mixture of gases through the culture,
good toxin production was obtained in from 48 to 72 hours, depending
upon the pH, the composition of the medium and the gas mixture used.

G21. The Titration of Bifermentans Antitoxin and the Relationship
between Clostridium bifermentans and Clostridium sordellii.
Sarah E. Stewart, National Institute of Health, Washington,
D. C.
In the course of the standardization of sordellii antitoxin the author was confronted with the problem of the relationship of Clostridium sordellii and Clostridium bifermentans. It is believed by various investigators that Clostridium sordellii, a proteolytic anaerobic bacillus which produces a strong exotoxin, and Clostridium bifermentans, a non-toxin-producing proteolytic anaerobe, may represent a single species.

For the titration of sordellii antitoxin, a dried toxin and antitoxin were prepared. The unit chosen was based on the one proposed by Alfredo Sordelli. Mice were used as the test animals and inoculations were made intravenously. Since Clostridium bifermentans does not produce a toxin, antisera were produced in rabbits against 4 strains and these antisera were tested against the dried sordellii toxin. The Clostridium bifermentans sera were found to give protection against 2-5 M.L.D. of sordellii toxin.

A comparative study was then made of strains from both of these species. They were found to be identical in morphology, colony formation and biochemical reactions. Cross-agglutination and cross-precipitation reactions were also obtained.

From the above studies, it appears that the two species are identical. Since the species name bifermentans has priority over sordellii, it is proposed that the designation Clostridium bifermentans should be used to cover the two species, and that the antitoxin made against the toxin be designated as bifermentans antitoxin.

G22. The Action of Chemical and Physical Agents on Clostridium welchii and Its Toxin. FRANCIS E. COLIEN, Creighton University, School of Medicine, Omaha, Neb.

The action of glutathione, cysteine, hydrogen peroxide, leucocytes and x-rays on the toxin of Clostridium welchii was studied in vivo and in vitro. The theories which have been given to explain the factors concerned with the destruction of toxin in cases of gas gangrene are various. Hence, an attempt was made to study in vitro the action on toxin of chemical substances found in living tissue. In addition, toxin, cultures of Clostridium welchii in broth containing sterile tissue, and toxin plus sterile tissue were subjected to x-rays. Pigeons were used in all cases to determine the resulting toxicity. The action of x-rays on guinea pigs, rabbits, roosters and pigeons injected with toxin or living organisms was also studied. Various methods were employed for inoculating the experimental animals with Clostridium welchii.

The possibility that certain chemicals, either alone or after release
from tissue by x-rays, act as detoxifying agents is discussed. We have little conclusive evidence on the detoxification of the toxin of *Clostridium welchii* by x-rays; there seems to be some evidence, however, that early treatment with x-rays may prevent the development of gas gangrene, and that it may even have some effect in treatment.

**G23. A Stable Hemolysin-Leucocidin Isolated from \( \beta \)-Hemolytic Streptococci.** E. J. Czarnetzky and Isabel M. Morgan, University of Pennsylvania School of Medicine, Philadelphia, Pa.

The isolation of a stable hemolysin-leucocidin from \( \beta \)-hemolytic streptococci has been reported. As hemolysin, it is active to a dilution of 1:40,000; it has been shown, by inhibition of the reduction of methylene blue by leucocytes, to be a leucocidin. The hemolysin has a molecular weight of 2260, and its crystalline derivative a molecular weight of 720. Further work has shown the empirical formula of the stable hemolysin to be \( \text{C}_{70}\text{H}_{140}\text{O}_{71}\text{P} \).

Intraperitoneal injection of the crystalline derivative into mice (Dr. H. Molitor) has shown that the minimal lethal dose for mice is of the order of 0.001 mgm. By the injection of single doses of various sizes into rabbits, we have found the minimal lethal dose to be of the order of 1 mgm. Repeated injection into rabbits of sub-lethal doses (of increasing size, approaching the lethal dose) produced no quantitative effect on the circulating leucocytes; however, an anemia developed with an erythrocyte count of about 3.5 million per cubic millimeter, compared with a count of about 5.5 million per cubic millimeter in a group of normal rabbits living under the same conditions. The rabbits did not develop a tolerance for the material, the final fatal dose in each case being of the same order as the minimal lethal dose for normal rabbits (the material has never been shown to be antigenic in the sense of producing antibodies on injection).


The experiments reported concern the antigenic and synergistic capacities of a toxic serum-extract of hemolytic streptococci (described by Weld). A special study was made of the ability of this toxic serum-extract to cause an animal's own serum to become irritating when injected into its peculiarly hypersensitized skin. The toxic serum-extract was prepared by shaking portions of pooled rabbit serum for one
hour at room temperature with sediment from broth cultures of a Group A hemolytic streptococcus (Strain C-203). The same strain was also used for animal inoculations and serological tests. Five groups of rabbits were studied: Group A, untreated control group; Group B, culture given intravenously; Group C, culture given intracutaneously; Group D, toxic serum-extract given intravenously; and Group E, toxic serum-extract given intracutaneously. Humoral antibodies for the homologous organism appeared in high titer in the sera from members of Group B, and irregularly in those from Group C, but not in other groups. The response to intracutaneous injection of culture was altered only in Groups B and C; an immune hypoergic reaction was elicited in the former, and a hyperergic response was demonstrable in the latter. Cutaneous hypersensitivity, both to the toxic extract and to normal untreated rabbit serum, developed in members of Group E and to a lesser degree in those of Group C. Such cutaneous lesions in Groups B and D were smaller than among the controls. The toxic serum-extract was most effective as a synergist in inducing cutaneous hypersensitivity in rabbits to normal (untreated) blood serum of that species.

G25. Demonstration of Bacterial Anticoagulants in vivo. ERWIN NETER, University of Buffalo and Children's Hospital, Buffalo, N. Y.

Of the two substances, fibrinolysin and anticoagulant, produced by the hemolytic streptococcus in vitro, the former has previously been demonstrated in vivo. Experiments are reported herewith which deal with the demonstration of bacterial anticoagulants in purulent exudates of lesions in man. The tests were performed by mixing the supernatant fluid of the exudate with plasma and a solution of calcium chloride. The anticoagulant continuously inhibited plasma coagulation.

Of 109 purulent exudates, 25—including empyema fluids, peritoneal and pericardial exudates, spinal fluids and abscess material—contained anticoagulants. The microorganisms implicated were: Streptococcus hemolyticus, Streptococcus fecalis, pneumococcus, staphylococcus, influenza bacillus, Escherichia coli and Clostridium welchii. These experiments show that anticoagulants may be found in exudates of man due to a variety of microorganisms.

In view of Goodner's observations that the edema fluid from dermal pneumococcal lesions in rabbits retards blood coagulation, pneumococcal exudates of man were tested for the presence of anticoagulant. Of 29 exudates, 3 due to pneumococcus (Types I, VII and XIII) were positive.

The nature of the anticoagulating factor found in vivo is not known.
It may be mentioned, however, that the anticoagulating action of purulent exudates may be due to at least two different mechanisms, because it was possible to inhibit the anticoagulating activity in some exudates (6 specimens) by means of normal horse serum and in others (4 specimens) by means of a 0.25 per cent solution of calcium chloride.

G26. The Systematic Relationships of the Autotrophic Bacteria. R. S. BREED AND H. J. CONN, New York State Agricultural Experiment Station, Geneva, N. Y.

The systematic relationships of autotrophic bacteria remain an interesting problem for speculation. The assumption that such bacteria represent the most primitive known forms of life was questioned by the authors in 1918. It is possible that these forms represent a specialized adaptation to inorganic food developed after certain types of bacteria had become adapted to life in soil. Bacteria with their highly complex protoplasmic structure can hardly be regarded as the simplest forms of life, even though their metabolism is simple in character. Research has shown that organized bodies exist which are apparently living but which have a much simpler protoplasmic structure than autotrophic bacteria.

Recent discussions of the systematic relationships of bacteria (Pribram, Kluvyver and Van Niel, Rahn) have clarified the relationships of autotrophic and other groups of bacteria in a helpful way, but the discussions are not in entire accord with each other nor in complete agreement with the ideas expressed by others.

The autotrophic organisms, in recent editions of Bergey’s Manual, have been placed in the family Nitrobacteriaceae, distinct from either Bacteriaceae or Coccaceae. As given, the family also contains certain parasitic and saprophytic bacteria (Rhizobium, Azotobacter and Acetobacter) which should be removed from the group. The present tendency not to recognize divisions on the basis of cell-form, makes it possible to consider the autotrophic bacteria as a family or tribe coordinate with the tribes of the present family Bacteriaceae. If the family Bacteriaceae is dropped, the tribes become families.

G27. Taxonomic Relationship of Lactobacillus bifidus [Bacillus bifidus (Tissier)] and Bacteroides bifidus (Eggerth). JAMES E. WEISS AND LEO F. RETTGER, Department of Biology, Brooklyn College, Brooklyn, N. Y. and Department of Bacteriology, Yale University, New Haven, Conn.

A taxonomic study was made of various strains of bacteria which
The organisms group themselves as follows:

**Lactobacillus bifidus,** Type I. Synonyms: *Bacillus bifidus* (Tissier); *Bacteroides bifidus,* Group I (Eggerth); *Lactobacillus bifidus* (Weiss and Rettger).

**Lactobacillus bifidus,** Type II, *(Lactobacillus parabifidus).* Synonyms: *Bacteroides bifidus,* Group II (Eggerth); *Bacterium bifidum* (Orla-Jensen).

G28. **Comparison of Bacterium necrophorum from Ulcerative Colitis in Man with Strains Isolated from Animals.** G. M. Dack, L. R. Dragstedt, Robert Johnson and N. B. McCullough, Department of Bacteriology and Parasitology, University of Chicago, Chicago, Ill.

A study has been made of 42 strains of non-sporulating Gram-negative anaerobes which we have called *Bacterium necrophorum.* Nineteen of these strains were isolated from the colons of patients with chronic ulcerative colitis. According to the literature some of these strains should be named *Bacterium funduliforme* and others *Bacterium necrophorum.* Twelve strains isolated from bovine liver abscesses would qualify as representatives of the latter group.

The purpose of this investigation was to compare the two groups in order to determine whether we were dealing with one or with two species. Accordingly, a study was made of the growth requirements of the strains, colony morphology, cell morphology on different media,
biochemical reactions, pathogenicity for rabbits and ability to produce ulceration of the colons of experimental animals. From this study no sharp line differentiating these bacteria was found. Strain variations in morphology were observed, but these were not sufficient for species differentiation. Likewise there was a difference in pathogenicity; only the 12 strains isolated from bovine liver abscesses were lethal to rabbits when injected subcutaneously. However, there were marked variations among the other strains in regard to ability to produce lesions. The type of lesion appeared to be similar with all strains, with a gradient from slightly virulent to highly virulent types, as represented by the strains isolated from the bovine liver abscesses.

Since there is no clear cut method for separating these two species, it appears better to retain the name, Bacterium necrophorum.

G29. Nitrogen Availability as an Aid in Differentiation of Bacteria in the Coli-Aerogenes Group. N. B. MITCHELL AND MAX LEVINE, Department of Bacteriology, Iowa State College, Ames, Iowa.

The availability of the nitrogen of nucleic acid and certain of its degradation products for the coli-aerogenes group of bacteria was investigated. The purpose was to secure an approach, other than by carbohydrate studies, to the systematic and physiological relationships of the organisms comprising this group. Previous work on uric acid suggested the possibility of employing the purines for this purpose.

The compounds tested included yeast nucleic acid, xanthine, adenine sulfate, uric acid, uracil, allantoin, hydantoin and urea. Over 350 coli-aerogenes strains were used. This collection was considered representative of the Escherichia, Aerobacter and “intermediate” groups.

Xanthine and adenine were attacked by all strains, and consequently had no differential value. The Aerobacter strains utilized the nitrogen of all the other compounds, whereas the Escherichia and “intermediate” strains were each able to utilize the nitrogen of only one of the compounds listed. Uracil was an available nitrogen source for Escherichia, but not for “intermediate” strains. Urea was an available nitrogen source for “intermediate,” but not for Escherichia strains. These data, when correlated with the results of Voges-Proskauer, citrate, hydrogen sulfide, and indol tests, strengthen the evidence for allocating the “intermediate” strains to a separate genus, Citrobacter. About 90 per cent of the “intermediates” gave reactions identical with those of a transfer of the original strain, Citrobacter freundii, the type species of the genus Citrobacter.
Studies of nitrogen availability provided a means for generic allocation of a number of strains which could not be adequately classified on the basis of carbohydrate dissimilation.

**G30. A Study of the Paracoli Group.** Jacob L. Stokes, R. H. Weaver and M. Scherago, Department of Bacteriology, University of Kentucky, Lexington, Ky.

Thirty-two strains of the paracoli group were isolated from human feces and their morphological and biochemical characteristics were studied. All strains produced some degree of fermentation of lactose on continued cultivation in 5 per cent lactose broth. From 22 strains, variants were obtained that were able to ferment 1 per cent lactose broth within 48 hours with the production of acid and gas. These variants, therefore, were indistinguishable from members of the cloilaerogenes group.

It is concluded that the strains of the paracoli group studied are variants of various members of the coliaerogenes group. On the basis of the methyl-red, Voges-Proskauer and sodium citrate tests, 30 of the strains appear to be variants of members of the *Escherichia* genus and 2, variants of members of the coliaerogenes "intermediate" group.

**G31. Comparative Studies of Methods for the Detection of Hydrogen Sulfide in the Coli-Aerogenes Group.** Charles A. Hunter and James E. Weiss, State Health Laboratory, University of South Dakota, Vermillion, S. D., Kansas Public Health Laboratories, Topeka, Kan., and Department of Biology, Brooklyn College, Brooklyn, N. Y.

The ability of the coliaerogenes group to produce hydrogen sulfide was studied, using 152 strains. Ninety-eight cultures were isolated from water supplies, 35 from human feces and 19 were stock cultures. The cultures were classified into three groups: *Escherichia coli*, *Aerobacter aerogenes* and "intermediates." The methods for detecting the formation of hydrogen sulfide were: Difco lead acetate, Difco peptone iron, semi-solid peptone iron (0.5 per cent agar), semi-solid bismuth mannitol agar (Hunter and Crecelius) and bismuth filter paper. Of the 78 cultures of *Escherichia coli*, only 2 gave a positive reaction in the lead acetate and peptone iron media, but in the bismuth medium all 78 were positive, and with bismuth paper 57 were positive. With the 16 aerogenes strains no hydrogen sulfide was formed in the lead acetate or peptone iron media, while all strains were positive in the bismuth
medium and 12 gave positive reactions with bismuth paper. There were 57 cultures classified as "intermediates," of which 19 were positive in the lead and iron media and 56 were positive with semi-solid bismuth mannitol agar. The bismuth paper showed that 34 cultures produced hydrogen sulfide. The semi-solid peptone iron medium failed to show any increase in the number of positive reactions and did not prove as satisfactory as the usual 1.5 per cent agar. These results show that practically all members of the coli-aerogenes group produce hydrogen sulfide, when tested with a sensitive indicator, the only differences in the organisms being the quantity of hydrogen sulfide produced.

G32. The Morphological Variation of the Tubercle Bacillus. HARRIETTE D. VERA AND LEO F. RETTGER, Department of Bacteriology, Yale University, New Haven, Conn.

The cellular variation of the tubercle bacillus was studied in microculture by means of hanging block preparations which permitted observation of single cells or microcolonies over considerable periods of time. Stained smears, impression preparations, filtration experiments, and cross sections of colonies were also used. An attempt was made to correlate acidfastness and growth conditions with the forms observed.

Morphological variants were induced readily and in great diversity by alteration of food supply and oxygen tension. They included club forms, spore-like bodies, granules of various sizes, coccoid, diphtheroid, and branching cells. Formation of granules in, or from, rods was observed repeatedly. Granules, "spores," and all cells that differed greatly from the typical rod failed to grow under conditions favorable to multiplication of bacilli. Branched cells, however, were observed to grow and segment so as to form bacilli indistinguishable from neighboring bacilli formed by simple division. Although branching was observed in the four strains studied, the usual method of reproduction was division. No evidence of reproduction by granular or filtrable forms was obtained.

G88. Notes on the History of Bacteriology. The Introduction of Agar-Agar into Bacteriology. ARTHUR PARKER HITCHENS AND MORRIS C. LEIKIND, Army Medical School, Washington, D. C. and Institute of the History of Medicine, Johns Hopkins University, Baltimore, Md.

Agar-agar was introduced into bacteriology by Frau Fanny Eilsheimer Hesse, wife of Dr. Walther Hesse, a district physician, of Schwar-
zenberg, Saxony. Although no exact date is ascertainable, agar was first used sometime during the early 1880's. Frau Hesse who was assisting her husband in his studies on the bacterial content of the air (begun in Koch's laboratory) suggested the use of agar as a solidifying agent to eliminate the difficulties arising from the use of gelatin. The idea apparently came to Frau Hesse from her use of agar in her kitchen in the making of fruit jellies. She had been given the recipe by her mother who in turn had received it from a Dutch family; they had brought it from Batavia in the Dutch East Indies, where they had lived before coming to America.

When agar was found to be successful in Dr. Hesse's experiments, he communicated the discovery to Robert Koch by letter. Koch recognized the value of this and adopted its use in his laboratory. No formal paper was ever published on this discovery.

Frau Hesse of German (Hanover) descent was born in Jersey City, N. J., in 1850. She met her husband while travelling in Germany and spent the rest of her life there. She died in 1934, in Dresden.

MEDICAL BACTERIOLOGY, IMMUNOLOGY AND COMPARATIVE PATHOLOGY

M1. Antigenicity, with Especial Reference to Infectious Agents. Michael Heidelberger, College of Physicians and Surgeons, Columbia University, and Presbyterian Hospital, New York City.

A brief discussion is given of what is known of the chemical basis of the immunological specificity of single antigens, such as crystalline egg albumin, crystalline serum albumin, and thyroglobulin. The multiplicity of antigens in most natural products and infectious agents is emphasized. An account is given of some of the chemical factors involved in the immunological behavior of pneumococci, streptococci, tubercle bacilli, and the Salmonella and cholera groups of microorganisms.


The virulence-enhancing effect of animal passage is well established. No investigations, however, have been made on the rôle of the different organs in the production of the antigenic changes correlated with the change in virulence. Experiments undertaken partly with Bacillus typhosus and partly with a newly isolated paratyphoid strain (Stanley)