of being entrusted with these young lives during the formative period.

Even in a school like ours the faculty can exert a strong personal influence for good and can, if they will, create an atmosphere of honesty which should be of special benefit to the students in connection with that vexed question of examinations. The responsibility for honest examinations first rests on the examiners. And we must remember that the man who is not honest in the class-room defrauds his *alma mater* and weakens and debauches his own character.

God grant that such an influence shall always be around the students of Stevens, and that so they may go out into the world not only honestly trained to take their places in the engineering profession, but also influenced to do their whole duty as citizens and self-respecting, God-fearing gentlemen.

ALEX. C. HUMPHREYS.

THE SOCIETY OF AMERICAN BACTERIOLOGISTS.

THE fourth annual meeting of the society was held at the Columbian University Medical School, Washington, D. C., on December 30, 31, 1902. Abstracts of papers* presented at the sessions of the society follow herewith:

Contribution to the Study of Agglutinins: W. W. FORD and J. T. HALSEY. (From the Pathological Laboratory, John Hopkins University.)

Experiments were undertaken to determine which constituent of the red blood corpuscle takes part in the production of lysins and agglutinins when the blood of one species of animal is used to immunize another species, Bordet stating that the stroma was responsible for the lysins, Nolf maintaining that the stroma was responsible for the agglutinins, the laked blood for the lysins. In the present experiments rabbits and guinea-pigs were immunized with the stroma and the laked blood of hens; guinea-pigs with the stroma and laked blood of rabbits; rabbits with the stroma, the laked blood, and the washings from the stroma, of the goose; and rabbits and guinea-pigs with the hæmoglobin of hens' and dogs' blood.

For the preparation of the stroma and the laked blood, the blood was washed with isotonic salt solution, laked with two to three times its bulk of water, made up to one per cent. salt solution, and centrifugalized to separate stroma from aqueous solution. Stroma was then washed repeatedly with water made up to one per cent. salt solution or with isotonic salt solution.

For the preparation of hæmoglobin the blood was collected in ammonium oxalate, washed, laked with distilled water, centrifugalized to get rid of the stroma, treated with 25 per cent. absolute alcohol, upon the addition of which the crystals of oxyhæmoglobin are deposited at 0° Centigrade. The dog's hæmoglobin crystallizes readily, the hen's hæmoglobin with some difficulty.

The results of the experiments showed that in all cases the animals immunized with the laked blood and the stroma from rabbits and from hens developed in their sera agglutinins and lysins both far beyond the limits of normal variation, so powerful that frequently in dilutions of 1–100, always in dilutions of 1–50, complete agglutination and lysis took place. The rabbits immunized with goose's blood stroma and aqueous solutions developed agglutinins only—no lysis taking place. The agglutinins were present in very high dilutions, at times 1–10,000, always in dilutions of 1–1,000. Normal rabbit's serum agglutinates goose's blood in dilutions of 1–250 or 1–330. The attempt to supply a complement for a hypothetical amboceptor

* The abstracts were prepared by the authors.

with hen's, rabbit's and guinea-pig's sera was unsuccessful. The animals immunized with dog's hæmoglobin possessed, after repeated injections, a serum not differing from the normal, while the animals treated with hen's hæmoglobin developed agglutinins and lysins present in dilutions of 1–100 parts.

On the Nature of 'Pyocyanolysin': E. O. Jordan, University of Chicago.

A number of bacteria, including pathogenic forms like the tetanus bacillus, and ordinary saprophytes like *B. megatherium*, have been reported as producing hæmolysins in their broth cultures. *B. pyocyaneus* is one of these forms, and 'pyocyanolysin' has been generally considered as belonging in the same general category with tetanolysin and staphylolysin.

The well-known laking effect of alkalies and the fact that old cultures of *B. pyocyaneus* possess a strongly alkaline reaction led to inquiry into the relation between the alkalinity of the bacterial filtrate and the power of the filtrate to produce hæmolysis. It was found that the filtrates from broth cultures of *B. pyocyaneus* (seven strains, one freshly isolated and quite virulent) produced no greater hæmolysis than NaCl solution, or sterile broth of the same degree of alkalinity. The alkalinity of *B. pyocyaneus* filtrate sometimes reaches as high as 2.6 per cent. normal alkali. If the alkalinity of the *B. pyocyaneus* filtrate be increased or diminished, the hæmolyzing power is correspondingly affected. The hæmolytic power is practically destroyed by neutralization (indicator, phenolphthalein). Most bacterial hæmolysins, like the hæmolysins of blood sera, are inactivated by exposure to a temperature of 56°; but 'pyocyanolysin' will withstand 125° for at least an hour. The statements regarding 'pyocyanolysin' made by Bullock and Hunter, Weingeroff, Breymann and Loew indicate that the hæmolyzing power observed by these writers in the filtrate of *B. pyocyaneus* is no greater than might be due to the simple alkalinity of the medium. It is possible that other strains of *B. pyocyaneus* may be found which produce some other hæmolysin than alkali, but it is evident that in any study of bacterial hæmolysins the superimposition of the effect of alkali upon that of any other hæmolyzing substance must be reckoned with, especially when corpuscles so sensitive to alkali as those of the dog are used for test objects.

A Fat-splitting Torula Yeast Isolated from Canned Butter: L. A. Rogers, Biochemic Laboratory, Washington, D. C.

The author has isolated from several samples of canned butter, a torula yeast, possessing to a limited degree, the ability to split up glycerides with the liberation of free fatty acid. The action of this torula is much weaker than that of the fat-splitting molds.

The acid number of a pure butter fat inoculated with a milk culture of the torula, increased in two weeks from 0.579 to 3.474.

The cells are elliptical, about 3.5 μ long and have little tendency to form chains or bunches.

The yeast ferments maltose slowly at 37° C., but does not ferment lactose, galactose, levulose, mannose or cane sugar.

A complete description will be given in a later paper.

Oligonitrophilic Bacteria of the Soil: Frederick D. Chester, Delaware Agricultural Experiment Station.

Reference is given to the early literature bearing upon the subject of nitrogen assimilation by lichens, aerophilous algæ, molds and bacteria.

Land may gain in nitrogen through the activities of soil bacteria. Oligonitrophilic

bacteria are those that grow in nitrogen-free or nitrogen-poor media, and that possess the power of utilizing the free nitrogen of the air. The oligonitrophiles belong to the *Clostridium* group, or to Beijerinck's *Granulobacter* group.

Clostridium Pasteurianum, which Winogradsky found to possess nitrogen-assimilating properties, is an anaerobe, but it also grows in symbiosis with aerobic forms; it is, therefore microaerophilic. The microaerophiles will grow luxuriantly under normal conditions under diminished oxygen pressure, effected by the active utilization of oxygen by the aerobes (macroaerophiles).

Nitrogen assimilation in the soil is not the result of the activities of a single organism, but of symbiosis of microaerophiles with macroaerophiles. Of the microaerophiles we have *Clostridium Pasteurianum,* several species of *Granulobacter* of Beijerinck, and *Radiobacter* of Beijerinck. Of the macroaerophiles we have *Azotobacter* of Beijerinck.

Azotobacter alone is without nitrogen-assimilating properties, and the same is true of the *Granulobacter* and *Radiobacter,* but mixed cultures of *Azotobacter* with the other forms showed marked gain of nitrogen of four to seven milligrams per gram of assimilated sugar in the medium. A form of *Azotobacter* isolated from Delaware soil was without the power of assimilating atmospheric nitrogen.

The Bacterial Flora of the Oyster's Intestine: CALEB A. FULLER, Brown University.

Of late there has been considerable difference of opinion regarding the significance of *B. coli* in drinking water and various foodstuffs. Some authorities do not consider this organism a certain indication of sewage pollution, while others look with suspicion on all food materials containing bacteria of the colon group. Oysters are especially liable to contamination by sewage, for many cities and towns discharge their waste matters into bays or other bodies of water where they are cultivated.

In some reports on the bacteriology of the oyster it was suggested that *B. coli* might be present normally in the intestines of oysters. This statement differs materially from the results of some previous work of mine on oysters and sewage in Narragansett Bay. These results seem to indicate that this organism does not occur in oysters obtained from perfectly clean sea-water. In order to throw some light on this point I examined the intestines of over two hundred oysters in October and November, 1902. These oysters were taken from a bed known to be free from any trace of sewage. A number of tests have shown that the sea-water above this bed does not contain *B. coli.*

The method of analysis was as follows: Two gelatin plates were inoculated, each with a large loop of material from the intestine of each oyster and grown at room temperature for three or four days. One of these plates was made from the usual nutrient gelatin and the second from gelatin containing carbolic acid (.05 per cent.). From the ordinary gelatin plates I separated sixteen species of bacteria; some of them common water forms, and others unidentified, that seem to be characteristic of the oysters of this locality. Of the carbol-gelatin plates, with but a single exception all remained sterile. The single colony that developed was not *B. coli.* If *B. coli* was present in the intestines of these oysters, even in small numbers, it would have developed in the above medium. Of the other species isolated, none resembled *B. coli* when tested by the usual methods.

From the results of these experiments it appears that the colon bacillus is not normally present in the intestines of oysters, and when present always indicates contamination from some outside source.

The Influence of Physical Conditions on the Character of Colonies on Gelatin Plates: A Preliminary Communication: Edward K. Dunham, New York University and Bellevue Hospital Medical College, New York.

Attention was called to the influence of physical conditions on the appearance of colonies by two sets of observations: (1) The same species of bacterium grown in different lots of gelatin made with the same ingredients and having the same reaction frequently produced colonies of widely divergent appearances; (2) colonies of different species often form colonies that are indistinguishable in some gelatins, but when grown in other lots of gelatin can be readily recognized as different. These variations were traced to differences in the stiffness of the gelatins, and this led to a study of the physical properties of nutrient gelatin. The melting points, penetrabilities and viscosities were determined and compared with the appearance of colonies on plates made with the gelatins. Attention was chiefly directed to the deep colonies, and the studies were confined to the colon bacillus, bacillus typhosus, bacillus dysenteriæ and a paratyphoid bacillus.

If the gelatin is very stiff the colon colony is lenticular in form and presents a tendency to form multicontours. In a somewhat softer gelatin the colony is spherical, with indications of concentric structure. In still softer gelatin, budding or root-like projections are formed on the surface. In a very soft medium, not a single colony but a federation of colonies, closely grouped together, is produced. Similar variations occur when typhoid colonies develop on plates. These may be small and spherical, or more or less thready with delicate filaments penetrating into the medium, according to whether the gelatin is stiff or relatively soft. In a very soft but still solid gelatin, the typhoid bacilli may penetrate the medium, disseminating themselves throughout its mass. Such plates appear sterile.

Variations in the stiffness of the gelatin may be produced by a reduction of its original stiffness with heat or by incubating the plates at different temperatures. A ten per cent. gelatin made with Compte Fils's or Heinrich's 'Gold Label' gelatin, cooked with an egg for thirty minutes and sterilized three times for fifteen minutes in the Arnold sterilizer, will melt at 29.5° to 30.3° and have a viscosity between eight and nine times that of water. Gelatin plates made with this gelatin and incubated at 27° will yield, *e. g.*, colon and typhoid colonies that can easily be distinguished from each other and fished within twenty-two hours.

In the author's opinion the physical properties of gelatin and temperature of incubation should receive fully as much attention as the ingredients and reaction in the standardization and use of gelatin, particularly when employed for plating with reference to species.

Milk-agar as a Medium for Demonstrating the Production of Proteolytic Enzymes: E. G. Hastings, University of Wisconsin.

If ten to twelve per cent. of sterile skim milk is added to ordinary nutrient agar, after the same has been allowed to cool to 50° C. after having been melted, an opaque medium is obtained which, when allowed to solidify in tubes in a sloping position, or poured into Petri dishes, has some advantages over gelatin for the determination of the liquefying properties of bacteria,

inasmuch as it can be incubated at high temperatures.

If cultures of a liquefying organism be made in this medium, the growth after a few hours' incubation will be surrounded by a transparent zone due to the liquefaction of the casein.

Its advantages over gelatin are that it can be incubated at any temperature; that the liquefying power of organisms whose optimum temperature zone lies above 20° C. can be determined in a much shorter time than by the use of gelatin.

It can also be used to determine the presence of proteolytic enzymes in plant and animal tissues by adding cubes of milk-agar to the extracts of such tissues, in the presence of suitable antiseptics, such as small amounts of carbolic acid or formalin. The presence of proteolytic enzymes is made apparent by the edges of the cubes becoming transparent.

Laboratory Notes: W. M. ESTEN, Middletown, Conn.

A new thermo-regulator for incubators heated with incandescent lamps was constructed from a description by Mr. H. E. Ward, of the Illinois Experiment Station. This was shown and its regulating quality demonstrated. Its advantages are that the heat is applied to the interior, and that incubators can be constructed of wood and danger from fire avoided.

New Method of Preparing Blue-litmus-lactose-gelatin.—The cooking and sterilizing of litmus with gelatin proves to be detrimental to the reaction of litmus. The litmus and gelatin media are prepared and sterilized separately, then mixed immediately before plating. Fifteen to twenty per cent. of litmus is digested in distilled water for several hours at 70° C., filtered, the reaction adjusted to + 1.5 per cent., and sterilized. A gelatin medium is prepared with 3 per cent. lactose and 25 per cent. less water than ordinary gelatin. Tubes are filled with 8 c.c. of gelatin.

Cheese-whey-gelatin is prepared by adding rennet to fresh skim milk. The whey is placed in an autoclave for thirty minutes at 105° C. Ten or eleven per cent. of gelatin is added and the medium cooked in open dish until one-fourth is evaporated; the reaction is adjusted to + 1.5 per cent., and tubes filled with 8 c.c.

To prepare the gelatin tubes for plate cultures, place in each tube of melted gelatin with sterile pipette 2 c.c. of the litmus solution, mix and add 1 c.c. of diluted milk, and plate.

The comparative values of the two kinds of gelatin are that the lactose-litmus-gelatin gives the maximum numbers while the cheese-whey-litmus-gelatin gives a strong differentiation of acid and non-acid species. To get the advantages of both kinds of gelatin mixing half and half proves very satisfactory.

It is possible by means of this mixed gelatin to classify the different kinds of bacteria on the plates by means of the colonies alone.

The 'Germicidal Property' of Milk: W. A. STOCKING, JR., Middletown, Conn.

Freudenreich, Park, Hunziker and others have shown that cows' milk, when a few hours old, contains a smaller number of bacteria than when freshly drawn from the cow. From this they conclude that milk possesses a 'germicidal property or action' during the first few hours. This conclusion was based on the results obtained from agar plate cultures, on which the total numbers of bacteria were determined. These investigators, however, were unable to explain the cause of this phenomenon. The purpose of the experiments described in this paper was to determine, if possible, the cause of this dropping out of the organ-

isms during the early part of the ripening period. For this work peptone-litmus-gelatin was used and the milk was plated at intervals of three hours. From these plates the total number of organisms, the number of acid-producing bacteria and the different species, as far as possible, were determined. The results of a long series of experiments seem to show that the decrease in numbers was due, not to any 'property or action' possessed by the milk, but to the natural dropping out of certain species of bacteria which do not find the milk a suitable medium in which to grow.

Fresh milk obtained under ordinary conditions contains a large variety of types and species of bacteria, while milk which has soured or curdled contains but few species, often not more than two or three. Fresh milk ordinarily contains but few of the typical lactic organisms which later cause souring and coagulation. When these species have once gained access to the milk their growth is constant and quite uniform from the first. Certain other acid-producing species, however, and many non-acid species do not find the milk a favorable medium in which to grow, and drop out. Some species appear only in the plates made from the fresh milk, while other species may continue for a few hours and then disappear. Usually the decrease in the numbers of the miscellaneous species is greater than the increase in the 'lactic' species, during the first few hours, so that plate cultures made when the milk is a few hours old will show smaller numbers of bacteria than were found in the fresh milk.

Summary of the Steps which must be Followed in Staining Flagella by Löffler's Method: W. R. COPELAND, Bureau of Filtration, Philadelphia, Pa.

The films of bacteria on the cover slips should be made from suspensions of bacteria obtained by immersing the cells in water for one or two hours in order to dissolve the outside gelatinous capsule.

Löffler's mordant should be made of the best grade of tannic acid with ferrous sulphate and Gruebler's basic fuchsin. This mordant should be heated to 70° or 75° C., until a stream of steam rises for a distance of two inches. The preparation should then be set aside for half a minute. The stain is made of the best grade of aniline oil, absolute alcohol and a saturated alcoholic solution of Gruebler's basic fuchsin. The stain should be applied cold, for from eight to ten seconds.

Finally Löffler's method of staining flagella is better and more powerful than either van Ermengem's, Pitfield's or Löwitz's methods. It magnifies the size of the cells and flagella in a manner that is especially favorable for class demonstration.

Egg Medium for the Cultivation of Tubercle Bacilli: M. DORSET, Biochemic Laboratory, Washington, D. C.

A further report of the results obtained by the use of this medium which had been previously described in *American Medicine,* April 5, 1902, and the 'Eighteenth Annual Report of the Bureau of Animal Industry,' 1901.

Cultures were made from more than seventy-five tuberculous rabbits and guinea-pigs, with almost uniform success, the few failures being traceable to a contamination of the culture tubes or the presence of very small numbers of tubercle bacilli in the tissues from which the cultures were taken. The medium seems to be specially well adapted for obtaining the first growth of tubercle bacilli from animals. Tubercle bacilli of bovine origin gave a slightly less abundant growth than the human tubercle bacilli, and the gross appearances of the cultures differed slightly. The morpho-

logical characters of human and bovine tubercle bacilli when grown on egg have been left for future report.

Studies on Quantitative Variations in Gas Production in the Fermentation Tube: C.-E. A. WINSLOW, Massachusetts Institute of Technology, Boston, Mass.

Experiments were made to determine the amount of variation in gas formation in a series of dextrose broth tubes filled with the same batch of culture medium and inoculated with the same organism. For inoculation, measured portions of an aqueous suspension of the surface agar growth of a strain of *B. coli* were used. A wide variation between individual tubes was observed. Thus in one case with tubes receiving the same amount of culture material the amount of gas varied from 20 per cent. to 62 per cent. of the closed arm after 16 hours, and from 38 per cent. to 86 per cent. after 64 hours. This was not simply a variation in the rapidity of the evolution of gas; for in this instance the maximum of gas formed in a given tube at any time varied from 42 per cent. to 86 per cent. By averaging the results obtained in a number of tubes more general relations became apparent. During the first 12 hours the amount of gas formed depended upon the amount of material used for inoculation, and the relative proportion of hydrogen was greater than at a later period. Between 24 and 48 hours the maximum of gas was generally formed with the classical gas formula of two to one, and after 48 hours a marked decrease of total gas occurred, due to the absorption of carbon dioxide. The principal point brought out was the wide variation in individual tubes due to some unknown factor, and apparently only to be avoided by making a series of duplicate analyses.

Preliminary Note on Chromogenic Cultures of B. diphtheriæ: HIBBERT WINSLOW HILL, Boston Board of Health Laboratory.

Six stock cultures of *B. diphtheriæ*, the originals of which had been isolated between March, 1901, and January, 1902, and since kept on serum, with reinoculation at intervals of one to two months, showed gradually increasing yellow color when streaked on serum.

Recently (December, 1902) this coloration became so striking as to attract definite attention. One of the six cultures (4014) isolated October 18, 1901, from a clinical case of diphtheria, and then typical morphologically and typically virulent to guinea-pigs, was selected for examination. The morphology and virulence, retested in December, 1902, were still typical.

Cultures from this stock developed the color on serum at 37° C., slightly in one day; by the third day the color was very marked—a clear bright yellow. The growth, removed by scraping, is treated with chloroform, which dissolves the pigment. After filtration to remove the bacilli, evaporation to dryness deposits the pigment, which is then found soluble in chloroform and in ether, but not in water. The same culture grown on agar for the same time yields only an ordinary dirty-white tint. When treated similarly, such dirty-white cultures yield a small amount of faint grayish-brown pigment. From fresh uninoculated serum of the same lot ether extracts a yellow pigment, but chloroform does not.

The writer has observed cultures of *B. diphtheriæ* showing a faint pink color, and others which, especially when old, show quite dark-brown or black coloration.

The Chemistry of Bacterial Pigments: M. X. SULLIVAN, Brown University.

While growing bacteria upon synthetic

media, I noticed that often chromogenic varieties became colorless. Accordingly experimenting to determine what salts, bases or acids in addition to the organogens, carbon, hydrogen, oxygen and nitrogen, are necessary for pigment production, I found, with Jordan, that for the formation of fluorescent pigment, sulphates and phosphates are required. Extending the research to other pigments, such as those produced by *B. pyocyaneus, B. prodigiosus, B. ruber balticus, B. rosaceus metalloides, B. janthinus* and *B. violaceus,* I found that the characteristic pigments were produced whenever there were present, in addition to suitable compounds of carbon, hydrogen, oxygen and nitrogen, phosphates together with sulphates, chlorides or nitrates, irrespective of the base. Suitable compounds of C, H, O, N, are asparagin, and the ammonium salts of succinic, lactic and citric acids. The solutions containing asparagin were the best, so that upon a medium consisting of asparagin 0.2 per cent., $MgSO_4$ 0.02 per cent., K_2HPO_4 0.1 per cent., glycerin 2 per cent., the pigments were quickly produced. Magnesium and potassium may be replaced by other bases, as sodium or ammonium. If the glycerin is left out the asparagin must be increased to 1 per cent. to get good pigment formation. Upon media consisting of $(NH_4)_3PO_4$ 0.1 per cent., $(HN_4)_2SO_4$ 0.1 per cent. and glycerin 2 per cent., there occurred a good production of pigment.

Replacing the asparagin and glycerin by ammonium salts of organic acids, 0.2 per cent. to 0.5 per cent., I found that while the succinate, lactate and citrate gave pigment, the tartrate, oxalate, urate and formate, though allowing growth, were unfavorable to chromogenesis.

By testing the chlorides and nitrates as to pigment formation, it was found that upon a solution consisting of asparagin 1 per cent., K_2HPO_4 0.02 per cent., NaCl or KCl 0.2 to 0.5 per cent., or KNO_3 0.02 per cent. the pigment was formed, though less abundantly than when $MgSO_4$ was present. On the other hand the sulphides, bromides and iodides were unfavorable to pigment production.

The conclusions to be drawn are that, in addition to suitable compounds of C, H, O, N, phosphates and sulphates are necessary for the fluorescent pigment, while for the pigments of *B. pyocyaneus, B. prodigiosus, B. rosaceus metalloides, B. ruber balticus, B. janthinus* and *B. violaceus,* the sulphates may be replaced by the chlorides or nitrates.

The Pyocyanin and Fluorescent Functions of Bacteria: M. X. Sullivan, Brown University.

Since Gessard's discovery in 1882 of a bacillus which produced a blue or blue-green pigment soluble in chloroform, many experiments have been carried on not only as regards the morphological characters of the bacillus to which Gessard gave the name of *B. pyocyaneus,* but also as to the nature of its pigments. In the study of *B. pyocyaneus,* many varieties have been isolated, so that at present we have kinds which produce pyocyanin alone, others which produce both pyocyanin and a greenish-yellow fluorescent pigment, insoluble in chloroform, but soluble in alcohol and ether, and further, some perhaps degenerate types, which produce a fluorescent pigment only. Now the question is, what is the relation between the different varieties of this bacillus? Are the varieties characterized by the ability to produce a different pigment or pigments, or can the same race be compelled to form different colored products according to the medium on which it is grown? That the latter view is the correct one would seem to be

the conclusion from the following experiments.

A variety which produces pyocyanin only on a medium consisting of asparagin 1 per cent., $MgSO_4$ 0.02 per cent., K_2HPO_4 0.1 per cent., can be made, by gradually increasing the phosphate to 0.5 per cent., to produce both pyocyanin and fluorescent pigment. In this case there is very little pyocyanin and a great deal of the fluorescent pigment. Another variety, which was producing both pyocyanin and the fluorescent pigment, was made to produce the fluorescent pigment alone on asparagin 0.2 per cent., $MgSO_4$ 0.02 per cent., K_2HPO_4 0.5 per cent. This same variety upon asparagin 1 per cent., $MgSO_4$ 0.05 per cent., K_2HPO_4 0.2 per cent., strongly acid, produced pyocyanin alone.

Turning now to the common *B. fluorescens liquefaciens,* which on asparagin 1 per cent., $MgSO_4$ 0.02 per cent., K_2HPO 0.1 per cent., produced the fluorescent pigment. I gradually lessened the phosphate and in another series the sulphate to determine whether or not this bacillus could be induced to take up the pyocyanin function. The fluorescent pigment disappeared and the growth became colorless, but no pyocyanin was produced.

The conclusions to be drawn are that the same variety of *B. pyocyaneus* can be made to produce pyocyanin alone, pyocyanin and a fluorescent pigment, or the fluorescent pigment alone, according to the medium upon which the bacillus is grown; but that the purely fluorescent bacilli can not be made to take up the pyocyanin function.

A Preliminary Chemical Study of Various Tubercle Bacilli: E. A. DE SCHWEINITZ and M. DORSET, Biochemic Laboratory, Washington, D. C.

Dr. de Schweinitz gave, for himself and Dr. Dorset, a brief résumé of the work carried on by the Biochemic Laboratory of the Department of Agriculture so far, upon a chemical examination of the following bacilli: bovine, horse, swine, avian, virulent human and attenuated human. He pointed out that the conclusions which might be drawn from these analyses indicate a closer resemblance in the composition of the germs between the moderately virulent human bacilli and the bovine and swine, than between the moderately virulent human and the very attenuated human bacilli. The analyses also indicate a closer relationship in composition between the attenuated human bacilli and the avian bacilli, than between the two varieties of human bacilli used. He also called attention to the fact that a similar comparative examination of human bacilli and bovine bacilli of various degrees of virulence was being carried out. Attention was called, further, to the fact that the large amount of phosphoric acid obtained from the germs indicated that this constituent was absolutely necessary for the proper development of these bacilli, and it was noted that for a number of years in all the work in the study of tubercle bacilli in the Biochemic Laboratory, culture media had been prepared with the addition of acid potassium phosphate, and that sodium chloride had been entirely eliminated. The results had been uniformly more satisfactory than with any liquid medium that has been used for the tuberculosis bacilli. The importance of a chemical study, not only of the tubercle bacilli themselves, but also of their products, was emphasized.

The authors further presented the history of a case of generalized tuberculosis in a child of five years of age that had been brought up on milk. The cultures obtained from the mesenteric glands of this child had produced generalized tuberculosis in a heifer, after subcutaneous inoculation, within about a month. Drawings which showed the appearance of the lung

from this calf, and also the appearance of the liver of a pig, which had also been submitted to subcutaneous inoculation with this germ, were shown. In addition, drawings showing the comparative results of a subcutaneous inoculation of bovine and human tubercle bacilli in monkeys were presented. These indicated that the bovine tubercle bacilli were very much more virulent for the monkey than the human tuberculosis bacilli used. In the discussion which followed this paper, Dr. de Schweinitz further stated that the cultural characteristics of the germ which had produced the tuberculosis in the heifer upon subcutaneous inoculation appeared to be those which some authors claim to be possessed only by the bacilli derived from the bovine species, and that further, whether the germ that killed the heifer was regarded as a bovine germ or a human germ, the conclusions naturally were of equal value; for if the germ was of bovine origin, then it seemed that tuberculosis in children could be produced by bovine bacilli. If, on the contrary, the germ was what is commonly called the human germ, then it was a germ which was virulent for cattle. He also called attention to the fact that the attenuated human germs used in the chemical study referred to were the offspring of the same attenuated germs which had been used a number of years ago for the purpose of producing immunity to tuberculosis in small animals, by subcutaneous inoculation. These results were published at the time, in the *Medical News,* December, 1894.

Reference was also made to the fact that tuberculin prepared from bovine bacilli, and tuberculin prepared from the virulent or attenuated human bacilli, when tested interchangeably on men and animals, seemed to give the same positive results. A résumé of these tuberculin tests was published in *American Medicine,* in January, 1902.

Further Evidence of the Apparent Identity of B. coli and Certain Lactic Acid Bacteria: S. C. Prescott, Massachusetts Institute of Technology, Boston, Mass.

Last year it was reported by the author that certain lactic acid bacteria isolated from grains and products of milling gave all the cultural reactions generally regarded as typical of *B. coli.* In the present work cultures of 'lactic acid bacteria' were isolated from various sources apparently free from contamination with fæcal matter, and were compared directly with 23 cultures of *B. coli* obtained either directly from fæces or from waters known to be sewage-polluted. Of these 61 cultures, 44 gave exactly the same reactions in the culture tubes, 25 of them being lactic acid bacteria, and 19 typical colon bacilli. These organisms were also found to be alike in their morphological characters.

A study of the fermentative power showed that the 'lactic acid bacteria' and 'colon bacilli' produced approximately the same amount of acid when grown under similar conditions, while organisms of different groups, as for example streptococci, gave results showing a marked difference in fermenting power.

As a final test the effect of inoculation into animals was noted, with the result that lactic acid bacteria and colon bacilli produced the same results when used in the same manner and with like amounts. Subcutaneous injection of 1 c.c. produced dullness and torpor, followed by rise of temperature, while intraperitoneal inoculation of 1.5 c.c. produced death within twenty-four hours.

As a result of the experiments the author believes that the organisms studied are not merely alike in certain characteristics, but are absolutely identical, and thus that or-

ganisms having the same characteristics as *B. coli* are very widely distributed in nature, and their presence, unless in considerable numbers, is not necessarily indicative of recent fæcal contamination.

On the Relative Viability of B. coli and B. typhosus under Certain Conditions: STEPHEN DEM. GAGE, Lawrence Experiment Station.

In various studies of both *B. coli* and *B. typhosus* at the Lawrence Experiment Station, a number of points of similarity in the behavior of the two species under certain conditions have been noted, which appear to have a bearing on the interpretation of tests for *B. coli*.

1. As regards sand filtration. With a water to which both species have been added, 99.9 per cent. of all the *B. coli* and 100 per cent. of the *B. typhosus* were removed by an intermittent filter, and 99.8 per cent. of *B. coli* and 99.9 per cent. of *B. typhosus* by a continuous filter.

2. As regards the persistence of the two organisms in a filter after infection of the applied water has ceased, *B. coli* was found to continue in the effluent from the intermittent filter for 24 to 36 hours, and *B. typhosus* only for two to three hours.

With the continuous filter *B. coli* continued for four to six days and *B. typhosus* for two days.

3. Effect of cold without freezing. In a water subjected to a temperature of 33° F., about 90 to 95 per cent. of both species were destroyed in 24 hours; a few organisms of each, however, may live for a considerable number of days.

4. Elimination by freezing and viability in ice. About 50 per cent. of the *B. coli* and 75 per cent. of the *B. typhosus* were destroyed by fifteen minutes' freezing; after one hour, 95 per cent. of the *B. coli* and 98 per cent. of *B. typhosus* were killed; and at the end of 24 hours over 99 per cent. of all the organisms had disappeared. Of the few organisms surviving, however, *B. coli* were found alive after three months, and *B. typhosus* after nine months, in the frozen condition, these experiments being still in progress at the present writing.

5. Resistance to heat. Both species resist temperatures up to 45° C. for five minutes. At between 45° and 55° C. all but a few individuals of each are destroyed, these few individuals, however, resisting temperatures up to 85° C. at which temperature all the organisms of both species were destroyed.

The effect of sunlight and the relative viability of both species in both sterile and natural waters are being studied, and from the data at hand a similarity between the two species will also appear.

The Germicidal Properties of Glycerine in Relation to Vaccine Virus: M. J. ROSENAU, Hygienic Laboratory, Washington, D. C.

The bacteriological examination of many dry points and capillary tubes of glycerinated virus bought upon the open market showed an excessive contamination, due to an over-confidence in the germicidal properties of glycerine. About one year ago, of 41 dry points examined, there was found an average of 4,807 organisms per point; of 51 glycerinated tubes examined, there was an average of 2,865 colonies per tube, some individual tubes running as high as 18,000. Following a publication of these facts and the warning given to manufacturers that glycerine is not a substitute for care, a great improvement in the bacteriological contents of glycerinated virus on the market resulted. Thus, of 89 tubes examined an average of only 28 organisms per capillary tube was found as a result of recent studies.

Glycerine has distinct antiseptic powers. It restrains the growth of most bacteria in dilutions of 35 per cent.; molds grow on the surface of bouillon containing 48 per cent.; no growth was observed above 50 per cent. Its germicidal properties are very feeble. It has practically no effect on spores, anthrax and tetanus being the spores tested. Tetanus, however, does not multiply in glycerinated lymph, nor in bouillon containing 60 per cent. of glycerine, the amount used by manufacturers in glycerinated virus.

It was found that the antiseptic and germicidal powers of glycerine varied somewhat with the kind of glycerine used, and also with the organisms tested. Cholera and plague were retarded by the presence of 21 per cent. to 24 per cent., while pus cocci grew in 31 per cent. and some molds grew on the surface in 48 per cent. Pus cocci are usually rendered sterile in 50 per cent. glycerine within five days, though they were kept alive as long as ten days in the ice-chest; they died more quickly at incubator temperature. In 80 per cent. and 90 per cent. glycerine Staphylococcus pyogenes aureus was kept alive in the ice-chest at 12° C., 41 days. Anthrax spores have been kept alive 247 days and the experiments are still going on. Tetanus spores were found viable in various percentages of glycerine after 135 days in the ice-chest.

The Reaction of Certain Water Bacteria with Dysentery-Immune Serum: D. H. Bergey, University of Pennsylvania, Philadelphia.

A Mold Pathogenic to Lobsters: F. P. Gorham, Brown University.

Complete Inhibition of the Cholera-Red Reaction by Impure Peptone. James Carroll, Army Medical Museum.

Demonstration of the Value of MacConkey's Medium for the Differentiation of B. coli from B. typhosus: N. MacL. Harris, Johns Hopkins University.

Edwin O. Jordan,
Secretary.

SCIENTIFIC BOOKS.

Ueber verschiedene Wege phylogenetischer Entwickelung. By O. Jaekel. Jena, Gustav Fischer. 1902. 8vo. Pp. 60; 28 text-figures.

Der Neo-Lamarckismus und seine Beziehungen zum Darwinismus. By R. von Wettstein. Jena, Gustav Fischer. 1903. 8vo. Pp. 30.

The intensity which a few years ago characterized the struggle between the opposing camps of Neo-Lamarckism and Neo-Darwinism has, fortunately, largely subsided. Some new standpoints have arisen, notably those afforded by the doctrine of organic selection and by the rediscovery of the Mendelian law, and there has been a general tendency to inquire more thoroughly into the laws of variation and to seek for the factors concerned in that phenomenon.

The first of the two pamphlets which form the subject of this notice represents a phase of this tendency, and is of interest as exhibiting the views of a paleontologist who has had access to and has made admirable use of an exceptional abundance of material bearing upon the questions he discusses. In his opening pages Professor Jaekel combats the idea that if the paleontological record were complete it would furnish evidence of almost insensible transition from species to species, so that no 'good' species could exist for the paleontologist, and points out that an exhaustive search for confirmation of this idea, extending through the last three decades, has brought to light only three more or less acceptable cases, namely, those of the Steinheim *Planorbis* and of the Pannonian and Kossian *Paludinas,* none of which shows any more gradation than may be found in variable species of recent land snails.