

# SCIENCE

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FRIDAY, MARCH 1, 1901.

THE AMERICAN SOCIETY OF BACTERIOLOGISTS.

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MSS. intended for publication and books, etc., intended or review should be sent to the responsible editor, Professor J. McKeen Cattell, Garrison-on-Hudson, N. Y.

THE American Society of Bacteriologists held its second annual meeting at Baltimore, at the end of December under the presidency of Professor Wm. T. Sedgwick, whose address has already been published in SCIENCE. The following papers were presented:

*Distribution of Bacillus aerogenes capsulatus*:  
W. H. WELCH.

Dr. William H. Welch presented the results of investigations of Mr. L. K. Hirshberg in the Pathological Laboratory of the Johns Hopkins University. There can be no question but that the bacillus discovered by Welch in 1891 and fully described by Welch and Nuttall in the following year is identical with Fraenkel's *B. phlegmones emphysematosae*, with Veillon and Zuber's *B. perfringens*, and with Schattenfroh and Grassberger's *Granulobacillus saccharobutyricus immobilis liquefaciens* described in 1900. It is possible that Klein had in his cultures *B. aerogenes capsulatus*, but his description of his *B. enteritidis sporogenes* can not be reconciled with the properties of the former bacillus, especially his statements as to motility and peptonization of milk. It has already been demonstrated by Welch, by Howard, and by Hitschmann and Lindenthal that *B. aerogenes capsulatus* is a widely distributed organism, its natural habitats being especially the intestinal canals of man, animals

and the soil. Mr. Hirshberg has, during the past summer, made a systematic study of the distribution of this bacillus in various situations. Various methods were employed for its isolation, one of the most useful being the inoculation into the circulation of rabbits, which were then killed according to the procedure described by Welch and Nuttall. In each instance the bacillus, if found, was isolated in pure culture, and identified by its characteristic properties. *B. aerogenes capsulatus* was found by Mr. Hirshberg regularly in the feces of man (being isolated from all parts of the intestinal canal), of swine, of dogs and of cats, and was found with varying frequency, as a rule, in 50 to 80 per cent. of the animals examined, in the feces of rabbits, guinea-pigs, mice, rats, chickens, pigeons and cows. It was likewise obtained from the excrement of flies hovering around the bodies of infected animals or human cadavers. It was isolated constantly from garden earth, rarely from street dust. It was detected four times out of eighteen examinations of dust swept from the floors of hospital wards, the dispensary or the laboratory. Once it was obtained in scrapings from the human skin. It was isolated twice from cesspools. The results of Schattenfroh and Grassberger concerning the presence of this bacillus in market milk were confirmed. In the light of these and previous investigations *B. aerogenes capsulatus* must be regarded as the most widely distributed of bacteria.

*The Bacterial Condition of City Milk and the Need of Health Authorities to prevent the Sale of Milk containing Excessive Numbers of Bacteria:* H. W. PARK, New York.

The author raised the question whether it is possible for health boards to set a limit to the number of bacteria which milk may contain, and above which its sale could be prohibited. During the coldest weather the milk in New York City averages about

250,000 bacteria per cc., during cool weather about 2,000,000 and during hot weather about 5,000,000. The milk in other large cities is, from all accounts, in about the same condition. The above statement does not apply to the special milks which contain only from 5,000 to 20,000 bacteria at the different seasons of the year. In answer to the question whether these enormous numbers of bacteria found in milk during the hot weather are harmful, reference need only to be made to the universal clinical experience that a great number of children in cities sicken on the milk supplied in summer; that those who are put on milk that is sterile, or contains few bacteria, as a rule, mend rapidly, while those kept on the impure milk continue ill, or die. We probably have, as yet, insufficient knowledge to state just how many bacteria must accumulate to make them noticeably dangerous in milk, but it is a safe conclusion that no more bacteria should be allowed than it is practicable to avoid. Any intelligent farmer can use sufficient cleanliness and supply sufficient cold, with almost no increase in expense, to supply milk 24 to 36 hours old which will not contain in each cc. over 100,000 bacteria, and no milk poorer than this should be sold. The most deleterious changes which occur in milk during its transportation are now known to be due to the changes produced by bacterial growth and activity. These add to the milk acids and distinctly poisonous bacterial toxins to such an extent that much of the milk, by the time it is used in summer, has become decidedly injurious to invalids and infants. While it is the universal custom of the health authorities to guard their milk in many ways, they nevertheless entirely fail to prevent the sale of milk rendered unfit for use through excessive numbers of bacteria and their products. This seems all the more remarkable when we consider how comparatively easy the

test, and how rapidly the farmer and middle-man could greatly improve the bacterial purity of their milk if only their dense ignorance and lack of desire to improve could be removed.

*Duration of Life of Typhoid Bacilli, derived from Twenty Different Sources, in Ice; Effect of Intense Cold on Bacteria; W. H. PARK, New York.*

Cultures derived from twenty different cases of typhoid fever were grown 28 hours in nutrient agar. From each one a loopful was inoculated into 300 cc. of sterile distilled water and this was poured into thirty glass tubes. These were kept in a room averaging 23° F. (-5° C.). From time to time a tube was removed and the number of bacilli which should develop in nutrient agar tested. The following table gives the results:

Number of weeks frozen.	Per cent. of bacilli living.	Per cent. of cultures living.
0 (Original)	100	20
½ week	42	20
1 week	14	20
2 weeks	7.50	20
3 "	.4	20
5 "	.11	18
7 "	.09	18
9 "	.05	17
12 "	.005	11
15 "	.002	8
18 "	.0001	3
22 "	none	0

Watery suspensions of typhoid, colon diphtheria and hay bacilli and of the *Staphylococcus pyogenes aureus* were placed in small tubes and dropped into liquid air. From time to time the tubes were removed and the contents plated in nutrient agar. The percentage living was as follows:

Per cent. living after exposure of	Typhoid.	Colon.	Staph.	<i>B. subtilis.</i>
3 min.	18	19		
20 "	10	11	85	80
60 "	7.5	8	51	67
130 "	3	5.5	27	55

The virulence of the organisms was only slightly diminished by this intense cold for two hours.

*The Use of Paraffin to exclude Oxygen in growing Anaërobic Bacteria; W. H. PARK.*

Nutrient glucose bouillon in tubes or flasks covered with a layer of paraffin, melting at 42° C., has proved very useful in the development of tetanus cultures and toxins and of other anaërobic bacteria. With bacteria not possessing spores the medium is quickly cooled and inoculated and then covered by a layer of very hot sterile paraffin. The accumulation of gas forces the paraffin up in the tube or flask, but does not allow the entrance of oxygen. When absolute exclusion of oxygen is desired, the tubes with their layer of paraffin are sterilized in an autoclave which renders them free from oxygen. Spore-bearing bacteria are inoculated through the liquid paraffin before the bouillon is fully cooled. Bacteria without spores are inoculated by breaking through the paraffin film or by heating the paraffin in a gas flame. A pipette can then be carried through the hot paraffin into the cool liquid below. The paraffin layer has also been found useful in preserving media from drying or from changes due to the absorption of gases of the air.

*Bacteria in the Ames Sewage Disposal Plant;*

L. H. PAMMEL, Ames, Iowa.

The author describes a sewage plant designed for the disposal of the sewage of about six hundred people. The plant is of the ordinary type, consisting of two beds, each covering about 0.2 of an acre. The filtration in the beds was at the rate of about 100 gallons a day, per acre. The whole plant was installed for about \$3,000. The efficiency of the filter bed was shown by bacteriological analysis. The effluent of the filter bed for 1899 showed an average of 5,127 bacteria per cubic centimeter, and at no time did it rise over 11,075 per cc.

The number of bacteria in the water in the tank varied largely with the temperature, rising in September to 9,000,000, and falling in colder weather to a little over a hundred thousand in March. The filter bed was, therefore, extremely efficient in removing bacteria. In the study of the species of bacteria found in the effluent, some of the common sewage types were found. The author found, also, in this effluent, *Bacillus prodigiosus*. This was interesting inasmuch as it made its appearance in the sewage after it had been introduced into the laboratory in Ames. It was not believed, however, to be a native of the locality, but an introduced species.

*Variations of Bacillus rosaceous metalloides (Dowdeswell)*: NELSON G. DAVIS, Lewisburg, Pa.

During the summer of 1896 a series of experiments was begun on the variations of *Bacillus rosaceous metalloides*. In making a pure culture of the organism, it was noticed that one colony was much paler in color than the others. No pigment appeared until the colony was some days old. Replating from this colony, all the daughter colonies were colorless until the fourth day, when a pale pink pigment appeared. After a time the characteristic metallic luster became visible. A continuation of the replating and selection of colonies was kept up for nine months. By that time cultures of the *Bacillus rosaceous* varying in color from colorless to a deep red, deeper than the original variety had been obtained. The darkest variety of all appeared as a 'sport'; so did also the first pale colony. The other variations appeared as gradual modifications. An attempt was made to produce a variety that would not liquefy in gelatin. This was unsuccessful, although in two instances the colonies were obtained, much slower than usual in their action. Similar selection experiments dem-

onstrated great varieties in the size of the organism. After about two hundred replatings, there appeared in one of the gelatin plates a colony in which the length of the elements was the same as the breadth. In other words, it appeared to be a coccus  $0.5\ \mu$  in diameter, and was so described by students. This variety was cultivated in various media, at various temperatures, in light and darkness. It remained constant in size.

*Some Varieties of Bacillus pyocyaneus found in the Throat*: F. P. GORHAM, Providence, R. I.

*Bacillus pyocyaneus* is a comparatively frequent form in the nose and throat. Two varieties can be distinguished, one producing both pyocyanin and a fluorescent pigment, the other producing only pyocyanin. These forms are often present in almost pure culture, and may persist in the same individual for several months. The cultures are virulent for guinea-pigs and rabbits.

*Demonstration of Photogenic Bacteria*: F. P. GORHAM.

Cultures of several varieties of phosphorescent bacteria were exhibited at the evening session. They were growing on fish, fish-agar and fish-bouillon. Some of the cultures were remarkably luminous.

*Bacillus Lactis Viscosus, a Cause of Ropyness in Milk and Cream*: ARCHIBALD R. WARD, Ithaca, N. Y.

The writer has closely observed the occurrence of the milk fault, known as 'ropy milk,' in the creameries of three different milk dealers in widely separated localities in New York State. *Bacillus lactis viscosus*—Adametz, has been found to be the cause of trouble in each outbreak. The identification of the organism found in the ropy milk was confirmed by Dr. Adametz, who studied a culture sent for identification, and pronounced it identical with the one first de-

scribed by himself. Attention is called to the fact that in several text-books there occurs an erroneous statement to the effect that the organism brings about the viscid condition in milk very slowly, and that it is, therefore, of no practical importance to dairymen. The statement is founded upon a misconstruction placed upon a sentence written by Dr. Adametz. The organism is found in water, and multiplies at a temperature as low as 8° C. These characteristics, together with the method of keeping the milk, account for the persistence with which ropy milk appears on a milk route when the creamery is once infected. In all the cases coming under my observation, the milk dealer has cooled the milk in long, open-topped cans standing in ice water. In each case the ice water was found to contain the organisms. These might readily be introduced into the milk by the spattering of water incident to the removal of cans, addition of ice, etc. That the ice water was the immediate cause of trouble was indicated by an experiment in which potassium bichromate was added to the ice water in the proportion of one part to one thousand parts of water. The trouble did not recur in those cans of milk which were placed in the water after the addition of the disinfectant. In this case scrupulous care was observed in sterilizing vessels which had been infected.

*Concerning the Presence of Streptococci in the Healthy Udder of a Cow:* R. C. REED and A. R. WARD.

At intervals between November, 1897, and July, 1900, the presence of streptococci in the freshly drawn milk of a cow in the Cornell University herd was noted. While some cases of mammitis, associated with streptococci, were known to have occurred in the herd during this period, yet we had no record that this cow ever suffered an attack. The fact that she led the herd in

butter production during the period in which the streptococci were observed in the milk indicates that the cow was not suffering from a chronic form of the disease. This fact is significant in view of the serious effect of mammitis on the secretion of milk. The slaughter of the animal in the summer of 1900 afforded an opportunity to study the bacterial flora of the udder by means of cultures made directly from all parts of the gland. In addition to some organisms commonly found in the udder, streptococci appeared in all the thirty-six cultures. In conjunction with the streptococcus under consideration one culture from a sporadic case of mammitis and one from an epizootic of the same disease were studied. In their cultural, morphological and pathogenic properties the streptococcus from this healthy udder was indistinguishable from those isolated from the cases of mammitis. None were pathogenic to guinea-pigs or rabbits, but all three induced mammitis when injected into a healthy udder. While these observations are incomplete, they can not but suggest the idea that the streptococci associated with mammitis may, like the specific organisms of diphtheria and pneumonia in the healthy throat, be harbored in the healthy udder without producing disease.

*Immunization of Animals to Rattlesnake Venom, and some studies of Antivenine:* JOSEPH MCFARLAND, Philadelphia, Pa.

In order to determine whether the experimental immunity to serpents' venom, upon which Calmette has done such interesting work, applied equally well to venoms of the cobra and rattlesnake. The endeavor was made about two years ago to immunize several horses to the venoms of American rattlesnakes. The problems encountered were more difficult than those with which Calmette had to contend, because of the intense local irritative action

of the venom. Cobra venom possesses this local irritative property in very slight degree, and Calmetti found that when the cobra venom was heated to 70°C., for an hour, it was completely set aside. It was found, however, that when rattlesnake venom was heated sufficiently to annul its irritative qualities its toxic properties were almost destroyed. The horses at first received heated venom, but later were injected with solutions of the dried venom in its normally active state. The injections were given subcutaneously and were followed by enormous edemata, necroses and sloughs, so that after determining that no immunity to the local action developed, this method was abandoned and the intravenous used. The interior of the vessels showed no sign of injury, probably because the well-diluted venom at once met with greater dilution in the circulating blood. No local or other irritative disturbances followed the intravenous injection, but the nervous impression was profound; the horses often fell, and remained unconscious for some minutes after injection, and, to prevent injury to themselves, required to be suspended. Two of three horses died before antivenine developed from the damage to their tissues caused by the irritation of subcutaneous injections. The third horse lived for a long time and developed a very marked immunity associated with the appearance of antivenine in the blood. The death of the horse finally resulted from the unfortunate accidental entrance of some venom into the sheath of the jugular vein during one of the injections. Not being immuned to its irritant effects, the venom produced a local edema which killed the animal by suffocation. The antivenine produced by this horse was of such strength that 2 cubic centimeters of the serum protected a rabbit—an adult rabbit—against a fatal dose of either rattlesnake (0.002 gram) or cobra (0.001 gram) venom.

*How can Bacteria be Satisfactorily Preserved for Museum Specimens?* H. W. CONN, Middletown, Conn.

A method of preparing museum specimens was described. A 2-per-cent. agar culture medium is placed in large test tubes and tilted so as to make agar slants. The tubes are left undisturbed for from six to eight weeks, in order to allow the surplus moisture to evaporate. They are then inoculated in long streaks and immediately sealed with plaster of Paris and paraffin. The cultures grow for a few days, then cease growing, and remain unaltered indefinitely. No disinfectant is needed. The cultures remain alive for many months, and possibly for years. The method is satisfactory except for one fact—the atmosphere in the tube becomes filled with moisture and this condenses on the inside of the tube with changes of temperature. No method has yet succeeded in avoiding this condensation of water, which in most cases renders the tube cloudy, and injures its value as a display specimen.

*The Effect of Salt Solution and other Fluids on Bacteria compared with Serum Reaction:* ADOLPH GEHRMANN.

The author described, first, a series of experiments to determine the effect upon bacteria (typhoid and colon bacilli) of transferring them from one solution to another in which the percentage of salt is less. These experiments showed that, if the salt was not stronger than one per cent., the solution did not materially injure the bacteria, and did not produce the plasmolysis and plasmotypsis described by Fisher. Solutions of one per cent. have an inhibiting action and cause typhoid cultures to develop long chains and to lose their motility. A second series of experiments tested the effect of salt in the diluting fluids which were used in making the serum tests. Distilled water, normal salt

solution, and a bouillon culture fluid, made both with .5-per-cent. salt and without salt, were compared side by side, and were found to be fully equivalent. Blood diluted with any of the above readily produced the agglutination test, about the same time elapsing in all cases before the agglutination occurred. A further series of experiments tested the influence of the viscosity of fluids upon motile bacteria, as aiding in explaining agglutination. These experiments showed that the typhoid bacillus becomes readily agglutinated in fluids having considerable viscosity. For testing this phenomenon, gelatin and egg albumen were used both of which caused the bacilli to adhere in clumps, which, however, were dissipated if the solutions were diluted. While these observations were regarded as having significance in interpreting the serum test, the author was of the opinion that, when properly conducted, the agglutination obtained in the serum test can readily be distinguished from that which is the result of such physical conditions.

*Growth of Bacteria in the Presence of Chloroform and Thymol:* ERWIN F. SMITH, Washington, D. C.

As an illustration of the frequent dependence of bacteriologists and physiological chemists upon chloroform as an antiseptic, the speaker cited various passages from the recent valuable English work of Green on 'The Soluble Ferments and Fermentation.' In this book there are many statements and implications that animal and vegetable infusions can be preserved from bacteria growths during their examination by the addition of chloroform. Twelve micro-organisms are known which grow readily in test-tube cultures of milk, beef bouillon, etc., to which an equal volume of chloroform has been added. This probably by no means exhausts the list.

Test-tube cultures of eight of these organisms growing readily in presence of chloroform were exhibited. Two organisms are also known which grow readily in beef bouillon to which thymol has been added. It would appear, therefore, that there is no general rule, but that each bacterial organism must be tested by itself as to the effect upon it of chloroform, thymol, etc. If chloroform is used to preserve fluids or macerations of animal and vegetable substances from the growth of micro-organisms, it would be well to seal the flasks and keep them constantly agitated. Moreover, if one would be certain of their continued sterility, the freedom from bacteria growth of the substances under examination must be determined from time to time by microscopic examination and by cultures made from the fluids or macerations, otherwise, especially where bacterial organisms are able to produce the same substances as those sought for in plant or animal tissue, *e. g.*, cytase, diastase, etc., there can be no certainty as to the exact origin of the substance in question.

*Infection by means of Modeling Clay:* M. O. LEIGHTON, Montclair, N. J.

The author's attention is drawn to the possibility of the distribution of infectious disease among school children by the common use of model-clay. In the ordinary schools such clay, after having been used by one student, is returned to the stock box and subsequently used again. Study of clay thus obtained from schools showed bacteria to be tolerably abundant in the clay. The species of bacteria identified were those which ordinarily occur in pus formations, thus showing that clay may be capable of distributing these organisms. An attempt to sterilize clay showed that the only efficient means of accomplishing this purpose is by the use of superheated steam under the pressure of 15-20 pounds for 45 minutes. Next, an attempt was made to

determine how long certain pathogenic bacteria could remain alive in the clay. Sterilized clay was inoculated under proper precautions, with the bacilli of typhoid, diphtheria and tuberculosis. The clay was then kept moist and warm, and studied periodically for the presence of these organisms. The results were, briefly, as follows: *B. Typhi abdominalis* grew vigorously after having been enclosed in the clay for 32 days. After that no colonies were found. *B. diphtheria* grew after having been enclosed in the clay for 18 days. *B. tuberculosis* was alive after 18 days. How much longer the latter two bacilli would remain alive in the clay the author did not determine. The experiments, however, sufficiently demonstrate that the indiscriminate use of modeling clay in the schools is unwise, and liable to distribute communicable diseases, if such are present among the pupils.

*A Preliminary Report upon a Hitherto Undescribed Pathogenic Anaërobie Bacillus:*  
NORMAN HARRIS, Baltimore, Md.

This organism was isolated, post-mortem, from one of several abscesses in the liver of a man who had entered the service of Professor Halsted in the Johns Hopkins Hospital of Baltimore. He complained of great pain in the hepatic region of the abdomen, accompanied by nausea, vomiting and jaundice. Blood examination showed a marked leucocytosis. Exploratory laparotomy was performed and the condition was found to be one of multiple abscess of the liver, and beyond radical treatment. The patient died the fourth day after operation. The autopsy disclosed the presence of numerous abscesses throughout the liver, as well as in the right lung and spleen. Petri dish cultures were made in plain and in hydrocele fluid agar and grown both aërobieally and in an atmosphere of hydrogen for a period of seventy-two hours. All dishes showed no growth,

except the undiluted hydrocele fluid culture which had been in the hydrogen atmosphere. This developed four colonies surrounded by a halo of growth, and these appeared to arise from small particles of liver detritus. Subcultures were successfully grown only when the media were made up with hydrocele fluid or human blood, and when oxygen was excluded. Characteristics: the organism is a bacillus which in general is not minute, although its size varies somewhat on the various media. It may occur as cocci diplococci, very short rods, longer rods, filaments, or more rarely as chains of cocci or very short rods. Occasionally some rods are seen to have swollen ends, or may show distinct polar granules, or may be slightly curved. It is non-motile; it is decolorized by Gram's method of staining; it does not liquefy gelatin; it does not appear to have spores; its thermal death point is an exposure of ten minutes at 50° C. In all media it gives off a very strong fecal odor, and forms gas from the ordinary and sugar-free beef broth, when made up with either hydrocele fluid or blood. It likewise actively ferments glucose, forming CO<sub>2</sub>, H<sub>2</sub>, and H<sub>2</sub>S, the gas igniting readily. Experimentally, lesions similar to those found in the human subject were produced in rabbits and guinea-pigs, and mice succumbed to subcutaneous inoculation with a local necrotic lesion only. The name proposed for the organism is *Bacillus mortiferus* or *Bacterium mortifer*. The organism differs essentially from any of anaërobie bacilli hitherto described.

*Concerning the Theories of Silage Formation:*

H. L. RUSSELL and S. M. BABCOCK, Madison, Wis.

The authors instituted along series of experiments to determine whether the changes that take place in the silo are due to microorganisms, as has been believed, or to other

kind of action. Their conclusions are: (1) Silage can be made under conditions that exclude bacterial activity. (2) The initial heating of the silage is due, mainly, to the respiratory processes of the cut plant tissues. (3) The peculiar characteristic of good silage is due, not to bacteria, but to changes inaugurated under the more or less direct control of the activity of the protoplasm of the plant tissues. The acids of silage seem to be for the most part a product of the intra-molecular respiration, and in quantity are roughly proportional to the length of time that ensues before the cells stop respiring. This fact explains the reason that silage from immature corn has a higher acidity, and is more likely to undergo putrefactive changes due to bacteria growing in the succulent tissues than silage made from mature corn. (4) The aroma of good silage can be produced under conditions in which all vital processes are suspended. This seems to point strongly to the idea that enzymes are operative in the production of this aroma. It has previously been shown that such ferment bodies are liberated from dying vegetable cells and that they continue to act after the cells lose their vitality.

*Demonstration of some New Laboratory Devices:*

F. P. GORMAN, Providence, R. I.

The following laboratory devices were demonstrated: (1) The application of the incandescent electric lamp to heating incubators, water and paraffin baths. (2) Culture tubes with etched surface for writing data. (3) Large slides for the examination of series of cultures. (4) Cotton 'silver' for plugging tubes, etc.

*A Low Temperature Incubator:* E. H. WILSON, Brooklyn, N. Y. (Read by title.)

*Preservation of Sputum for Microscopic Examination; A New Fermentation Tube:* A. ROBIN, Newark, Del.

The author has experimented with some of the active germicides with a view to pre-

serving tuberculosis sputum. Carbolic acid, 5-per-cent. solution; trikresol, 2-per-cent.; formaldehyde, 5-per-cent., and hydrochloric acid, 10-per-cent., were added to sputum containing large numbers of tubercle bacilli. The coagulation resulting from the addition of carbolic acid or trikresol to sputum containing pus was largely overcome by vigorous shaking, the coagulation being thus finely broken up. The sputum was examined at the end of 24 to 48 hours. Weekly and then monthly examinations were made for a period of four months. Except when hydrochloric acid was used, the bacilli were found well preserved and, if anything, stained much more deeply. HCl, on the other hand, seemed to have either so disorganized the bacilli or so changed their staining properties that they could not be

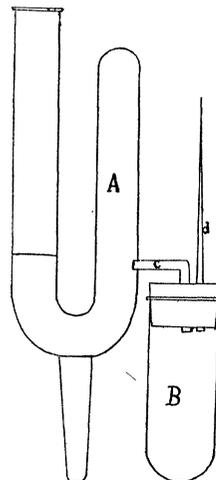


FIG. 1. A New Fermentation Tube.

found at the end of 24 hours. As a result of these experiments the author recommends the addition of an equal volume of a 5-per-cent. solution of carbolic acid to the sputum, which should be vigorously shaken up in the bottle so as to break up the lumpy coagulation. The apparatus is illustrated by the accompanying drawing. The side-tube *c* is packed with non-absorbent cotton; the arm *A* of the U-tube is filled with mer-

cury, the tube *B* is filled with the culture, when the rubber stopper holding the side-tube *d* and straight tube *d* is tightly inserted. When this is done the end of *d*, which serves for the escape of air displaced by the stopper, is sealed in the flame. The gas generated in *B* escapes into the closed arm *A*, displacing the mercury. To determine the  $\text{CO}_2$  ratio, the tube *B* is filled to the rubber stopper. Two fermentation tubes are used. In one, the arm *A* is filled with mercury, and the other half of the arm is filled with a saturated solution of sodium or potassium hydrate, this being readily accomplished by inclining the *U* tube towards the operator. The  $\text{CO}_2$ , passing through the caustic solution, is absorbed, and the unabsorbed gas (*H*) is left. The ratio between the two is then determined. This apparatus is manufactured by Eimer and Amend, New York.

*A New Method of applying the Rabies Test:*  
CHAS. F. DAWSON, Detroit, Mich.

The author, in his work upon rabies, was dissatisfied with the current method of inoculating animals in the cerebrum, which involved trephining the animals, with occasional death from hemorrhage, possibility of self-inoculation and other difficulties. He has, therefore, devised a method of inoculation in which these difficulties are reduced or eliminated. The method is as follows: A bit of the brain of the suspected animal is ground in a mortar containing sterilized 6-per-cent. sodium solution, and is then filtered through sterilized cotton. Two minims of this solution are then injected on the base of the anterior cerebrum by way of the optic foramen. To accomplish this, full grown rabbits are used which are thoroughly anaesthetized with ether. A hypodermic syringe is used with a needle seven-eighths of an inch long. The inoculation is made by lifting the nictitating membrane out of the way by means of the syringe

needle, and then forcing the needle upwards and backwards through the orbital tissues until it enters the optical foramen. The contents of the syringe barrel are then injected and the needle carefully withdrawn. By this means the solution is injected underneath the cerebrum and the chances of injury to the animal are much lessened. In a series of comparative tests made with this and the ordinary method, the author reaches the conclusion that the new method is fully as satisfactory as the old, and much easier to apply.

*The Use of Carbohc Acid in isolating the Bacillus Coli Communis from River Water:*  
WILLIAM B. COPELAND, Pittsburg, Pa.

The author described a method which he had devised for the purpose of separating the coli bacillus in river water, by the use of solid media. For this purpose he used Wurtz's agar, inasmuch as it could be incubated at  $37^\circ$  and the acid colonies were readily distinguishable by the reddening of the litmus. Inasmuch, however, as many other bacteria are present in river water, especially after a rain, which can develop at  $37^\circ$ , it is quite desirable to devise some means by which they may be reduced without affecting the colon bacillus. The author accomplishes this by adding to the agar two-tenths cc. of a 2-per-cent. solution of carbolic acid. Experiments showed that such addition of carbolic acid reduced the total number of bacteria about 45 per cent., while it had no effect, apparently, upon the colon bacillus. This makes it possible to determine the number of colon bacillus in water much more readily than if all bacteria are allowed to grow. By the use of this method, a study of the relation of the muddiness of river water and the number of colon bacilli was made. The result showed that, leaving out certain irregularities due to abnormal conditions, the number of colon bacilli increased with the turbidity of the water, a

relationship pointing to an increase in sewage pollution at times when the water of the river becomes turbid. The author recommends the use of carbolic acid as described in the employment of solid culture media for the determination of the number of colon bacilli present in surface waters without dilution.

*A Few Experimental Data on Hypodermic Injections:* S. J. MELTZER, New York City.

From two series of experiments, Meltzer arrived at the conclusions: (1) That the effect of subcutaneous injection depends to a very large degree upon the concentration of the injected fluid, and very little, if any, upon its bulk; (2) that the effect is distinctly increased by a distribution of the injected quantity over several areas. The author employed crystalloid solutions, and restricts his conclusions to this kind of liquids.

*The Utility of a Supply of Live Steam in the Laboratory:* H. A. HARDING, Geneva, N. Y.

The expense connected with cooking and sterilizing in the bacteriological laboratory is usually great, because of the high cost and low efficiency of gas. As a saving of time and money, the advantage of using steam, generated directly by coal, is obvious. In fitting up the bacteriological laboratory at the New York Agricultural Experiment Station, the following devices have been tried and found satisfactory: In the case of the Arnold sterilizer, a steam pipe was introduced through the wall of the passage in which the steam normally rises into the sterilizing chamber, and an elbow screwed to the end of this pipe and turned downward. With this connection, the Arnold can be brought to a temperature of 99° C. within five minutes, without any unpleasant noise or undue waste of steam. An autoclave was constructed, differing from

the ordinary type in that steam was introduced from a high pressure boiler. By means of a reducing valve the steam pressure and, consequently, the temperature within the autoclave, can be held within very narrow limits. A ten-minute exposure at 120° C. suffices to render tubes of gelatin and other media sterile. Steam cups were installed, having the shape of an ordinary water-bath, except that their depth was considerably increased. A steam inlet was placed at the bottom, and a waste pipe provided for carrying off the condensation. In these cups water is heated and agar is melted much more quickly than it could be done over an ordinary Bunsen burner, and in cooking media there is no possibility of boiling over or burning. The above pieces of apparatus, together with the hot air sterilizer, are placed upon an eight-foot bench, and nearly all the heat radiated is carried off by a galvanized iron hood. These devices have been in use for nearly two years and are giving good satisfaction.

H. W. CONN,  
*Secretary.*

*WORK AND EXPENDITURES OF THE AGRICULTURAL EXPERIMENT STATIONS FOR THE YEAR ENDED JUNE 30, 1900.*

THE Secretary of Agriculture has recently transmitted to Congress the annual report on the work and expenditures of the Agricultural Experiment Stations, made by A. C. True, Director of the Office of Experiment Stations. The following paragraphs are taken from the introduction to this report:

THE WORK OF THE STATIONS AS RELATED TO PRACTICAL AGRICULTURE.

In making our examination of the work of the experiment stations during the past year we have particularly inquired whether their operations are conducted with special reference to the agricultural needs of their