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SECTION OF MICROSCOPY.

May 27th, 1886.

The President in the chair.

The following Paper was read by Mr. Geo. W. Rafter:

ON THE USE OF THE MICROSCOPE IN DETERMINING THE SANITARY VALUE OF POTABLE WATER,

WITH SPECIAL REFERENCE TO THE BIOLOGY OF THE WATER OF HEMLOCK LAKE.

An abundant supply of pure water has always been considered an important desideratum of civilized communities. So thoroughly was this want felt and recognized by the ancient civilizations, that in order to afford such a supply conduits of the most expensive character were constructed. At Rome alone a daily supply of over 300,000,000 gallons was furnished. To bring this large quantity several aqueducts were required, some of them exceeding fifty miles in length.

In modern times this want is equally recognized, and usually our municipal expenditures for water supply are on a liberal scale. Indeed, excepting a few towns fortunately situated with reference to an abundant supply, the expenditure for this purpose is the most important item going to make up the sum total of municipal expenditures.

The financial value of water supply is therefore a matter of considerable importance, and methods of determining its sanitary value of necessity rank high in the economics of modern life.

It is the purpose of this paper to give a brief account of some of the methods in use for determining the sanitary value of a potable water.

By sanitary value is meant, in general terms, the purity of the water, its fitness for domestic use, etc., and the use of the term sanitary value may be understood by considering that such value is inversely as the amount of impurity; pure waters having high sanitary value, impure waters low sanitary value.

By pure water, is meant, again, water which is unobjectionable, from a sanitary point of view, for absolutely pure water, *i. e.*, chemically pure water, does not exist in nature. Earth and air both contribute organic and inorganic impurities, and the sanitary examination of a sample of water will in effect consist in determining whether or not such impurities are hurtful to those consuming it.

Let us first consider, briefly, the methods by chemical analysis. *According to Frankland, "The *exhaustive* chemical examination of a sample of water is one of the most tedious and troublesome operations known to chemists. It requires weeks, sometimes even months for its completion. This arises partly from the great multiplicity of separate substances which may be present in the water, both in solution and in suspension, partly from the very minute proportion in which those substances sometimes exist, and partly on account of the difficulties attending their exact determination. Such an exhaustive examination includes:

1. The extraction and separate volumetric measurement of the dissolved gases.

2. The separate determination of the weight of each constituent of the saline matters in solution.

3. The determination of the two chief elements of the organic matters in solution.

4. The separation of the suspended matters, if any, and the determination of their total weight when dry.

5. The separation and determination of each mineral constituent of the suspended matters.

6. The separation and determination as far as possible of each organic constituent of the suspended matters."

In sanitary examinations, however, the question is not what are *all* the constituents, and what their proportional weight, but rather, what, if any, are the unsanitary constituents and what are their amounts, and finally, what effect will they have upon human beings who use the given sample for domestic purposes?

Putting the question in this form immediately limits the range of an ordinary sanitary analysis very considerably. We know for instance that the volumetric determination of the dissolved gases will throw little or no light on the sanitary value of potable water. We know also that the separate determination of most of the saline matters in solution is rarely required as with few exceptions they have no appreciable influence upon the wholesomeness of a water. On no account, however, should certain of them be omitted. These

*Water analysis for Sanitary Purposes, with hints for the Interpretation of Results, by E. Frankland, Ph. D., D. C. L., F. R. S., pages 9 and 10.

are ammonia, nitrates, nitrites and chlorides. The mineral constituents should also be examined and if lead, copper, arsenic and barium exist in more than mere traces their respective quantities should be determined by weight. Finally the degree of hardness should always be determined.*

†The methods of determining the organic matter have been the subject of considerable discussion among chemists and widely varying opinions have been advanced on both sides of the question. On one side we find Dr. Frankland who aims at the quantitative determination of the *whole* of the organic carbon and nitrogen present, although confessedly, these, or an unknown proportion of them, may occur in harmless states of combination. On the other side we find the equally eminent Wanklyn attempting to determine such portions of one or both of these elements as most readily undergo change, as for instance the oxidation of carbon, or the formation of ammonia from nitrogen, the supposition being that such forms of organic matter as alter readily under the conditions of experiment are most likely to be the forms chiefly concerned in putrefactive decay, and likely in consequence to involve danger to health. Neither of these suppositions have yet been satisfactorily proved, although an elaborate, and in many respects admirable, system of chemical analysis has been built upon them. Dr. Frankland who has, as analyst for the Rivers Pollution Commission, had an opportunity to give this whole subject a very thorough study, has in conjunction with Prof. Armstrong devised the system of analysis which bears his name, and which is also known as the "combustion" process. This method is by far the most elaborate of any that has been proposed and aims as above stated at a determination of all the organic elements. This is accomplished by evaporating a given quantity of the water and in submitting the residue to a process of organic analysis.

The method devised by Wanklyn in conjunction with Profs. Chapman and Smith is known as the "albuminoid ammonia" process and while containing many points of interest must be considered as having on the whole relative value only. In the introduction to their work on water analysis its authors say :—‡"It is a received fact that the drinking of water which contains an undue proportion of organic

*See Frankland as above, pages 10-12

†See Reports (1), (2), (3) on Results of an Investigation Made by Direction of the National Board of Health, as to the Chemical Methods in use for the Determination of Organic Matter in Potable Water, by Prof. J. W. Mallet, F. R. S., University of Virginia.

‡ Water Analysis; a Practical Treatise on the Examination of Potable Water, by J. Alfred Wanklyn and Ernest T. Chapman.

filth is injurious to health. Such being the case, the first question which the sanitarian asks respecting a given specimen of water which is proposed for use as "potable" water is this :—Is it abnormally charged with organic filth? To this question the water analyst is enabled by the ammonia process to answer in many cases by an absolute No. In other cases he can show that the water *is* abnormally filthy ; and in some cases that there is danger of specific poison."

These two systems of analysis substantially cover all that has yet been devised in this direction of value. There are other systems among which may be mentioned the permagnanate of potash method, and the method by ignition, which while of value in certain directions lack generally the exactness of the first two and need not be specially considered here.

The following will serve to illustrate the value of results and the method of interpreting them for the Wanklyn and Frankland systems respectively:

*"If a water yield no "albuminoid ammonia" it may be passed as organically pure, despite of much free ammonia and chlorides ; a water giving less than 0.005 part of "albuminoid ammonia" in 100.000 parts may be regarded as very pure. A water containing 0.005 part of "albuminoid ammonia" together with a considerable quantity of free ammonia is suspicious, but in the absence of free ammonia the "albuminoid ammonia" may be allowed to amount to something like 0.010 part; above 0.010 part should be regarded as very suspicious, and according to Wanklyn over 0.015 part should condemn the water.

The Rivers Pollution Commission in interpreting the results of Frankland's process, make the following classification· "Surface water or river water which contains in 100.000 parts more than 0.2 part of organic carbon or 0.03 part of organic carbon or 0.03 part of organic nitrogen is not desirable for domestic supply and ought, whenever practicable, to be rejected. Spring and deep well water ought not to contain in 100.000 parts more than 0.1 part of organic carbon or 0.03 part of organic nitrogen."

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*Wanklyn, Water Analysis as above, page 67. See also Water Supply Considered Mainly from a Chemical and Sanitary Standpoint, by Wm. Ripley Nichols, Professor at the Massachusetts Institute of Technology.

†Frankland, Water Analysis as above, page 143. See also Nichols' Water Supply, Chemical and Sanitary.

Prof. Mallet, of the University of Virginia, has recently made for the National Board of Health an exhaustive comparison of these various methods of water analysis together with a determination of their relative value. This report appears in full in the National Board of Health report for 1882 and is on the whole the best recent study of this subject.

In this report may be found a very full account of the different methods of water analysis together with the detail of each.

The report is too elaborate to go into at length here. The conclusions as given by Prof. Mallet are of great interest, as throwing light on the unsatisfactory results attending the examination of a sample of water by chemical means alone. His conclusions are as follows:

*" 1. It is not possible to decide absolutely upon the wholesomeness or unwholesomeness of a drinking water by the mere use of any of the processes examined for the estimation of organic matter, or its constituents.

2. I would even go further and say that in judging the sanitary character of a water, not only must such processes be used in connection with the investigation of other evidence of a more general sort, as to the source and history of the water, but should even be deemed of secondary importance in weighing the reasons for accepting or rejecting a water not manifestly unfit for drinking on other grounds.

3. There are no sound grounds on which to establish such general 'standards of purity' as have been proposed, looking to exact amounts of organic carbon or nitrogen, "albuminoid ammonia," oxygen of permanganate consumed, etc., as permissible or not. Distinctions drawn by the application of such standards are arbitrary and may be misleading.

4. Two entirely legitimate directions seem to be open for the useful examination by chemical means of the organic constituents of drinking water, namely, first, the detection of *very gross* pollution, such as the contamination of the water of a well by accidental bursting or crushing of soil pipes, extensive leakage of drains, etc.; and secondly, the periodical examination of a water supply, as of a great city, in order that, the normal or usual character of the water having been previously ascertained, any suspicious changes which from time to time may occur shall be promptly detected and their cause investigated.

*Report of National Board of Health for 1882.

5. In connection with this latter application of water analysis, there seems to be no objection to the establishment of *local* 'standards of purity' for drinking water, based on sufficiently thorough examination of the water supply in its usual condition."

We may derive from the above the general conclusion, then, that chemical analysis alone, cannot in the present state of chemical science give full information as to the sanitary value of a potable water, and we are ready, therefore, to determine in what degree the microscope can be relied upon in obtaining such information.

The present writer is of the opinion that *ultimately* the decision of questions relating to the sanitary value of potable water may nearly all be determined by methods essentially biological in their character.

The examination of the organisms in the water itself will cover an extensive field and will afford ample opportunity for testing all the resources of our microscopes. *If either animal or vegetable life be found we have at once evidence of organic impurity, and the kind of animal or plant life discovered will give a clue to the class of water under examination. We know for instance that certain forms both plant and animal are only found in dead and decaying organic infusions, while other forms are equally the accompaniment of fresh and wholesome waters. The microscope, therefore, enables us to state not only whether the suspended matter is animal or vegetable but also whether or not it is dangerous.

The substances found are of the most diverse character, and may include in water containing sewage contamination minute portions of almost every article entering into the domestic economy of human beings. Such for instance as cotton, woolen and linen fibers, fragments of paper, muscular tissue, portions of epithelia from man, hairs of animals, starch grains and many others. A water containing these necessary concomitants of human existence is at once open to grave suspicion and should, unless accidental or temporary contamination can be shown, be in all cases rejected.

A complete description of the animal life which may be observed in potable water would require volumes, and our space will permit of mere mention of a few found in contaminated and impure water.

Among rhizopods we may mention the Amœbae which is considered to indicate impure water chiefly from the fact that its favorite habitat is found in bogs and morasses.†

*See paper on The Sanitary Examination of Drinking Water by Dr. Albert J. Wolff, in the Eighth Annual Report of the Connecticut State Board of Health.
†Dr. Wolff.

Of infusoria the paramæcia are usually found in water contaminated with sewage and their presence may be safely taken as indicating either the presence of sewage or other objectionable organic matter in large quantities. The Paramæcium aurelia is frequently met with and should be studied until easily recognized by every microscopist interested in the biological examination of potable water. Indeed the whole family of paramæcia should be familiar to one engaged in this line of investigation. The writer has recently seen paramæcia present in large numbers in water which had no suspicion of sewage contamination. While several different species of paramecia were present there was no trace of the Paramæcium aurelia. In other cases of water free from suspicion of sewage contamination he has found the Paramæcium aurelia present. All such waters have contained, however, large quantities of decaying vegetable matter, and as an index of serious organic impurity the paramæcia may stand as the representative infusoria.

*Other infusoria are frequently met with as, for instance, euglena, especially the species E. viridis and E. pyrum. Also the kolpoda, coleps, stentor, kerona and others are found. The presence of these bodies indicates that their food exists in the water and that this must be either animal or vegetable. As yet, however, the most of these animals have not been studied sufficiently to enable it to be stated what the food of each species is. The settling of this question may possibly throw some light on certain obscure changes in the quality of water noted at various places.

Certain species of the entomostraca are frequently present in large quantities. The most commoh are Daphnia pulex and the Cyclops tenuicornis. In our own water supply there have been during the early part of the present spring a considerable number of the Diaptomus castor. In addition to the Diaptomus there have also been observed the Bosmina, Chydorus, Cypris and Canthocamptus. Entomostraca are of great interest as they are quite highly organized animals, and their study becomes quite fascinating from that fact. They have recently been considered as having possibly important relations to certain diseases,† the discovery having been made that Cyclops tenuicornis is infested with an *endo-parasite* which after passing through two moults in the abdominal cavity of the parent entomostracan is transferred to the intestinal canal of food fishes. This much is positively known. The inference may be drawn that these parasites pass either through the water or from the fish used

*Parke's Practical Hygienne, 6th Edition, Chapter on Physical Examination of Drinking Water.
†See A Final Report on the Crustacea of Minnesota by C. L. Herrick.

as food into the human system and there cause if not actual disease at least serious disturbance. Two such parasites of the Cyclops are known, namely, the *Cucullanus elegans* and the *Fillaria medinensis.** The Daphnia schæfferi has also been observed to have nematoid worms parasitic in the blood sinus in front of the heart, subsisting upon the nutriment in the blood which constantly bathes the animal.† It seems to be a fair inference, then, that the pathological conditions prevailing among the microscopic animals infesting our water supply are of importance and well worthy of careful study and investigation. This is brought home to us even more forcibly when we consider that probably many of these endo-parasites are but the immature forms of parasites of animals much higher in the scale.†

This branch of our subject is as yet hardly touched and the present writer predicts rich returns to whoever will give it patient cultivation.

The question presents itself: Of what use after all are these various little animals in the final economy of nature? Dr. Baird in the introduction to his "British Entomostraca" has answered this question so far as the entomostraca are concerned substantially as follows:

Referring to Cypris he says:

"The greater number of these little creatures are furnished with branchiae, either to their feet or maxillae, and when noticed in their native habitats may be seen to have them in constant motion, their action being seldom interrupted. One chief use, therefore, of them in the economy of nature may be, as Muller says, to ventilate the water day and night, and as they chiefly reside in standing pools, they may thus be of great use in preventing them from becoming putrid. And again, I have no doubt that most of the entomostraca are essentially carnivorous, and I have frequently seen specimens of Cypris in their turn, as soon as dead, attacked immediately by quantities of Cyclops, who, in a few minutes, had fastened themselves upon the dead animal and were so intent upon their prey that they were scarcely frightened away from it by being touched with a brush In a short time the Cypris might be seen lying at the bottom of the vessel, the valves of the shell separated and emptied of their contents. When, indeed, we consider the amazing quantity of ani-

* See A Final Report on the Crustacea of Minnesota, by C. L. Herrick.
† See Report as above by C. L. Herrick.

mals which swarm in our ponds and ditches, and the deterioration of the surrounding atmosphere which might ensue from the putrefaction of their dead bodies, we see a decided fitness in these entomostraca being carnivorous, thus helping to prevent the noxious effects of putrid air which might otherwise ensue; whilst they, in their turn, become a prey to other animals, which no doubt serve their purposes also in the economy of nature."

*The most of the rotifera are not considered to be generally harmful. The common Rotifer or Rotifer vulgaris may, however, be taken as an index of organic impurity, being usually found, in the present writer's experience, accompanying decaying vegetable matter. A few others are also found in impure waters.

†Concluding the subject of animal organic contamination the question may be asked, are there any cases in which the origin of epidemics or diseases of any sort have been traced to the presence of either infusoria, entomostraca or other microscopic animal life in drinking water? In answer two cases are cited which have been noted in recent reading. The city of Dorpat in Russia suffered an epidemic outbreak of a serious form of fever which was traced to the presence of *paramæcia*. Whether they were the agents producing the disease or simply accompanied more serious causes is immaterial. The one fact for microscopists to remember is that the outbreak of a serious epidemic traced directly to the drinking water was accompanied by their presence in the water in large quantities.

The second case is that of the city of Boston where outbreaks of diarrhœa, dysentry and other bowel complaints are stated to have been accompanied by the presence in the water supply of certain of the entomostraca in large quantities.

‡The vegetable forms met with are numerous and among the more important may be mentioned first of all the bacteria. We will also find confervoid algæ of various sorts, including occillatoria, nostocs, palmellaceæ, desmids, diatoms and others. The vegetable forms will, as we have already seen in the case of animals, present many different characteristics, and in order to fully comprehend their real import we must cultivate a hitherto neglected branch of botany. The confervoid algæ are found in ponds and pools and may gener-

*See The Rotifera, by Hudson & Gosse. Now publishing in parts by Longmans & Co. 4 parts out. The chapter on Habits and Haunts of the Rotifera is of special interest to the general biologist.

†See Dr. Wolff's paper on Sanitary Examination of Drinking Water as above.

‡See paper by Dr. Wolff above referred to, Parke's Hygienne and Nichols' Water Supply, Chemical and Sanitary.

ally be distinguished by their greenish color. Some varieties are, however, nearly colorless. Many of them are not considered to be specially harmful. The oscillatoria and nostocs when decaying are the cause of the pig-pen odors which have been frequently observed. That portion ot the water supply of the city of Boston which includes the Mystic ponds has on several occasions been infected with smells very much such as arise from an illy kept pig-pen. Such investigation as has been made traces this odor to the presence of the above mentioned algæ. The same thing has been met elsewhere and traced to the same cause.

Professor Farlow, of Harvard, has studied this whole subject very carefully, and his exhaustive paper in the Massachusetts State Board of Health Reports * is cited as a model for this kind of study. In his paper he states, in effect, that the most injurious vegetable substances found in drinking water are the blue-green nostocs above referred to.

The bacteria are of special interest to every biologist who makes the sanitary value of potable water the subject of investigation. Not only because of their relation to certain infectious diseases, but because of the unique system of biological analysis, dependent upon their presence, recently devised by Dr. Koch. The bacteria stand at the foot of the scale of vegetable life. For a long time their true position was a matter of uncertainty, and the subject of controversy. It is now, however, generally admitted that they are algae, and may be referred to the domain of botony. They were discovered two hundred years ago by Leuwenhoeck, but their correct position in the general system of classification has only been made out within the last few years by Cohn and Warming. Pasteur, Koch and others have investigated their pathological relations, and recent discoveries relative to the part which these microscopic algae play as producers of disease have rendered them of vast importance.

The bacteria are mainly unicellular, devoid of chlorophyl, and are classified by Cohn into four genera, as follows :

1. Spherobacteria or globular forms.
2. Microbacteria or minute rods.
3. Desmobacteria or filaments.
4. Spirobacteria or twisted or spiral filaments.

Of the first we have the well known genus micrococcus, of which there are three species. The second has but one genus, bacterium,

*Supplement to Report for 1879.

of which two species are known, the Bacterium termo and Bacterium lineola. In the third variety we have the species Bacillus and Vibrio. The fourth class spirobacteria includes the two species, Spirillum and Spirochaete. Some farther sub-divisions are made, but the above answers our present purpose. Those who desire the complete classification should consult Dr. Sternberg's translation of Magnin, where Cohn's classification may be found complete.*

The literature of the bacteria has become very extensive, and as it is so comparatively recent, some interested members of the Section may desire to know where it can be obtained. The attention of such is directed to the work published last fall by Dr. Dolly, a former resident of Rochester, on the † "Technology of the Bacteria," where the complete bibliography of the subject may be found.

The method of water analysis designed by Dr. Robert Koch, is one of the many ingenious devices in this field by the greatest of the modern biological workers. Its detail has been recently described in the "*Lancet*" by Dr. G. Bischof, from whose paper the following account is extracted : ‡

"I.—Collection and transport of samples of water.

1. A number of flasks of 100 cubic centimetres capacity are plugged with cotton-wool and sterilized by heating in an air-bath for one hour to 150–180° C. They are used after cooling for the collection of samples of water. Pipettes, glass plates, etc., are similarly sterilized by being inclosed in sheet-metal boxes, with a tightly fitting lid of suitable dimensions, and heated in the air-bath as above. 2. When filling a flask so prepared with a sample of water, the plug of cotton-wool is carefully taken out and held between the fingers of the left hand, without touching the lower part of the stopper or turning it upward. Some thirty cubic centimetres of the samples are filled in and the cotton-wool is at once replaced. 3. The following precautions should be taken in filling the flasks : (*a.*) From a tap or the like the water is made to run to waste for three or four minutes, and then directed into the flask, if possible without touching its side. (*b.*) From a reservoir, stream, etc., the water is collected by means of a sterilized pipette and then filled into the flask as above. 4. The flasks should be so carried during transport as to prevent contact between the water and cotton-wool. 5. As

*The Bacteria, by Dr. Geo. M. Sternberg, Surgeon U. S. Army.
†Published by S. E. Casino & Co.
‡See Sanitary Engineer for Dec. 31, 1885.

far as possible, all samples should be mixed with the nutritive gelatine, etc., (II., 2,) within a few hours after their collection.

II.—Examination of a mixture of the water to be tested and nutritive gelatine spread on a glass plate.

1. Preperation of nutritive gelatine : One kilogram of raw minced beef is mixed with two litres of distilled water in a flask, and left to stand for twenty-four hours surrounded by ice. The liquid is then squeezed, by means of a suitable press, into a flask, where it is mixed with 200 grains of ordinary white gelatine, twenty grains of peptone, and ten grains of common salt. After standing for a short time, the ingredients are dissolved by gently heating the mixture, which is next neutrilized by sodic carbonate. It should be rather alkaline than acid. The flask is plugged with cotton-wool, and the contents are heated in Dr. Koch's apparatus for sterilizing by steam on two consecutive days for half an hour each day. The contents are now passed through a paper filter in a hot-water funnel, and about ten cubic centimetres of the light yellowish filtrate filled into each of a number of sterilized test tubes provided with cotton-wool plugs. These test tubes must once more be sterilized by steam on each of three consecutive days for rather less than fifteen minutes. Nutritive gelatine thus prepared solidifies at the ordinary temperature, and is kept in test tubes ready for use. If the latter remain perfectly clear after a few day's standing, sterilization has been complete. This can be further confirmed by a blank experiment. 2. Application of the nutritive gelatine for testing water : (*a.*) The contents of some of the above test tubes are liquefied by immersion in water of about 30° C. Mix the water in the sample flask by gentle agitation, remove the plug of cotton-wool as above, (I., 2,) and introduce as rapidly as possible into the test tube by means of a sterilized pipette, the quantity of water intended to be used for the experiment, at once replacing the cotton-wool. The water and gelatine must now be intimately mixed by bringing the test tube repeatedly into an almost horizontal position, imparting to it at the same time a screwing motion. It does not matter if the mixture at this stage touches the cotton-wool. (*b.*) A sheet-iron box is filled with glass plates which have been rinsed thoroughly and sterilized as above, (I., 1.) After cooling, the box is placed on its side and opened. A plate is taken out, reversed, and placed in a perfectly horizontal position on another glass place which forms the cover of a glass dish. The latter has been *completely* filled with water and ice, and rests on a tripod, which can be leveled

by means of screws. (*c*.) The cotton-wool is removed from the test tube, the open end of the latter passed once or twice through a Bunsen flame and the liquid mixture poured on a glass plate in such manner as to form, after solidifying, as nearly as possible a rectangle, the surface of which can be readily measured, and is at least half an inch distant from the sides of the plate. Place a bell-shaped glass cover at once over the plate for a few minutes, until solidification has taken place. (*d*.) The plate is transferred to a moist chamber, consisting of a glass dish with a dish of moistened filter-paper at the bottom. A glass rest is placed on the paper to support the gelatine plate, and covered by an inverted glass dish slightly less in diameter than the other and resting on the paper. (*e*.) The sample thus prepared is kept for two or three days at a moderate temperature, until the colonies have sufficiently developed. A basement-room answers well for the purpose, on account of uniformity of temperature. It would probably he convenient to lay down the rule that the development of colonies be always determined after an incubation in the moist chamber of *three days;* only should there be danger, in the case of very impure waters, of the colonies growing into each other, their number must be determined on the second day, which then should be stated. The approximate temperature during incubation may be noted with advantage. (*f*.) The number of colonies is conveniently calculated per cubic centimetre of water. It is determined by placing the plate after incubation on a dark ground. A glass plate, divided into square centimetres and one-ninth of a square centimetre, is so arranged on a hinge that it can readily be made to cover the gelatine plate without touching it. If there are many colonies their number is counted in some of the smaller squares, the average being calculated on the entire area of the gelatine. With few colonies the larger squares should be made use of, or indeed the colonies counted all over the plate. A lens sliding on the top of the graduated glass plate assists in the discovery of the smaller colonies. Recently glass plates have been manufactured, which are provided with a slightly projecting rectangle of enamel of uniform area. I have no experience with them, but if they stand the sterilization well they would save the estimation of the total area, provided it be covered all over with gelatine. (*g*.) Lastly, the number of *liquifying* colonies over the whole or part of the area of gelatine has to be counted as before. These colonies are probably due to micro-organisms which are in connection with putrefactive changes, and they are distinguished, after a little practice, by holding and moving the glass plate slightly inclined before the eye.

III.—Remarks on the preceding method, translated from Dr. Becker's treatise.

1. The germs contained in the water under examination are *separated* by mixing with the nutritive gelatine. When the latter solidifies they are deposited and fixed, each becoming the centre of a colony. 2. Each distinct colony thus obtained is a pure culture resulting from one single germ. 3. The number of developed colonies, therefore, bears a a ratio to the germs contained in the sample. 4. Each sample of water differs quantitatively and qualitatively as to the bacterial germs it contains. Developed colonies show differences, visible even to the naked eye, in their color, their mode of development, liquefaction of the gelatine, formation of gases, etc. 5. The micro-organisms forming each colony may be examined more minutely by abstracting a minute portion (if necessary, with the assistance of the microscope) by means of platinum wire, which has been previously heated to redness. Such matter is mixed with a drop of distilled water on a thin glass cover and further treated as explained hereafter (V.) 6. Certain forms of micro-organisms are invariably found in each sample of water, and with some practice a colony foreign to it may be detected without any great difficulty. 7. Water which is rich in products of decomposition is not suitable for drinking; similarly, water rich in the agents causing decomposition is unsuitable. It is not impossible that a sample of water may appear quite unfit for drinking because it contains an unusually large number of micro-organisms, whilst chemical analysis fails to give warning. 8. The method appears peculiarly suited to the examination of the efficiency of filters, the germs in the water before and after filtration being compared."

Such examinations have lately been made at Berlin, and the results are given in the following table, showing the monthly average of bacteria in one cubic centimetre of water before and after filtration.*

MONTH.	BEFORE FILTRATION.	AFTER FILTRATION.	IN ONE OF THE CITY HOUSES.
July	1064	265	409
August	1440	277	157
September	2496	63	144
October	3251	21	50
November	466	27	26
December	811	57	34
January	864	39	29
February	865	98	84
March	1843	16	34

* Paper in the *American Monthly Microscopical Journal* for April, 1886, "Notes on the Biological Examination of Water, etc."

As to the number of bacteria allowable in what is called good water, Dr. Koch has said:

* "A large number of micro-organisms indicate that the water has received admixtures in a state of decomposition and loaded with micro-organisms, impure tributaries, etc., which might contribute, in addition to the many harmless bacteria, also pathogenic forms, that is, infectious matter. Experience thus far has shown that in good waters the number of germs capable of development varies between 10 and 150. As soon as the number considerably exceeds this limit, the water must be suspected of receiving contributions from polluted sources. If the number reaches or exceeds 1,000 I should not permit its use as drinking water, at least not in times of a cholera epidemic. The number 1,000 is chosen by me as arbitrarily as has been the case in selecting the limiting values in chemical analysis, and I allow each one to change it according to his convictions."

An excellent test of the presence of organic matter, is that known as the sugar test of Heisch. For this purpose ten grains of pure granulated sugar are added to about five ounces of the water to be tested. A perfectly clean bottle should be filled with this and tightly corked to exclude the air. Ground glass stoppered bottles should be used when they are available. Place the bottles in a window where they are exposed freely to daylight but not to the direct rays of the sun, and maintain as nearly as possible a uniform temperature of 70° F. If the water contains much organic matter an abundance of whitish specks will be seen floating about in the course of a day or to, and the more organic matter there is, the more marked will be this appearance. After several days these little particles settle to the bottom of the bottle, where they appear as white floculent masses. If the water is *very* bad after a week or two the odor of rancid butter may be perceived.

By this simple test in conjunction with the use of the microscope much information relative to the quality of potable waters may be gained. The present writer deems it of special value for purposes of comparison. For instance, it is his habit to put up at the same time and under the same conditions several bottles, some of them representing waters whose constituents are well known while others are the unknown samples whose sanitary values are to be determined. After several days samples of each are taken out with proper precautions as to sterilization of pipettes, etc., and examined under

*See paper as above in *Microscopical Journal.*

the microscope. Such examination has always thrown light on the real character of the water under consideration.

In the present writer's experience as Sanitary Engineer some very interesting incidents have occurred in connection with examinations looking towards the selection of a source of water supply. At a town in Texas, for instance, of from 12,000 to 14,000 inhabitants, the city authorities proposed at one time to turn a sewer into the Trinity river just above the crib in the middle of the stream from whence the water supply was to be derived. The Trinity at that point is a stream varying from a dry weather flow of almost nothing to a flood flow four to six times as great as that of the Genesee here at Rochester. No special reason existed for turning a sewer into the river above the water works source of supply, and indeed, all the sewerage of the town was finally turned in at a point far below, and from what is known of the topography it may be stated that this was done without special additional expense. The fact rhat a proposition of this sort is seriously considered by the municipal authorities of any town simply illustrates the great lack of popular information as to the sanitary value of potable water.

Another case: A town in the neighboring State of Pennsylvania, called upon the writer about a year and a half ago to advise as to the selection of its water supply and as to the design of works for utilizing such supply. Arriving on the ground it was found that the local controlling board had proposed to use the water of a small stream which in the nature of things must take all the ground drainage from a cemetery of considerable extent. The cemetery being located on a side hill, sloping towards the stream with gravelly soil and hard pan underneath. The controlling board had pointed out to it some of the evil results likely to follow such a selection, and was advised to look farther for a source of supply.

At Westfield, N. Y., a town of 2,500 inhabitants, a system of water works has been in use since 1874. A few months ago the writer was called upon to advise the town authorities either as to increasing the capacity of the works now in existence or else as to the construction of new works.

The present supply is derived from a well sunk ten to twelve feet into what was originally a bog, the water therefrom being pumped by *windmill* into a shallow reservoir, (depth of from four to five feet) from whence it is distributed to the consumers The question before the Board of Trustees was as to the propriety of sinking another well in the bog and as to the construction of another shallow reservoir. It was claimed by the town authorities that this well was in reality supplied from a deep-seated spring and not from the surface and ground water of the bog. An examination, however, revealed the fact that the well was carried down through the peaty matter forming the soil of the bog to a close-textured shale rock and it was thought to be very improbable that any spring rose through this shale. Immediately south of the well, the limit of the bog in that direction was reached with an abrupt rise of from ten to twelve feet, beyond which the ground rose rapidly to a street with several houses, surrounded by barns, pig-pens, privies, etc., and at a distance of about 200 feet from the well. From the abrupt rise near the well to these houses and outbuildings there is a gravelly soil with the same impervious shale a few feet below. It was found on inquiry that diseases of the zymotic type were prevalent wherever the water from the public supply was used. With these facts gained as the result of examining the matter, the writer predicted that the water of their public supply would be shown on analysis to contain more organic matter than was compatible with safety, and the trustees were advised to abandon this well entirely and construct works with the Chautauqua creek, a clear mountain stream near by, as the source of supply.

An examination with the microscope in conjunction with an application of the sugar test above detailed more than justified the opinion originally expressed. As matter of interest it may be stated that at the time of the general examination the reservoir contained literally millions of tadpoles and the interesting biological question was at once raised as to the real import of this fact in settling the sanitary value of the Westfield water.

But time presses and we must pass to the final conclusions, which are to this effect: that chemical analysis can determine but little relative to the dangerous character of the organic contamination found in potable water, that this part of the question can generally be settled by the use of the microscope. Farther, if we possessed full information as to the real significance of all the substances, both organic and inorganic found in the sediment, the determination of the sanitary value of potable water would become a microscopic study, with the use of chemical processes to clear up occasional uncertainties, instead of being as at present a chemical study with the microscope as an accessory.*

* See Report by Dr. Chas. Smart, U. S. A. in National Board of Health Report for 1882.

Relative to our own water supply the writer suggests the following as worthy of consideration:

* The thorough chemical study of the Hemlock lake water made by Prof. Lattimore several years ago has shown it to be, so far as chemical analysis can, of exceptional purity. Such microscopical analysis as Prof. Lattimore was able to make at that time also exhibits the same truth. We should remember, nevertheless, that Hemlock lake is a comparatively small body of water, its mean area being rather less than 1,850 acres. Its depth, however, is great, being for a considerable portion of the lake from 40 to 70 feet. The water works conduit now takes the supply at a depth of 30 feet and may be extended as occasion demands. The whole body of water is still comparatively small even though the depth is considerable. The water shed is fairly well settled and sources of organic contamination are likely on the whole to increase somewhat from year to year. From whence it follows that we may expect changes in the character of the microscopic life. It seems a reasonably evident proposition, therefore, that the biology of our water supply may be profitably studied. It is to be regretted that the means at Prof. Lattimore's command did not permit of making such a study in connection with his chemical study of ten years ago. It may be said, however, that in the last ten years biological methods have advanced greatly in completeness and in consequence of this advance any study made at that time would be considered incomplete now.

We may further make note of the general proposition that at Hemlock lake relatively *small* amounts of contamination will be *likely* to produce more serious effects than they would if the whole body of water were larger.

A determination of the sanitary value of this water would require the careful and systematic study of a number of important biological questions, some of which are perfectly general in their character. A few such questions have been jotted down without special regard to order as follows:

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* See Reports as follows:

1°)—Report on the Recent Peculiar Condition of the Hemlock Lake Water Supply. By S. A. Lattimore, Ph. D., L. L. D., Professor of Chemistry in the University of Rochester, in the Annual Report of the Executive Board of the City of Rochester for 1876.

2°)—Report on the Water Supply of the City of Rochester, by Prof. S. A. Lattimore, in Annual Report of the Executive Board for 1877.

3°)—Also See Report of J. Nelson Tubbs, Chief Engineer Rochester Water Works for same years. To be found in Annual Report of Executive Board.

1°)—Do any of the ordinary bacteria of putrefaction ever change into pathogenic bacteria, and if so under what conditions? *

2°)—It has been asserted that pathogenic bacteria cannot exist in the presence of putrefactive bacteria. Is this statement at all true, and if so what are the specific cases?

3°)—Under what conditions are infusoria developed in our water supply and does it now contain any which are pathologically objectionable? If so, what are they and what is the life history of each?

4°)—Aside from the bacteria is it probable that any of the other algæ which exist in our water supply are pathologically objectionable?

5°)—What is the real sanitary significance of the entomostraca, rotifera and other microscopic life in our water supply, and what probability is there of their transmitting endo-parasitic diseases to human beings?

6°)—What is the habit, period of recurrence and life history of all endo-parasites affecting the entomostraca, rotifera and all other minute life in our water supply, if any?

7°)—There should be made for a number of years a series of observations to determine whether the presence of any of these organisms in large quantity has any effect on the general public health, and if so, what effect?

8°)—Make a systematic study of the fish inhabiting the water supply, their diseases, etc., in order to determine whether their presence ever effects the public health injuriously, and if so, ascertain the nature of such effect.

9°)—There is at the head of Hemlock lake a swamp of about 120 acres, which at the time of high water each year is practically sub-

* In asking this question the writer had in mind the quite generally received opinion that the ordinary bacillus of a hay infusion may, under certain conditions, be transformed into the bacillus anthracis. This opinion is based primarily on the observation of Dr. Buchner, who states in a paper published in 1880, that he succeeded under certain detailed conditions in producing such transformation.

The hay bacillus and bacillus anthracis are both classified as bacillus subtilis. (See Plate 3. Fig. 14.)

Since penning this question the writer has received a copy of the third edition of Dr. Klein's Micro-Organisms and Disease. In the chapter "On Relations of Septic to Pathogenic Organisms" Klein casts considerable doubt on the accuracy of Dr. Buchner's observations. His objections are based chiefly upon observed technical differences in the two bacilli, and considers that the results obtained by Dr. Buchner from inoculation experiments were due to accidental contamination of the cultures, cultivations of genuine bacillus anthracis having been carried on in his laboratory while the experiments with hay bacillus were in progress.

Klein concludes his argument with the following:

"If Buchner could show us that in a laboratory in which for some considerable time anthrax cultures, anthrax animals, and examinations of anthrax bacilli, had not been carried on, cultivation of hay bacillus ultimately yields a fluid which produces typical anthrax, then I should be, perhaps, prepared to concede his proposition of a transmutation of hay bacillus into bacillus anthracis. Such a proposition is of the widest importance, and, therefore, its proof ought to be beyond cavil; there ought to be no chance of a possibility of error. Such proof Buchner has not given, and I cannot, therefore, accept his interpretation."

merged, while in low water it is nearly dry. This swamp is probably the breeding place of rhizopods, infusoria algæ, and in fact of nearly everything in the way of minute life found in the water. Does the presence of this swamp affect the water as received for consumption here at Rochester injuriously? If so, what is the specific injury and how may it be prevented?

10°)—There are about 80 cottages around the margin of the lake, with barns and other outbuildings. A portion of the refuse of these cottages, barns, etc., has previous to last year, gone directly into the lake and possibly some of it finally passed down the throats of our citizens.

Last year, however, under authority of a general State law, a system of sanitary protection was inaugurated by which the wastes from these various places are carried away to a point below the foot of the lake and treated. Are there still any undiscovered sources of contamination about the buildings, and if so, what are they?

11°)—In the course of his ordinary duties as assistant engineer of the Rochester Water Works, the writer made last spring a sanitary survey of the various sources of contamination about the lake. At that time hogs were kept at several points along the lake in such manner as to allow their refuse to drain into the lake. This seemed to him a flagrant violation of elementary sanitary principles. In addition to hogs, which possibly as a matter of prejudice merely, the writer considers the worst, there were several places where the drainage of large farm barnyards passed into the lake.

Under authority of the general law above referred to, the State Board of Health has framed a code of rules for the sanitary protection of the lake and most of the more serious sources of contamination have been removed.

The topography is such, however, that the absolute prevention of such sources of contamination will in the nature of things be difficult, at any rate so long as the water shed is used for farming and as habitation for human beings generally.

Ultimate relief from this difficulty may be obtained by purchasing the water shed and laying it waste. This heroic treatment has already been adopted in the case of water sheds supplying important foreign cities. The Hemlock water shed is not densely populated and such treatment will not be necessary if the inhabitants are properly impressed with the importance of cleanliness. It should, however, be determined what effect a diseased condition of domestic animals would have, if any, together with an investigation as to special remedies.

In all such matters we need greatly to have established a series of limiting values and for this purpose it appears that Dr. Koch's method of quantitative determination of the bacteria has special value.

12°)—* Prof. Pumpelly has shown that the filtering capacity of various soils has important bearing on many questions, the solution of which is here indicated as of interest and probable value. A complete biological survey would therefore include an investigation of this matter with special reference to the Hemlock drainage area.

13°)—The question has been raised as to whether the paramæcea are really indicative, *in any case*, of sewage contamination, and whether they are not frequently the accompaniment of *harmless* organic contamination. It appears to the present writer that light can be thrown on this subject by a series of experiments. For this purpose proper infusions of various kinds may be prepared artificially, and a daily study of such for a considerable period ought to throw light on the question of their special occurrence in sewage contaminated waters.†

The above is sufficient to indicate a few of the questions, of which a rational explanation is essential, for a full understanding, of some of the complex problems arising in the practical management of a public water supply.

In the writer's opinion the Section of Microscopy of the Academy of Science could hardly find a more fascinating or more prolific field than to enter upon a year's study of the biology of our water supply. It is reasonably evident that in such a study the microscope is the one necessary instrument of precision, and it is hoped that it has been shown that such a study is a matter of utility and hence well worthy of the best efforts of the Section.

To the objection that the investigation here outlined presupposes an elaborate and expensive laboratory at the command of the Section, it is answered that this is an error, and the statement of ‡ Prof. Leidy, that an instrument costing $50 will do everything required of a microscope, in the production of such a work as his memoir on the Fresh Water Rhizopods, is cited as proof of the cor-

* Report on the Results of an Investigation into the Filtering Capacity of Soils. By Raphael Pumpelly. National Board of Health Reports for 1881 and 1882.

† See Kent's Manual of the Infusoria.

‡ Fresh Water Rhizopods of North America, by Joseph Leidy, M. D., page 3 of Introduction.

rectness of this opinion, and it may be safely concluded that the instruments and accessory apparatus now owned by the individual members of this Section will suffice for such a study as is here contemplated.

The farther objection may be made by some medical men that the field here proposed to be covered is one pertaining especially to the work of the specialist in pathology and hygiene. The attention of such may be called to the fact that the finest studies of recent times in this direction have been made by Pasteur, the chemist, and Tyndall, the physicist, neither of whom have any claim to the special training of a physician. Their attention may be further directed to the fact that modern biological literature contains no more magnificent generalizations than those of Herbert Spencer, the engineer.

What is really required is the patience to observe one object until its whole life history is determined, together with an analytical turn of mind to insure proper interpretation of phenomena.

It has already been shown in general terms that many different lines of investigation require to be pursued in order to make such a study really effective. This presupposes a number of observers working on the various lines. Such a study, therefore, if undertaken by the municipality and paid for at the current rates for such service would become very expensive. In the present condition of the municipal finances, and of public intelligence relative to the importance and real value of such work, there is no probability of obtaining any municipal appropriation.

An investigation of this character if made at all must then be under the auspices of some scientific body, and made as a contribution to the scientific literature of the day. No such body exists in Rochester except the Academy of Science, and the Section of Microscopy of the Academy would naturally undertake such a work.

In conclusion the writer takes occasion to express his appreciation of the honor conferred upon him by the invitation to read a paper berore this Section on the biology of our water supply, especially since he is neither a member of the Academy nor a person known as a microscopist, and he sincerely hopes he has repaid in part the indebtedness which he is under to the Section for valuable information received by attending several of the monthly meetings.

Explanation of Plates.

PLATE 1.

All the species figured in this plate are grass-green, and produce no injurious effect on drinking water.

Figs. 1-3. Chara coronata, Var. Schweinitzii, A. Br. Fig. 1, life size; Fig. 2, branch with antheridia, a, and sporangia, b, magnified slightly; Fig. 3, sporangium containing spore magnified 50 diameters.

Figs. 4, 5. Lemna polyrrhiza, L. Fig. 4, life size; Fig. 5, the same seen from the under side, and slightly magnified.

Figs. 1-5 were drawn from life by Mr. C. E. Faxon.

Fig. 6. Cosmarium Botrytis, Menegh. From Ralf's British Desmidieæ; a and b are two unicellar individuals, which are represented in c and d respectively as having ruptured, their contents having united to form the spore, s. Diameter of spore, $\frac{1}{375}$ of an inch.

Fig. 7. Spirogyra from Luerssen's Handbuch; c, chlorophyl bands in the cells; s, spores formed by the union of the contents of two cells. Magnified 240 diameters.

PLATE 2.

All species figured in this plate are of a bluish-green color, and in decay give off a pig-pen ordor.

Fig. 1. Coelosphaerium Kuetzingianum, Naeg., and Anabaena Flos-aquæ, var. circinalis, Kirchner. From the surface of Basin No. 3, South Framingham, October, 1879. Magnified 300 diameters.

Fig. 2. The same, showing details of Anabæna; a, spores; b, heterocysts.

Fig. 3. A portion of Fig. 2, magnified 600 diameters.

Fig. 4. Coelosphærium. From Fig. 1, magnified 600 diameters. A gelatinous halo is usually found round the colonies when seen with this power.

Fig. 5. Clathrocystis æruginosa. Henfrey. From Fresh Pond, Cambridge, October, 1879. Mature colony magnified 400 diameters.

Fig. 6. Lyngbya Wollei, Farlow. From Horn Pond, Woburn. A portion of two filaments magnified 400 diameters, showing the bluish-green disk-shaped cells, surrounded by a colorless sheath.

PLATE 3.

Eig. 1. Micrococcus prodigiosus. (Monas prodigiosus. Ehr.) Spherical bacteria of the red pigment aggregated in pairs and in fours; the other pigment bacteria are not distinguishable from this one.

Fig. 2. Micrococcus vacinæ. Spherical bacteria from pock-lymph in a state of growth, aggregated in short four to eight-jointed straight or bent chains, and forming also irregular masses.

Fig. 3. Zooglea-form of micrococcus, pellicles or mucus strata characterized by granule-like closely set spherules.

Fig. 4. Rosary-chain (Torula-form) of micrococcus ureæ, from the urine.

Fig. 5. Rosary-chain and yeast like cell masses from the white deposit of a solution of sugar of milk which had become sour.

Fig. 6. Saccharomyus glutinis, (Cryptcoccus glutinis. Fersen.) a polluting yeast which forms beautiful rose-colored patches on cooked potatoes.

Fig. 7. Sarcina spec, * from the blood of a healthy man; **from the surface of a hen's egg grown over with Micrococcus lutens, forming yellow patches.

Fig. 8. Bacterium termo, free motile form.

Fig. 9. Zooglea-form of Bacterium termo.

Fig. 10. Bacterium pellicle, formed by rod-shaped bacteria arranged one against the other in a linear fashion from the surface of sour beer.

Fig. 11. Bacterium lineola, free motile form.

Fig. 12. Zooglea-form of B. lineola.

Fig. 13. Motile filamentous Bacteria, with a spherical or elliptical highly refringent "head," perhaps developed from gonidia.

Fig. 14. Bacillus subtillus, short cylinders, and longer, very flexible motile filaments, some of which are in process of division.

Fig. 15. Bacillus ulna, single segments and longer threads, some breaking up into segments.

Fig. 16. Vibrio rugula, single or in process of division.

Fig. 17. Vibrio serpens, longer or shorter threads, some dividing into bits; at * two threads combined.

Fig. 18. Swarm of V. serpens, the threads felted.

Fig. 19. Spirillum tenue, single and felted into swarms.

Fig. 20. Spirillum undula.

Fig. 21. Spirillum volutans, * two spirals twisted around one another.

Fig. 22. Spirochoete plicatilis.

All the figures in plate 3 were drawn by Dr. Ferdinand Cohn with the immersion lens No. 9 of Hartnack, Ocular III. representing a magnifying power of 650 diameters.

Plates 1 and 2 with the explanations are from a paper "On Some Impurities of Drinking-water Caused by Vegetable Growths," by Prof. W. G. Farlow, of Harvard University, printed with the Report of the Massachusetts State Board of Health for 1879.

Plate 3 with the explanation is from the *Microscopical Journal.* Vol. XIII. N. S., Pl. V.

The writer has already called attention by foot notes to his indebtedness to the paper of Dr. Wolff in the Eight Annual Report of the State Board of Health of Connecticut, "On the Sanitary Examination of Drinking-water." This paper is an excellent resume of the subject, and while the writer believes that in the present state of knowledge Dr. Wolff is not justified in a few of his opinions, still the paper as a whole is so valuable, that great pleasure is taken in specially acknowledging a considerable indebtedness in the preparation of the present paper.

Dr. Wolff's paper on Park River Nuisance, in the Seventh Annual Report of the same Board, is of interest and value to students of the Bacteria.