THE MICROSCOPICAL EXAMINATION OF POTABLE WATER.

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PREFACE.

THE publishers of the SCIENCE SERIES having asked the writer to prepare a monograph on the Microscopical Examination of Potable Water, I will endeavor to comply; though that a civil engineer should be expected to prepare anything of value on a subject which has engaged the attention of the most eminent chemists and biologists is something of a surprise. Possibly, however, the fact that studies in this direction have occupied my leisure hours for a number of years, and have led to the production of a number of more or less useful papers thereon, may be to some extent an explanation; but it is sincerely hoped that the expectations of neither publishers nor readers will be raised too high.

This little book has, it is believed, one merit frequently absent in formal works of more pretension. It may be taken as fairly representing the state of the art, of which it professes to treat, at the date of issue, namely, at the beginning of the year 1892; that this is a real merit will be appreciated by every serious-minded student who has

had occasion to travel in new roads. To all such who may be interested in the advancement of public sanitation this monograph is presented, albeit somewhat hesitatingly, in the hope that it may be of essential use in actual work.

The perfected method of making the quantitative enumeration of the microscopical organisms in potable water, which is here described, is the joint work of Prof. William T. Sedgwick and myself, though both Mr. A. L. Kean and Desmond FitzGerald, C. E., have contributed useful ideas. To Professor Sedgwick must, however, be assigned the credit of working out a really practical method of making these examinations, and to him must be assigned the honor of giving the method a name. The author took the method, as will be shown in the body of the volume, after Professor Sedgwick had put it on a working basis, and added certain refinements of technique. Professor Sedgwick has deemed these refinements of sufficient value to justify coupling the author's name with his own, and has accordingly described it in the Massachusetts Health Reports as the Sedgwick-Rafter method. This I acquiesce in, though the statement may be made, that a complete balancing on my part of the account between the biologists of the Massachusetts State Board of Health and myself, would show on the whole transaction a considerable amount still to the credit of that Board.

In preparing this monograph, I have assumed

that the reader possesses a fairly complete knowledge of the optical part of the microscope and of micrometric measurements. On these heads, therefore, only such information is given as is necessary to elucidate the special work in hand. In the same way the technique of collecting, preserving, and mounting, as treated in Part I., have been only briefly given. The full detail leads too far away from the special subject. Those not possessing the necessary preliminary information, and still wishing to fit themselves for an intelligent use of the method, may find abundant references to standard literature of the microscope, either in the explanatory foot-notes or at the end of the volume.

In the same way a knowledge of the fundamental ideas in relation to modern water analysis is assumed on the part of the reader. Those who do not fully possess such knowledge can hardly do better than to consult the recent Special Reports of the Massachusetts State Board of Health.

G. W. R.

ROCHESTER, N.Y., Dec. 24, 1891.

NOTE. — The small figures throughout the text, thus: MacDonald's Water Analysis,[14] refer to the number of the volume cited in the list of literature following Part II.

MICROSCOPICAL EXAMINATIONS.

PART I. — QUALITATIVE.

How to Study the Biology of a Water Supply.

OTHER things being equal, there is in every community having a public water supply, a relation between the degree of purity of such supply and the public health. Indeed, it may be broadly stated, that in a community with a water supply of a high standard of purity, there will exist a lower death-rate than if the standard of purity be materially lower.

No argument is necessary, then, to establish the proposition, that information relative to the biology of a public water supply is of vast importance, both to individuals and communities; and a study having for its object the solution of biological problems may be safely counted as worthy of intelligent effort.

WORKING-TOOLS.

For the general study of the biology of a water supply certain handy tools are requisite.

These are the compound microscope, fitted with about the following list of objectives: one-inch, one-half inch, or four-tenths, one-fourth, and one-eighth. The one-half and the one-fourth inch are the most useful. A one-half inch Gundlach, of 50° angular aperture, and a one-fourth inch Bausch & Lomb professional, of 110° angular aperture, have been found satisfactory. For this work the moderate-angled objectives are preferred to those of wider angle. They have better working distance than the wide-angled lenses of recent make. The one-inch, one-fifth inch, and one-eighth inch are sometimes used, but the use of these latter is infrequent compared with the two above mentioned; and it may be said, in passing, that a very complete study of the biology of a water supply can be made with only two objectives; namely, the one-half inch and the one-fourth. In studying sup-

plies containing many of the larger forms, such as *Hydra*, *Cyclops*, *Daphnia*, *Dioptamus*, etc., either a two-inch, or one and one-half-inch would, however, be very convenient.

While thus somewhat radical in expressing a preference for moderate-angled objectives for ordinary work, it is but fair to say that for the highest class of biological work wide-angled objectives are nearly indispensable. For any immersion objective above one-sixth inch focal distance, one should purchase those of large angular aperture. There is, however, in all such objectives, a considerable sacrifice of working distance to aperture, so that the specific use to which an objective is to be put must to a great extent determine what to purchase. Of one thing we may be certain, that the very wide-angled immersion lenses require a perfection of movement in the microscope stand, and a delicacy of manipulation, which add considerably to the amount of time required to complete an examination;*

* For *very* high-power objectives, cover glasses, even of the thinest glass, are too thick, and ordinary talc split into

so that for the working microscopist to whom time is of value, the question of just what shall be the limit is an important one.

Of eye-pieces one is indispensable, and two or more are desirable. Where only two are purchased, they should be one of an inch, and one of an inch and a half focal distance. The Huyghenian eye-piece gives somewhat clearer definition than the periscopic, but the periscopic has the advantage of doubling the field. A fairly complete battery would consist of a one-inch periscopic, in addition to the two Huyghenian eye-pieces above suggested.

LIFE-CAGES AND CULTURE-CELLS.

The catalogues are filled with life-cages, growing and culture cells of divers and various sorts, and the beginner in biology is likely to conclude that considerable ex-

thin laminæ may be used. W. Saville Kent, in his "Manual of the Infusoria," speaks of using laminæ of such extreme tenuity that they may be blown away with the lightest touch. With such films Kent says the investigation of the infusoria, with 1-16, 1-25, or even 1-50 inch objectives, becomes a comparatively easy task.

penditure is necessary for apparatus of this sort. The author's experience, after trial of the various life-slides and life-cages, is that for ordinary examinations a plain slide, with a ring of cement forming a shallow cell, covered with a cover glass, is, on the whole, preferable. Holman's siphon-slide and Holman's siphon life-cage are of use where it is desired to observe the same object continuously for several days. Their expense, however, is considerable, and fairly satisfactory results may be gained with the following device, which has the merit of costing almost nothing. A plain glass slip is taken, and to one side of it two three, four, or more thicknesses of chemical filter paper are pasted, the number of thicknesses depending upon the depth of cell required. The cell is made by cutting out the centre, either round or square, according to the taste or fancy of the operator. In this cell is deposited the organism which it is desired to study. The placing of a cover glass, and the securing of it with a little cement, completes the operation so far as the construction of

the growing-cell is concerned. The cell is placed upon the stage of the microscope, and supplied with water by a rubber tube, acting as a siphon, from a jar standing on a shelf above the stage. The supply of water is controlled by a brass cock placed at the lower end of the rubber siphon tube, set so as to allow water to drop very slowly upon the paper composing the cell, just outside the edge of the cover glass.

In preparing such a cell, care should be taken to cement the several layers of the paper together at the inner edges, in order to prevent the more minute objects from passing in between the layers. In using it, the slide should be dipped in water, thoroughly wetting the paper before filling the cell, and the delivery of water from the siphon brought to such a rate as to keep the paper constantly wet, just supplying the loss from evaporation. The best results will be obtained by setting the microscope vertically. Filter paper is used in its construction, in order to insure that the paper contains nothing likely to kill the object whose life-history it is desired

to study. For deeper cells the best quality of white cotton blotting-paper * may be used, the precaution having been taken to soak it for several days in frequent changes of pure water. For low-power objectives this cell may be constructed of two thin slides, with a layer of blotting-paper between them, the slides held in place by rubber bands at the ends.

Recklinghausen's growing cell † is stated by Frey to be an efficient device for preventing the evaporation of fluids. It consists of a glass ring cemented to an ordinary slide, forming a cell, in which the organism to be examined is placed in a little water. Blotting-paper is folded over the edges of the glass ring, and a tube of thin rubber slipped over this, and connected with the objective, being held in place by compression bands. Around the outside of the glass cell several thicknesses of moist blotting-paper are wrapped, and

* Such a quality of blotting-paper, which is claimed to be entirely free from chemicals, and composed of nothing but pure cotton fibre, may be obtained from the stationers.

† Frey, on Microscopes and Microscopical Technology,[90] p. 99.

to these additional moisture occasionally added.

For studying the life-history of very minute organisms, an efficient cell may also be made by inverting a cover glass over a glass cell, with a little water at the bottom, the organism to be studied being contained in a drop of liquid on the under side of the cover glass.

The moist chamber used by Dallinger and Drysdale in their investigation of the monads is of interest and value,* and illustrations of it can be found in Kent's "Manual of the Infusoria."

Maddox's growing slide is also worth trial. Carpenter is a convenient reference for a description.†

Weber's annular cell is an American invention, and worth trial.‡

* This cell was originally illustrated in *Monthly Microscopical Journal* for March, 1874.

† "The Microscope and its Revelations," by Wm. B. Carpenter, sixth edition, p. 145.[86] Originally described by Dr. Maddox in his paper on Cultivation of Fungi, in *Monthly Microscopical Journal* for 1870, vol. iii.

‡ Carpenter, *loc. cit.*, p. 147.

SUB-STAGE CONDENSER.

For assisting the illumination, a good sub-stage condenser is at the present day indispensable, and of the several forms the Abbe may be considered the best. It admits of the greatest range of adjustment without loss of time; and when it is desired to examine an object under all conditions of illumination, this is a matter of considerable importance. It is provided with a swinging ring carrying diaphragms of various apertures. The blue glass which accompanies it, for furnishing monochromatic light when working by lamplight, is also of value.* By its use the objectionable and trying yellow glare of lamplight is entirely obviated; and if one works by lamplight, a considerably larger amount can be done without over-fatiguing the eye than can possibly be accomplished without it. The value of the condenser may be readily shown by a trial of it on one of the test

* The opticians also furnish a blue-glass mounting for sub-stage, which is adapted to any microscope without the condenser. A blue-glass chimney for the lamp may be made to give substantially the same results.

diatoms; and probably the most decisive test will be by resolution of the *Pleurosigma angulatum,* with one-fourth of medium angular aperture. Such an objective will only make the resolution "clearly" without the condenser, when the light is somewhat oblique. Moreover, the resolution is not made instantly, but, even with a fairly expert operator, will require a little expenditure of time in manipulation. With the condenser, on the contrary, such an objective will resolve the test instantly, or, at any rate, as nearly so as one can rack the condenser to its proper position in the sub-stage; and this, too, with the smallest diaphragm in place, so that the resolution is in reality made with nearly central light.*

The foregoing are the more important tools to be used in a study of the biology of a water supply. For dissections, knives, needles, watch-glasses, tweezers, a dissecting microscope, and other accessories, will be required.

* For hints on the use of the condenser, see "Manipulation of the Microscope,"[83] by Edward Bausch.

COLLECTING FROM WATER MAINS.

Whoever undertakes to unravel the problem of the biology of a water supply, will find it necessary to investigate in many directions; and a few hints on the subject of general collecting are therefore included.

In the first place, when it is desired to collect samples from the mains of a public water supply without reference to the method of quantitative enumeration, described in Part II., the simple method of fastening a single thickness of fine cotton cloth over an ordinary cock, and allowing the water to flow freely, will answer every purpose. It is necessary to allow the water to flow full size of opening, in order that the various suspended objects may be carried along and brought into the filter; and two hours of such flow will ordinarily be sufficient. For cleaning the specimens from the filter it should, after removal from the cock, and after turning wrong side out, be either dabbled in a small quantity of water contained in a

deep dish, or washed off with an ordinary laboratory wash bottle. In this way the filterings of a number of hours may be concentrated into an amount of water not more than enough to fill a medium sized beaker. After standing for a short time, samples may be selected, either from the sediment at the bottom, or from other portions of the liquid, depending upon what particular class of organism the operator may be looking for. Further details of this operation, with references to the literature, are given in Part II.

TRANSPARENT ORGANISMS.

In the examination, organisms are frequently encountered so nearly transparent that the eye fails to discern the structure. In such cases the examination may be materially assisted by the use of some staining reagent which, added to the sample, has the effect of bringing out the hidden structure. For such purpose the various aniline dyes are useful, though probably hæmatoxylin is most frequently applied. For the infusoria, a solution of

iodine, in iodide of potassium, and osmic acid have both been successfully used. They color the structure, leaving the cilia extended.

The use of first quality objectives, however, by reason of their superior definition, renders the application of staining reagents less necessary than with ordinary objectives.

COLLECTING FROM STREAMS AND RESERVOIRS.

Collections from streams, reservoirs, lakes, or ponds, used as sources or parts of public water supplies, will have to be made by different methods, and the particular one used will depend upon what it is desired to collect.

For fresh-water algæ, the implements needed, in addition to long rubber-boots, are substantially as follows: —

1. A small iron or tin ladle, two inches across, and provided with teeth for one-third of the circumference opposite the handle. The handle is a hollow ferrule, and serves to attach the ladle to

one's walking-stick. The teeth are bent inward, in order to catch masses of algæ beyond arm's-length.

2. A small sieve is necessary for intercepting floating masses of desmids, etc.

3. A common iron spoon for removing thin layers of mud along the margins where the presence of desmids or diatoms is for any reason suspected.

4. An iron rake for bringing up samples from the bottom is of value. It should have enough strong cord attached to reach the bottom of the deepest body of water to be examined.

The foregoing, with a number of bottles, a good pocket magnifier, and a strong jack-knife, will be the principal tools required for collecting the fresh-water algæ.* Indeed, the author's own collections have thus far been all made with the help of a few bottles, a walking-stick, a rake, and for objects at a distance on the surface, such means of reaching them as could be readily improvised on the ground.

* "Collector's Handy-Book," by Johann Nave.[96]

Mr. Wolle's outfit for collecting desmids consists of four or five tin cans (tomato or fruit), one within the other for convenience of carriage; ten or a dozen wide-mouthed bottles, and a ring net similar to that described below for collecting the entomostraca.*

According to the late Dr. Leidy, rhizopods are best collected by the use of a small tin ladle, as above described. Instead of a walking-stick, Dr. Leidy carried on his collecting tours a jointed pole of two or three pieces, each about five feet long. The ladle, or dipper, was used by slowly skimming the edge along the bottom of the water, so as to take up only the most superficial of the ooze, which was then gently raised from the water and transferred to a glass jar.†

Dr. Leidy states that he was most successful in finding rhizopods in the ooze near the shores of lakes and ponds, possibly due to the fact that the ooze near the

* "Desmids of United States," by Rev. Francis Wolle,[42] p. 13.

† "Fresh-Water Rhizopods of North America," [47] pp. 7 to 13 inclusive, are of great interest to the collector.

shores could be better seen, thus enabling the collector to get the desired material.

The infusoria are the most widely distributed of any class of microscopic life. Infusions of every sort and kind, and waters of every degree of purity, contain them.* Even falling rain and dew provide a home for extensive series. Certain classes are found only in salt and brakish water, and others in putrid infusions. These may be excluded as beyond the limits of the present inquiry.

Weedy ponds, or weedy nooks in reservoirs or lakes, and slowly running water, are the most favorable collecting fields for the species we are at present interested in. In such places one may profitably examine finely divided living plants for specimens of the more sedentary species, such as the Ciliata Flagellata. Dead and decaying leaves in the water should be examined for colonies of *Vorticella* and *Euglena*. Certain of the entomostraca, as, for instance, *Cyclops* and *Canthocamptus*, and the higher

* Kent's "Manual of the Infusoria,"[64] ("The Distribution of the Infusoria,") p. 107, and following.

crustacean forms, *Assellus* and *Gamarus*, are likely to be covered with some of the parasitic species.

For the collection of the infusoria one needs most a dipping-bottle, and some means of reaching beyond arm's-length, together with several small bottles to which to transfer the collections from the dipping-bottle.

A dipping bottle, as used by the author in his collecting-trips, consists of an ordinary two-ounce morphine bottle, fastened to the end of a walking-stick by a strong rubber band around the neck of the bottle, with the end of the stick passing between the band and the neck. For concentrating the dippings, a large-mouthed twelve-ounce bottle with two funnels, one of them small enough, when the stem is thrust through a small hole in the cork, to pass down into the body of the bottle in an inverted position, has been used. The mouth of this funnel is covered with a piece of light cotton cloth. The other funnel, which is larger, also has its stem passing through the cork, but in an up-

right position. The dippings are poured into the upright funnel, pass down through the stem of same into the bottle, where the inverted cloth-covered funnel acts as a strainer, allowing the water to flow up out of the bottle, but retaining whatever of microscopic life may have been brought up by the dipping.* This apparatus has been found of use on several occasions where the particular organism desired existed only in small numbers, and sparingly distributed through a considerable volume of water.

The rotifera may be collected by means of the collecting-bottle and concentrating apparatus just described. They are likely to be found in all varieties of water, and a formal enumeration of their habitats would transcend the limits of the present chapter. The reader is referred to Hudson and Gosse's † new work for detailed information on this point.

* For illustrations of this device see "Practical Microscopy," by Geo. E. Davies, second edition, p. 130, chapter on "Collection of Objects."

† "The Rotifera, or Wheel Animalcules,"[60] by C. T. Hudson, assisted by P. H. Gosse. Chapter iv., on the "Haunts and Habits of the Rotifera."

Certain worms of the class *Annulata* are indicative of badly contaminated sewage waters. Where it is desired to collect and preserve them for purposes of comparison, they may be found in any stream receiving sewage, and are easily obtained by use of a simple dipping-bottle.

For collecting the entomostraca from ponds, lakes, and reservoirs, the dipping-net is indispensable.* This is made by an iron ring, about one foot in diameter, attached by a strong ferrule to a pole ten to fifteen feet in length. The iron ring has a bag fitted to it (a flour-sack answers every purpose), and the pole should be strong enough to allow of lifting some considerable amount of water. This net may be used, not only in shallow water and among weeds, but also for towing behind a boat in deeper water.† It is emptied by allowing it to drain through

* "A Final Report on the Crustacea of Minnesota," by C. L. Herrick, [88b], chapter iv., on "Collecting, Preservation, and Miscellaneous Notes."

† For illustration of modification of this net, especially adapted for towing in deep water, see "Practical Microscopy," chapter on "Collection of Objects," above referred to.[89]

the meshes of the cloth, and then, when only a small amount of water is left in the bottom, transferring the same to a wide-mouthed bottle by quickly inverting the bag.

The above covers the principal methods and appliances for collecting. Other methods will, no doubt, suggest themselves as one progresses in biological studies.

In concluding the subject of collecting, it is desired to impress upon the reader the importance of keeping a full and complete record of everything relating to each collection, as, for instance, where it was found, and under what general and special conditions. Information of this kind will possess great interest when making a final judgment of the value of any given sample for sanitary purposes. For such a record the author usually carries several small blank gummed labels, such as are used for slides. These are numbered, and one is pasted to each bottle; the number being made to correspond with that of the entry in a small memorandum-book, which completes this part of the collecting-record.

These numbers can be carried in a series through an entire season's work, and further used for the record of the examination in the laboratory or at home.

PRESERVATION OF MATERIAL.

Having collected the material, it becomes an exceedingly important question how to best preserve it for future reference. Much ingenuity has already been expended in devising methods of separating microscopic material from the various degrading contaminations gathered with the original collections, and much still remains to be done.

Diatoms and desmids are usually separated by methods depending essentially upon differences in the specific gravity of various objects. For detailed description of such methods, and the necessary apparatus, the reader is referred to Nave's "Collector's Handy-Book," already mentioned.*

The smaller species of diatoms, when

* Also to "Practical Microscopy," [89] pp.138,139-288-290. See also article "Diatomaceæ," in fourth edition of "Micrographic Dictionary" [94] ("Collection"), p. 252.

living, may be separated by placing the material containing them in a shallow dish, with a little water, and laying over them a thin cloth. The tendency to move toward the light, which seems inherent in all these minute organisms, will cause them to creep through the meshes of the cloth, appearing on the upper side, frequently in such quantities as to be easily scraped off with a thin knife, and entirely free from the degrading material.

FILAMENTOUS ALGÆ.

The filamentous algæ can be cleansed by washing; but when in fruit this needs to be done with the greatest possible care, otherwise the operator is certain to lose that which he most desires to retain. Indeed, certain of them are so delicate, when in fruit, that the slightest disturbance will inevitably cause them to break up; and, as a measure of safety, it is often best to mount them without any attempt at washing. This becomes especially important when we consider that it is absolutely impossible to identify numerous species

of fresh-water algæ except when in fruit; and as the fruiting season with many species is very short, extending over only a few days in some cases, we are obliged to accept one horn or the other of the dilemma, — either to run the chances of losing that which makes certain the identification, or else to get into our mounts a little dirt. In many cases we are obliged to accept the dirt with algæ as inevitable, and run no chances.

FRESH-WATER ALGÆ FLUID.

Until a few years ago no satisfactory medium for mounting the fresh-water algæ was known in this country, and even now there seems to be a chance for slight improvement. King's Fresh-Water Algæ Fluid * has, however, the merit of preserving many species almost perfectly, and all species fairly well. Its chief fault is that the endocrome in some of the algæ shrink slightly, and unfortunately such shrinking usually has the effect of obscuring just the

* Prepared by Rev. John D. King.

features one desires most to see. On the other hand, it preserves the chlorophyl perfectly, so that even after the lapse of years the green color remains as distinct as on the day of collection. Mr. King has stated * that desmids mounted four years ago are still as bright as when first mounted. This is an excellent test of the preservative properties of this fluid, as the desmids are, on the whole, the most delicate, so far as the chlorophyl is concerned, of all the fresh-water algæ.

The following is the formula for this fluid: † —

Camphor water . . .	50.00 grammes.
Distilled water . . .	50.00 grammes.
Glacial acetic acid . .	0.50 grammes.
Crystal copper chloride .	0.20 grammes.
Crystal copper nitrate .	0.20 grammes.

This should be filtered after solution.

The following preservative fluid is sim-

* Private communication.

† This is really Petit's formula; but it has acquired the name of King's Fluid in this country, from the fact that it was introduced by Mr. King, and first used by him in his classes.

ilar to Mr. King's, and for many species works equally well: * —

Dissolve fifteen grains of acetate of copper in a mixture of four fluid ounces of camphor water and four fluid ounces of distilled water, add twenty minims of glacial acetic acid and eight fluid ounces of † Price's glycerine, and filter.

This fluid is said to answer well for preserving algæ in tubes, and for mounting.

MOUNTING IN FLUIDS.

The use of a fluid medium, however well it may preserve the distinctive features of the algæ, has the serious disadvantage that the mounts, if not made with the greatest care, are liable to leak, and this means, of course, the loss of the prepara-

* This is Morehouse's formula as given in vol. iv. of *The American Monthly Microscopical Journal.* Mr. Morehouse suggests varying the specific gravity by change of proportion of glycerine; and systematic study in this direction would probably result in the finding of a series of fluids adapted to nearly all the fresh-water algæ. Hantzsch's method, described in Nave's "Handy-Book," is a hint for such a study.

† Bower's glycerine, which is the standard article in this country, will answer equally well. It is more truly neutral than Price's.

tion. Mr. King has experimented with cements to meet this difficulty, and his Lacquer Cell and Finish * is claimed to furnish a tolerably safe remedy. With this cement thin cells are run on slides with a turn-table; and after drying, the mounting may be proceeded with in the usual manner.

Mr. King's directions for mounting in fluids are as follows : † —

1. Allow the cell to harden perfectly. It can be hardened with artificial heat in a few hours.

2. Bring the cell to an even surface with a fine file, or by warming and pressure with a smooth, flat metallic or glass surface.

3. Ring the outer half of the flattened surface with King's White Cement.

4. Lay on the cover and press it firmly to its place, and be sure that it adheres to the cell at every point.

* For sale by the Bausch & Lomb Optical Company. The cements may also be obtained directly from Mr. King.

† These directions are intended by Mr. King to apply particularly to mounting in cells composed of his Lacquer Cell and Finish Cements.

5. To seal the cell, pass it two or three times slowly over the flame of a spirit-lamp to soften it, then apply just pressure enough to the cover to imbed it slightly in the cell. To do this nicely may require a little practice.

6. Finish with the same, or another color, to fancy.

It is a good plan to put a ring of the white cement around the edge of the cover before applying the final coat of lacquer finish; or, if preferred, a good finish can be made with the white cement alone.*

King's Fluid, diluted with one-half water, answers well as a medium for mounting the infusoria.

GLYCERINE JELLY.

Glycerine jelly is also an excellent medium for mounting many species of fresh-water algæ. Indeed, Dr. Cooke † considers it, on the whole, the best. The glycerine jelly has the advantage of making

* For formula for King's cements, see Behrens' "Guide to the Microscope in Botany," [35] p. 235, 236.

† "British Fresh-Water Algæ," by M. C. Cooke.[35]

mounts safe from the danger of leaking, but delicate filaments are badly distorted. It has been used, however, for *Nostoc* and *Ulothrix* with good results.

Mounting with glycerine presents some difficulties of technique; and the following, on glycerine jelly and its use in mounting, is from Mr. King, who is an expert in its use. Mr. King says: * —

"I put up a jelly after Kaiser's formula, † with the improvement of a specially selected gelatine made from the swimming bladder of the sturgeon, that requires less glycerine, and is less objectionable, than any other I have ever used. . . . It is a splendid jelly, and will stand hot weather without melting.

"As to my methods of using, I melt it on the slide, in the quantity needed to nearly fill the cell, placing the object where I want it, and taking off every air-bubble that can be seen with the naked eye; after which it is put by and allowed to harden. I then melt a little of the jelly on the cover glass, breathe hard on

* Private letter. † Behrens', [85] p. 220.

the slide, turn the cover over quickly and put it on the object, being sure that no air-bubbles are caught under the cover. I then put on a delicate clip, pass it over a spirit-lamp till it warms enough to come to its bearings; let it harden; clean off the greatest part of the jelly with a pine stick sharpened, after which the slide is put into a dish of water and washed off clean with a small bristle-point brush. The slide is then carefully wiped dry and finished." *

In using glycerine jelly, the author has found it desirable to have the jelly one or two inches deep, in a five or six inch test-tube. This tube is stopped with a cork, in which is secured a glass rod about one-eighth of an inch in diameter, drawn to a blunt point at the lower end, and of such length as to reach just short of the bottom of the tube. In mounting, the object is first placed in position in the cell, and having warmed the jelly in the test-tube,

* Additional hints on mounting in glycerine jelly may be found in "Practical Microscopy," [89] chapter xiii., "The Preparation and Mounting of Objects," and in Behrens' "Guide to the Microscope in Botany." [85]

over the chimney of the lamp, which furnishes illumination for the microscope, the glass rod with a drop of the melted jelly upon it is brought to the object. Air-bubbles are removed, and the balance of the operation proceeded with substantially as described by Mr. King. This is found less troublesome than the cutting of small pieces of the jelly, as practised by Mr. King, and the difficulty of getting rid of the air-bubbles from the melted jelly is no greater.

KILLING AND FIXING.

* Glycerine and glycerine jelly are also the most useful mediums for mounting the entomostracan crustacea. They work admirably for all the species included in the order Copepoda, but for the Cladocera they shrink the tissue unless it is first submitted to special treatment; namely, the crustacean should be instantaneously killed with some reagent, which, while producing death, leaves the body in all its parts en-

* "Final Report on the Crustacea of Minnesota," by C. L. Herrick.[58b]

tirely unaltered. For this purpose osmic acid has been most used; but this is not entirely successful, due to the fact that it discolors the tissue.

Prof. Herman Fol has discovered that muriate of iron (ferric perchloride) produces not only instantaneous death, but a fixation of all the parts, with very little discoloration or shrinkage.* According to Herrick, the alcoholic solution is diluted to about two per cent, and applied to a small quantity of water, in which the animal is swimming. The water is poured off and the crustacean washed with seventy per cent alcohol, to which a few drops of nitric acid may be added to remove the iron salts.

Osmic acid is highly recommended by Kent † for killing and fixing infusoria. By its use he says they may be preserved as naturally as though living; and the matter of securing permanent mounts of nearly all types of infusoria becomes merely a ques-

* C. L. Herrick (*loc. cit.*).

† "Manual of the Infusoria." [64] "Preservation of the Infusoria," p. 113.

tion of patient manipulation. Coloring reagents may also be used in connection with the osmic acid, so that all the structures, such as Cilia and Flagella, the internal endoplast, and in *Euglena* the colors also are preserved; "the animalcules, excepting for the absence of motion, being scarcely distinguishable from the living organisms."

For killing and fixing hydra, Huxley and Martin * recommend first placing the animal in a small quantity of water, and after the hydra has extended its tentacles, adding boiling water.

The author has used for this purpose the muriate of iron, as recommended by Fol, for the crustacea, and was successful in killing the hydra in an extended condition; but the structure soon broke down, so that, from present information, it appears that the muriate of iron cannot be used where permanent preparations of hydra are desired.

The rotifera may be mounted in glycerine jelly, and for killing and fixing, both

* "Practical Biology," [101] by T. H. Huxley, assisted by H. N. Martin; chapter, "The Fresh-Water Polypes," p. 104.

osmic acid and muriate of iron have been found to work well.

The foregoing includes a few of the elementary facts. Whoever wishes to pursue the subject extensively, may find abundant references to literature in the list following Part II.

PART II. — QUANTITATIVE.

The Microscopical Examination of Potable Water.

LIMITATION OF THE SUBJECT.

In a paper on "Recent Progress in Biological Water Analysis,"[21a] by Prof. Wm. T. Sedgwick, of the Massachusetts Institute of Technology, is found a clear definition of the classes of micro-organisms as occurring in potable waters. The definition there given has been again used by Prof. Sedgwick, in a "Report on the Biological Work of the Lawrence Experiment Station,"[21d] as published in 1890; and it may be taken as representing the latest views, both in this country and abroad, in relation to the classification of this subject.

Tabulated, it assumes the following form : —

MICRO-ORGANISMS.

Plants or animals, either invisible or barely visible to the naked eye.

1. — MICROSCOPICAL ORGANISMS.
   - *a.* Not requiring special cultures.
   - *b.* Easily studied with the microscope.
   - *c.* Microscopic in size, or slightly larger.
   - *d.* Plants or animals.

2. — BACTERIAL ORGANISMS.
   - *a.* Requiring special cultures.
   - *b.* Difficultly studied with the microscope.
   - *c.* Microscopic or submicroscopic in size.
   - *d.* Plants.

The present monograph will deal exclusively with the microscopical organisms, without reference to the bacterial organisms. The latter have been treated so extensively in the last few years as to greatly obscure the former, with the result of entirely neglecting a promising branch of water analysis. At the present time it is proposed to give a brief history of, and describe a new method of quantitatively determining, the microscopical organisms.

The sanitary significance and relative economic importance of the microscopical forms will be treated in another volume.

COMPLETE SANITARY ANALYSIS.

By way of illustrating the importance of a quantitative determination of the microscopical organisms, we will briefly discuss the requirements of a complete study of potable water from the sanitary point of view.

In the first place, it has been proven many times that a single analysis, whether chemical or biological, is entirely without significance in determining the sanitary value of ordinary potable waters. The evidence grows stronger from day to day, that in selecting sources of supply for towns, public institutions, large manufacturing establishments, or any other place where an error in judgment would involve the health of a number of human beings, complete studies from every possible point of view should be made. If the case in hand is important enough to justify the expense (and it always will be in the case

of large town supplies), the examinations should extend over a whole season, and in difficult cases over two or more seasons. This conclusion is the plain teaching of experience, as exhibited in the water supplies of most of the cities of this country.

Assuming that a given source is either from a deep pond or lake, or from a creek or river, or involves the impounding of large bodies of water in storage basins, it is premised that the authorities in charge thereof should be possessed of definite information as to a number of points in relation to what may be termed the natural history of the water in question. In order to determine the said points, four distinct lines of investigation may be carried out, as exhibited in the following: —

1. — A STUDY OF THE ENVIRONMENT.

Including detailed statement of topographical and geological conditions of drainage area, together with observations on extent and character of population and industries of the region as special sources

of pollution, with study of normal samples by (2), (3), and (4).

2. — PHYSICAL PROPERTIES.

This will include a systematic study, with tabulation of results, including a statement of the following: —

a. Depth from which samples are taken.
b. Temperature.
c. Specific Gravity for actual depth and temperature.
d. Color.
e. Turbidity.
f. Sediment.
g. Taste and odor.

3. — CHEMICAL ANALYSIS.

a. Albuminoid Ammonia. { a_1 dissolved. a_2 suspended.
b. Free Ammonia.
c. Nitrites.
d. Nitrates.
e. Chlorine.
f. Hardness.
g. Total Solids.
h. Loss on Ignition.

4. — BIOLOGICAL EXAMINATION.

(4*a*.) PLANTS.

1. Chlorophyceæ.
2. Cyanophyceæ.
3. Diatomaceæ.
4. Fungi, including Bacteria.

(4*b*.) ANIMALS.

1. Crustacea.
2. Vermes (Rotifera etc.).
3. Polyzoa.
4. Infusoria.
5. Rhizopods.
6. Spongidæ.
7. Miscellaneous.

(4*c*.) AMORPHOUS ORGANIC MATTER.

The microscopical examination will include the determination of everything under (4), except the bacteria. It is thus seen to occupy an important place in a scheme for a complete sanitary analysis; and having remarked that a large number of examinations made during the last three years by (1) the biologists of the Massachusetts State Board of Health, (2) the

biologists of the Connecticut State Board of Health, and (3) by the present writer, have put the matter on a fair working basis, we may proceed to describe the present state of the art of making such examinations: —

HISTORICAL.

The first systematic examination of a water supply ever made was by Dr. Hassall of the water supply of London, in 1850.[7] The results were given in an illustrated memoir, and stand unique, as furnishing a beginning for scientific study of this important and interesting subject.

In 1865 L. Radlkofer[19] published an account of an examination of well waters in Munich.

In 1870 Prof. Cohn, of Breslau, published a paper on the Microscopical Analysis of Well Waters, and indicated clearly therein the significance of such studies.[3½] In this paper Prof. Cohn made the following generalizations, which are interesting as showing the advanced views to which he had arrived: —

1. Diatoms and green algæ, *Conferva*, *Protococcus*, *Scenedesmus*, etc., indicate a water to which light has had access, and one poor in organic matter.

2. Certain of the larger infusoria, especially the ciliated forms, *Nassûla*, *Loxodes*, *Urastyla*, etc., feed on these algæ; while upon both the infusoria and the algæ feed —

3. Entomostracans, — *Daphnia*, *Cyclops*, *Cypris*, — worms, such as naids and rotifera and insect larvæ.

Prof. Dr. L. Hirt, also of Breslau, published a paper upon the Principles and Methods of the Microscopical Investigation of Waters, in 1879.[8]

Dr. F. Hulwa published, in 1885,[8a] a paper giving tabulated results obtained by the methods described by Hirt.

The first edition of MacDonald's "Guide to the Microscopical Examination of Drinking Water,"[14] appeared in 1875. The second edition (1883) contains a method of examination which will be referred to hereafter.

In 1884 Dr. H. C. Sorby published a

paper on the Detection of Sewage Contamination by the Use of the Microscope, and on the Purifying Action of Minute Animals and Plants.[23]

In February, 1889, Mr. A. L. Kean [9] published, in *Science*, a method of making the quantitative examination of the microscopical plants and animals in potable water.

In September, 1889, Prof. William T. Sedgwick published, in the paper on Recent Progress in Biological Water Analysis already referred to,[21a] what is known as the sand method of making these examinations.

In September, 1890, the present writer published a paper on the Biological Examination of Potable Water.[20c]

About Jan. 1, 1891, the Special Reports of the Massachusetts State Board of Health appeared.[13c] Part I., Examination of Water Supplies, contains a method of making the quantitative examination as devised by Mr. G. H. Parker, formerly biologist to that Board.[13c] Part II., Purification of Sewage and Water, contains an

account of the various methods, and an abstract of the literature to the date of publication.[13c]

The foregoing are the more important sources of information to be consulted by one desiring to pursue the general subject of the microscopical examination of water historically. The titles of a few other papers and references of less importance may be given.

Mr. C. M. Vorce published papers on Forms observed in Water of Lake Erie,[25] in 1881 and 1882. These papers contain some useful generalizations as to persistency of certain forms in large bodies of water at all seasons of the year.

Mr. Henry Mills published a paper on the Micro-Organisms, in the *Buffalo, N. Y. Water Supply,* in 1882.[15] This paper contains an estimate, based on actual observation, of the amount of microscopic life in the Niagara River.

Dr. C. A. Chamberlain published a paper on Organic Impurities in Drinking Water, in 1883.[3]

Dr. Arthur J. Wolff published a paper

on the Sanitary Examination of Drinking Water, in 1886.[28]

The present writer published a paper on the Micro-Organisms in Hemlock Water, in 1888.[20a]

In 1889 Tiemann and Gärtner's Chemical, Microscopical, and Bacteriological Investigation of Waters[24] appeared. In it may be found an abstract of the useful foreign literature of the subject, and many hints of value in interpreting results.

THE METHOD OF DR. HASSALL.[7]

Dr. Hassall, although the first to apply the microscrope to the determination of the organic constituents of water, has not in any of his papers made it clear just how he obtained his results; though we may infer from what he has said, that his process was, essentially, to examine a drop of the sediment contained in the bottom of a test-tube, in which a sample of the water had been allowed to stand long enough for thorough sedimentation to occur.

Radlkofer[19] leaves us entirely in the dark as to the methods used in his examination of the well-waters of Munich; but we may infer that his method, like that of Cohn in the examination of the waters of Breslau, consisted in a direct microscopical study of either a few drops of water or of the sediment.

Hirt, in his paper, recommends the following course of procedure:[8] —

1. The direct observation of fresh samples of the water, a drop at a time; as many as twenty or thirty drops being successively scrutinized.

2. An examination of the sediment obtained after standing at least two days.

3. A study of the surface pellicle, should any form after the sample has stood for a few days.*

In this way Hirt finds it necessary to make as many as thirty to forty examina-

* This third course of procedure was used previous to the introduction of the modern methods of bacteriology, and was, until about 1881, the approved method of studying the bacteria in their relation to potable waters.

tions before completing the study of any given sample.

THE METHOD OF DR. MACDONALD.[14]

For this method of examination tall, cylindrical glass vessels with small rounded bottoms are recommended. In the absence of the special vessels a litre or half-litre measuring-glass may be used. For the examination of a sample, one of the tall glass vessels is first filled with water, after which a circular disk of glass, resting on a horizontal loop at the end of a long aluminium wire is lowered to the bottom. The tall glass is covered and set aside for twenty-four or forty-eight hours, as the case may be. After standing the specified time the water is siphoned off with tubing, leaving only a thin stratum over the disk, which is then raised and laid upon blotting-paper to remove surplus moisture. The disk is then covered with a large cover glass, and transferred at once to the stage of the microscope for direct examination.

Dr. MacDonald further says, an ordinary watch-glass may in some cases be substi-

tuted for the disk, with advantage, as being less likely to permit the loss of sediment by overflow. Another plan suggested is to siphon off the water until only enough remains to just permit the sediment to be shaken up with it, and turned into a conical-shaped glass, from which, after standing for a short time, portions may be taken for examination. By proceeding as outlined in the foregoing, a judgment could be formed as to the amount and kind of the organic impurity present. Independent, however of the impossibility of establishing numerical relations, the practical difficulties of examinations on either the plain glass disk or in the curving watch-glass are considerable. The chief sources of possible error in final judgment may be enumerated as follows: —

1. During the time of standing for purpose of sedimentation, a diminution of the number and kind of species originally present may be expected to take place, by reason of one form eating another up.

2. Impossibility of knowing that at the

end of the time allowed for sedimentation, all the forms originally present in the sample have fallen to the bottom, and are present in the sediment.

3. In using the plain glass disk, the liability of losing some of the sediment when placing the cover-glass.

4. In using the watch-glass, difficulty of fully examining the sediment, on account of the curved bottom.

5. In taking drops of sediment with a pipette for examination on a slide; uncertainty as to whether samples containing all the forms present in the sediment are obtained.

Dr. MacDonald's work must, nevertheless, be considered of the greatest possible value. His general definition of technique is good, especially the directions in reference to collection of samples, and methods of insuring microscopical cleanliness at every stage of the operation. His book should be in the hands of every person using the microscopical method.

THE METHOD OF DR. H. C. SORBY.[23]

In 1884 Dr. H. C. Sorby, of England, made a study of different samples of river and sea water, with the object of ascertaining the number per gallon of the various small animals large enough to be held back by a sieve with meshes about one two-hundredths of an inch in diameter. Amounts of water varying from ten gallons downwards, according to the number of forms present, were passed through such a sieve. The retained organisms were subsequently washed off into a dish, and an enumeration made, — just how, Dr. Sorby does not state. The forms which he was studying were comparatively large (*Cyclops* and other Entomostracan Crustacea); and an enumeration could be easily made, either in a watch-glass with a low-power objective of considerable penetrating power, or in a large flat cell of special construction, by the use of an ordinary hand magnifier. Dr. Sorby gives numbers per gallon of the various forms found in different waters; and we may conclude from what he has said.

that so far as the larger microscopical forms are concerned, he considered that a fairly accurate method of enumeration had been used. He also enumerated the infusoria, and states, that at low water in the Medway, above Chatham, in the first half of June, the average number per gallon has been about 7,000, but sometimes as many as 16,000. The average size of the infusoria so enumerated, he states at one one-thousandth of an inch. Clearly a much smaller mesh than was used for the entomostraca, and special methods of gathering would be required; but just how all this was accomplished Dr. Sorby has failed to tell us. The paper is, however, suggestive as to possibilities of future results, and may be profitably consulted by the student of the advanced methods.

METHODS USED IN THE UNITED STATES PRIOR TO 1889.

Of the several papers which have appeared in this country previous to 1889, it may be said, in a general way, that the authors of all have apparently gone no

further towards a quantitative determination of the microscopical organisms than to examine a sediment usually obtained by allowing the water to flow through a strong cotton cloth. When public water supplies were the subject of examination, a bag of such material was attached to an ordinary laboratory, or domestic cock, and the water simply allowed to run, until, as a matter of judgment, enough had been filtered through the cloth to insure a prolific sediment. The flow being stopped, the bag was removed, and, after the water still contained in it had drained through, the bag was turned wrong side out, and the organisms removed by dabbling and lightly washing in a small amount of water from the supply under examination, contained in a clean shallow dish. After so removing the organisms from the cloth, the water containing them was turned into a conical-shaped glass, from which, after subsidence of the organisms to the bottom, samples were taken by the use of a pipette for examination in the usual manner on a slide. In this way the results, as detailed

by Mr. Vorce,[25] Mr. Mills,[15] and by the members of the Microscopical Section of the Rochester Academy of Science, as detailed in the paper published by the present writer [20a] in 1888, were obtained.

THE METHOD OF MR. G. H. PARKER.[13c]

In 1888 Mr. Parker, as Biologist to the Massachusetts Board, worked out and applied a method for examination somewhat different from any of the preceding. A small piece of cloth is taken, and firmly tied to the stem of a common glass funnel, in such manner that the sample of water when poured into the funnel would filter through only that portion of the cloth directly in front of the end. All being arranged, two hundred cubic centimetres of the water to be examined are poured into the funnel. At the end of the filtration, the organisms contained in the water are found collected on the cloth on a small area of the size of the bore of the stem of the funnel. The cloth is now removed, and inverted over a glass tube of somewhat greater area than the circular spot

of arrested organisms. The tube is so held that the end with the inverted cloth is just above the middle of an ordinary glass slide; and a sharp blast of air through the tube dislodges the sediment with a small amount of water upon the slide, where, after covering with a cover glass, it may be examined under the microscope. Mr. Parker states, that this method gives good results, so far as determining the kind of organisms is concerned; but, from a quantitative standpoint, it yields only rough approximations.

The principal sources of inexactness are: —

1. A few of the smaller organisms pass through the cloth.

2. Impossibility of removing all the organisms from the cloth to the slide.

3. Difficulty of distributing the sediment on the slide equally enough to permit accurate estimates of the number of the organisms.

The method of Mr. Parker has been known as the "cloth method," and is so referred to in the Massachusetts reports.[13c]

THE METHOD OF MR. A. L. KEAN.[9]

In this method a known quantity of water (Mr. Kean says 100 cubic centimetres is a convenient unit) is put into a funnel, in the tube of which is half an inch in depth of coarse sand (24 to 30 grains to the inch). The sand is held in place by a plug of wire gauze in the foot of the funnel, which, while allowing the water to pass, still holds the sand back. After all the water has passed through, the plug is removed, and one cubic centimetre of distilled or freshly filtered water thrown into the stem of the funnel, by means of a pipette. This washes the sand and contained organisms down into a watch-glass, placed to receive it. The grains of sand sink to the bottom, leaving the organisms mostly suspended in the water. Stirring produces more even distribution, and liberates any caught between the grains of sand. A cell of one cubic millimetre volume is provided, and to it a portion of the water from above the sand in the watch-glass, sufficient to just fill it, is transferred

for examination. The organisms contained in the cell are then counted, and from the result the total number in the original sample computed.

This method was described by Mr. Kean in his original communication [9] in considerable detail; and to him, therefore, pertains the credit of having first published a quantitative method of determining the microscopical organisms in water. Credit for first using such a method seems to pertain to Dr. H. C. Sorby, though with what measure cannot be determined in the absence of the detail.

The chief defect of Mr. Kean's method is in the smallness of the amount actually examined, a difficulty which limits the accuracy of the method in four ways: —

1. The amount of one cubic millimetre is so small that important forms are easily overlooked.

2. The cubic millimetre is only the one one-thousandth part of the cubic centimetre, and the necessity of multiplying by 1,000 leads, with an error of one in the count to an error of 1,000 in the result, as

applying to the one cubic centimetre in the watch-glass. With an original quantity of 100 cubic centimetres, this again leads to an error of 10, per cubic centimetre, in the final result.

3. If only one of a given form appears in the count, it must be interpreted as indicating 1,000 in the one cubic centimetre in the watch-glass, whereas it may possibly have been the only one there.

4. The impossibility of getting in the reductions a number between 0 and 10 per cubic centimetre. To get one per cubic centimetre would require the filtering of 1,000 cubic centimetres (one litre) in every case.

THE SAND METHOD OF PROFESSOR SEDGWICK.[21a, 13c]

The various practical objections to the method of Mr. Kean having been developed by comparison and experiment, Prof. Sedgwick, in the spring of 1889, worked out what is known as the sand method. In this, the controlling idea was to provide a remedy for the excessively

high results attained by the method of Mr. Kean. The filtration is made as in Mr. Kean's method, 100 cubic centimetres being likewise the quantity of water operated upon. A cell 50 millimetres in length, 20 millimetres in width, and about 2.5 millimetres in depth is provided. This cell is formed by cementing a brass border upon an ordinary three by one glass slide. Before setting this brass border, the upper surface of the slide is ruled into 1,000 squares, each one millimetre in area. The filtration being completed, the sand, together with the organisms, is washed by a small stream from a wash-bottle containing distilled water, into the cell just described, where they are evenly distributed over the bottom by the use of a needle or fine wire. We now have the organisms from 100 cubic centimetres of water evenly distributed in a cell of 1,000 square millimetres area; and theoretically it would be possible, by using a low-power objective, and taking in order each square millimetre of the area, to actually count all the organisms in the cell; that is to

say, to count all the organisms in the one hundred cubic centimetres of water operated upon. In practice, however, the counting of 1,000 squares would consume so much time as to make the labor of the counts a serious burden. Moreover, it was found by experience that a count of twenty representative squares gave a fairly accurate average of the whole; and twenty squares was accordingly adapted as the unit count. In this way 50 is obtained as a factor of multiplication, instead of 1,000, as in the method of Mr. Kean. By counting a larger number of squares a still smaller factor results; thus fifty squares counted gives a factor of multiplication of only 20.

For further details of this interesting and valuable advance in methods of making the microscopical examination of water, the reader is referred to either Prof. Sedgwick's original paper,[21a] or to his further discussion of it in the Massachusetts Board's Special Report.[13c]

THE PERFECTED SEDGWICK–RAFTER METHOD.

In June, 1889, the present writer organized, at the instance of Desmond FitzGerald, C. E., Resident Engineer of the Western Division of the Boston Water Works, a series of investigations of the Boston Water Supply. Considerable trouble has occurred at various times in this water supply, by reason of excessive growths, at irregular periods, of microscopical organisms; and the Boston Water Board deemed it desirable to study the matter broadly enough to presumably make some definite addition to existing knowledge of the laws governing such growths. A small laboratory was erected, the necessary appliances secured, and arrangements made for a systematic study extending, possibly, over a number of years. In July, Prof. Sedgwick kindly demonstrated his sand method to Mr. FitzGerald and the author in his laboratory, and finished one of his counting plates. The author's previous studies had made him familiar with the method

of Dr. MacDonald and the few American workers, and he was able to appreciate the advanced ground reached by Prof. Sedgwick. His sand method, while far in advance of that of any other worker, seemed, however, somewhat unsatisfactory in this, that the sand and organisms are both allowed to pass into the cell together; and inasmuch as the finest grains of sand are much larger than many of the organisms, it follows that the enumeration, however carefully made, is only an approximation to the number actually present, and usually falls short of the number present.

In the method as now used by the author, the sand is supported upon a plug of wire cloth, placed at the lower end of the funnel stem. After placing the plug, the sand is run into the funnel, lightly pressed to place with a glass rod, and from 20 to 40 cubic centimetres of freshly filtered water allowed to run through, in order to insure thorough settling of the sand before actually beginning the filtration. The amount of water to be filtered is gauged by the number of

organisms which it contains, as ascertained by preliminary inspection. Generally,

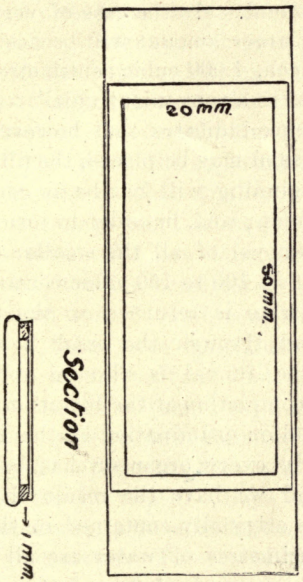

FIG. 1.—PLAN AND SECTION OF CELL WITH COVER-GLASS.

however, as large a quantity should be used as can be conveniently filtered without clogging the sand so much as to render the completion of the process too pro-

longed; and for ordinary samples 500 cubic centimetres has been fixed upon as the proper amount. In the case of very pure waters a larger amount will be desirable; and, for such, 1,000 cubic centimetres may be adopted as a convenient unit.

Experience indicates that however carefully the sand may be placed, the filtration at the beginning will not be as complete as further on; and, in order to insure the certain removal of all the smaller organisms, the first 100 to 150 cubic centimetres of the filtrate is returned to the funnel and passed through the sand a second time. The funnel is allowed to stand until the completion of the filtration, when it is found on examination of the filtrate that nearly every organism has been removed, and we have the result that the organisms originally contained in the 500 cubic centimetres of water are all in the sand at lower end of funnel stem. The plug of wire cloth is now removed, and the sand and contained organisms washed with 5 cubic centimetres of freshly filtered water, run from a 5 cubic centimetre pipette,

into a 5 or 6 inch test-tube. The test-tube is slightly shaken, in order to wash all the organisms clear from the sand. The sand, by reason of greater specific gravity, sinks quickly to the bottom, leaving the organisms distributed through the water. At the instant of the completion of the settling of the sand the supernatant water is turned into another smaller test-tube, leaving the clean sand at the bottom of the first tube. We now have the organisms from 500 cubic centimetres of water concentrated into 5 cubic centimetres in the second tube, from which, after slight stirring, to insure uniform distribution, 1 cubic centimetre is taken with a 1 cubic centimetre pipette, and transferred to a cell 50 by 20 millimetre area, and exactly 1 millimetre in depth. Such a cell, of course, contains 1,000 cubic millimetres, or 1 cubic centimetre. The top of the metal cell is ground perfectly smooth, and with a little practice one can float a thick coverglass to place without losing a drop.

The next step is the enumeration. This is accomplished by transferring the cell to

the stage of a microscope, the eye-piece of which is fitted with a micrometer, so ruled as to cover, with a given objective and fixed tube length, a square millimetre on the stage. The microscope itself is fitted with a mechanical stage with millimetre movement in both directions; and for this purpose certain simple additions have been made to the new mechanical stage of the Bausch & Lomb Optical Company, by means of which the desired result is obtained at slight expense. The count is made by beginning at one corner of the cell and going systematically over the area, in accordance with such a formula as will insure the count of squares selected from every part of the slide The number of squares actually counted, will depend upon the degree of accuracy which it is desired to attain. It is obviously impossible to count the 1,000 squares composing the entire area of the slide; and the practical question arises as to just what multiple of 1,000 shall be used to secure a correct average. This can only be determined by trial and comparison upon a number of sam-

ples. In any case, not less than 20 squares should be counted, and if time will possibly permit, the preference should be in favor of always counting at least 50.

In order to illustrate the matter, a table has been prepared, which represents the area of the cell divided into 1,000 squares. Brief inspection of this table will show the difficulty of obtaining true averages when only 20 squares are counted, and exhibits the value of counting the larger number, in order to obtain true averages.

The precise millimetre movement of the mechanical stage is considered a matter of considerable importance, and, indeed, insisted upon as an integral part of the method. Without it the tendency will be to sometimes select squares for counting which are contiguous; while at other times one will pass over squares containing few or no organisms in a search for more prolific ones, making, in either case, an error in the final result. By use of the mechanical stage, with a definite formula for passing over the slide, personal errors of this

sort are eliminated, leaving only those which are due to irregularity of distribution of the organisms in the water; and by always stirring thoroughly, before taking the portion for examination, with 1 cubic centimetre pipette this error may also be reduced to a small degree, provided as many as 50 squares are counted as the basis of the final average. Additional uniformity of distribution of organisms in the cell, may also be obtained by stirring gently in the cell itself with the pointed end of the pipette, before floating the cover-glass to place; but the precaution should always be taken in these stirring operations to proceed gently, in order to guard against breaking up unnecessarily the particles of amorphous organic matter, which are nearly always present in any sample of water in which algous growths and decay are taking place.

The definite estimation of the amorphous organic matter is a thing of some difficulty; and in the author's use of this method he has formed a sort of mental standard as to the unit of area covered by

one mass of the amorphous matter. Mr. Geo. C. Whipple, who assisted in the work for the Boston Water Works, has suggested that this unit be made definite for all persons, by taking it a fixed number of square microns; and for this purpose 20

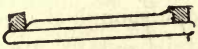

FIG. 2. — SECTION OF OPEN CELL, SHOWING CURVE OF SURFACE OF FLUID DUE TO CAPILLARY ATTRACTION AT SIDES.

seems to be the desirable unit. By careful comparison with a stage micrometer for a few times this unit can be firmly fixed in mind, and an estimate of the amount of amorphous matter made with considerable precision.

The advantage of a cell of such depth as to just contain the quantity taken for examination is illustrated by Fig. 2, which represents the *open* cell, and shows the meniscus form taken by the liquid, by reason of capillary attraction at the sides. This curvature is so considerable as to render a count in the squares near the

edges of the cell impracticable, for optical reasons, which every user of the microscope will readily understand. With the covered cell, on the contrary, the count may be made up to the sides as easily and with as much certainty as in the middle.

The placing of the cover-glass is easily accomplished, although the careful observance of certain details are essential to uniform success. Thus, the cover-glass should be perfectly clean, and just before placing should be moistened. The operation of putting it to place consists in laying one end, held in a horizontal position, in contact with the ground upper surface of the metallic portion of the cell, and, while keeping it in close contact at all points, gradually sliding it forward until the whole cell is covered.

In this connection it may be noted that cleanliness is quite essential in all these operations; and the hints given by Dr. MacDonald in his Water Analysis fully cover the case.

In the original cell, as designed by Prof. Sedgwick, the division into squares,

for the purpose of obtaining the relation of organisms to area, was arrived at, as already stated, by ruling square millimetres upon the upper surface of the glass slide on which the cell is based. This, however, gives a unit square only for the bottom of the cell; and for all organisms at the top of the liquid no unit of area is obtained; inasmuch as the considerable change of focus required in order to see them at all, renders it impossible to distinguish the ruled lines and such floating objects at the same time. With the eye-piece micrometer, however, this difficulty is removed, and the unit square is clearly in the field of vision without reference to the plane in the cell upon which the objective is focused.

The working objective for these counts may be either a two-thirds or one-half inch; and for identification of minute unknown forms, a one-fourth or one-fifth water immersion capable of working through a thick cover-glass, and cell one millimetre in depth, would be useful. I have, however, no experience with a high-

power objective of this character, and can only cite the opinion of the Rochester opticians that such an objective of satisfactory correction and definition can be made.

In this connection it may be mentioned that Mr. E. Gundlach has made, at the author's request, a dry fourth which possesses the necessary working distance, and is, therefore, easily used to determine objects at the very bottom of the cell, even when a thick cover-glass is in place. Such an objective is, however, doing its work through three mediums, all varying in refractive index; namely, air, glass, and water, and, as may be easily predicated, the trial objective is not entirely a success. The angle is, of course, very narrow, though this defect is inseparable from long working distance. The greatest difficulty is the imperfection in the correction of the chromatic aberrations. It is improvement in this direction which is chiefly expected to result from the use of a water immersion. The dry objective is, however, of considerable use in assisting in de-

termining very minute objects which present only a simple structure; it fails utterly when those requiring any resolving power are encountered.

The following table shows the comparative value of the open cell with mixed sand and organisms, and the covered cell with sand and organisms separated. The results are in number of organisms per cubic centimetre, and represent only the plant forms present in the given samples.

NAME OF ORGANISMS.	(1)		(2)	
	Open cell with sand.	Closed cell without sand.	Open cell with sand.	Closed cell without sand.
Asterionella .	14	30	7	23
Tabellaria . .	11	21	4	15
Cyclotella . .	1	1	0	2
Anabaena . .	2	16	7	13
Clathrocystis .	4	6	1	8
Cœlosphaerium	5	12	5	3
Nostoc . . .	0	2	1	1
Melosira . . .	2	20	1	1
Totals . . .	39	108	26	66

A number of similar comparative counts have been made, with the result of uni-

formly getting a larger number of organisms by the Sedgwick-Rafter method.

In the recently published Twenty-Second Annual Report of the Massachusetts Board, it is stated as the result of the various comparisons made by the biologists of that Board, that the improvement in processes has resulted not only in an increase in the total number of organisms found in a given water, but also in the number of genera. Thus, in a general way, the number of organisms observed by Prof. Sedgwick's original sand method is several times that observed by the cloth method of Mr. Parker; likewise the number observed by the Sedgwick-Rafter method is probably from two to four times the number observed by the sand method.

The foregoing indicates rather briefly the several steps taken by different workers before we could be said to have a practicable method of making the microscopical examination and enumeration of the living organisms in potable water, which fairly met all the conditions.

The author hopes that some worker of

the immediate future will be able to still further advance the method along the road to ultimate perfection.

VALUE OF METHOD.

The value of a method of this character will be readily recognized by all who understand the limitations of chemical analysis, as applied to the decision of questions relating to the sanitary value of potable water. The most useful of the various chemical methods recognizes in effect only two classes of organic impurity; namely, free and albuminoid ammonia; and groups every organic substance occurring in water as one or the other of these. This has resulted in the condemning of the waters of mountain streams by chemists, who ventured positive opinions as to sanitary value on the evidence of chemical analysis alone. The use of the biological method, by exhibiting clearly the character of the organic contamination, will, therefore, lead to a more accurate knowledge of potable waters than can be gained by chemical analysis.

Moreover, as we gain more knowledge of

the real sanitary significance of the various forms of plant and animal life, the daily or weekly fluctuations in quality of a public water supply can be quickly obtained by the use of this method of biological analysis; and it is probable that, in future, public water supplies will be regularly subject to such examinations.

RESULTS.

The following table shows a number of counts of samples from different localities, and illustrates the variations in number

No of Sample.	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Sponge Spicules	1	1	0	0	0	1	1	0	3	0
Rhizopods . .	0	2	0	0	0	1	0	1	1	0
Infusoria . .	5	2	6	80	21	50	16	0	10	11
Rotifera . . .	1	1	0	0	3	6	1	0	2	3
Crustacea . .	0	0	0	0	0	1	0	0	0	0
Total Animals .	7	6	6	80	24	59	18	1	16	14
Desmidieæ . .	0	1	1	0	3	4	5	0	1	1
Diatomaceæ .	50	6	12	2	19	45	50	8	17	35
Zoospores . .	130	51	73	280	244	88	2400	26	132	90
Chlorophyceæ	2	5	4	1	55	13	1	0	1	5
Cyanophyceæ .	15	38	70	4	157	110	0	0	0	10
Fungi . . .	3	1	0	0	0	0	0	0	1	2
Total Plants .	200	102	160	287	478	260	2456	34	152	143
Amorphous Matter . . .	80	75	140	180	238	230	45	165	170	240

and kind of organisms found in various waters. In this table the results are grouped in classes to save space, and are the number of organisms per cubic centimetre, as before.

In these samples (8) is from a spring, and represents very pure water. All of the samples except (5), (6), and (7) are from water supplies, and represent water of medium quality. The large amount of Cyanophyceæ in (5) and (6) might, of itself, in the present state of our knowledge, lead to the rejection of those two waters as unfit for domestic purposes, especially if continuous observation, extending over two or more seasons, showed that such extensive growths occurred frequently. In all such cases, however, a study of the environment would be desirable before making a final decision, and it is not intended to say positively that a given sample can be definitely rejected on the evidence of the microscopical examination alone. The statement may, however, be made that the microscopical examination will, by itself, quite as frequently furnish

definite evidence relative to the suitableness or unsuitableness of a given sample as can be obtained from a chemical examination alone; while the microscopical examination in conjunction with a study of the environment may easily furnish decisive evidence for or against. This latter statement may be applied with equal force to chemical examinations in conjunction with studies of the environment; and the conclusion is therefore reached, that in rational water analysis, the microscopical examination stands on a par with the chemical. This interesting and highly important conclusion has been fully recognized, as already stated, by the chemists and biologists of the Massachusetts Board, and we accordingly find the two side by side in their recent reports. The following tabulations illustrate a typical series of such compound analyses of water from Reservoir No. 4 of the Boston Sudbury River Supply, extracted from the Twenty-Second Annual Report of that Board. Moreover, this particular series is of special interest by reason of giving the results continuously, by

months, for a year and a half; and the movements of the plant and animal life in conjunction with the variation in the ammonias, as determined chemically, is clearly shown, not only in a plane one foot below the surface, but at mid-depth and near the bottom.

In order to illustrate the question under discussion fully, the series also includes the examination of water from Cold Spring Brook, the influent stream to Reservoir No. 4.

SUDBURY RIVER SUPPLY. — *Chemical Examination of Water from Cold Spring Brook, at Head of Reservoir No. 4, in Ashland.*

[Parts per 100,000.]

Number.	Date of Collection.	Date of Examination.	Appearance. Turbidity.	Appearance. Sediment.	Appearance. Color.	Residue on Evaporation. Total.	Residue on Evaporation. Loss on Ignition.	Ammonia. Free.	Ammonia. Albuminoid. Total.	Ammonia. Albuminoid. Dissolved.	Ammonia. Albuminoid. Suspended.	Chlorine.	Nitrogen as Nitrates.	Nitrogen as Nitrites.	Hardness.
	1889.														
4772	June 4	June 5	Slight.	Slight.	2.70	5.50	3.00	.0038	.0454	.0410	.0044	.19	.0040	.0001	1.6
4887	July 1	July 2	V. sl't.	V. sl't.	1.30	–	–	.0050	.0336	.0322	.0014	.27	.0040	.0002	–
5012	Aug. 5	Aug. 6	V. sl't.	V. sl't.	3.50	–	–	.0024	.0714	.0642	.0072	.22	.0020	.0000	–
5131	Sept. 3	Sept. 4	None.	V. sl't.	2.30	–	–	.0020	.0368	.0364	.0004	.35	.0050	.0001	–
5209	Oct. 2	Oct. 3	V. sl't.	V. sl't.	2.50	–	–	.0016	.0380	.0354	.0026	.34	.0020	.0001	–
5292	Nov. 4	Nov. 5	V. sl't.	V. s'lt.	1.90	–	–	.0024	.0344	.0336	.0008	.32	.0050	.0000	–
5393	Dec. 2	Dec. 3	V. sl't.	V. sl't.	1.50	–	–	.0004	.0272	.0264	.0008	.26	.0170	.0000	–

	1890.														
5493	Jan.	2	Jan.	3	None.	V. sl't.	0.90	–	–	.0016	.0192	.0178	.0014	.27	.0220	.0000	–
5575	Feb.	3	Feb.	4	V. sl't.	Slight.	0.75	–	–	.0000	.0168	.0132	.0036	–	.0090	.0001	–
5727	Mar.	3	Mar.	4	V. sl't.	Cons.	0.75	–	–	.0000	.0200	.0180	.0020	.24	.0150	.0002	–
5827	Apr.	1	Apr.	2	V. sl't.	V. sl't.	0.70	–	–	.0006	.0184	.0156	.0028	.22	.0070	.0000	–
5924	May	1	May	2	Slight.	Slight.	1.20	–	–	.0024	.0272	.0244	.0028	.23	.0090	.0000	–
6018	June	2	June	3	V. sl't.	Slight.	1.80	5.20	2.55	.0012	.0322	.0190	.0032	.20	.0100	.0000	–
6147	July	1	July	2	Slight.	Slight.	0.90	4.20	–	.0030	.0318	.0310	.0008	.21	.0030	.0001	1.4
6341	Aug.	4	Aug.	5	V. sl't.	Slight.	0.30	5.10	3.00	.0024	.0272	.0222	.0050	.22	.0070	.0002	1.1
6453	Sept.	2	Sept.	3	Slight.	Slight.	0.35	3.95	1.25	.0002	.0234	.0178	.0056	.24	.0050	.0001	1.7
6549	Oct.	1	Oct.	2	Slight.	Slight.	0.50	3.80	1.45	.0000	.0200	.0146	.0054	.25	.0050	.0001	1.4
6672	Nov.	4	Nov.	5	None.	V. sl't.	1.60	4.80	2.05	.0004	.0294	.0266	.0028	.30	.0060	.0001	1.7
6771	Dec.	1	Dec.	2	V. sl't.	V. sl't.	1.20	4.05	1.75	.0010	.0264	.0214	.0050	.29	.0100	.0001	1.7
Av.	.....		.....		.....	.....	1.04	4.48	2.01	.0016	.0305	.0274	.0031	.26	.0077	.0001	1.5

Odor, distinctly vegetable, sometimes faintly vegetable, occasionally none.— The samples were collected from Cold Spring Brook, at head of Reservoir No. 4, at a depth of one foot beneath the surface.

Microscopical Examination. (*Water from Cold Spring Brook.*)

[Number of organisms per cubic centimeter.]

	1889.							1890.		
	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Day of Examination	–	2	6	6	3	–	3	4	4	4
Number of sample	4772	4887	5012	5131	5209	5292	5393	5493	5575	5727
PLANTS.										
Diatomaceæ	–	**3**	**0**	**pr.**	**4**	–	**8**	**3**	**3**	**5**
Ceratoneis	–	0	0	0	0	–	0	0	1	2
Melosira	–	0	0	0	0	–	8	0	0	0
Navicula	–	0	0	pr.	0	–	0	pr.	0	pr.
Nitzchia	–	0	0	0	0	–	0	0	0	0
Synedra	–	2	0	0	4	–	pr.	3	1	3
Tabellaria	–	1	0	0	0	–	0	0	1	0
Algæ (several genera)	–	**0**	**0**	**pr.**	**2**	–	**0**	**1**	**pr.**	**1**
Fungi. Crenothrix	–	**0**	**20**	**11**	**7**	–	**0**	**0**	**2**	**0**
ANIMALS.										
Rhizopoda. Actinophrys	–	**0**	**0**	**0**	**0**	–	**0**	**0**	**0**	**0**
Infusoria	–	**0**	**0**	**0**	**0**	–	**0**	**0**	**0**	**0**
Peridinium	–	0	0	0	0	–	0	0	0	0
Trachelomonas	–	0	0	0	0	–	0	0	0	0
TOTAL ORGANISMS		3	20	11	13	–	8	4	5	6

	1890.								
	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
Day of examination	2	2	4	2	5	3	2	5	3
Number of sample	5827	5924	6018	6147	6341	6453	6549	6672	6771
PLANTS.									
Diatomaceæ	**8**	**31**	**41**	**7**	**16**	**6**	**3**	**7**	**76**
Ceratoneis	0	5	6	1	1	0	0	0	0
Melosira	0	0	0	0	0	4	0	0	17
Navicula	pr.	0	1	1	1	2	1	pr.	4
Nitzchia	0	0	2	0	0	0	0	3	30
Synedra	6	25	23	4	11	0	1	3	25
Tabellaria	2	1	9	1	3	0	1	1	pr.
Algæ (several genera)	**1**	**1**	**2**	**6**	**2**	**9**	**3**	**pr.**	**0**
Fungi. Crenothrix	**pr.**	**2**	**33**	**47**	**113**	**0**	**0**	**0**	**0**
ANIMALS.									
Rhizopoda. Actinophrys	**0**	**0**	**0**	**0**	**0**	**28**	**0**	**0**	**pr.**
Infusoria	**1**	**0**	**pr.**	**pr.**	**1**	**4**	**0**	**0**	**0**
Peridinium	1	0	pr.	pr.	1	0	0	0	0
Trachelomonas	0	0	0	0	0	4	0	0	0
TOTAL ORGANISMS	10	34	76	60	132	47	6	7	76

SUDBURY RIVER SUPPLY. — *Chemical Examination of Water from Reservoir No. 4, in Ashland, collected one foot beneath the surface.*

[Parts per 100,000.]

Number.	Date of Collection.	Date of Examination.	Appearance. Turbidity.	Appearance. Sediment.	Appearance. Color.	Residue on Evaporation. Total.	Residue on Evaporation. Loss on Ignition.	Ammonia. Free.	Ammonia. Albuminoid. Total.	Ammonia. Albuminoid. Dissolved.	Ammonia. Albuminoid. Suspended.	Chlorine.	Nitrogen as Nitrates.	Nitrogen as Nitrites.	Hardness.
	1889.														
4775	June 4	June 5	Dist't.	V. sl't.	0.80	–	–	.0014	.0254	.0218	.0036	.18	.0020	.0002	–
4888	July 1	July 2	Slight.	V. sl't.	0.80	–	–	.0006	.0298	.0226	.0072	–	.0050	.0002	–
5013	Aug. 5	Aug. 6	Slight.	V. sl't.	0.60	–	–	.0022	.0234	.0202	.0032	–	.0040	.0002	–
5132	Sept. 3	Sept. 4	V. sl't.	V. sl't.	0.80	–	–	.0018	.0274	.0256	.0018	–	.0030	.0000	–
5210	Oct. 2	Oct. 3	Slight.	V. sl't.	1.20	–	–	.0026	.0248	.0230	.0018	–	.0040	.0001	–
5293	Nov. 4	Nov. 5	Slight.	Slight.	1.00	–	–	.0042	.0254	.0236	.0018	–	.0050	.0001	–
5394	Dec. 2	Dec. 3	V. sl't.	V. sl't.	1.30	–	–	.0024	.0270	.0244	.0026	–	.0140	.0002	–

1890.

5494	Jan. 2	Jan. 3	V. sl't.	V. sl't.	0.80	–	–	.0022	.0228	.0226	.0002	–	.0180	.0002	–
5576	Feb. 3	Feb. 4	V. sl't.	V. sl't.	0.75	–	–	.0002	.0196	.0186	.0010	–	.0070	.0001	–
5728	Mar. 3	Mar. 4	V. sl't.	Slight.	0.75	–	–	.0008	.0222	.0186	.0036	.25	.0180	.0001	–
5828	Apr. 1	Apr. 2	Slight.	V. sl't.	0.70	–	–	.0000	.0214	.0182	.0032	.21	.0090	.0000	–
5925	May 1	May 2	Slight.	Slight.	0.70	–	–	.0024	.0202	.0182	.0020	.24	.0090	.0001	–
6019	June 2	June 3	V. sl't.	V. sl't.	0.50	3.50	1.15	.0002	.0186	.0176	.0010	.24	.0050	.0000	–
6148	July 1	July 2	Slight.	Slight.	0.50	3.40	–	.0008	.0248	.0200	.0048	.21	.0020	.0001	–
6342	Aug. 4	Aug. 5	Slight.	Slight.	0.35	3.40	1.10	.0000	.0278	.0194	.0084	.22	.0120	.0002	1.4
6454	Sept. 2	Sept. 3	Slight.	Slight.	0.35	3.35	1.55	.0000	.0206	.0180	.0026	.24	.0100	.0001	1.9
6550	Oct. 1	Oct. 2	Slight.	Slight.	0.40	3.55	1.50	.0000	.0204	.0150	.0054	.24	.0080	.0001	1.7
6673	Nov. 4	Nov. 5	V. sl't.	Slight.	0.85	4.00	1.45	.0012	.0256	.0230	.0026	.27	.0070	.0002	1.7
6772	Dec. 1	Dec. 2	V. sl't.	Slight.	0.70	4.20	1.65	.0012	.0222	.0198	.0024	.26	.0100	.0001	1.7
Av.	.....	.....	.....	.....	0.73	3.67	1.40	.0013	.0237	.0205	.0031	.23	.0080	.0001	1.7

Odor, generally very faintly vegetable or none; seldom disagreeable. The samples were collected from the reservoir, near the gate-house, at a depth of one foot beneath the surface.

Microscopical Examination. (*One foot beneath surface, Reservoir No.* 4.)

[Number of organisms per cubic centimeter.]

	1889.							1890.		
	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Day of examination	–	2	6	6	3	–	3	4	4	4
Number of sample	–	4888	5013	5132	5210	–	5394	5494	5576	5728
PLANTS.										
Diatomaceæ	–	**0**	**0**	**0**	**pr.**	–	**2**	**7**	**0**	**4**
Cyclotella	–	0	0	0	0	–	0	0	0	pr.
Melosira	–	0	0	0	0	–	0	1	0	0
Stephanodiscus	–	0	0	0	pr.	–	0	pr.	0	0
Synedra	–	0	0	0	0	–	0	4	0	4
Tabellaria	–	0	0	0	0	–	2	2	0	0
Cyanophyceæ	–	**0**	**0**	**0**	**0**	–	**0**	**0**	**0**	**6**
Aphanocapsa	–	0	0	0	0	–	0	0	0	6
Chroöcoccus	–	0	0	0	0	–	0	0	0	0
Algæ	–	**0**	**23**	**62**	**97**	–	**21**	**24**	**2**	**5**
Chlorococcus	–	0	23	62	97	–	0	6	0	3
Closterium	–	0	0	0	0	–	21	18	2	2
Conferva	–	0	0	0	0	–	0	0	0	0
Raphidium	–	0	0	0	0	–	0	0	0	0
Staurogenia	–	0	0	0	0	–	0	0	0	0
ANIMALS.										
Rhizopoda. Actinophrys	–	**0**	**0**	**0**	**0**	–	**0**	**0**	**0**	**0**
Infusoria. Dinobryon	–	**0**	**0**	**pr.**	**1**	–	**pr.**	**0**	**0**	**0**
Vermes (several genera)	–	**0**	**2**	**pr.**	**pr.**	–	**0**	**pr.**	**0**	**0**
Porifera. Sponge spicules	–	**0**	**0**	**0**	**0**	–	**0**	**0**	**0**	**0**
TOTAL ORGANISMS	–	0	25	62	98	–	23	31	2	15

	1890.								
	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
Day of examination	2	2	4	2	6	3	2	5	3
Number of sample	5828	5925	6019	6148	6342	6454	6550	6673	6772
PLANTS.									
Diatomaceæ	**7**	**16**	**107**	**19**	**42**	**1**	**1**	**3**	**62**
Cyclotella	0	0	92	14	42	0	0	0	0
Melosira	0	0	8	3	0	0	0	0	0
Stephanodiscus	0	5	0	0	0	0	0	3	25
Synedra	5	9	5	2	pr.	1	1	pr.	36
Tabellaria	2	2	2	0	0	0	0	pr.	1
Cyanophyceæ	**4**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**28**
Aphanocapsa	4	0	0	0	0	0	0	0	0
Chroöcoccus	0	0	0	0	0	0	1	0	28
Algæ	**2**	**114**	**108**	**1**	**2**	**28**	**9**	**11**	**62**
Chlorococcus	0	92	104	0	2	10	9	8	5
Closterium	2	22	2	1	0	0	0	0	pr.
Conferva	0	0	0	0	0	18	0	0	0
Raphidium	0	0	2	0	0	0	0	3	46
Staurogenia	0	0	0	0	0	0	0	0	11
ANIMALS.									
Rhizopoda. Actinophrys	**0**	**0**	**0**	**0**	**0**	**14**	**0**	**0**	**0**
Infusoria. Dinobryon	**0**	**2**	**pr.**	**0**	**0**	**0**	**0**	**0**	**1**
Vermes (several genera)	**0**	**pr.**	**0**	**0**	**0**	**0**	**0**	**0**	**pr.**
Porifera. Sponge Spicules	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**
TOTAL ORGANISMS	13	132	215	20	44	44	11	14	153

SUDBURY RIVER SUPPLY. — *Chemical Examination of Water from Reservoir No. 4, in Ashland, collected twenty feet beneath the surface.*

[Parts per 100,000.]

Number.	Date of Collection.	Date of Examination.	Appearance. Turbidity.	Appearance. Sediment.	Appearance. Color.	Residue on Evaporation. Total.	Residue on Evaporation. Loss on Ignition.	Ammonia. Free.	Ammonia. Albuminoid. Total.	Ammonia. Albuminoid. Dissolved.	Ammonia. Albuminoid. Suspended.	Chlorine.	Nitrogen as Nitrates.	Nitrogen as Nitrites.	Hardness.
	1889.														
4773	June 4	June 5	Slight.	Slight.	0.70	–	–	.0026	.0260	.0218	.0042	.20	.0020	.0002	–
4889	July 1	July 2	V. sl't.	V. sl't.	0.70	–	–	.0058	.0224	.0188	.0036	–	.0060	.0002	–
5014	Aug. 5	Aug. 6	V. sl't.	V. sl't.	0.70	–	–	.0054	.0294	.0272	.0022	–	.0020	.0001	–
5133	Sept. 3	Sept. 4	V. sl't.	V. sl't.	1.20	–	–	.0038	.0312	.0258	.0054	–	.0040	.0001	–
5211	Oct. 2	Oct. 3	Slight.	Slight.	0.90	–	–	.0054	.0262	.0230	.0032	–	.0930	.0001	–
5294	Nov. 4	Nov. 5	V. sl't.	Slight.	1.00	–	–	.0038	.0244	.0214	.0030	–	.0040	.0001	–
5395	Dec. 2	Dec. 3	Slight.	V. sl't.	1.20	–	–	.0028	.0274	.0218	.0056	–	.0090	.0002	–

		1890.														
5495	Jan.	2	Jan.	3	V. sl't.	V. sl't.	0.80	–	–	.0036	.0128	.0212	.0016	–	.0200	.0000	–
5577	Feb.	3	Feb.	4	V. sl't.	V. sl't.	0.75	–	–	.0000	.0174	.0166	.0008	–	.0100	.0001	–
5729	Mar.	3	Mar.	4	V. sl't.	Slight.	0.90	–	–	.0008	.0228	.0176	.0052	.25	.0120	.0001	–
5829	Apr.	1	Apr.	2	Slight.	Slight.	0.70	–	–	.0004	.0190	.0160	.0030	.22	.0150	.0001	–
5926	May	1	May	2	Slight.	Slight.	0.75	–	–	.0026	.0176	.0130	.0046	.23	.0070	.0001	–
6020	June	2	June	3	Slight.	Slight.	0.60	3.65	1.45	.0016	.0186	.0156	.0030	.23	.0080	.0000	–
6149	July	1	July	2	Slight.	Slight.	0.50	3.20	–	.0036	.0284	.0188	.0096	.19	.0030	.0001	–
6343	Aug.	4	Aug.	5	Slight.	Slight.	0.40	3.90	2.05	.0048	.0218	.0188	.0030	.20	.0150	.0003	1.4
6455	Sept.	2	Sept.	3	Slight.	Slight.	0.40	4.45	1.75	.0000	.0216	.0176	.0040	.26	.0150	.0001	1.8
6551	Oct.	1	Oct.	2	Slight.	Slight.	0.45	3.30	1.05	.0002	.0196	.0154	.0042	.24	.0080	.0001	1.7
6674	Nov.	4	Nov.	5	V. sl't.	Slight.	0.85	3.90	1.65	.0000	.0222	.9172	.0050	.26	.0080	.0002	1.6
6773	Dec.	1	Dec.	2	V. sl't.	Slight.	0.70	4.00	1.70	.0018	.0238	.0202	.0036	.25	.0100	.0001	1.6
Av.	.....	.....	.....	.....	.....	.....	0.75	3.87	1.61	.0024	.0233	.0194	.0039	.23	.0085	.0001	1.6

Odor, very faintly vegetable or none. — The samples were collected from the reservoir, near the gate house, at a depth of 20 feet beneath the surface.

Microscopical Examination. (*Twenty feet beneath the surface, Reservoir No.* 4.)

[Number of organisms per cubic centimeter.]

	1889.							1890.		
	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Day of examination	–	2	6	6	3	–	3	4	4	4
Number of samples	–	4889	5014	5133	5211	–	3395	5495	5577	5729
PLANTS.										
Diatomaceæ	–	**pr.**	**0**	**0**	**11**	–	**0**	**6**	**0**	**pr.**
Cyclotella	–	0	0	0	0	–	0	0	0	0
Melosira	–	0	0	0	9	–	0	0	0	0
Stephanodiscus	–	0	0	0	0	–	0	0	0	0
Synedra	–	pr.	0	0	2	–	0	2	0	pr.
Tabellaria	–	0	0	0	0	–	0	4	0	0
Cyanophyceæ. Chroöcoccus	–	**0**	**0**	**0**	**0**	–	**0**	**0**	**0**	**0**
Algæ	–	**0**	**8**	**95**	**142**	–	**28**	**19**	**3**	**0**
Chlorococcus	–	0	8	95	140	–	3	pr.	0	0
Closterium	–	0	0	0	2	–	25	19	3	0
Raphidium	–	0	0	pr.	pr.	–	0	0	0	0
Staurogenia	–	0	0	0	0	–	0	0	0	0
ANIMALS.										
Rhizopoda. Actinophrys	–	**0**	**0**	**0**	**0**	–	**0**	**0**	**0**	**0**
Infusoria	–	**0**	**0**	**0**	**6**	–	**0**	**pr.**	**0**	**0**
Dinobryon	–	0	0	0	0	–	0	pr.	0	0
Vorticella	–	0	0	0	6	–	0	0	0	0
TOTAL ORGANISMS	–	0	8	95	159	–	28	25	3	0

	1890.								
	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
Day of examination	2	2	4	2	6	3	2	5	3
Number of sample	5829	5926	6020	6149	6343	6455	6551	6674	6773
PLANTS.									
Diatomaceæ	**5**	**28**	**119**	**252**	**27**	**0**	**1**	**1**	**72**
Cyclotella	0	0	104	250	27	0	0	0	0
Melosira	0	12	11	0	0	0	0	0	1
Stephanodiscus	0	5	0	pr.	0	0	0	0	41
Synedra	5	7	2	2	0	0	1	1	30
Tabellaria	0	4	2	0	0	0	0	0	pr.
Cyanophyceæ. Chroöcoccus	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**26**
Algæ	**4**	**27**	**10**	**26**	**pr.**	**0**	**5**	**5**	**97**
Chlorococcus	0	21	8	26	pr.	0	3	5	6
Closterium	4	6	2	0	0	0	0	0	0
Raphidium	0	0	0	0	0	0	2	0	66
Staurogenia	0	0	0	0	0	0	0	0	25
ANIMALS.									
Rhizopoda. Actinophrys	**0**	**0**	**0**	**0**	**0**	**7**	**0**	**0**	**pr.**
Infusoria	**0**	**17**	**0**	**6**	**0**	**1**	**0**	**0**	**pr.**
Dinobryon	0	17	0	6	0	1	0	0	pr.
Vorticella	0	0	0	0	0	0	0	0	0
TOTAL ORGANISMS	9	72	129	284	27	8	6	6	195

SUDBURY RIVER SUPPLY. — *Chemical Examination of Water from Reservoir No. 4, in Ashland, collected near the bottom.*

[Parts per 100,000.]

Number.	Date of Collection.	Date of Examination.	Appearance. Turbidity.	Appearance. Sediment.	Appearance. Color.	Residue on Evaporation. Total.	Residue on Evaporation. Loss on Ignition.	Ammonia. Free.	Ammonia. Albuminoid. Total.	Ammonia. Albuminoid. Dissolved.	Ammonia. Albuminoid. Suspended.	Chlorine.	Nitrogen as Nitrates.	Nitrogen as Nitrites.	Hardness.
	1889.														
4774	June 4	June 5	V. sl't.	V. sl't.	0.60	–	–	.0038	.0206	.0182	.0024	.22	.0050	.0002	–
4890	July 1	July 2	Dist't.	V. sl't.	1.00	–	–	.0058	.0230	.0206	.0024	–	.0050	.0002	–
5015	Aug. 5	Aug. 6	Slight.	V. sl't.	0.60	–	–	.0030	.0208	.0204	.0004	–	.0080	.0001	–
5134	Sept. 3	Sept. 4	Slight, milky.	V. sl't.	0.70	–	–	.0018	.0196	.0180	.0016	–	.0080	.0001	–
5212	Oct. 2	Oct. 3	Slight.	V. sl't.	1.00	–	–	.0016	.0214	.0192	.0022	–	.0050	.0000	–
5295	Nov. 4	Nov. 5	V. sl't.	Slight.	1.10	–	–	.0034	.0242	.0212	.0030	–	.0040	.0001	–
5396	Dec. 2	Dec. 3	V. sl't.	Slight.	1.10	–	–	.0032	.0278	.0236	.0042	–	.0180	.0002	–

1890.															
5496	Jan. 2	Jan. 3	V. sl't.	V. sl't.	0.80	–	–	.0022	.0230	.0214	.0016	–	.0200	.0000	–
5578	Feb. 3	Feb. 4	V. sl't.	V. sl't.	0.75	–	–	.0000	.0182	.0162	.0020	–	.0120	.0002	–
5730	Mar. 3	Mar. 4	Slight.	Cons.	0.90	–	–	.0010	.0212	.0176	.0036	.23	.0150	.0002	–
5830	Apr. 1	Apr. 2	Slight.	Slight, fibr'us.	0.70	–	–	.0004	.0208	.0156	.0052	.23	.0040	.0001	–
5927	May 1	May 2	Slight.	Slight.	0.70	–	–	.0026	.0178	.0154	.0024	.24	.0070	.0001	–
6021	June 2	June 3	Slight.	Slight.	0.70	3.80	1.25	.0024	.0160	.0142	.0018	.25	.0080	.0001	–
6150	July 1	July 2	Slight.	V. sl't.	0.40	3.20	–	.0050	.0168	.0156	.0012	.20	.0075	.0002	–
6344	Aug. 4	Aug. 5	V. sl't.	Slight, fibr'us.	0.35	4.00	1.85	.0014	.0200	.0166	.0034	.17	.0150	.0003	1.3
6456	Sept. 2	Sept. 3	Slight.	Cons.	0.50	3.95	1.65	.0008	.0220	.0168	.0052	.26	.0150	.0001	1.8
6552	Oct. 1	Oct. 2	Slight.	Cons.	0.55	3.80	1.50	.0024	.0170	.0124	.0046	.24	.0150	.0002	1.7
6675	Nov. 4	Nov. 5	V. sl't.	Slight, fibr'us.	0.85	4.05	1.50	.0002	.0226	.0198	.0028	.25	.0080	.0002	1.7
6774	Dec. 1	Dec. 2	V. sl't.	Slight.	0.70	4.20	1.50	.0016	.0234	.0198	.0036	.27	.0120	.0001	1.6
Av.	.....	.....	.....	.....	0.74	3.97	1.54	.0022	.0209	.0180	.0029	.23	.0103	.0001	1.6

Odor, very faintly vegetable or none.— The samples were collected from the reservoir, near the bottom, just above the gate-house. When the reservoir is full, the sample is collected 40 feet beneath the surface.

Microscopical Examination. (*Near bottom, Reservoir No.* 4.)

[Number of organisms per cubic centimeter.]

	1889.							1890.		
	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Day of examination	–	2	6	7	3	–	3	4	4	5
Number of sample	–	4890	5015	5134	5212	–	5396	5496	5578	5730
PLANTS.									1	
Diatomaceæ	–	**0**	**0**	**0**	**0**	–	**pr.**	**2**		**58**
Cyclotella	–	0	0	0	0	–	0	0	0	0
Melosira	–	0	0	0	0	–	0	0	0	0
Stephanodiscus	–	0	0	0	0	–	0	1	0	0
Synedra	–	0	0	0	0	–	pr.	1	1	58
Cyanophyceæ	–	**0**	**0**	**0**	**0**	–	**0**	**0**	**0**	**0**
Chroöcoccus	–	0	0	0	0	–	0	0	0	0
Microcystis	–	0	0	0	0	–	0	0	0	0
Algæ	–	**0**	**12**	**39**	**44**	–	**17**	**27**	**3**	**0**
Chlorococcus	–	0	12	39	44	–	0	0	0	0
Closterium	–	0	0	0	0	–	17	27	3	0
Staurogenia	–	0	0	0	0	–	0	0	0	0
Fungi. Crenothrix	–	**0**	**1**	**12**	**pr.**	–	**pr.**	**0**	**0**	**pr.**
ANIMALS.										
Rhizopoda. Actinophrys	–	**0**	**0**	**0**	**0**	–	**0**	**0**	**0**	**0**
Infusoria	–	**0**	**0**	**0**	**1**	–	**0**	**0**	**0**	**0**
Dinobryon	–	0	0	0	0	–	0	0	0	0
Monas	–	0	0	0	1	–	0	0	0	0
TOTAL ORGANISMS	–	0	13	51	45	–	17	29	4	58

	1890.								
	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
Day of examination	2	2	4	2	6	3	2	5	3
Number of sample	5830	5927	6021	6150	6344	6456	6552	6675	6774
PLANTS.									
Diatomaceæ	**3**	**11**	**22**	**27**	**22**	**2**	**0**	**3**	**88**
Cyclotella	0	0	15	26	12	0	0	0	0
Melosira	0	0	3	0	9	0	0	0	0
Stephanodiscus	0	4	0	0	1	0	0	3	58
Synedra	3	7	4	1	pr.	2	0	pr.	30
Cyanophyceæ	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**pr.**	**29**
Chroöcoccus	0	0	0	0	0	0	0	0	19
Microcystis	0	0	0	0	0	0	0	pr.	10
Algæ	**1**	**21**	**10**	**0**	**0**	**0**	**0**	**12**	**50**
Chlorococcus	0	14	7	0	0	0	0	12	2
Closterium	1	9	3	0	0	0	0	0	0
Staurogenia	0	0	0	0	0	0	0	0	48
Fungi. Crenothrix	**0**	**pr.**	**0**	**1**	**0**	**3**	**6**	**0**	**0**
ANIMALS.									
Rhizopoda. Actinophrys	**0**	**0**	**0**	**0**	**0**	**14**	**0**	**0**	**0**
Infusoria	**1**	**9**	**0**	**0**	**0**	**1**	**0**	**0**	**0**
Dinobryon	0	9	0	0	0	0	0	0	0
Monas	1	0	0	0	0	1	0	0	0
TOTAL ORGANISMS	5	41	32	28	22	20	6	15	167

DEDUCTIONS FROM THE OBSERVATIONS AT RESERVOIR NO. 4.

A detailed study of the foregoing series of chemical and microscopical analyses at Reservoir No. 4 reveals a number of important points, the apparent significance of which will be briefly pointed out.

Reservoir No. 4, situated in the town of Ashland, on Cold Spring Brook, is about three-quarters of a mile in length, and when filled to the ordinary flow-line, has a depth near the dam of about forty-five feet. It is nearly two thousand feet wide at the dam, and preserves a width of perhaps twelve hundred feet to near the upper end. The construction was completed in 1885, and the reservoir filled for the first time in April, 1886. The bottom was thoroughly cleaned of all loam, stumps, and vegetable matter, and the margins deepened wherever the original marginal depth at high water was less than eight feet. The water-shed is 6.06 square miles area, with few inhabitants, but is somewhat swampy. The storage capacity is about 1,100,000,000

gallons when filled to the ordinary flow level.

The foregoing series of analyses of water from the Cold Spring Brook, are of samples taken from the flowing stream a short distance above the head of Reservoir No. 4.

The samples from the reservoir itself were taken at the depths indicated, from a point about mid-way in the reservoir, not far from the dam.

As stated in the report, the microscopical examinations from June, 1889, to November, 1890, inclusive, were made by Prof. Sedgwick's sand method; while those for December, 1890, were made by the Sedgwick-Rafter method. This may be taken as partly accounting for the considerable increase in the number of organisms tabulated for the month of December, 1890, over that observed in November, and the months immediately preceding.

While the results obtained are of great interest, and strongly indicate the value of the microscopical analysis in conjunction with the chemical, it may nevertheless be

observed that the work of the present year will be likely to more decisively exhibit such value; not only by reason of the improvement in method of examination, but because of the increased experience of the observers. The field of cryptogamic botany and zoölogy necessary to be covered, in order to make such examinations at all, is large; and the biologists who undertook to make these studies were literally exploring an unknown world. That their work gives very satisfactory results at this early date, can only be taken as the highest possible evidence of their painstaking industry. The work itself is the best exposition of the new views, and it is unnecessary to consume any large amount of space in pointing out the details. A few deductions may, however, be briefly noted:—

The running water of Cold Spring Brook contained a number of species of Diatomaceæ [*Navicula, Nitzchia, Synedra*], which are either not found at the lower end of the reservoir at all, or in much smaller number. Of these, *Navicula* and *Nitzchia*

are apparently entirely absent from the reservoir, while *Synedra*, except at the bottom, is present only in smaller quantity. Thus in Cold Spring Brook the average number per month of *Synedra*, per cubic centimetre, for the twelve months of 1890, is found to be 8.8. At a depth of one foot below the surface in Reservoir No. 4, the average number per month of *Synedra* for the same period is 5.6. At mid-depth we find 4.0, and near the bottom 9.0, *Synedras* per cubic centimetre.

Collating the showing for *Cyclotella* in the same way, we find the form entirely absent from the Cold Spring Brook. In the reservoir this form is present at all depths, only in the months of June, July, and August; 1890, the average per cubic centimetre per month for the three months being, at one foot below the surface, 49.0, at twenty feet, 127.0, and at the bottom 18.0.

Taking the whole number of Diatomaceæ the average per cubic centimetre per month in Cold Spring Brook is 17.0; at one foot below the surface in the reservoir,

22.4; at twenty feet, 42.5; and near bottom, 20.0.

Taking the total number of plant forms and tabulating, we make the following showing: —

Locality.	Jan.	Feb.	Mar.	April.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.	Average.
Cold Spring Brook	3	5	6	9	34	76	60	131	15	6	7	76	35.7
Reservoir No. 4, 1 foot below surface	31	2	15	13	130	215	20	44	29	11	14	152	56.3
Reservoir No. 4, mid-depth . .	25	3	0	9	55	129	278	27	0	6	6	195	61.1
Reservoir No. 4, near bottom . .	29	4	58	4	32	32	28	22	5	6	15	167	33.5

From this table it is clearly apparent that the greatest activity of the microscopical plants is in the months corresponding to the highest temperature. December we may leave out of this comparison, as by reason of the increased number due to the change in method, the record for that month is probably abnormal so far as the balance of the series is concerned. February appears as a period of minimum plant life, while October and November are also low. This is as might be expected in February, though

the record here gives a different result in October and November from the author's experience with other bodies of water in which the microscopical plant life has been found especially vigorous in these months. The explanation must be looked for in further study of samples from Reservoir No. 4, though a partial explanation may be inferred by studying the relations of the chemical constituents to the cryptogamic growths. Such relations can be more conveniently shown by diagrams than by either tabulations, or written descriptions; and Plates I. to IV. have accordingly been prepared for this purpose. Studying them, it appears that in Cold Spring Brook, and also in the reservoir, at both surface and mid-depth, the period of greatest development of plant life bears a clear relation to the maximum point reached by the nitrates; the same may also be affirmed of the results of the examinations of samples near the bottom, though probably somewhat different forces are at work near the bottom of a deep body of water from those either near the surface or at mid-depth,

and points between. The nature of these forces may be now indicated: —

EFFECT OF LIGHT.

The chlorophylaceous and amylaceous plants require light as the primary condition of growth; and if we assume, for the time being, a fixed condition of the water at various planes, we may say that the varieties of cryptogamic plant life, which depend upon light, will decrease as we go deeper in any given body of water, in accordance with some law bearing a relation to the intensity of light at the given plane. Changes in quality of light as depth increases may result from two causes, or, rather, from a combination of two causes. There will always be either an increased opacity of the water itself, due to increase of coloring matter as the depth increases; or where the coloring matter is constant at all depths, the decrease in intensity of light will be in accordance with the general law, that intensity decreases geometrically as distance increases arithmetically. By way of illustration, we may assume the

opacity of a given body of water to be such as to cut off $\frac{1}{20}$ of the total intensity of light at the depth of one foot. We have then, after passing through one foot of such water, $\frac{19}{20}$ of the original intensity at the surface. In passing through the second foot, the light again loses $\frac{1}{20}$ of the total quantity of light entering the second foot, or $\frac{1}{20}$ of $\frac{19}{20}$. At the depth of two feet, the total intensity is therefore $\frac{361}{400}$ of the original intensity, and so on for any depth whatever.

On examining the color column in the tables of results, or the profile of the same on the diagrams, we find that great variations in color occurred in the water of Cold Spring Brook, the color determinations running all the way from 3.50 in August, 1889, to 0.30 in August, 1890. Also, that in June, 1890, the color scale stands 1.80. This of itself represents an enormous difference in the plant-producing capacity of the water of this stream between June and August, 1890, and by itself affords a partial explanation of why the plant development in August, 1890, was so greatly in excess of that in June. Partial explanation is

stated in the foregoing, because many other circumstances tend to modify the results, and no one cause can be assigned as a full explanation.

If we consider the color scale in the results for Reservoir No. 4, one foot below the surface, we find that the range in variation in 1890 was from 0.35 in August and September, to 0.85 in November; 0.50 being the figure for July and August in that year. At mid-depth the figures for 1890 show 0.60 for June; 0.50 for July; 0.40 for August and September; 0.45 for October; and 0.85 for November. Near the bottom they are 0.70 for May and June; 0.40 for July; 0.35 for August; 0.50 for September; 0.55 for October; and 0.85 for November. The relation of these to the other results are also clearly indicated on the diagrams.

Examining the tabulations further, it appears while a considerable number of chlorophylaceous algæ (Chlorophyceæ) are found at mid-depth, the number is still much less than at one foot below the surface; and near the bottom, the numbers

run generally even smaller. This is as may be predicated from what is known in reference to the influence of light on vegetation. In white light the rays of different intensity of wave motion are commingled in such manner that the various-colored bands of the spectrum are not apparent. Such light is normal so far as related to physiological action on the processes of vegetation. The chemical changes in growing plants, however, are chiefly due to the rays of inferior wave motion; as, for instance, the red, orange, yellow, or green. The mechanical changes, on the other hand, are produced by the rays of high-wave motion, — the blue, violet, or ultra-violet. The former are chiefly concerned in the production of chlorophyl, the decomposition of carbon dioxide, and the formation of starch. The latter influence the rapidity of growth, alter the movements of protoplasm, compel swarm-spores to adopt a definite direction in their motion, change the tension of the tissues of the motile organs, and hence affect their position, etc.*

* Sachs' Botany, p. 778.[32a]

Again, the action of light on plants is in proportion to its intensity. This question is one with more than theoretical interest, as is sufficiently shown by considering that the production of starch in the chlorophyllaceous algæ is dependent upon the quantity of light which the plants receive. All the free-floating forms are from a variety of causes; as, for instance, changes of temperature, cessation of the production of gas by the plants themselves, etc.; quite susceptible to changes in specific gravity, and, therefore, at different times, occupy different levels in the water. In light of less than a certain degree of intensity the starch is not formed; the protoplasmic matter, which, with sufficient intensity of light, would go to the production of starch, remains protoplasmic. Again, if algæ, in which starch is fully formed, are placed in the dark, or in light of less than the starch-producing degree of intensity, the starch already formed will disappear; such changes taking place as restore the starch material to its original state. On being again brought into strong light, the starch will

reform, and by treatment in a suitable culture-cell, all these formations can, under proper gradation of light, be observed for a considerable length of time.

The application to be made of these observations is in relation to the changes in intensity of the light which will exist at different depths in any given body of water, and, consequently, in relation to the varying quality of the water itself at different depths. In this connection it is important to clearly understand that the production of chlorophyl and starch is very intimately related to the chemical composition of water, and that if such conditions obtain as preclude the continuance of their formation, changes in chemical composition may be expected to result.

This phase of the subject could be pursued indefinitely, but the limits of a volume of the Science Series clearly will not permit. The foregoing is a skeleton merely; and the reader who cares to pursue the subject further must consult the great works of Sachs'.[32a and b]

EFFECT OF TEMPERATURE.

The most important law of temperature in relation to plant life with which we are concerned, in an investigation of the relation of cryptogamic growths to the purity of a natural water, is that affirming, that in plant growth the exercise of every function is restricted to certain definite limits of temperature, within which it alone can take place.*

A corollary to the foregoing may be stated as follows: the functions of a plant are assisted and accelerated in their intensity when the temperature rises above the lower limit for that function; on reaching a definite higher degree, a maximum of intensity is attained, the activity then decreases with a further increase of temperature, until it entirely ceases at the upper limit.†

As a brief deduction from the foregoing law, it may be assumed that some plants (and the assumption is apparently entirely true as applied to cryptogams) will

* Sachs' Botany, p. 727. † *Loc. cit.*, p. 729.

grow best in low temperatures, others in high temperatures, the latter being much the more numerous in this latitude. Ordinarily, therefore, the lowering of the temperature of a body of water, as winter approaches, will be accompanied by a decrease in the amount and variety of microscopical life. Exceptions to this rule may, however, be expected by reason of certain forms flourishing in a low temperature.

Again, in very large and deep bodies of water it is necessary to go only a few feet (50 to 80) below the surface before a level is reached in which the temperature is practically constant throughout the whole year. In such a body the few observations that have been thus far made, indicate an abundant development of both plant and animal life in winter. This is finely shown by Mr. Vorce in his paper on Forms Observed in Water of Lake Erie,[25] where are figured and described in the first part, one hundred and ninety-two species of plants and animals, all observed between Dec. 25, 1880 and Jan. 22, 1881. A study of Lake Erie water for several years by Mr. Vorce,

indicated the appearance of certain forms at about the same time every year. This observation as to periodicity of forms has been verified by the author in his studies of the water of Hemlock Lake.

In smaller bodies of water, like Reservoir No. 4 of the Boston supply, it is uncertain that any such permanency and periodicity of the winter forms are maintained. Additional study is necessary to elucidate this point.

PHYSICAL CONDITION OF THE WATER.

From the preceding, it is evident that in generalizing the results of these examinations, it must be borne in mind that April, May, and June are months of increasing temperature; July, August, and September months of maximum temperature; October, November, and December months of decreasing temperature; and January, February, and March the months of minimum temperature. The general effect of these changes on the quantity and quality of the microscopical life has been already briefly indicated; it now remains

to point out an important series of changes in the body of water itself, due to the fluctuations of temperature.

In the first place, in summer the mean temperature of shallow bodies of water will generally be higher than that of deeper ones; this temperature will be more quickly reached in the months of increase, and more quickly lost in the months of decrease. In winter the shallow body will usually exhibit a somewhat lower temperature than the deeper one.

Again, as cold weather approaches, in the fall, the upper layers of a body of water become cooler than the layers immediately below; and there accordingly results, by reason of gravitation, a complete vertical circulation, through the influence of which the relative positions of the top and bottom layers are reversed, down to a depth where the temperature may be expected to remain uniform for the whole year. By way of illustrating the extent of the force producing this overturning in the fall, the following table of relative

density and weight of a cubic foot of water, at different temperatures, is inserted.*

Temperature.	Relative Density.	Weight of a Cubic Foot in Pounds.
32°	.99987	62.416
35°	.99996	62.421
39°.3	1.	62.424
45°	.99992	62.419
50°	.99975	62.408
55°	.99946	62.390
60°	.99907	62.366
65°	.99859	62.336
70°	.99802	62.300
75°	.99739	62.261
80°	.99669	62.217

July 4, 1889, Desmond FitzGerald, C. E., and the author made a number of measurements of the temperature of Lake Cochituate, at a depth of sixty feet. A mean of several of the observations gives a

* Smith's Hydraulics, p. 14.

temperature at that depth of 45°.4; the change at points a short distance apart being from 44°.2 to 47°0. The surface temperature at the point of making the observations was 75°.6, the air being 77°.2. From the foregoing table, it appears that a cubic foot of water at 45° weighs 62.419 pounds; and at a temperature of 75° the weight is 62.261 pounds, giving a difference at these temperatures of 0.158 pounds.

It is clear, therefore, that at this time of year the bottom layers were certain to remain at the bottom, by virtue of superior gravity. On the approach of cold weather, however, the decrease in temperature at the surface increases the density there, gradually leading to a complete vertical circulation, as already indicated.

Again, in shallow bodies of water we may further occasionally have a vertical circulation, — from bottom to top, — due to the influence of heavy winds; and the upper layers of a large and deep body may also be expected to respond to the same source of discrepancy.

All these various modifying influences must be taken into account in studying the results of water-supply examinations.

LUDLOW RESERVOIR, SPRINGFIELD.

The main source of supply to the city of Springfield, Massachusetts, is derived from the Ludlow Storage Reservoir. The area when filled, is about 445 acres, and the total content 1,992,000,000 gallons. The reservoir was constructed about 1875, and during every summer since, the water has been unpleasantly affected with bad tastes and odors. The greatest depth is about twenty-four feet, with an average of nearly fourteen feet. Of the area flowed by the reservoir, two hundred and eighty-one acres were covered with forest, a portion of which was swampy land with peaty deposits, from six inches to four feet in depth. The peaty areas are all at least twelve feet below the flow-line, and many of them sixteen feet below. All trunks of trees and brush were burned, and stumps cut low and charred. Nearly six and one-half acres of the most objectionable portion of

the swamp were sanded over to a depth of about one and one-half feet. The shores are mostly abrupt, the only exception being a small shallow area at the upper end.

The original plan included the uniting of several water-sheds by canals; and the total tributary area was 6,484 acres. In 1886 a portion of this was cut off, leaving at the present time 4,358 acres, on which there is only a very small population.*

The chemical and microscopical analyses recorded in the following tables are of the greatest interest, as showing the relation between the high ammonias and the excessive development of plant, and, at times, of animal life. These relations are so clearly shown by the tables and diagrams that an extended discussion may be omitted. A few points only will be noted. In the first place, the reader's attention is directed to the fact, that during the whole time covered by these tables, this water has been in constant use for domestic purposes in the city of Springfield; the total consumption for all purposes being about 4,000,000 gallons per day.

* Special Report, Part I., pp. 296-7.[13c]

Chemical Examination of Water from Ludlow Reservoir at a depth of six feet beneath the surface.

[Parts per 100,000.]

Number.	Date of Collection.	Date of Examination.	Appearance. Turbidity.	Appearance. Sediment.	Appearance. Color.	Residue on Evaporation. Total.	Residue on Evaporation. Loss on Ignition.	Ammonia. Free.	Ammonia. Albuminoid. Total.	Ammonia. Albuminoid. Dissolved.	Ammonia. Albuminoid. Suspended.	Chlorine.	Nitrogen as Nitrates.	Nitrogen as Nitrites.	Hardness.
	1889														
4768	June 3	June 4	Dec'd.	Heavy.	0 10	–	–	.0006	.0698	.0248	.0450	.10	.0040	.0001	–
4811	June 10	June 11	Dec'd.	Cons.	0.10	–	–	.0022	.0602	.0242	.0360	–	.0020	.0000	–
4846	June 17	June 18	Dist't.	Cons.	0.20	–	–	.0128	.0746	.0402	.0344	–	.0020	.0003	–
4873	June 24	June 25	Slight.	Cons.	0.15	–	–	.0004	.0688	.0356	.0332	–	.0020	.0000	–
4918	July 8	July 9	Slight.	Heavy.	0.30	–	–	.0140	.0604	.0366	.0238	–	.0050	.0001	–
4942	July 15	July 16	Dist't.	Cons.	0·30	–	–	.0060	.0.60	.0328	.0572	–	.0000	.0002	–
4965	July 22	July 23	Dist't.	Cons.	0.10	–	–	.0002	.0.00	.0302	.0598	–	.0040	.0001	–
4990	July 29	July 30	Slight.	Cons.	0.15	–	–	.0000	.0754	.0288	.0466	–	.0020	.0001	–
5029	Aug. 5	Aug. 6	Dec'd.	Slight.	0.10	–	–	.0000	.0786	.0246	.0540	–	.0020	.0001	–
5052	Aug. 12	Aug. 13	Dist't.	Slight,	0.15	–	–	.0008	.0758	.0248	.0510	–	.0040	.0000	–
5078	Aug. 19	Aug. 20	Dec'd.	V. sl't.	0.15	–	–	.0012	.0670	.0224	.0446	–	.0040	.0001	–
5118	Aug. 30	Aug. 31	Dist't.	Cons.	0.10	–	–	.0002	.0634	.0270	.0364	–	.0050	.0001	–
5156	Sept. 9	Sept. 10	Slight.	Slight.	0.03	–	–	.0006	.0534	.0262	.0272	–	.0020	.0000	–
5186	Sept. 16	Sept. 17	Dist't.	Cons.	0.10	–	–	.0032	.0636	.0298	.0338	–	.0000	.0000	–
5207	Sept. 30	Oct. 1	Dist't.	Cons.	0.20	–	–	.0014	.0570	.0280	.0290	–	.0020	.0001	–
5243	Oct. 9	Oct. 10	Slight.	Cons.	0.10	–	–	.0002	.0554	.0276	.0278	–	.0050	.0000	–
5280	Oct. 24	Oct. 25	Slight.	Cons.	0.30	–	–	.0006	.0540	.0274	.0266	–	.0040	.0001	–
5330	Nov. 11	Nov. 12	Dist't.	Cons.	0.20	–	–	.0056	.0510	.0304	.0206	–	.0040	.0001	–
5382	Nov. 25	Nov. 26	Slight.	Cons.	0.10	–	–	.0044	0522	.0318	.0204	–	.0040	.0001	–
5448	Dec. 16	Dec. 18	Slight.	Cons.	0.05	–	–	.0028	.0398	.0238	.0160	–	.0050	.0001	–

	1890														
5551	Jan. 20	Jan. 21	Slight.	Cons.	0.10	–	–	.0016	.0282	.0186	.0096	–	.0060	.0001	–
5661	Feb. 13	Feb. 14	Dist't.	Slight.	0.10	–	–	.0000	.0204	.0162	.0042	–	.0030	.0001	–
5785	Mar. 12	Mar. 13	Slight.	Cons.	0.10	–	–	.0000	.0254	.0170	.0084	–	.0020	.0001	–
5820	Mar. 26	Mar. 27	Slight.	Cons.	0.20	–	–	.0000	.0188	.0094	.0094	–	.0030	.0002	–
5875	Apr. 10	Apr. 11	Dist't.	Cons.	0.05	–	–	.0002	.0240	.0126	.0114	–	.0020	.0000	–
5909	Apr. 24	Apr. 26	Dist't.	Cons.	0.05	–	–	.0000	.0248	.0138	.0110	.12	.0000	.0000	–
5961	May 12	May 13	Slight.	Cons.	0.05	–	–	.0016	.0212	.0150	.0062	–	.0020	.0000	–
6010	May 28	May 29	Dist't.	Cons.	0.05	–	–	.0002	.0632	.0174	.0458	–	.0020	.0000	–
6115	June 23	June 24	Dist't.	Cons.	0.20	–	–	.0444	.0594	.0318	.0276	–	.0020	.0004	0.8
6218	July 14	July 15	Dist't.	Slight.	0.50	–	–	.0036	.0752	.0412	.0340	.11	.0020	.0000	0 8
6422	Aug. 18	Aug. 20	Slight.	Cons.	0.05	–	–	.0000	.0674	.0286	.0388	–	.0030	.0001	0.9
6518	Sept. 16	Sept. 17	Dist't.	Cons.	0.15	–	–	.0000	.0442	.0180	.0262	.11	.0050	.0001	1.2
6620	Oct. 20	Oct. 21	Slight.	Cons.	0.25	–	–	.0008	.0492	.0268	.0224	.09	.0070	.0003	1.2
6733	Nov. 17	Nov. 18	Slight.	Slight.	0.15	2.55	1.40	.0010	.0762	.0230	.0132	.12	.0070	.0001	0.8
6825	Dec. 15	Dec. 16	Slight.	Slight.	0.10	–	–	.0002	.0418	.0272	.0146	.09	.0200	.0001	0.9
Av.	.....	.....	.....	.....	0.15	–	–	.0037	.0491	.0248	.0243	.11	.0047	.0001	0.9

Odor, generally vegetable and grassy; increased by heating; occasionally disagreeable.—The samples were collected from near the middle of the reservoir, at a depth of 6 feet beneath the surface, with the exception of No. 5448, which was collected at the filter-dam pier, 3 feet beneath the surface, and Nos. 5551, 5661, and 5785, which were collected at depths of 3 feet or less beneath the surface. During the period from June, 1889, to December, 1890, the reservoir was kept at from 2 to 11 feet below high water.

Microscopical Examination. (*Ludlow Reservoir, six feet beneath the surface.*)

[Number of organisms per cubic centimeter.]

	1889.											
	June.	June.	June.	June.	July.	July.	July.	July.	Aug.	Aug.	Aug.	Aug.
Day of examination	–	11	18	25	11	16	23	30	6	13	20	31
Number of sample	4768	4811	4846	4873	4918	4942	4065	4990	5029	5052	5078	5118
PLANTS.												
Diatomaceæ	–	**30**	**2**	**2**	**3**	**0**	**19**	**16**	**50**	**34**	**192**	**46**
Asterionella	–	0	0	0	0	0	0	1	2	0	0	0
Melosira	–	30	2	2	3	0	19	15	48	34	190	46
Synedra	–	pr.	pr.	0	0	0	0	0	0	0	2	0
Tabellaria	–	pr.	0	0	0	0	0	0	0	0	0	0
Cyanophyceæ	–	**122**	**23**	**9**	**337**	**362**	**805**	**1300**	**654**	**634**	**1588**	**560**
Anabæna	–	120	23	5	12	24	17	0	0	0	2	0
Aphanocapsa	–	0	0	0	0	0	0	0	0	0	0	0
Chroöcoccus	–	0	0	0	0	0	0	0	0	0	0	0
Clathrocystis	–	2	pr.	4	2	5	26	6	6	6	10	68
Cœlosphærium	–	0	0	0	323	333	762	1294	648	628	1576	492
Microcystis	–	0	0	0	0	0	0	0	0	0	0	0
Nostocaceous spores	–	0	0	0	0	0	0	0	0	0	0	0
Algæ	–	**7**	**3**	**10**	**257**	**63**	**45**	**39**	**8**	**4**	**22**	**126**
Chlorococcus	–	5	3	3	163	10	0	14	0	0	0	114
Closterium	–	pr.	0	0	0	0	0	0	0	0	0	0

Cœlastrum	–	0	0	2	15	2	0	0	0	0	0	0
Dictyosphærium . .	–	0	0	0	0	0	0	0	0	0	0	0
Pediastrum	–	0	pr.	pr.	3	2	0	0	0	0	0	0
Protococcus	–	0	0	0	0	0	0	0	0	0	0	0
Raphidium	–	0	0	0	0	0	0	0	0	0	0	0
Scenedesmus	–	2	pr.	4	5	2	2	0	0	0	6	0
Sorastrum	–	0	0	0	0	0	0	0	0	0	0	0
Staurastrum	–	pr.	pr.	1	71	47	43	25	8	2	16	12
ANIMALS.												
Rhizopoda. Actinophrys	–	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Infusoria	–	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**2**	**0**
Dinobryon	–	0	0	0	0	0	0	0	0	0	0	0
Monas	–	0	0	0	0	0	0	0	0	0	0	0
Peridinium	–	0	0	0	0	0	0	0	0	0	0	0
Trachelomonas . . .	–	0	0	0	0	0	0	0	2	0	2	0
Vermes	–	**pr.**	**pr.**	**pr.**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**4**
Anurea	–	pr.	pr.	0	0	0	0	0	0	0	0	2
Polyarthra	–	0	0	pr.	0	0	0	0	2	0	0	0
Rotifer	–	0	0	0	0	0	0	0	0	0	0	0
Rotatorian ova . . .	–	0	0	0	0	0	0	0	0	0	0	2
Crustacea	–	**pr.**	**pr.**	**pr.**	**pr.**	**pr.**	**0**	**0**	**0**	**0**	**0**	**0**
Cyclops	–	pr.	pr.	pr.	pr.	0	0	0	0	0	0	0
Daphnia	–	pr.	pr.	pr.	0	pr.	0	0	0	0	0	0
TOTAL ORGANISMS.	–	159	28	21	597	425	869	1355	716	672	1804	736

Microscopical Examination. (*Ludlow Reservoir, six feet beneath the surface.*)

[Number of organisms per cubic centimeter.]

	1889.								1890.		
	Sept.	Sept.	Oct.	Oct.	Oct.	Nov.	Nov.	Dec.	Jan.	Feb.	Mar.
Day of examination	10	17	2	12	26	12	27	18	22	15	15
Number of sample	5156	5186	5207	5243	5280	5330	5382	5448	5551	5661	5785
PLANTS.											
Diatomaceæ	**92**	**193**	**1196**	**372**	**228**	**612**	**1390**	**940**	**660**	**465**	**557**
Asterionella	0	9	8	18	106	94	432	598	166	276	428
Melosira	90	184	1188	354	122	516	952	340	344	49	119
Synedra	2	0	0	0	0	2	6	2	150	140	4
Tabellaria	0	0	0	0	0	0	0	0	0	0	6
Cyanophyceæ	**1028**	**920**	**242**	**160**	**392**	**374**	**96**	**7**	**0**	**1**	**60**
Anabæna	30	56	60	0	260	272	88	7	0	0	0
Aphanocapsa	0	0	0	0	0	0	0	0	0	0	4
Chroöcoccus	0	0	0	0	0	10	0	0	0	0	56
Clathrocystis	10	10	10	8	24	4	0	0	0	1	0
Cœlosphærium	988	854	156	20	80	88	8	0	0	0	0
Microcystis	0	0	0	0	0	0	0	0	0	0	0
Nostocaceous spores	0	0	16	60	28	0	0	0	0	0	0
Algæ	**46**	**52**	**522**	**3272**	**666**	**116**	**196**	**76**	**44**	**12**	**72**
Chlorococcus	36	15	272	3224	598	88	160	43	21	9	70
Closterium	0	0	0	2	6	10	8	4	1	0	0

Cœlastrum	0	1	0	2	2	0	0	0	0	0	0
Dictyosphærium	0	0	46	0	30	10	0	0	0	0	0
Pediastrum	0	1	0	0	0	2	0	0	0	0	0
Protococcus	0	0	0	0	0	0	0	0	0	0	0
Raphidium	0	30	196	36	20	0	0	4	0	0	0
Scenedesmus	6	1	2	4	4	6	26	24	8	3	2
Sorastrum	0	0	0	0	2	0	0	0	0	0	0
Staurastrum	4	4	6	4	4	0	2	1	14	0	0
ANIMALS.											
Rhizopoda. Actinophrys	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Infusoria	**4**	**6**	**4**	**2**	**2**	**2**	**18**	**17**	**35**	**3644**	**2891**
Dinobryon	0	0	0	0	0	0	12	5	21	3644	2890
Monas	0	0	0	0	0	0	0	2	0	0	0
Peridinium	0	1	0	0	0	0	0	8	14	0	1
Trachelomonas	4	5	4	2	2	2	6	2	0	0	0
Vermes	**2**	**4**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**2**	**0**
Anurea	0	1	0	0	0	0	0	0	1	0	0
Polyarthra	2	0	0	0	0	0	0	0	0	1	0
Rotifer	0	0	0	0	0	0	0	0	0	0	0
Rotatorian ova	0	3	0	0	0	0	0	0	0	1	0
Crustacea	**0**	**0**	**0**	**0**	**pr.**	**pr.**	**0**	**0**	**0**	**0**	**0**
Cyclops	0	0	0	0	pr.	pr.	0	0	0	0	0
Daphnia	0	0	0	0	0	0	0	0	0	0	0
TOTAL ORGANISMS	1172	1175	1964	3806	1288	1104	1700	1040	740	4124	3580

Microscopical Examination. (*Ludlow Reservoir, six feet beneath the surface.*)

[Number of organisms per cubic centimeter.]

	1890.											
	Mar.	Apr.	Apr.	May.	May.	June.	July.	Aug.	Sept.	Oct.	Nov.	Dec.
Day of examination	28	12	26	13	31	25	15	21	17	22	19	18
Number of sample	5820	5875	5909	5961	6010	6115	6218	6422	6518	6620	6733	6825
PLANTS.												
Diatomaceæ	**1268**	**4056**	**4704**	**1824**	**410**	**143**	**68**	**1940**	**1304**	**1022**	**542**	**240**
Asterionella	490	2016	2280	1492	150	0	0	0	24	356	190	110
Melosira	328	2040	2424	332	225	143	68	1940	1280	668	350	96
Synedra	0	0	0	16	0	0	0	0	0	0	2	34
Tabellaria	0	0	0	0	35	0	0	0	0	0	0	0
Cyanophyceæ	**8**	**8**	**36**	**52**	**1120**	**506**	**710**	**54**	**644**	**97**	**220**	**19**
Anabæna	0	0	8	38	1110	490	200	0	56	5	4	0
Aphanocapsa	0	0	0	0	0	0	0	0	0	26	24	pr.
Chroöcoccus	8	0	16	0	0	0	0	28	26	44	158	13
Clathrocystis	0	2	4	10	10	3	155	12	52	6	0	6
Cœlosphærium	0	6	8	4	0	13	355	14	508	4	14	0
Microcystis	0	0	0	0	0	0	0	0	2	12	20	0
Nostocaceous spores	0	0	0	0	0	0	0	0	0	0	0	0
Algæ	**58**	**36**	**20**	**196**	**50**	**13**	**241**	**184**	**20**	**115**	**141**	**46**
Cholorococcus	50	34	8	168	35	8	38	44	2	87	86	0
Closterium	0	0	0	2	0	0	0	0	0	0	0	0

Cœlastrum	0	0	0	0	0	0	0	0	0	0	0	0
Dictyosphærium	0	0	0	8	0	0	0	0	0	0	0	0
Pediastrum	0	0	0	0	0	0	5	22	2	2	1	1
Protococcus	0	0	0	0	0	0	0	0	0	0	0	18
Raphidium	0	0	0	0	0	0	0	0	0	2	0	0
Scenedesmus	8	0	12	12	10	5	95	28	6	13	6	5
Sorastrum	0	0	0	4	0	0	13	44	4	0	0	0
Staurastrum	0	2	0	2	5	0	90	46	6	11	48	22
ANIMALS.												
Rhizopoda. Actinophrys	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**28**	**8**	**24**
Infusoria	**934**	**192**	**34**	**322**	**5**	**0**	**0**	**68**	**12**	**4**	**68**	**171**
Dinobryon	932	168	14	314	5	0	0	0	0	0	58	132
Monas	0	0	0	8	0	0	0	0	0	0	0	0
Peridinium	2	18	18	0	0	0	0	0	0	0	4	36
Trachelomonas	0	6	2	0	0	0	0	68	12	4	6	3
Vermes	**2**	**0**	**0**	**4**	**0**	**0**	**3**	**0**	**2**	**3**	**2**	**1**
Anurea	2	0	0	0	0	0	0	0	0	1	0	0
Polyarthra	0	0	0	2	0	0	0	0	0	2	2	1
Rotifer	0	0	0	2	0	0	3	0	2	0	pr.	0
Rotatorian ova	0	0	0	0	0	0	0	0	0	0	0	pr.
Crustacea	**0**	**0**	**0**	**pr.**	**0**	**0**	**0**	**pr.**	**0**	**pr.**	**pr.**	**0**
Cyclops	0	0	0	pr.	0	0	0	pr.	0	pr.	pr.	0
Daphnia	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL ORGANISMS	2270	4292	4794	2398	1585	662	1022	2246	1982	1269	981	501

DEDUCTIONS FROM THE LUDLOW RESERVOIR DIAGRAM.

A study of the diagram of the results of the chemical and microscopical examinations, Plate V., of water from six feet below the surface of Ludlow Reservoir, further elucidates a number of points of interest in relation to the connection between the light transmitting capacity of water, and the production of excessive growths of microscopical plants and animals. One explanation of such growths has been what may be termed the theory of sufficiency of food; according to which, it is assumed that excessive developments of any given organism can only occur in a particular locality, when the kind of food required by the organism is present in that locality in sufficient abundance to nourish the developing form. This theory, while, as a general proposition essentially true, is still, by reason of the existence of a number of modifying elements, hardly a full explanation of all the attending phenomena, as may be briefly pointed out.

The so-called free ammonia and the mineral nitrates, are the two chemical constituents which contribute most extensively to the nourishment of minute plants in potable water. Both of these are relatively low in Ludlow Reservoir, for the whole time covered by these observations. If, however, we examine the relation of the color line on the diagram to that of the Diatomaceæ, Chlorophyceæ, Cyanophyceæ, and Infusoria, we discover that the several maximum developments of microscopical life have all occurred, either when the color scale was decreasing, or at or near a minimum. In no case during the period from June, 1889, to December, 1890, has there been an excessive development of life while the color scale was high. Again, a rise in the color scale has apparently been followed, usually, by a decrease in the number of organisms. Again, in August, 1890, a rise of the Diatomaceæ to 1,950 per cubic centimetre, was nearly coincident with a fall of the color scale from 0.50 to 0.15. If we examine other tabulations of large developments of minute life,

as given in the Report of the Massachusetts Board, we find a number of confirmations of the general law here indicated. Some exceptions are also found, but the evidence in favor is apparently somewhat in excess of that against.

A study of the several tables, in reference to the point under discussion, indicates, further, that the excessive growths of microscopical life have usually, thus far, occurred in Massachusetts in waters of relatively low color scale; a point which, if found true on further study, must be set down to the credit of the colored waters, as indicating that they are somewhat less liable to sudden deteriorations of taste and odor than the colorless or so-called white waters. The whole subject of plant and animal development in potable waters is, however, still in its infancy, and provisional conclusions can only be drawn at present. It is not intended, therefore, to assert that the law of development of minute forms in an inverse ratio to the amount of color is yet fully proven. Its demonstration, if made at all, will follow

from further, and more accurate and elaborate, tabulations of the amount of minute life.

The question may, however, be very appropriately asked, why the theory of sufficiency of food does not fully explain the cause of the excessive development which regularly takes place not only in the Ludlow Reservoir, but in many other similar bodies of water?

A complete answer will lead to the consideration of somewhat profound questions in relation to the reproduction and development of the Protophyta and the Protozoa, and will indeed lead, figuratively, into rather deep water. The question was, however, ably discussed by Alexander Braun, forty years ago, in his "Rejuvenescence in Nature."[99] Braun takes up the question more especially in its relation to the life and development of plants, and shows that among the cryptogams, at any rate, there are alternating periods, on the one hand, of moderate reproduction, and, on the other, of extraordinary reproduction; that during the first period, the life

forces of the plant are gradually conserving themselves for the necessarily excessive effort required in the second. There is, therefore, an alternation of generations, the respective periods of which are as yet indeterminate; and we may conclude that the excessive development of minute life which has characterized water-supplies suffering from bad tastes and odors, is merely a manifestation of one phase of such alternation; but why, in many cases, occurring at irregular intervals, we are, as yet, unable to definitely say.

An explanation of this irregularity of appearance of these troubles may be found in the case of some of the Cryptogams, in the consideration that the spores, after a period of activity, enter into a resting state, and only re-awaken to a new life after more or less complete desiccation and resubjection to moisture. It is quite possible, in this view, that many years may intervene between periods of such disturbances of a water supply by any given cryptogam.

Returning for a moment to the subject

of colored water, the statement can be now made, that while a given colored water may be the subject of an excessive development of plant life, nevertheless, other things being equal, the development would be for many species more pronounced, when once started, in either a colorless water or one of low color scale, than in waters of high, or relatively high, color scale. The decrease in intensity, or the modification in quality, of light, resulting from the presence of the coloring matter may be expected to exert a modifying influence on the activity of any given cryptogamic development.

In reference to the Ludlow Reservoir it may be said, by way of concluding the subject, that the old, swampy bottom of this reservoir must be considered as unfavorable for maintaining a high degree of freedom from cryptogamic growths. Such a location may be expected to contain the accumulated resting spores of various Cryptogams for many years. The original flooding of the reservoir started this accumulation of resting spores into vigor-

ous life; the energy of the development being possibly proportionate to length of the period of rest. The original construction should, therefore, have included the sanding of the entire bottom to the depth of at least two feet, instead of the worst portions to the depth of a foot and a half. The existing conditions can probably be improved by correcting the shallow flowage at the upper end, and keeping the reservoir as nearly full as possible: we thereby eliminate the opportunity which now exists for an annual desiccation and revivification of spores. Systematic observations of the kind, made in 1889 and 1890, will be likely, then, to determine if any marked decrease in number and variety of organisms is taking place, as may be expected if the foregoing theory is approximately true. It is, however, exceedingly doubtful if there will be any marked improvement so long as the alternate covering and uncovering of the shallow portions at the upper end furnishes annually the ideal conditions for active cryptogamic development. All that can

be hoped for, with present conditions, is that the generations of little plants and animals may in time exhaust themselves: as yet there is absolutely no data for predicting when this will take place.

The water supply of Brockton, Mass., presents a case of a water in which the color is usually high (the minimum from June, 1889, to December, 1890, is 0.45, the maximum 1.30, and the mean 0.85), and which is also the source of an abundant cryptogamic life. A comparison of the tabulations indicates that here, also, the general law of relation of the development of the plants to intensity of light, as indicated by the color scale, is found to fairly hold good as shown in the case of Ludlow Reservoir.

The foregoing is a skeleton of the new art of the quantitative microscopical examination of potable water. The list of literature will show the several sources of useful information.

LITERATURE.

THE following list of books, journals, and miscellaneous papers does not in any sense exhaust the several subjects. With three or four exceptions it includes only those either in the author's own collection, or to which he has, at various times, had access. A considerable number, both of books and papers of little value for actual work at the present time, have been omitted; and the list may, therefore, be taken as including, so far as the author can judge from his own experience, only those likely to be of utility, either in studying the sanitary relations and biology of a public water supply and cognate questions, or in making the microscopical examination of potable water. Those who desire a more complete bibliography are referred to the volumes in the following list, which are specially indicated by the double asterisks thus,** where exhaust-

ive lists of the several special subjects may be found. The Natural History Catalogues of W. P. Collins, 157 Great Portland Street, London, W., may also be profitably consulted for lists of books and papers on the various forms of microscopical life.

A very few books on the microscope are included in this list, to which the objection may be made, that they are of a popular character rather than scientific. To this objection it may be stated, that all such which are included have been of use to the author by furnishing some fact not found elsewhere; and it is with the expectation that they may be of similar use to others that they appear here.

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(*b*) Report of the Biologist, G. H. Parker. Nineteenth An. Rept.

(*c*) Special Report on Water Supply and Sewerage, 1890. Part I. contains: (1) The Chemical Examination of Waters and the Interpretation of Analyses, by Dr. T. M. Drown; (2) Report upon the Organisms, excepting the Bacteria, found in the Waters of the State, by G. H. Parker; (3) Summary of Water Supply Statistics, by F. P. Stearns; (4) Classification of Drinking Waters of the State; (5) Special Topics Relating to the Quality of Public Water Supplies, by Messrs. F. P. Stearns and Dr. T. M. Drown; and (6) the Pollution and Self-Purification of Streams, by F. P. Stearns.

Part II. contains: (1) A Report of the Chemical Work of the Lawrence Experiment Station, including Methods of Analysis, and some Investigations of the Process of Nitrification, by Messrs. Dr. T. M. Drown and A. Hazen; (2) A Report of the Biological Work of the Lawrence Experiment Station, including an account of Methods Employed and Results Obtained in the Microscopical and Bacteriological Investigations of Sewage and Water, by Wm. T.

Sedgwick; (3) Investigations upon Nitrification and the Nitrifying Organism, by E. O. Jordan and Ellen H. Richards. (In addition, the general subject of water supply, its purification, and the purification of sewage, are all treated exhaustively in this Special Report.)

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