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EXAMINATION OF WATER.

(CHEMICAL AND BACTERIOLOGICAL.)

BY

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PREFACE.

KNOWLEDGE of ordinary quantitative analysis is here necessarily assumed; therefore the merest suggestions are given for determination of the mineral matters present in a water, while the items properly lying within the scope of a sanitary examination are dealt with more at length.

Upon the bacteriological side, only so much is touched upon as has been demonstrated to be of real service to the water examiner; leaving the great field of ultimate differentiation to be further explored, and rendered still more practically useful, by the professed bacteriologist.

RENSSELAER POLYTECHNIC INSTITUTE,
TROY, N. Y.,
FEBRUARY, 1899.

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EXAMINATION OF WATER.

CHAPTER I.

INTRODUCTORY.

A GREAT deal of popular misconception exists upon the subject of the analysis of potable water, and it is commonly supposed that such an examination may be looked upon from practically the same point of view as the analysis of an iron ore. That this belief is founded on fallacy may, however, be readily shown. When an iron ore is submitted for analysis, the chemist determines and reports upon the percentages of iron, phosphorus, sulphur, etc., found therein ; and at that point his duties usually cease, inasmuch as the ironmaster is ordinarily capable of interpreting the analysis for himself. Even should the analyst be called upon for an opinion as to the quality of the ore, the well-known properties of the several constituents make such a task an easy one, and, assuming the sample to have been fairly selected, the opinion may be written without any inquiry as to the nature of the local surroundings whence the ore was taken.

A water-analysis, on the other hand, is really not an analysis at all, properly so called, but is a series of experiments undertaken with a view to assist the judgment in determining the potability of the supply. The methods of conducting these experiments are largely influenced by the individual preferences of the analyst, and are far from being uniform or always capable of comparison, thus often introducing elements of confusion where two or more chemists are employed to analyze the same water. Some of the substances reported—"albuminoid ammonia," for instance—do not exist ready formed in the water at all, and are but the imperfect experimental measures of the objectionable organic constituents, which our present lack of knowledge prevents our estimating directly.

Thus the numerical results of a water-analysis are not only unintelligible to the general public, but are not always capable of interpretation by a chemist, unless he be acquainted with the surroundings of the spot whence the sample was drawn, and be posted as to the analytical methods employed.

It is very common for water to be sent for analysis, with the request that an opinion be returned as to its suitability for potable uses, while at the same time all information as to its source is not only unfurnished, but is intentionally withheld, with a view of rendering the desired report unprejudiced in character.

Such action is not only a reflection upon the moral quality of the chemist, but it seriously hampers him in his efforts to formulate an opinion from the analytical results.

For instance, a large quantity of common salt is a cause for suspicion when found in drinking-water, not because of any poisonous property attaching to the salt itself, but because it is usually difficult to explain its presence in quantity except upon the supposition of the infiltration of sewage. Thus an amount of salt sufficient to condemn the water from a shallow well in the Hudson valley could be passed as unobjectionable if found in a deep-well water from near Syracuse, N. Y.

Hence we see how important it is for the chemist to be fully acquainted with the history of the water he is to examine in order that he may compare his results in " chlorine " with the " normal chlorine " of the section whence the sample is taken.

A knowledge of the history of the water is no less important in order to interpret the remaining items of a water-analysis. Some time since a water was sent from Florida to the author for examination, and was found to contain 1.18 parts " free ammonia " per million. Much " free ammonia " commonly points to contamination from animal sources, and had it not been known that the water in question was derived from the melting of artificial ice made by the ammonia process the enormous quantity of ammonia found would have condemned it beyond a peradventure. As it was, the water was pronounced pure, the other items of the analysis having been found unobjectionable.

Analytical results which would condemn a surface-water may be unobjectionable for water from an artesian well, for the reason that in the latter case high figures in " free ammonia," " chlorine," or " nitrates " are often capable of an explanation other than that of sewage-infiltration. Even though such a water should, at a previous period, have come in contact with objectionable organic waste material, yet the intervening length of time and great distance of underground flow would probably have furnished abundant opportunity for thorough oxidation and purification.

" Deep " samples taken from the same lake, at the same spot and depth, will greatly vary in analytical results if the temperature of the water at the several dates of sampling should be markedly different, owing to the disturbing influence of vertical currents.

Again, suppose it is desired to determine whether or not the water of a large stream is so contaminated with up-stream sewage as to be unfit for a town supply. An analysis of the water taken from the site of the proposed intake would very possibly be valueless, because the enormous dilution to which the admitted sewage would have been subjected would remove from the analytical results everything of an absolute character. Examinations of any real value in such cases should always be of a comparative nature. Samples should be taken above and below the point of contamination and again at the proposed intake. If the difference between the first and second samples, which is a measure of the pollution, be maintained, or nearly so, at the point of intake, then the water should be condemned, no matter how completely the analytical results fall within the limits of the so-called standards of organic purity.

Thus it is that a chemist must be in full possession of all the facts concerning the water which he is asked to examine, in order that his opinion as to its purity may be based upon the entire breadth of his past experience, for in no branch of chemical work are experience and good judgment better exercised than in the interpretation of a water-analysis.

As Nichols has well said, " It is a great mistake to suppose that the proper way to consult a chemist is to send a sample of water in a sealed vessel with no hint as to its source. On the contrary, the chemist should know as much as possible as to the history and source of the water, and, if possible, should take the samples himself."

However faithfully the various laboratory tests may be applied to decide the question of the fitness or unfitness of a certain water for dietetic purposes, there is nothing upon which greater stress should be laid than a thorough personal knowledge of the surroundings of the source of supply. In other words, it is essential to make a careful and thorough " sanitary survey."

It was years ago laid down as a golden rule " never to pass judgment upon a water the history of which is not thoroughly known," and the nearer this maxim can be lived up to to-day the fewer will be the mistakes in the reports issued.

CHAPTER II.

CHEMICAL EXAMINATION OF WATER.

In the taking of samples for so important a matter as a town supply the chemist should unquestionably personally superintend their collection ; but for individual outlying waters printed instructions frequently have to be depended upon. Those issued by the author are as follows:

DIRECTIONS FOR TAKING A WATER-SAMPLE.

Large glass-stoppered bottles are best for sampling, but as they are seldom at hand, a two-gallon *new* demijohn * should be employed, fitted with a *new* soft cork. Be careful to notice that no packing-straw or other foreign substance yet remains in the demijohn, and thoroughly rinse it with the water to be sampled. Do not attempt to scour the interior of the neck by rubbing with either fingers or cloth. After thorough rinsing, fill the vessel to overflow-

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* Stoneware jugs are not admissible for collecting water samples. Instances are known of masses of the salt used for glazing remaining in the interior.

ing so as to displace the air, and then completely empty it.

If the water is to be taken from a tap, let enough run to waste to empty the local lateral before sampling; if from a pump, pump enough to empty all the pump connections; if from a stream or lake, take the sample well out from the shore, and sink the stoppered sampling-vessel towards mid-depth before removing the stopper (or cork), so as to avoid both surface-scum and bottom mud.

In every case fill the vessel nearly full, leaving but a small space to allow for possible expansion, and cork securely. Under no circumstances place sealing-wax upon the cork, but tie a piece of cloth firmly over the neck to hold the cork in place. The ends of the string may be afterwards sealed if necessary.

Bear in mind, throughout, that water-analysis deals with material present in very minute quantity, and that the least carelessness in collecting the sample must vitiate the results. Give the date of taking the sample, as full a description as possible of the soil through which the water flows, together with the immediate sources of possible contamination.

Having secured the sample, the analysis should be begun at once, for the reason that water is liable to rapid changes in character during laboratory storage. For instance, the following analyses are of the same sample of water from the laboratory tap, drawn

November 10, and allowed to stand in the sampling-bottle at ordinary room temperature :

	Nov. 10	Nov. 12	Nov. 13	Nov. 14	Nov. 15	Dec. 15
Free ammonia..........	.037	.042	.042	.050	.075	.060
Albuminoid ammonia....	.220	.178	.191	.175	.155	.205
Chlorine...............	4.5	......	......	......	......	......
N in nitrites...........	trace	trace	trace	trace	trace	none
N in nitrates...........	.50	.525	.55	.60	.60	.60
Required oxygen........	4.35	4.6	4.2	4.4	4.1	4.6
Total solids.............	140	......	......	......	......	......

This water shows gradual oxidation of the nitrogen contents to nitrates, but on the whole is fairly stable. As showing, on the other hand, how rapid and how irregular the storage changes may at times be, the following analyses by Liversidge are given :*

	Horse-pond.		Fish-pond.		Peaty Water.	
	Free Ammonia.	Albuminoid Ammonia.	Free Ammonia.	Albuminoid Ammonia.	Free Ammonia.	Albuminoid Ammonia.
December 11............	10.00	7.00	0.12	0.90	0.72	0.19
" 12............	2.00	2.00	0.11	0.92	1.12	0.04
" 13............	8.00	4.00	0.16	1.04	1.12	0.13
" 15............	7.00	4.00	0.16	1.03	1.08	0.12
" 16............	6.00	2.00	0.38	0.69	0.03	0.04
" 19............	5.00	2.00	0.52	0.56	0.02	0.03
" 20............	4.00	1.00	0.70	0.38	0.01	0.01
" 21............	2.00	0.50	0.90	0.30	......	......
January 8	0.50	0.25	1.38	0.06	......	......
" 10	0.07	0.07	1.50	0.04	......	......

These are, of course, exaggerated cases containing high ammonias, but they serve to point out the neces-

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* *Chem. News,* lxxi. 249.

sity of avoiding delay between the collection of the sample and the beginning of the analysis. At the most, very few days should intervene.

Another example, chiefly interesting as showing the successive steps in the oxidation of organic nitrogen, is here given.* Eighty samples of water, including all classes of surface-water, were examined at various intervals after standing, and gave the following results :

“ The organic matter in suspension decays in about seven days, as is shown by the increase in ‘ free ammonia.’ In about fourteen days this ‘ free ammonia ’ has disappeared, and ‘ nitrite ’ has taken its place, reaching a maximum in about twenty-one days. Later the ‘ nitrite ’ also disappears, and in twenty-eight days, or more, all the nitrogen has been converted into the form of ‘ nitrate.’ When the suspended matter is removed by filtration through paper, or by precipitation with alumina, no change occurs, unless free ammonia were present at the outset.”

Hitherto no small confusion has existed, on account of the many ways in which the results of water-analyses were stated, but this difficulty, it is to be hoped, will be gradually done away with by the acceptance of the method recommended in the report

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* Report of the Massachusetts Board of Health, 1890, 365.

of the committee of the American Association for the Advancement of Science, appointed to examine into this and other water questions.*

The committee recommended that all results be given in *parts per million in weight.* This method has the advantage that a litre, or fraction thereof, of water, having been operated upon, and the substances found having been determined in milligrammes, no long arithmetical calculations will be required.

Of course the assumption is made that a litre of water weighs a kilogramme—a true enough statement for potable waters, but one capable of introducing error where mineral waters are dealt with, whose specific gravities are appreciably above unity. In such a case the water to be analyzed is actually weighed, or else its weight is estimated from the known specific gravity and volume.

Water should not be filtered before analysis. If sediment be present, it should be equally diffused by thorough shaking before measuring.

The reason for this is that a water-analysis should represent the water as the consumer uses it, and not in a condition improved by filtration.

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* The preliminary report of the committee may be found in the *Journal of Analytical Chemistry*, vol. iii., page 398.

Water-analysis cannot be properly conducted in a general laboratory, because many of the tests would be ruined by the fumes common to such a locality. A separate room, reserved exclusively for water-work, is the best arrangement. The author finds it convenient to have the woodwork therein painted white and to have a broad titration shelf fixed across the window, with a black curtain capable of being pulled down to the level of the titration-dish. Excellent conditions are thus given for noting slight differences in colors.

The appearance and turbidity of a water is best determined by using a brass tube 2½ inches in diameter and 2 feet long, closed at the ends by disks of quarter-inch plate glass held in place by screw-caps (Fig. 1).* Such tubes are easily cleaned and give excellent satisfaction. For the purpose of measuring the amount of turbidity, a standard is prepared, consisting of one gramme of exceedingly fine kaolin (obtained by elutriation) suspended in one litre of distilled water. Each c.c. of this preparation will contain one milligramme of suspended clay.

Having nearly filled the duplicate observation-tube with distilled water, enough of the "clay standard"

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* Such tubes may be obtained from Richards & Co., No. 30 East 18th Street, New York City.

is added to make the "turbidity" equal to that of the water under examination in the other tube. Knowing the volume of water operated upon and the amount of "clay standard" used, the "turbidity" expressed in parts per million can readily be calculated. It should be noted that although the tube containing the "clay standard" is, of necessity, not completely full, yet by inclining its barrel towards a horizontal position the required observation depth of two feet is easily obtained.

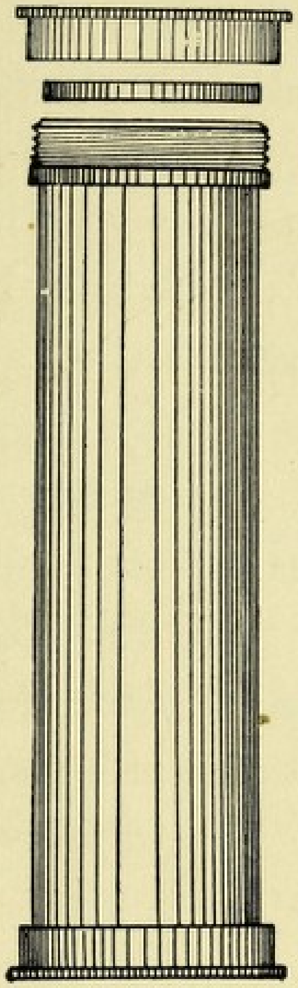

FIG. 1.

Any quickly subsiding material present should be classed as "sediment" rather than "turbidity." To determine the same it would be best to decant the water from above such deposit and then catch it upon a weighed filter or in a Gooch crucible.

Odor and Taste.—It is customary to report such odor and taste as a water may possess, although in the great majority of cases very little information is derived from such examination, because of the frequency of negative results. A good water may be possessed of a slight marshy odor, while one of extremely dangerous character may be limpid, tasteless, and odorless. The test is best applied before and after heating the water nearly to the boiling-point, and after thorough shaking in each case.

Both taste and odor are sometimes very pronounced, as when such organisms as "Asterionella" are present in quantity. Fortunately, disease has never been traced to such occurrence, however objectionable the water may be from an æsthetic standpoint. Organisms of this character are revealed by a microscopical examination.

Temperature.—A cool water should, if possible, be supplied for public use, but studies of temperature are rare, for the sufficient reason that considerations of much greater weight determine the selection of a source of supply.* Should many temperature readings in deep water, as in a lake, be decided upon, no better device could be chosen for the work than the "thermophone," invented by Warren and Whipple. The following is clipped from a description issued by the present makers, E. S. Ritchie & Sons, Brookline, Mass.:

"The thermophone is an electrical thermometer of the resistance type. It is based upon the principle that the resistance of an electrical conductor changes with its temperature, and that the rate of change is different for different metals.

"The operation of taking a reading is as follows: Having connected the leading wires to the proper binding-posts of the indicator-box, the current is turned on and the telephone held to the ear. A buzzing sound in the telephone is found to increase or diminish according as the pointer is made to approach or recede from a certain section of the dial. By moving it back and forth a position may be found where the telephone is silent. When at this point, the hand indicates the temperature of the distant coil. Instruments of ordinary atmospheric range, i.e., from 15° to 115° F., may easily be read to 0.1° even by an

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* The extreme variation of temperature for Croton water, as delivered by the street hydrants in New York City, for the year 1894 was:

On February 24	34° F.
On August 4	76° F.

inexperienced observer. With a smaller range, or with an instrument having a larger dial, a greater precision may be obtained.

“It is more sensitive than a mercurial or other expansion thermometer, because the rate of change of resistance per degree is greater than the rate of expansion of liquids or solids, and, moreover, slight changes in resistance may be more easily and accurately measured than slight changes in length or volume.

“It sets quicker than most mercurial thermometers. In obtaining the temperature of water of various depths one minute has been found to be sufficient time to allow for setting.

“It is independent of pressure.” [*]

The reaction of potable water is commonly slightly alkaline, although waters holding much free acid in solution, usually sulphuric, are by no means rare.

Determination.—Place 100 c.c. of the water in a casserole, and titrate with N/10 hydrochloric acid, using methyl orange as an indicator. Should the water be originally acid, make it slightly alkaline with a known

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[*] The deepest sounding found on the Challenger expedition was in lat. 11° 24′ N., long. 143° 16′ E. The depth was 4475 fathoms.

Temperature of bottom-water.................. 33.9° F.
“ of surface-water.................. 80° F.

Most of the thermometers employed were crushed by the great pressure of five tons per square inch.

amount of potassic hydrate before titration. It is convenient to report alkalinity as representing so many parts of $CaCO_3$ per million of water, and to note that such a form of result is quickly obtained by multiplying the number of c.c. of hydrochloric acid, used in the above titration, by fifty.

Acidity is best stated in the same terms using a negative sign.

Color.—Prof. A. R. Leeds has proposed the most convenient method for stating the color of a water.* Observations are made by the use of 50-c.c. "Nessler" jars, and unity of color is that caused by "nesslerizing" 1 c.c. of the standard ammonium chloride solution, diluted to 50 c.c. with ammonia-free water, in exactly the same manner as in the determination of "free ammonia." (Page 53.)

Turbid waters should be filtered before reading the color.

The investigations of the Boston Water Board show that both iron and manganese often enter largely as a cause of color in water from the stagnant layer of a deep pond, but the color of a purely surface water is mainly due to solution of organic material.†

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* For cases of special investigation the Hazen "platinum" standard gives most excellent results, although somewhat more difficult to apply. (*Am. Chem. J.*, xiv. 300.)

† An excellent paper by Mrs. Ellen H. Richards on "The Coloring Matter of Natural Waters" is published in *J. Am. Chem. Soc.*, January, 1896.

Having accomplished the foregoing preliminary observations, the examination proper comes now in order ; but before going further a word should be said upon the vexed question of "standards for interpretation of analytical results."

A hard-and-fast "standard" is simply an impossibility, as was indicated at the opening of this chapter. Results which would be considered satisfactory for one locality might be entirely inadmissible in another. Local standards are the proper ones by which to be guided, and it is to be regretted that local "normals" are not more frequently found on record.

For Massachusetts and Connecticut the information is more full, as is instanced by the fine charts of "normal chlorine" prepared by the State Boards of Health.

Following the description of each analytical process to be given hereafter there will be found a paragraph headed "Comparates," but the expression must not be permitted to mislead. The author's intention is simply to place before the reader sundry data and the opinions of various authorities, and he absolutely disclaims any desire to set boundaries to the free use of the analyst's good judgment. The term "comparates" is possibly a poor selection, but, with the above explanation, it will serve in place of a more lengthy expression.

TOTAL SOLIDS.

Source.—Material dissolved or suspended in water is naturally derived from the strata through which the water passes, or the surface over which it flows. Thus are obtained waters of all degrees of hardness (see "Hardness") and of great variety of color and turbidity.

Determination.—Thoroughly shake the vessel containing the sample and then measure out 100 c.c. of the unfiltered water by means of a pipette into a weighed platinum dish.

Evaporate to dryness on the water-bath, being careful to place a filter-paper between the dish and the water in the bath in order to prevent any deposit of impurities on the under side of the dish. (A better plan is to make use of a porcelain water-bath filled with distilled water.) When dry, place the dish and contents in an air-bath and maintain the temperature at 105° C. for half an hour.* Cool in a desiccator and weigh. Replace in the air-bath and repeat the weigh-

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* M. Albert-Levy, of the Montsouris laboratory, Paris, dries this residue at 180° C. for twelve hours before weighing. He gives the following illustration of how differently various waters act when dried at 125° C. and 180° C.:

	125°.	180°.
Vanne	231	231
Ourcq	483	445
Marne	328	289
Drain St. Maur	300	290

ing at intervals of half an hour until a constant weight be obtained. The final weight, less the known weight of the dish, will give the amount of total solids. This weight multiplied by ten will give the weight of solids per litre of water, which, expressed in milligrammes, will represent parts per million.

The "*loss on ignition*" is obtained by gradually raising the dish and its contents to redness and reweighing after cooling in a desiccator.

It is important to note that while no quantitative results of much value are to be expected from the ignition in question, yet considerable insight may often be obtained as to the character of the water by observing the intensity of the charring and the presence or absence of fumes.

As Dr. Smart says: "The blackening during the process is of more interest than the mere loss of weight. No matter how few parts are lost, if the lining of the capsule blackens all over and the carbon is afterward dissipated with difficulty, the water is to be viewed as suspicious. What are called 'peaty' waters here constitute the exception." *

Angus Smith pointed out that "in waters containing nitrates and nitrites no organic matter would be apparent on burning unless more should be present than these salts could oxidize"—a fact always to be borne in mind.

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* Report Nat. Board of Health, 1880.

*Comparates.**

Average in sundry waters known to be pure....	69
" " " " " " " polluted..	725

These averages are really of but small sanitary value, for the reason that a polluted water may be low in total solids, or *vice versa*, according to the character of the soil through or over which the water flows.

The Rivers Pollution Commission of Great Britain gives as averages out of 589 samples of unpolluted waters analyzed for total solids:

Rain	29.5
Upland surface................	96.7
Deep well....................	432.8
Spring	282.0

Dr. Smart (Nat. Board of Health, 1880) :

Safe limit....................	300
To be condemned.............	1000

A. R. Leeds (Water Depart. Wilmington, 1883) :

Standard for American rivers...	150 to 200

Wanklyn regards as permissible.	575

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* See page 18.

HARDNESS.

Before entering into the question of quantitative estimation, let it be premised here that "hardness" may be classified under two heads, viz., "Permanent" and "Temporary." The former is occasioned by the presence of calcium sulphate, and other soluble salts of calcium and magnesium, not carbonates, held in solution by the solvent action of the water itself; such a water cannot be materially softened by boiling under ordinary pressure.

"Temporary" hardness is caused by carbonates of calcium and magnesium held in solution by carbonic acid present in the water. Boiling such a water expels the carbonic acid, whereupon the salts separate from solution.*

Many samples of water possess both "permanent" and "temporary" hardness, and the analyst is at times called upon to report each separately; but

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* With some it is considered that the calcium is present as a soluble bicarbonate which breaks up upon boiling into carbonic acid gas and insoluble normal carbonate; but, as A. H. Allen says, it is not necessary to assume the existence of calcium bicarbonate (which compound has never been isolated) in order to account for the solubility of calcium carbonate. One water which he examined evolved very small quantities of carbon dioxide on boiling, and yet the precipitated calcium carbonate was large in amount. He considers it "probable that calcium carbonate is capable of existing in a soluble colloid condition, changing, on boiling the liquid, to the ordinary insoluble modification." (*J. Soc. Chem. Ind.*, vii. 801.)

more commonly the total hardness covers all that is required.

Ordinary hard soap is somewhat complex in structure, but for practical purposes we may consider it to consist of sodium stearate, $NaC_{18}H_{35}O_2$. This salt, coming in contact with the calcium carbonate or sulphate contained in a hard water, is immediately decomposed, with formation of insoluble calcium stearate acording to the following equations :

$$CaCO_3+2NaC_{18}H_{35}O_2=Ca(C_{18}H_{35}O_2)_2+Na_2CO_3$$

or

$$CaSO_4+2NaC_{18}H_{35}O_2=Ca(C_{18}H_{35}O_2)_2+Na_2SO_4.$$

Of course none of the soap can be depended upon for detergent purposes until all the calcium salts present have been thus provided for; hence the enormous waste resulting from the use of some waters may readily be imagined.*

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* "While no exact rule can be given for estimating the increased expense to a community caused by the use of hard water, in general it may be said (*Eng. News*, January 31, 1885) that each grain of carbonate of lime per gallon of water causes an increased expenditure of 2 ounces of soap per 100 gallons of water. The Southampton water contains about 18 grains of lime and magnesian salts per gallon. With such hard water it is probable that the increased expense for soap in a household of five persons would amount to at least $5 to $10 yearly; hence the inhabitants could afford to pay a higher water-rate by the amount of this difference for a soft-water supply." (*Engineering News*. April 16, 1892.)

In undertaking the estimation of hardness advantage is taken of the reaction above stated. A solution of soap of known strength is prepared, and is then poured into a given quantity of the water to be examined, until a permanent lather is formed, whereupon, from the known quantity of soap used, the amount of " hardening " salts present may be calculated.

This soap test, commonly known as Clark's, is not accurate, and is in some respects unscientific; but it is not without value, especially in a locality such as Troy, N. Y., where the enormous laundries use soap by the ton.

Soap Solution.—From a new cake of Castile (Syria) soap scrape ten grammes of shavings. Dissolve them in one litre of dilute alcohol ($\frac{1}{3}$ water). If not clear, filter, and keep tightly stoppered.

Standardizing the Soap Solution.—Carefully weigh out one gramme of pure $CaCO_3$. Dissolve in a little HCl. Neutralize with a *slight* excess of NH_4OH and dilute to one litre. Each cubic centimetre of this solution will contain an amount of calcium salt equivalent to one milligramme of $CaCO_3$.

Place 10 c.c. of this solution in an eight-ounce glass-stoppered bottle, make the volume up to 100 c.c. with pure water, and run in the prepared soap solution from a burette, little by little (shaking after each addition), until a lather be formed which persists for five minutes. Even when the amount of soap solution required is approximately known, never add more than half a cubic centimetre at once, and never fail to shake after such addition.*

Note the amount of soap solution used. Now repeat the experiment, using 100 c.c. of pure water only (no calcium salt solution), and again note the amount of soap solution required. This second reading will give the amount of soap solution (no inconsiderable quantity) used up by the 100 c.c. pure water, and by subtracting the same from the reading obtained in the first instance knowledge will be reached of the quantity of soap required for the calcium salt alone. Estimate now the value of 1 c.c. soap solution in terms of calcium carbonate and record the result on the bottle. Perhaps an example would be in keeping:

8.2 c.c. soap solution are required for 10 c.c. $CaCO_3$ solution + 90 c.c. water.

0.6 c.c. soap solution are required for 100 c.c. water.

Hence

7.6 c.c. soap solution are required for 10 mg. $CaCO_3$ only.

Hence

1 c.c. soap solution corresponds to 1.316 mg. $CaCO_3$.

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[*] See *Chem. News*, August, 1886.

Always place the date of standardizing on the bottle, and re-standardize frequently, as the soap solution is not permanent.

Determination.—Place 100 c.c. of the water in the eight-ounce bottle, run in the standard soap solution in the manner already stated, read off the amount required, multiply by the known value for 1 c.c. soap solution, multiply this again by ten, and there will be obtained the hardness expressed in so many parts of $CaCO_3$ per million of water.

It was formerly customary to report hardness in "degrees" rather than parts per million, but the difficulty of deciding which of the several systems of degrees was referred to provoked so much confusion that a change was made to the present simpler mode of expression.*

Should a report of both temporary and permanent hardness be called for, the soap test must be made both before and after boiling.

Any loss in volume due to the boiling must be, of course, made up, and any precipitate of calcium carbonate removed by filtration before adding the soap solution.

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* In England the Clark scale is still in use. Each degree corresponds to one grain of $CaCO_3$ per imperial gallon of water, i.e., one part in seventy thousand. Below 6 degrees is considered soft.

If the hardness due to salts of magnesia be required separately, shake the water up with a little solid ammonium oxalate, filter off the precipitated calcium oxalate on a dry filter, and determine the hardness in the filtrate.

When a water is so hard as to require a greater amount of soap solution for the 100 c.c. of the water than suffices to saponify 23 mg. $CaCO_3$, better results are obtained by diluting the water with an equal bulk (or more, if necessary) of pure water, inasmuch as too heavy a precipitate of the calcium stearate appears to interfere with the proper lathering. Of course the influence of the additional quantity of water must be allowed for.

For constant results the hardness of a water should be taken at a temperature of 15° C.*

Leeds's method † for determining "permanent hardness" is very convenient: The measured water is boiled with a known excess of Na_2CO_3. Precipitated $CaCO_3$ is filtered off, and the remaining Na_2CO_3 determined by titration with standard acid and methyl-orange. The loss in Na_2CO_3 is calculated to a corresponding amount of $CaSO_4$.

"Temporary hardness" is considered equal to the "alkalinity," previously determined. See page 16.

Comparates.—The average hardness of good waters

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* *J. Chem. Soc.*, lxiv. ii. 347.

† Modification of Hehner's method, see *Analyst*, viii. 77.

as given by the British Rivers Pollution Commission stands :

Rain	3
Upland surface	54
Deep well	250
Spring	185
Wanklyn allows	575

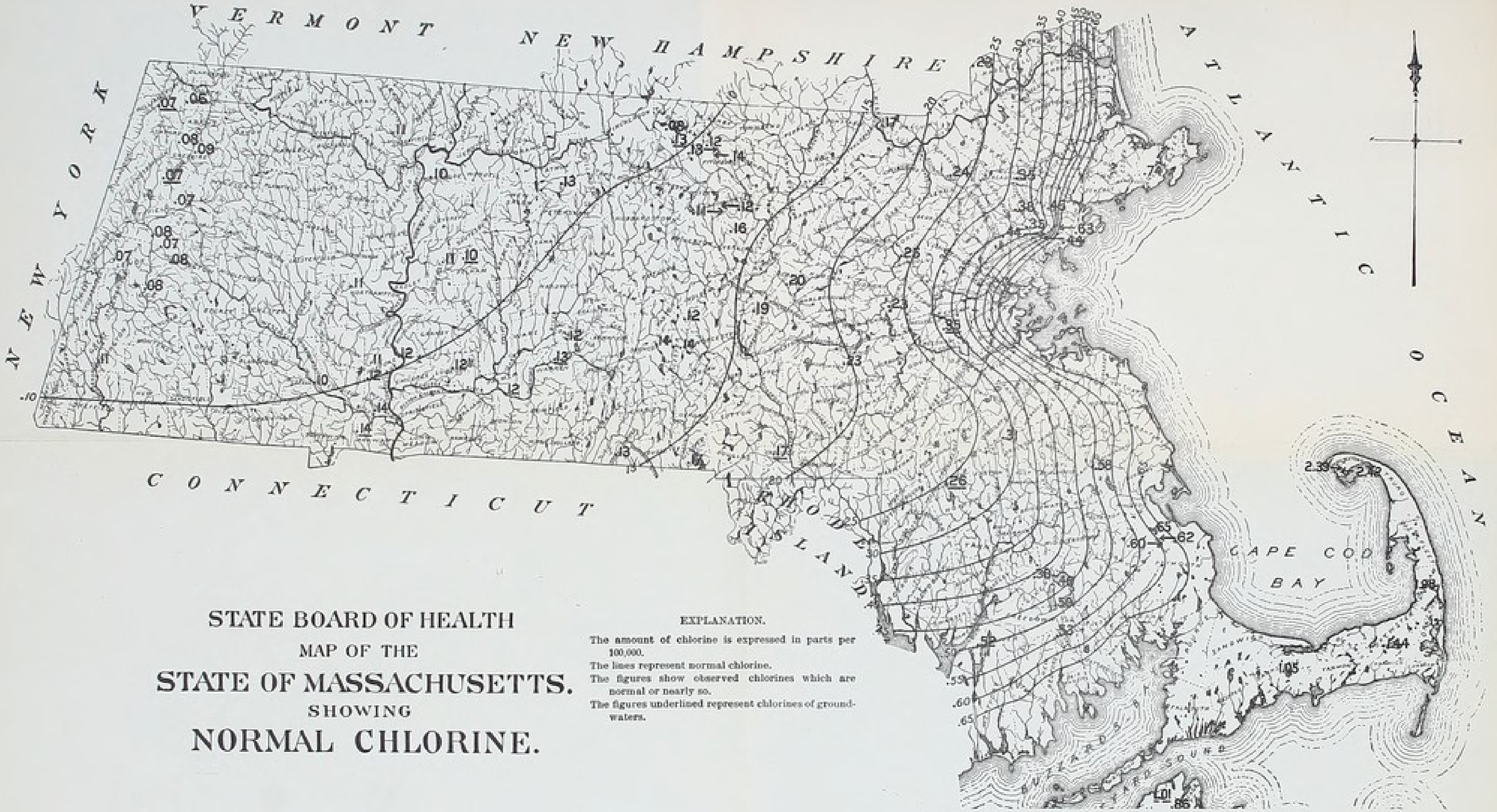
Leeds's standard for American rivers, 50 for soft, 150 for hard.

CHLORINE.

Water is rarely found free from chlorine, yet, notwithstanding its almost constant presence, there is hardly a factor in the sum total of water-analysis towards which attention is more quickly turned, or regarding which there is closer scrutiny.

Excepting in unimportant instances, chlorine is present in the form of common salt, washed from the air or soil, or added as one of the constituents of sewage. Salt itself is, of course, unobjectionable in the quantity usually present, but, it being so largely used in our food, there is always warrant for suspecting sewage contamination where the figures for chlorine run high.

True it is that those figures are at times misleading, but they, like other data in water-analysis, must



STATE BOARD OF HEALTH
MAP OF THE
STATE OF MASSACHUSETTS.
SHOWING
NORMAL CHLORINE.

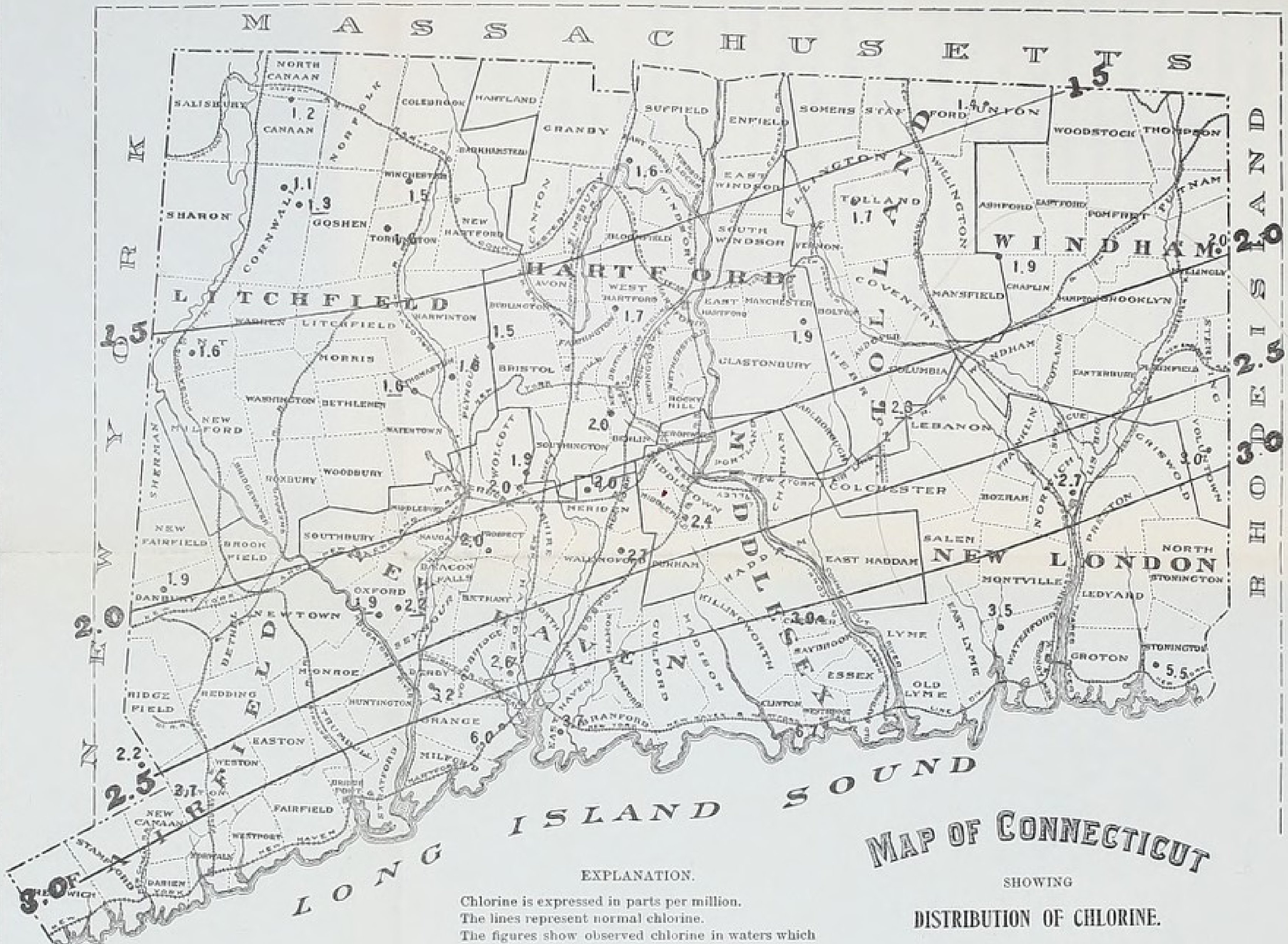
EXPLANATION.

The amount of chlorine is expressed in parts per 100,000.

The lines represent normal chlorine.

The figures show observed chlorines which are normal or nearly so.

The figures underlined represent chlorines of ground-waters.



MAP OF CONNECTICUT

SHOWING

DISTRIBUTION OF CHLORINE.

SCALE—7½ miles per inch.

EXPLANATION.

Chlorine is expressed in parts per million.
The lines represent normal chlorine.
The figures show observed chlorine in waters which are normal or nearly so.
Figures underlined are from ground-waters.

be considered with judgment, and due weight be accorded the character of local surroundings.

If the district whence the water comes be naturally rich in salt, as is the case with the deep-seated waters of Central New York, such fact must be borne in mind when formulating an opinion as to quality. Comparison should be made with a local water, of the same general character, known to be pure ; and for that purpose State maps, such as those issued for the States of Massachusetts and Connecticut, are most valuable, and their construction is well worth the expenditure of public money.

The influence of the sea upon the "normal chlorine" of these two States is made apparent by the charts referred to.

Such influence is naturally more marked in an insular country like England. Thus, Prof. Kinch reports the average amount of chlorine in the rain-water collected at Cirencester, England, during a period of twelve years to be 3.36 parts per million.

Variation in the chlorine-contents of rain-water always occurs inland, although not to the same degree as upon the coast. For instance, the mixed monthly rain and melted snow at Troy, N. Y., contains the following amounts of chlorine :

January............	2.50	per	million.
February............	1.07	"	"

March	1.55	per million
April	0.75	“ “
May	1.25	“ “
June	1.15	“ “
July	1.05	“ “
August	2.00	“ “
September	0.60	“ “
October	3.00	“ “
November	2.25	“ “
December	2.50	“ “
Mean	1.64	“ “

While not strictly city rain-waters, the Troy samples were doubtless somewhat affected by the neighborhood of the city.

Ground-water is more directly influenced than rain-water by the presence of human habitation. Thus the Massachusetts Board of Health (1890 [1], 680) found that twenty persons per square mile will add, on the average, 0.1 part per million of chlorine to the water flowing from such district.

The determination of chlorine in water is extremely simple. It depends upon the fact that if to a solution of a chloride which has been colored yellow by addition of a little potassic chromate a solution of silver nitrate be added, white silver chloride will be produced until the last trace of chlorine be disposed of, whereupon red silver chromate will begin to appear.

The reagents required are :

Standard Silver Solution.—Prepared by dissolving 4.8022 grammes of crystallized silver nitrate in one litre of water. Each cubic centimetre of such a solution is of a strength sufficient to precipitate one milligramme of chlorine. A sample of this reagent a year old gave exactly the same results as when freshly prepared. In common with all other reagents for water-analysis, it should be kept in bottles having caps covering the stoppers, such as are used for volatile liquids.

Potassium Chromate, Indicator.—Dissolve 2 grammes of the pure salt in 100 c.c. of distilled water.

Saturated Sodium Carbonate Solution.—Dissolve 50 grammes of the pure salt in 300 c.c. of distilled water.

Determination.—One hundred c.c. of the water to be examined are placed in a large "Nessler" jar; 1 c.c. of the potassic chromate solution is added, which will give a distinct yellow color, and then the standard silver solution is run in from a burette, until the red tint of the silver chromate just appears. From the known amount of silver solution used the amount of chlorine present is obtained, and this, multiplied by ten, will give the chlorine in milligrammes per litre or parts per million.

To determine with accuracy the first appearance of the red tint, it is best to make the examination in yellow light. The writer uses a photographic "dark room" lantern with a front of yellow glass. Reflection from a porcelain tile throws the light through the length of the "Nessler" jar, and side light is cut off by a black screen.

For the sake of accuracy it is better, during the titration, to have a second jar of the water, also colored with potassic chromate, in order that the formation of the red tint in the dish operated upon may be, by contrast, more readily detected.

Different waters, equally clear, and containing the same amount of chlorine, differ greatly in their ability to give a sharp "end-reaction." It therefore often aids the eye to prepare a third tube containing distilled water to which has been added the chromate indicator and 1/10 c.c. of the silver solution. This "overdose" having been matched by operating upon the unknown water, allowance is made by subtracting 1/10 c.c. from the burette reading.

Many waters possess such deep color, or such turbidity, as to interfere with proper titration ; under such circumstances it is best to shake the water with recently precipitated and washed aluminum hydrate and then filter it, or allow it to stand twenty-four hours in a tall glass cylinder. The coloring matter or turbidity is thus sedimented, and the water cleared for use.

With waters high in chlorine it is often very difficult to decide just when the red color begins to appear, for the reason that it is hard to compare the clear yellow liquid of the comparison-vessel with one which has become turbid from precipitation of silver chloride.

In such a case it is well to roughly determine the chlorine present, and then to make a second determination, using for comparison 100 c.c. of the water to which has been added not only the chromate indicator, but also an amount of silver nitrate solution just short of that necessary to satisfy the chlorine present.

By these means the eye is greatly aided in noting the slightest appearance of red tint, for in respect of turbidity both vessels are practically alike.

It makes no difference at what rate the silver solution is added during titration.

Circumstances sometimes demand, for purposes of special comparison, a closer reading for chlorine than is possible when only 100 c.c. of the water are employed. For such purpose it is best to place the measured quantity of water in a large porcelain casserole and to make it slightly alkaline with Na_2CO_3 before concentration. After reduction of the volume to 100 c.c. the process is continued as already described.

It is important that the same volume (100 c.c.) be always secured before running in the silver nitrate solution ; therefore distilled water must be added if the concentration should have been carried too far.

The material loss of chlorine that may occur should the evaporation be conducted without the addition of sodium carbonate is illustrated by the following experiment :

To each of two litres of water 3.35 milligrammes of chlorine were added, in the form of common salt. To one of them was likewise added 0.1 c.c. sodium carbonate solution. Upon evaporating each to 100 c.c. and titrating they gave the following results :

Water with carbonate added........	3.35 mg. of Cl.
Water with no carbonate added.....	3.25 " " "

The porcelain of the dish does not interfere with this determination, but it is very important to carefully scrub and wash down its sides after evaporation.

Comparates.

Average in sundry waters known to be pure...	2.75
" " " " " " " polluted.	58.3

The Rivers Pollution Commission reports the average amount of chlorine in 589 samples of unpolluted English water as follows :

Rain	8.22
Upland surface................	11.3
Deep well.	51.1
Spring	24.9

(Great Britain being an island, chlorine would naturally run high.)

Wanklyn considers 140 as possibly suspicious.
Frankland places the permissible limit at 50.
Leeds's standard for American rivers, 3 to 10.
Ordinary sewage, about 110 to 160.
Human urine (average of 24 samples), 5872.

NITROGEN AS NITRITES.

Frankland writes: "When fresh sewage is added to water already containing nitrates, the latter are generally reduced to nitrites," and it may be there are none to disagree with him; but when he adds that "when nitrites occur in shallow wells or river-waters, it is highly probable that these waters have been very recently contaminated with sewage," Wanklyn opposes such a view and declares that "nitrates and nitrites have been erroneously regarded as measuring the defilement of water." Finally, in the report of the National Board of Health for 1882, Mallet concludes: "With the facts of this investigation before me I am inclined to attach special and very great importance to the careful determination of the nitrites and nitrates in water to be used for drinking."

This statement of Prof. Mallet so entirely accords with the uniform experience of the writer as to force him to regard it as conclusive. It is based on most carefully conceived and executed experiments which "point strongly to the production of nitrites by oxidation of organic nitrogen, and their subsequent conversion into nitrates by further process of oxidation."

Whether, therefore, the presence of nitrites be considered due to reduction of pre-existing nitrates in presence of organic matter, or caused by direct oxidation of organic nitrogen, it becomes a necessity to estimate their quantity, for in either case the initial cause is probably contamination.

Of the several methods used of late for the determination of nitrites the second one suggested by Griess seems to be the most deserving of favor. It depends in principle upon the red coloration ("azobenzolnaphthylamine sulphonic acid") produced whenever "sulphanilic acid" and "naphthylamine hydrochloride" are added to an acidified solution of nitrite. The test is exceedingly delicate and is capable of distinguishing one part of nitrogen as nitrous acid in one thousand million parts of water.

The reagents are prepared as follows :

Sulphanilic Acid.—Dissolve 1 gramme of the salt in 100 c.c. hot water. The solution keeps well.

Naphthylamine Hydrochloride.—Boil ½ gramme of the salt with 100 c.c. water for ten minutes, keeping volume constant. Place in glass-stoppered bottle.

The solution tends to grow slightly pink on standing, but not sufficiently so to interfere with its use.

Standard Solution of Sodium Nitrite.—Sodium nitrite may be bought, but its purity is always to be questioned, and moreover it is too deliquescent a salt to be weighed with ease and accuracy. It is better, therefore, to prepare the silver salt, which may be readily handled, and from it the solution required may be made.

To a cold solution of commercial sodium or potassium nitrite add a solution of silver nitrate as long as a precipitate appears. Decant the liquid and thoroughly wash the precipitate with cold water. Dissolve in boiling water. Concentrate and crystallize the silver nitrite from the hot solution. Dry in the dark at the ordinary temperature (using vacuum is better) and keep it in a black bottle.

Weigh out .22 gramme of the dry silver nitrite. Dissolve in hot water. Decompose with slight excess of sodium chloride, cool if necessary, and dilute to one litre. Allow the precipitated silver chloride to settle, remove 5 c.c. of the clear solution, and dilute the same to one litre. This second dilution (which is the standard solution to be used) will contain an amount of nitrite per cubic centimetre equivalent to .0001 milligramme of nitrogen.

Determination.—In order to undertake the determination of nitrites place 100 c.c. of the water to be examined in a "Nessler" jar. Acidify with one* drop concentrated HCl. Add 1 c.c. of the sulphanilic acid solution, followed by 1 c.c. of the solution of hydrochloride of naphthylamine, mix,† cover with watch-glass, and set aside for thirty minutes. Prepare at the same time other "Nessler" jars containing known amounts of the standard solution of sodic nitrite and diluted to the 100-c.c. mark with *pure* distilled water (see page 51), adding the reagents as above. At the end of the time stated (thirty minutes) examine the depth of the pink color formed, and by comparing the unknown with the known an accurate determination of the amount of nitrogen present as nitrites may be made.

Comparates.—In a report upon the presence of nitrites in eighteen "natural waters, believed from actual use to be of good, wholesome character," and collected from every variety of source, Mallet's determinations show an average of .0135 part nitrogen as nitrites per million parts of water. The average, by the same investigator, for nineteen waters "which there seems to be fair ground for believing have actually caused disease" is .0403 part per million.

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* Addition of too much acid might cause *nitrates* to react as well. (*Analyst*, xii. 51.)

† To accomplish this mixing it is best to use a stout glass rod ten inches long, at one end of which is fused a cross, composed of two pieces of glass rod ¾ inch in length. The mixer is used as a plunger.

The author's experience has been that the average amount of nitrites found in good waters is very much less than the value given by Mallet.

In this connection it would be well to bear in mind Frankland's statement that "the presence of these salts in *spring* and *deep-well* water is absolutely without significance; for although they are in these cases generated by the deoxidation of nitrates, this deoxidation is brought about either by the action of reducing mineral substances, such as ferrous oxide, or by that of organic matter which has either been imbedded for ages, or, if dissolved in the water, has been subjected to exhaustive filtration." This is merely another instance of how careful the analyst should be to become familiar with the source of the water before undertaking to pass judgment upon its quality.

Nitrites should always be looked upon with suspicion if found in ground- or surface-waters.

The absence of nitrites, moreover, proves nothing. The author has recently had a most foul cistern-water for analysis which showed but a trace of nitrites and no nitrates, and yet the water was contaminated with the entire house drainage, and produced most serious illness.

Leeds's standard for American rivers, 0.003.

NITROGEN AS NITRATES.

Taking Mallet's statement as final, that there is every reason for assuming that nitrates present in water may be but the further step in the oxidation of nitrogenous organic matter, it necessarily becomes important to obtain an estimate of this constituent, which, in Frankland's opinion, is a factor in the measurement of "previous sewage contamination."

Nitrates are more liable to indicate putrefaction of animal rather than of vegetable tissue, not only because of the greater quantity of nitrogen present in the former, but also on account of its more ready decomposition.

Stoddart claims that "natural waters can, at most, obtain but from 1/10 to 2/10 grain of nitrogen as nitrates per imperial gallon (1.43 to 2.86 per million) from sources other than animal matter; and practically the whole of the nitrogen of sewage may be oxidized into nitric acid without diminishing the risk involved in drinking it."

"The proposal to consider a water safe so soon as the nitrogen has assumed the oxidized condition, irrespective of the quantity that may be present, is entirely irrational." *

Rain-water washes a very considerable amount of

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* *Analyst*, xviii. 293.

nitric nitrogen from the atmosphere ; thus an official report gives the following amounts of nitrogen as nitrates in sundry rain-waters, showing at the same time the tendency of neighboring towns to increase this item :

England, interior	.19
" cities	.22
Scotland, near the coast	.11
" interior	.08
" cities	.30
" Glasgow	.63
Montsouris, Paris, average of 18 years	.73

Nitrogen in the soil is increased by the fixing of atmospheric nitrogen through the agency of the roots of certain plants, such as peas, the process being aided by bacterial action.*

Such fixed nitrogen eventually enters the ground-water, and a knowledge of the local "normal" for nitric nitrogen is consequently of advantage when studying the domestic well-waters of a neighborhood.†

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* An interesting experiment to show this was recently made in France. Peas were grown in a closed space, and the nitrogen lost by the confined air was found equal to what was gained by the ground and plants. No such fixation of nitrogen was obtained when the soil was previously sterilized.

† Surface- and ground-waters of good quality are low in nitrates, for the reason that such material is quickly absorbed by growing vegetation.

After having tried many ways for the determination of "nitrates" in potable water the writer has adopted a modification of the old "picric acid method," as giving, on the whole, the greatest satisfaction.

Phenol-sulphonic acid is made by the action of phenol on sulphuric acid :

$$C_6H_5OH + H_2SO_4 = C_6H_4(OH)SO_3H + H_2O.$$

This reagent, reacting with nitric acid, forms tri-nitro-phenol,

$$\begin{aligned} &C_6H_4(OH)SO_3H + 3HNO_3 \\ &\qquad = C_6H_2(OH)(NO_2)_3 + H_2SO_4 + 2H_2O, \end{aligned}$$

which in turn forms yellow ammonium picrate when acted upon by ammonium hydrate: *

$$\begin{aligned} &C_6H_2(OH)(NO_2)_3 + NH_4OH \\ &\qquad = C_6H_2ONH_4(NO_2)_3 + H_2O. \end{aligned}$$

The intensity of this yellow color, produced in the water under examination, is compared with standard colors of known strength, and the quantity of nitrate present thus determined.

The interference of chlorides with this process, resulting in readings decidedly lower than the truth, is well known, but the method is so easy and convenient

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* See *Analyst*, x. 200.

that it occurred to the writer to try the addition of sodium chloride to the comparison standards rather than abandon the process.

The "chlorine" in the water under examination having been previously determined, an appropriate volume of standardized sodium chloride solution is added to each evaporation of standard potassium nitrate solution. Thus the water to be examined, and the nitrate solutions with which it is compared, all contain the same quantity of chlorine. The results are very satisfactory. If the chlorine be below six parts per million it does not interfere with the nitrate determination.

The solutions required are:

Phenol-sulphonic Acid.—

Sulphuric acid, pure and concentrated.	148 c.c.
Distilled water......................	12 c.c.
Pure phenol.........................	24 grammes

Standard Potassium Nitrate Solution.—Dissolve .7221 gramme pure KNO_3 in 1 litre distilled water. Dilute 100 c.c. of this solution to 1 litre with distilled water. This weaker solution, which is the standard employed, contains .01 milligramme of nitrogen as nitrate in each cubic centimetre.

Standard Sodium Chloride Solution. — Dissolve 1.6497 grammes pure NaCl (made from metallic sodium) in 1 litre distilled water. Each cubic centimetre will contain 1 milligramme of chlorine.

Determination.—Evaporate 100 c.c. (or less, according to nitrate-contents) of the water to dryness on the water-bath, having previously added 1/10 c.c. sodium carbonate solution (see page 31) to prevent loss from volatilization of nitric acid. Thoroughly moisten the residue with 2 c.c. of the sulphonic acid. Add an excess (about 15 c.c.) of ammonium hydrate. Make up to 100 c.c. in a " Nessler " jar, and compare the depth of color with those produced by operating upon different amounts of the standard nitrate solution, which have been evaporated and treated under precisely similar conditions.

To each selected volume of standard nitrate solution there should be added before evaporation 1/10 c.c. sodium carbonate solution and an amount of standard sodium choride solution sufficient to correspond with the amount of chlorine previously found to exist in the water.

These evaporations, both of the water and the comparison standards, are best made in deep evaporating-dishes of glass 4½ inches in diameter, and easily holding 100 c.c.

After dryness is reached the dish, with its contents, should be at once removed from the water-bath.

It is of the greatest importance that the conditions governing the operations to which the water is subjected should be strictly followed in preparing the comparison solutions.

In order to economize time, when dealing with waters low in chlorine, the writer makes use of a standard "nitrate color solution." This is made by evaporating 25 c.c. of the standard potassium nitrate solution, followed by addition of the other reagents in the way already detailed.

The yellow liquid produced is diluted to 1 litre and kept in stock. Each c.c. thereof corresponds to .0025 mg. nitrogen as nitrate. Much time is saved by diluting measured volumes of this "standard color" to 100 c.c. for the preparation of the comparison tubes.

The solution keeps its normal strength of color quite well, but should not be trusted after having been a few weeks in stock.

Before evaporating for the nitrate determination it is best to clear the water with aluminum hydrate as under "chlorine." (See page 32.)

Comparates.

Average in sundry waters known to be pure....	.47
" " " " " " " polluted.	7.19

Referring again to Mallet's report before quoted,* we find a very marked difference between the average amount of nitrates present in good, as compared with the quantity found in bad, waters.

In thirteen samples of water "known to be pure"

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* Report National Board of Health, 1882.

the nitrogen present as nitrates averaged 0.42 (the extreme limits being 0 and 1.04), while in twenty samples of water believed to be objectionable the average figures ran 7.239 (the extreme limits being 0 and 28.403). Such differences justify Mallet's statement that he regards the determination of nitrates as of great importance.

Elkin : dangerously polluted if in excess of....	6.00
Vienna Commission allows..................	1.04
Hanover " "	2.60
Brandes " "	7.00
Fischer (*Jour. für Prakt. Chem.*)..............	7.00
Leeds's standard for American rivers...	1.11 to 3.89

The Rivers Pollution Commission gives the following averages from 589 unpolluted English waters for nitrogen as nitrites and nitrates *together:*

Rain	0.03
Upland surface.................	0.09
Deep well....................	4.95
Spring	3.83

As illustrating how widely the nitrates may vary in deep wells of good character the following list is taken from the *Analyst*, xx. 84 :

Depth of Well in Feet.		N as Nitrate.
200.	Stratford	0.00
200.	Wimbleton	0.43

Depth of Well in Feet.		N as Nitrate.
490.	Chatham	6.85
900.	Southend	0.71
600.	Witham	6.43
160.	Mistley	0.71
430.	Braintree	0.28
305.	Colchester	0.00
400.	Norwich	11.43

Fresh sewage is often found entirely free of either nitrites or nitrates simply because the organic nitrogen present has had, as yet, no sufficient opportunity to become changed to the oxidized form. For instance, the sewage of Troy, N. Y., contains (sample of December, 1895):

	Parts per Million.
Free ammonia	.875
Albuminoid ammonia	.675
Nitrogen as nitrates	none
Nitrogen as nitrites	trace
Chlorine	31
"Required oxygen"	89
Total residue	489
Loss on ignition	315

A curious case of pure water with very high "nitrates" recently came under the writer's observation. The water was from a deep rock-drilled well, which had been "torpedoed" by fifty pounds of nitroglycerine. Note how important the "history of the case" was to a proper interpretation of the analytical results in this instance.

ORGANIC MATTER.

A revolution has been wrought during recent years in the determination of organic matter in potable water. Methods have arisen and disappeared. Authors of the highest rank have combated each other in print, with a success in establishing their views that has not always been commensurate with their positiveness in stating them.

It was in an effort to throw a little unprejudiced light upon the several processes of rival writers that Mallet undertook the investigation from the report of which we here so often quote—an investigation that required a period of years for its accomplishment, and which marks an era in the history of water-analysis. As therein referred to there are three methods of estimating organic pollution worthy of special mention, viz.: (*a*) the combustion process of Frankland; (*b*) the albuminoid ammonia process of Wanklyn; (*c*) the "Forschammer" process as modified by subsequent investigators.

Concerning the first, which is a direct combustion of a water residue, after the manner of an ultimate organic analysis, we note the following in the *Analyst* for September, 1885: "It is subject to many causes of error, and is of so extremely delicate a nature as to be almost abandoned at the present time." In his report to the Philadelphia Water Board for 1884 Dr. Leeds, referring to this method, says: "The determinations were discontinued, because the amount of information which they afforded did not appear commensurate with the great labor which they involved."

This remark of Dr. Leeds is almost identical with statements made to the author by Albert-Levy and other French authorities.

In short, Frankland's combustion method is difficult, liable to error in unpractised hands, and its results are not indispensable for forming a correct opinion of the sanitary value of a water.

A far more general method for obtaining information as to organic impurity is Wanklyn's

Albuminoid Ammonia Process.—By the employment of this method a knowledge of the amount of "free ammonia" present is also obtained.

We may outline the process as follows: The "free ammonia" is distilled from a measured quantity of the water, and its amount is determined by what is known as "Nessler's" method, which will be described later. A strongly alkaline solution of potassic permanganate is then added to another portion of the water and the distillation is repeated. Nitrogenous organic matters are thereby broken up and the resulting ammonia ("albuminoid"), which distils over with the steam, is determined by the "Nessler" method in like manner as before. It must be noted that the so-called "albuminoid" ammonia does not exist ready formed in the water, but is a product of the decomposition of organic nitrogenous substances by the alkaline permanganate. The term is derived from the fact that "albumen" gives off ammonia in like manner when similarly treated.

The reagents necessary are:

"*Nessler's*" *Solution.*—Dissolve 16 grammes mercuric chloride ($HgCl_2$) in about half a litre of pure water. Dissolve 35 grammes potassic iodide (KI) in about 200 c.c. pure water. Pour the first solution into the second until a faint show of excess is indicated. Add 160 grammes solid potassium hydrate (KOH). Dilute to one litre, and finally add strong solution of mercuric chloride, little by little, until the red mercuric iodide just begins to be permanent. Do not filter from excess of mercuric iodide, but let the same settle to the bottom of the vessel. The finished reagent should have a pale straw color. It is improved by age.

"Nessler's" solution will give a distinct brownish-yellow coloration with the most minute traces of ammonia or ammonium salts. If the quantity of ammonia be at all considerable, a brown precipitate will appear. The reaction in case of either precipitate or coloration will be

$$2(2KI,HgI_2) + NH_3 + 3KOH = \overline{NHg_2IH_2O} + 7KI + 2H_2O.$$

Pure Water.—This must be prepared with great care, in a room free from the usual laboratory fumes. In short, as has been already said, the entire examination of potable water should be undertaken in a locality other than a general working laboratory. The most suitable retort for this purpose is of copper, three gallons in size, and with a tin condensing worm. Fill it with good spring-water, distil, collect distillate in 50-c.c. "Nessler" jars, and to each successive jarful so collected add 2 c.c. "Nessler" solution.* After waiting five minutes, should a brown tint be observed upon looking through the liquid (*longitudinally*) at a white porcelain tile or piece of white paper, the presence of ammonia is indicated.

Continue the distillation and the "nesslerizing" of the successive 50-c.c. portions of the distillate until no coloration is obtained even after standing for five minutes. When ammonia ceases to be detected, the distilled water may be collected for use. The distillation should not be pushed too far, both on ac-

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* The "Nessler" jars here used are carefully prepared, so as to have a uniform distance (8½ inches) between the bottom of the jar and the 50-c.c. mark. No mixer or stirrer is ever employed, as the high gravity of the "Nessler" solution causes it to quickly sink into and mix with the comparatively light distillate.

count of danger to the retort and of possible production of ammonia from decomposition of the organic material remaining in the bottom.

Alkaline Potassic Permanganate.—Dissolve 200 grammes solid potassic hydrate (KOH) and 8 grammes crystallized potassic permanganate ($K_2Mn_2O_8$) in 1250 c.c. of pure water. Boil down to one litre and keep for use.

Sodic Carbonate Solution.—Dissolve 50 grammes of the pure salt in 300 c.c. pure water.

Standard Ammonia Solution.—Dissolve 1.5706 grammes of pure dry ammonium chloride in half a litre *pure* water. Dilute 5 c.c. of this solution to half a litre with *pure* water. This second solution will represent a strength of .01 mg. of NH_3 per cubic centimetre, and is the standard solution used.

DETERMINATION OF FREE AMMONIA.

Fit a one-quart glass tubulated retort to a large Liebig condenser,* letting the neck of the retort pass well into the condensing-tube (3 or 4 cm.) and through a large-size soft-rubber stopper. This connection must be thoroughly tight. Place 200 c.c. *pure* water in the retort and add 10 c.c. of the sodic carbonate solution. Distil off two 50-c.c. jars of water, and "nesslerize" the second in order to be

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* For a description of the retorts, condensers, etc., used by the author see page 67.

sure that no ammonia yet remains in the retort. Any ammonia that may have resulted from the imperfect cleaning of the apparatus, or that may have been present in the sodic carbonate solution, will usually all go over in the first 50 c.c. of distillate, but the same quantity (i.e., 100 c.c.) must be distilled off in all cases in order that when the actual analysis of the unknown water is started upon the condition as to volume may be always constant.

In fact, it may be conveniently stated here that *perfect uniformity of conditions* is a requisite for success in water-analysis.

To the contents of the retort is now added half a litre of the water to be examined.

Distil and catch the distillate in 50-c.c. " Nessler " jars. The rate of the distillation should be so managed as to allow about fifteen minutes for the filling of each 50-c.c. jar. Add 2 c.c. " Nessler " reagent to each jarful, and continue the operation with each successive portion of the distillate until no further reaction for ammonia is apparent after waiting five minutes. Usually four jars will be sufficient to carry off all free ammonia, but it is the author's custom to always distil off six.

From a small burette measure definite amounts of the *standard ammonia solution* into several clean " Nessler " jars. Dilute each to the 50-c.c. mark with pure water, add 2 c.c. " Nessler " solution, and after standing for five minutes compare as to depth of tint with the distillates already "nesslerized." With a little practice it will be found easy, by varying the amounts of standard ammonia solution used, to produce tints corresponding to those existing in the distillates, and thereby a most accurate knowledge of the quantity of ammonia actually present may be obtained. Such ammonia existed ready formed in the water, either free or as an ammonium salt, and passed over unchanged with the steam ; it is therefore technically known as "*free ammonia.*"

To make clear the calculation of results let us cite an example : Suppose the first jarful to have required 9 c.c. standard ammonia solution (diluted to 50 c.c.) to match its color when "nesslerized," the second one 3 c.c., and the third 1 c.c. Then, since each cubic centimetre of the standard ammonia solution corresponds to .01 mg. NH_3, the whole amount of "free ammonia" present in the original half-litre of water would be :

1°	.09
2°	.03
3°	.01
4°	.00
	.13 mg.

Multiplying this by two to obtain the quantity for an entire litre, and remembering that 1 mg. is the millionth part by weight of a litre of water, we find the total "free ammonia" present in the water to be 0.26 *part per million.*

ALBUMINOID AMMONIA.

Throw out the residue remaining after the distillation for *free ammonia,* clean the retort thoroughly, and refit it to the condenser. Place in the retort 200 c.c. pure water and 50 c.c. of the *alkaline permanganate solution.* Distil off three 50-c.c. jars, and "nesslerize" the third one in order to insure freedom from ammonia. Add half a litre of the water under examination, and proceed with the distillation, and the "nesslerizing" of the successive 50-c.c. portions of the distillate, as in the determination of *free ammonia.* The distillation is to be continued until six 50-c.c. jars are filled. The ammonia determined by this distillation will be *total* (i.e., "free" plus "albuminoid"); therefore from the Nessler reading of each jarful of distillate must be subtracted the reading for the corresponding jarful for "free ammonia": the difference will give the "albuminoid ammonia" for that jar.

The calculation is entirely similar to that for *free ammonia,* as stated.

Interpretation of Results.—Concerning the interpretation of results, Wanklyn, the inventor of the method, is very dogmatic, and says : " The analytical characters, as brought out by the ammonia process, are very distinctive of good and bad waters, and are quite unmistakable. There is, indeed, hardly any branch of chemical analysis in which the operator is less exposed to the risk of failure." This statement is altogether too strong. Waters of great organic purity or those of great pollution are undoubtedly easy to classify, but with the great mass of cases which lie about the boundary-line between " good " and " bad " the greatest care is to be exercised in the reading of results and the passing of judgment. One rule, already mentioned, and upon which too much stress cannot be laid, is never to give an opinion concerning a water whose history and surroundings are not thoroughly known.

The " free ammonia " in artesian wells is often excessive, under circumstances that make animal contamination an impossibility, and even rain-water, freshly collected after periods of long drought, will often exhibit properties calculated to mislead the analyst.

C. B. Fox gives the following determinations in pure *deep-well* waters :

	Free Ammonia.	Albuminoid Ammonia.
Well 230 feet deep........	0.80	0.05
" 250 " "	0.76	0.04
" 300 " "	0.74	0.03
" 330 " "	0.37	0.06
" 385 " "	0.59	0.04
" "very deep"........	0.41	0.07

This excess of free ammonia may be due either—

"1. To entrance of rain-water ;

"2. To the beneficial transformation of harmful organic matter into the harmless ammonia, through the agency of sand, clay, and other substances which act on the water in a manner similar to the action of a good filter ;

"3. To some salt of ammonia existing in the strata through which the water rises ; or,

"4. To the decomposition of nitrates in the pipes of the well. Mr. H. Slater suggests that the agent concerned in this reduction may, in the case of the deep-well waters, be the sulphide of iron which is found in the clay.

"We conclude, then, that the presence of free ammonia in such comparatively large quantities in these deep-well waters is due to the reduction of nitrates and nitrites by sulphide of iron, or some kinds of organic matter, or some other agent, such oxidized nitrogen salts having been produced in past ages by the oxidation of organic matter." *

Free ammonia in deep-well water may, however, be derived from very objectionable sources; as when surface pollution is admitted because of cleavage and fracture cracks in friable rocks, and because of the "dip" of the strata being nearly vertical. The writer has seen a number of such cases.

Take, for instance, the water from a rock-drilled well in friable shale. The boring was 57 feet deep and was located in a city containing many privy-vaults, the nearest of which was 75 feet distant. The "free ammonia" reached the very high figure of 2.025, and curiously enough there was no "albuminoid ammonia" whatever. An additional item condemning this water was the large amount (69.) of chlorine present.

Free ammonia is at times very high in the rain-water collected near large cities, and is liable to run higher in winter than in summer. Of course high figures under such conditions are without objection, assuming a clean roof and a clean cistern; but when dealing with a rain-water it must be always borne in mind that storage cisterns are often very foul.

Dr. Drown points out the low values commonly found for both "ammonias" in ground-waters of

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* Fox, "Sanitary Examinations of Water, Air, and Food."

good quality, and places that for albuminoid ammonia as rarely exceeding .025. He shows the influence of growing plants in reducing free ammonia, and quotes as illustration the great difference in this item in the water of Mystic Lake with change of season ; thus two readings for free ammonia were :

August	.000
January	.573

A further point that is mentioned by the same observer is the liability to high free-ammonia readings in water from wells sunk in ferruginous,* swampy regions, because organic matter associated with oxide of iron furnishes in absence of oxygen favorable conditions for development of ammonia.†

Wanklyn would clear away all difficulty of interpretation by holding that " albuminoid ammonia above .10 part per million begins to be a very suspicious sign ; and over .15 it ought to condemn a water absolutely." Such a hard-and-fast rule is too severe for general application.

The author has seen many an excellent water

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* Water passed through newly laid and rusty mains will often become materially changed in chemical character as well as in physical appearance. The influence of the iron-rust is to reduce the nitrates present and increase the nitrites and free ammonia. A good water might thus be very readily condemned upon the analytical results alone did the analyst not know its antecedents.

† Mass. Board of Health, 1892, 324.

greatly exceed these limits, particularly the brown waters supplying some of our Eastern towns. Many peaty waters, of proved wholesomeness, far exceed Wanklyn's limits. As has already been pointed out, waters of a brown or peaty character are always to be looked upon very narrowly, but some of them are unquestionably of good quality, and all of them would be condemned by the proposed standards.

The analyst must here again use his good judgment and decide whether or not there is natural and harmless cause for the high ammonia readings. The depth of color of the water will be a material guide to his decision.

The writer has recently analyzed the water from a mountain lake, situated far away from all possibility of sewage contamination, with the following results :

Free ammonia..............	.01
Albuminoid ammonia........	.34

An excellent mountain stream that the writer recently recommended for a city supply, although but slightly colored, ran :

Free ammonia..............	.055
Albuminoid ammonia.......	.230

As further comparates it may be of interest to give the following :

Averages in sundry waters known to be pure :
Free ammonia, .041 ; albuminoid ammonia, .079
Averages in sundry waters known to be polluted :
Free ammonia, .875; albuminoid ammonia, .479

As a result of the analysis of fifteen drinking-waters from widely scattered sources, many of them city supplies, and all of them believed to be wholesome, Prof. Mallet gives figures for " albuminoid ammonia " that show an average of .152 part per million (highest = .325, lowest = .020). Most of these would be condemned by the Wanklyn standard.

In his report to the Water Department of the City of Wilmington for 1882 Dr. Leeds, as the outcome of his experience in the analysis of American waters, says : " I should venture to propose, as an aid in determining whether a water-supply, derived (as most of our American cities' water-supplies are) from a flowing stream, is good and wholesome, the following highest limits as a standard of purity :

Free ammonia.......	.01 to .12	per million
Albuminoid ammonia.	.10 to .28	" " ."

Some years ago Dr. Smart pointed out that the *rate* at which the ammonia is evolved as of an importance at least equal to, if not greater than, the total amount of the same ; he holds that : " *Gradual* evolution of albuminoid ammonia indicates the presence of organic matter, whether of vegetable or animal origin, in a fresh or comparatively fresh condition, while *rapid* evolution indicates that the organic matter is in a putrescent or decomposing condition."

This is entirely in accord with the present writer's experience. Thus the evolution of albuminoid ammonia was found as follows when analyzing the water of a mountain lake in which was a considerable growth of pond-lilies and other water-plants :

"Nessler" jar No.	1	.0600
" " "	2	.0450
" " "	3	.0250
" " "	4	.0150
" " "	5	.0100
" " "	6	.0075
" " "	7	.0050
" " "	8	.0025
		.1700×2=.34

Water giving such results can be looked upon with much more favor than one presenting an albuminoid record such as the following :

"Nessler" jar No.	1	.1000
" " "	2	.0350
" " "	3	.0125
" " "	4	.0025
" " "	5	.0000
		.1500×2=.30

Thus we see that the reading of results is entirely a question of opinion and sound judgment, and in this connection Mallet's conclusion cannot be read without marked interest ; he says : " It is impossible to decide absolutely upon the wholesomeness or unwholesomeness of a drinking-water by the mere use of any of the processes for the estimation of organic matter or its constituents. I would even go further, and say that, in judging the sanitary character of a water, not only must such processes be used in connection with the investigation of other evidence of a more general sort as to the source and history of the water, but should even be deemed of secondary importance in weighing the reasons for accepting or rejecting a water not manifestly unfit for drinking on other grounds. There are no sound grounds on which to establish such general standards of purity as have been proposed." *

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* " The question of a standard by which to judge the quality of any particular sample of water has frequently been discussed, but as yet no generally satisfactory conclusion has been arrived at. Several standards have indeed been proposed, but none has been generally adopted, and we cannot say that we regret the result. The laying down of any one general standard by which to judge the great variety of waters met with in different parts of the country and in different geological formations is, in our opinion, at once impossible and undesirable.

" By all means take into consideration and, on suitable occasions, make use of such general standards as have been laid down by chemists of high ability and large experience; but use these standards cautiously and with discrimination, and

As a further aid to judgment the following analyses are given of sundry waters, in different parts of the country, condemned by the writer, several of them having caused disease. Also a few instances of waters of reliable quality. As elsewhere throughout the book, the results are in parts per million.

	Number.		Free Ammonia.	Albuminoid Ammonia.	Chlorine.	N as Nitrate.	N as Nitrite.	Required Oxygen.	Total Solids.
Impure	1	Shallow city well...............	.025	.08	122	17.38	trace	1.4	554
	2	City well 30 ft. deep (caused typhoid)...........	.005	.035	146	10	0	1	769
	3	Rock-drilled city well 57 ft. deep	2.025	0	69	.025	0	.85	487
	4	Spring-water (caused repeated cases of dysentery)	.01	.025	6	7	0	.8	35
	5	Well near city.................	.005	.045	24	9	0	1.1	215
	6	Country well, strong salty taste	.59	.245	2803	......	.25	......	5225
	7	Town well.	.815	.075	36	0	trace	......	421
	8	City well 250 ft. deep..........	1.59	.395	102	0	trace	......	681
	9	" " 255 " "	.31	.02	58	0	0	6.45	635
	10	" " 226 " "	1.11	.08	199	0	0	1.3	779
	11	Deep well in large stock-yard, Kansas City................	1.725	.025	80	0	trace	......	.. ..
	12	Hudson River, at Troy, during freshet......................	.42	1	3	.5	trace	......	205
	13	Deep city well, in "made ground"................ ..	excessive	excessive	47	.875	0	2.5	637
Pure	14	Peaty mtn. stream (autumn). .	.055	.23	2.4	0	0	7.4	34
	15	Same stream in winter.........	.055	.117	1.9	.08	0	6.6	47 5
	16	Mountain spring...............	.04	.048	4	1.404	trace	.3	228
	17	Town supply, Elizabethtown, N. Y..........................	.048	.002	1.05	.05	0	.35	106
	18	Large well-situated spring.....	.027	.006	2.2	1.6	0	0	90
	19	High mountain lake (peaty)...	.01	.34	2	0	0	6.6	43
	20	Lake Erie (middle of lake). ...	.045	.112	3.5	.08	trace	1.25	134
	21	Lake Superior (40 miles from shore)....................	.03	.02	1	.1	0	1.15	54
	22	Flowing wells (N. J. coast)....	.023	.05	9	.5	trace	.4	30
	23	Driven wells (Hempstead, N. Y.)	.013	.004	2.5	1.25	0	.35	22
	24	Domestic well (Catskill Mtns.).	.016	.007	.75	.175	0	.35	32

judge every case on its own merits. Judge by its conformity to, or divergence from, the general character of the waters of the district from which it comes; in other words, have district standards instead of a general standard." (A. Dupré, *Analyst*, April, 1883.)

It will be observed that a goodly proportion of the impure waters quoted have figures for free ammonia higher than those for albuminoid ammonia. This is always a suspicious sign, unless both numbers in question be low.

One of the worst waters in the list, number two, would not have been condemned upon the ammonia items at all, thus showing the importance of judging from the completed analysis. Water number three, from Erie, Pa., is a rare case in the writer's experience, showing no albuminoid ammonia. The well is drilled in friable shale and within short distance of city privies. Water number four was from an isolated country summer residence. The water is materially higher in "chlorine" and "nitrates" than the local "normals," and is exposed to drainage from outhouse and stables.

Water number six was from Coxsackie, N. Y., and was sent for analysis ; it is impossible to account for such an amount of chlorine except by assuming exceedingly careless sampling. Water number nine was from a well drilled into Hudson River shale, and was protected from immediate surface drainage. The chlorine rose from 58 to 64.3 some fifteen hours after emptying a bushel of salt into a privy-vault fifty feet distant.

Water number ten was from a drilled well, in shale rock, constructed with much more care than usual.

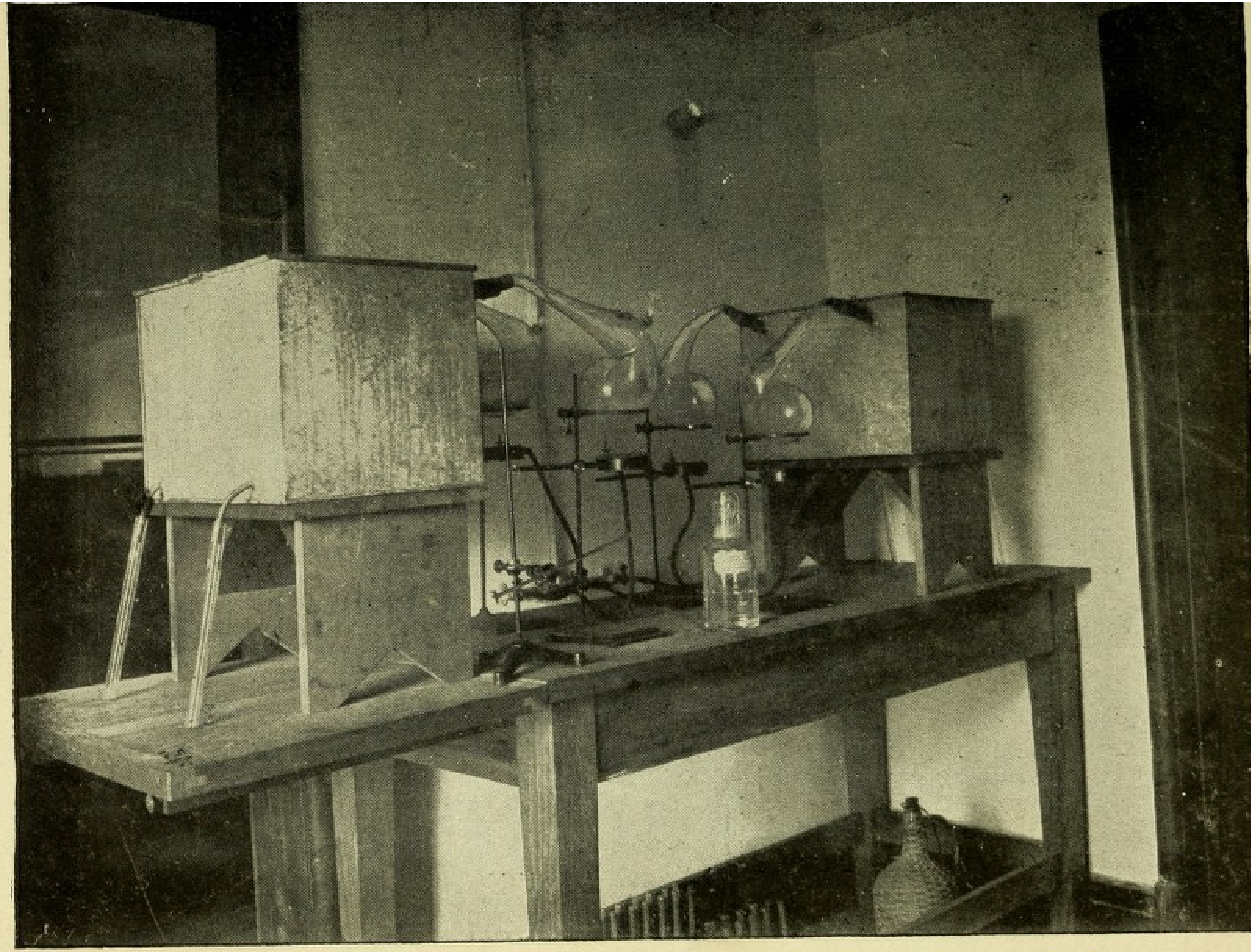
Extreme precautions were taken to shut out all immediate surface drainage, and they were undoubtedly successful. Nevertheless the neighboring privies contributed their seepage, raising the "free ammonia" and "chlorine" tremendously above the local "normals." Such results show us how unsafe it is to trust to the purity of rock-drawn water, when, owing to the seamy character of the rock, and the direction and angle of its "dip," almost direct connection may be established between the bottom of the well and the surrounding sources of surface pollution.

A comparison of waters fourteen and fifteen shows the influence of freezing weather in tying up the fountains of "peaty" contamination.

In working the "albuminoid ammonia" process it is of importance that sundry minor details should be observed in order that concordant results may be obtained ; attention is therefore called to the following points :

Use a tubulated glass-stoppered retort, and connect the same with the condenser by a large-bore soft-rubber stopper. The retort-neck should project 3 to 4 cm. into the condenser-tube, and the rubber stopper must make a perfectly tight joint.

Trouble is often experienced with certain waters, owing to their tendency to "bump," and thus permit



SHOWING RETORTS, CONDENSERS, AND "NESSLER" JARS FOR "ALBUMINOID AMMONIA" PROCESS.

of a portion of the retort contents being mechanically carried over into the condenser-tube. We avoid this difficulty by bending the neck of the retort through an angle of about twenty degrees at a point a little beyond its middle. Upon fitting such a bent retort to the condenser the contained liquid rests well towards the back, and splashing over is practically impossible.

The condensers used in this laboratory are galvanized iron tanks 14 inches deep, 14 inches broad, and 20 inches long. They rest upon wooden benches of the same superficial area and 12 inches high. The two condensing-tubes for each tank are of block tin, ¼ inch inside diameter, and they enter the side of the tank 1½ inches from the top, dip immediately to near the bottom, and then slope gently to the exit-point one inch above the bottom on the opposite side. Just before entering the tank the tin tube is suitably enlarged to receive the neck of the retort.

Block-tin tubes are much better than those of glass. They conduct heat better, do not break, and are less acted upon by the distillate.*

The "Nessler" jars used are long and narrow, being 11½ inches total length, and 8½ inches from

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* *J. Am. Chem. Soc.*, xix. 154.

the bottom to the 50-c.c. mark. A very convenient lamp for heating the retorts is the broad flat "Bunsen" ($3\frac{1}{2}$ inches diameter) with numerous small jets over its surface.

* * *

Keep the current of cooling water passing through the condenser at a velocity such that the difference between the temperature of the inflowing and outflowing water shall not exceed one degree centigrade.

This is very easily done with condensers as large as those described.

* * *

Be very careful to have the "standard ammonia" solutions and the distillates at the same temperature when the "Nessler" solution is added; otherwise equal strengths of ammonia would strike different shades of color and produce error. This end is best achieved by allowing the distillates to attain the temperature of the room before adding the "Nessler" solution.

* * *

Even with the utmost precaution some ammonia will be lost through imperfect condensation, and this loss will be greater in proportion as the rate of distillation is made more rapid; for instance, the following different results for "free ammonia" were obtained from the same tap-water by varying the time required to fill a 50-c.c. " Nessler " jar :

Jar Number.	5 Minutes.	10 Minutes.	15 Minutes.	20 Minutes.
1...........	.0050	.0075	.0250	.0250
2...........	.0025	.0050	.0075	.0075
3...........	trace	.0050	.0060	.0065
4...........	0	trace	.0025	.0025
5...........	0	trace	trace	trace
6...........	0	0	0	0
	.0075	.0175	.0410	.0415
	2	2	2	2
	.0150	.0350	.0820	.0830

The amount of ammonia in the distillate being therefore a function of the time employed, it becomes necessary to eliminate, so far as may be, any error that might arise from this source by conducting all distillations as nearly as possible at the same rate. So manage the lamp, therefore, as to fix the time required for the distillation of each 50 c.c. at *fifteen minutes.*

It is not sufficient to note the *total* amount of " free " and " albuminoid " ammonias, but the full notes of the " nesslerizing " process must be retained. that the *rate* at which the ammonia passes over may be determined. Important findings may be deduced therefrom (see page 61).

Do not observe the tint of a " nesslerized " solution until five minutes after the addition of the reagent. After the expiration of that time the color may be considered constant, no further material change taking place in twelve hours. Consequently, in the case of the examination of many successive samples, the " nesslerized " standard solutions need not be made up for each water, but those prepared in the morning may be used during the entire day, proper care being taken to protect them from the action of the atmosphere by covering them when not in use. (Standards of .01, .02, .03, .04, .05, .06, .07, .08, and .09 mg. of NH_3 in 50 c.c. water were prepared and " nesslerized," and after an interval of three days were compared with fresh preparations. It was observed that .03, .05, and .07 had not changed. The remainder had darkened to a very slight degree, but less than .0025 in each case.)

The routine standards made by the writer are: .00, .0025, .005, .0075, .01, .015, .02, .03, .04, .05, .06, .07, .08, .09, .1, and higher if necessary.

Much trouble was formerly experienced from the low standards becoming clouded or " smoky." This was found to have been due to the fact that, in making the distilled water, the water in the copper retort had been allowed to run too low, resulting in volatile decomposition products being evolved from the concentrated organic matter (see page 51).

As has been already pointed out, water-samples will not keep many days ; whence the necessity for a speedy analysis after the collection is once made.*

With many waters the tendency is for " free ammonia " to disappear upon keeping ; and, as a rule, the " albuminoid ammonia " also diminishes, but this rule is by no means uniform. For instance, the writer found that a certain brown water, after ten days' storage, showed an increase in " free ammonia " from .125 to .28 and a decrease in " albuminoid ammonia " from .255 to .235. From observations made upon the appearance and disappearance of nitrites there seems to be little doubt that the loss of " free ammonia " is to be accounted for by a process of nitrification. Nitrites are formed at the expense of the ammonia, and they, in their turn, are converted into nitrates by further oxidation. Nitrogenous organic matter in water may be considered as belonging to two classes : *first,* " that which passes readily into the condition of ' free ammonia ' through putrefactive agencies," and which is consequently easily acted upon by the alkaline permanganate solution ; and *second,* that which is more

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* The changes which take place in water upon keeping have been carefully investigated by Smart and Mallet (Nat. Board of Health, 1882).

stable, and from which no ammonia is evolved during distillation with the above reagent. Upon standing for any considerable time this latter class becomes slowly converted into the less stable variety, which in its turn is gradually converted into "free ammonia," the ammonia in turn becoming finally nitrified, as already stated. Thus we have a perfect system of changes, from the stable nitrogenous organic matter on the one hand to the fully oxidized nitrate on the other. Of course we are citing but a typical case, and must be prepared to see all manner of departures therefrom in special instances, according as the character and amount of materials and the nature of the environment may differ.

It is good practice to redetermine the albuminoid ammonia after the sample has stood a number of days. By such means an idea may be obtained of the stability of the nitrogenous organic material whence such ammonia is derived.

OXYGEN-CONSUMING CAPACITY.

("REQUIRED OXYGEN.")

The third and last method for estimation of organic matter that we shall touch upon is Kubel's modification of the old permanganate process of Forschammer. The original mode of procedure was published in 1850 and "consisted merely in adding a solution of potassic permanganate of known strength, without any other reagent, to a measured amount of water to be examined, until the liquid had acquired a faint permanent tinge, and then noting the quantity used. It was afterwards ascertained that more uniform results could be obtained, and with less expenditure of time, by causing the permanganate to act in the presence of free acid or free alkali." Kubel uses a boiling temperature.

The form of determination recommended is that employed by Dr. Smart. The necessary solutions are:

Standard Potassic Permanganate Solution.—Dissolve 0.3952 gramme of the salt in one litre of distilled water. Each cubic centimetre of such solution will contain 0.1 mg. of oxygen available for oxidation. The available oxygen of the permanganate in presence of sulphuric acid may be represented by the equation

$$K_2Mn_2O_8 + 3H_2SO_4 = K_2SO_4 + 2MnSO_4 + 3H_2O + 5O.$$

Dilute Sulphuric Acid.—One part of the strong acid to three of distilled water.

Solution of Oxalic Acid ($H_2C_2O_4 . 2H_2O$).—Dissolve 0.7875 gramme of the crystallized acid in one litre of distilled water. This solution if titrated against the permanganate solution (while hot, and in presence of H_2SO_4) should correspond to it c.c. for c.c. In practice, however, this correspondence will be found to be approximate only. The equation is

$$\begin{aligned} K_2Mn_2O_8 + 3H_2SO_4 + 5(C_2H_2O_4 \,.\, 2H_2O) \\ = K_2SO_4 + 2MnSO_4 + 10CO_2 + 18H_2O. \end{aligned}$$

The solution tends to grow weaker quite rapidly with lapse of time, and must be restandardized every time it is used. This is, however, but a slight inconvenience, and is accomplished as follows :

Ten c.c. of the oxalic acid solution, diluted with 200 c.c. pure water and 10 c.c. of the dilute sulphuric acid, are titrated, boiling, with the standard potassic permanganate solution, and the amount of the latter required to produce a faint pink tinge is recorded.

Determination.—Place in a porcelain casserole 200 c.c. of the water under examination, and add 10 c.c. of the dilute sulphuric acid. Heat rapidly to incipient boiling, and run in the standard permanganate solution from a burette until the water has a very marked red color. Boil *ten minutes,* adding more permanganate from the burette from time to time, if necessary, in order to maintain the intensity of red color observed at the start. Do not let the color fade nearly out, and then add the permanganate in quantity at once, but strive to keep the color as nearly constant as possible by gradual addition.

Remove the lamp, add 10 c.c. (or more, if necessary) of the oxalic acid solution to destroy the color, and then add the permanganate solution from the burette until a faint pink tinge again appears. From the total permanganate used deduct that corresponding to the 10 c.c. (or more) oxalic acid employed, and from the remainder calculate the milligrammes of "required oxygen" consumed by the organic matter present in the water. Correction must be made for nitrites, ferrous salts, or hydrogen sulphide if any of them be present.

Example :

	c.c.
Total permanganate solution used	25.
Less that required for the oxalic acid	9.7
Hence that required to oxidize organic matter	15.3

corresponding to 1.53 mg. oxygen.

Therefore "required oxygen" is 1.53 × 5 = 7.65 per million.

Comparates.—As this determination deals principally with the organic carbon present, the readings are naturally high in the cases of brown peaty waters, and surface-waters carrying organic matter in suspension. (See the list of analyses, page 64.)

Leeds's for *American rivers* .... 5 to 7

Averages from determinations by Dr. Smart :

Impure (14 samples)	5.880
Doubtful purity (5 samples)	3.073
Medium purity (15 samples)	1.414
Pure (18 samples)	0.581

The severe character of the following French classification is due to the fact that spring-waters are popular in France, and surface-waters are filtered before use :

Very pure	1
Potable	2
Suspected	3 to 4
Impure	above 4

LEAD AND COPPER.

It at times becomes necessary to examine water for these poisonous metals, and the ease with which their dark sulphides may be formed provides a ready method. (Miller.)

Prepare a standard solution of lead nitrate, $Pb(NO_3)_2$, by dissolving 1.5990 grammes of the salt in one litre of distilled water. Each cubic centimetre will contain 1 mg. metallic lead.

Place the water in a 100-c.c. " Nessler " jar ; add 4 drops of concentrated HCl, followed by 1 c.c. of colorless ammonium sulphide, and match the tint by operating in a similar manner with measured amounts of the standard lead solution diluted to 100 c.c.

This will not, of course, distinguish between copper and lead, but, inasmuch as either is objectionable, distinguishing is not commonly necessary.

In order to test the action of the water upon *lead pipe,* permit it to remain in contact with both bright and dull lead (in separate vessels) for twenty-four hours, and then examine the water as above.

IRON.

This metal is objectionable if in considerable quantity,* particularly in water to be used for washing white goods, and for dyeing. A knowledge of the presence of iron will, moreover, aid in guarding against an invasion of iron-secreting algæ such as crenothrix.

To determine its quantity acidify a suitable volume of the water with aqua regia ; concentrate to 100 c.c.; place in a 100-c.c. " Nessler " jar ; add 2 c.c. of ammonium sulpho-cyanate solution, and compare the depth of color produced with known amounts of standard iron solution diluted to 100 c.c. and similarly treated with ammonium sulpho-cyanate solution.

The *standard iron solution* is prepared by dissolving 0.1 gramme pure iron in a little HCl to which a few drops of HNO_3 have been added, and then diluting to one litre.

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* Wanklyn believes that a drinking-water should not contain over 3 parts of iron per million.

Iron which tends to increase in a well-water as the draught upon the underground supply grows in volume is a discouraging symptom ; for the probabilities are strong that the water will eventually become unfit for use without removal of the ever-increasing iron.

ZINC.

Zinc is not a cumulative poison, but its presence in a water is nevertheless distinctly objectionable. Galvanized iron pipe is attacked by certain waters, and spring-water is at times zinc-bearing, as has been especially noticed in Southern Missouri.*

For the determination of the metal it is best to evaporate considerable of the water, and make the usual gravimetric determination after precipitation as a sulphide.

For qualitative purposes, such as showing action of a water on galvanized iron pipe, Allen's test is

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* Zinc-bearing spring-water from Missouri:

	Parts per Million.		Parts per Million.
$PbSO_4$	trace	$CaSO_4$	109.9
$CuSO_4$	0.5	$MgSO_4$	19.0
$CdSO_4$	0.9	K_2SO_4	5.6
$ZnSO_4$	297.7	Na_2SO_4	5.9
$FeSO_4$	1.6	$NaCl$	4.3
$MnSO_4$	6.3	$CaCO_3$	72.0
$Al_2(SO_4)_3$	2.5	SiO_2	13.7
			539.9

(Hillebrand, Bul. 113, U. S. Geol. Sur.)

useful: Render the water slightly alkaline with ammonium hydrate; boil; filter, and add a few drops of potassium ferro-cyanide. A white precipitate will form in presence of a trace of zinc.

When applying this test it must be remembered that only the zinc in solution is detected. That portion which is present in insoluble form, suspended in the water, is often the larger of the two.

Arsenic occurs in some waters naturally, and *chromium* may be present from industrial waste. Should the presence of these elements be suspected, their determination should be undertaken, in the concentrated water, by the usual gravimetric methods.

ALUM.

When examining the effluent from mechanical filters, it becomes essential to determine if any undecomposed coagulant (i.e., alum) passes into the filtrate. For such purpose the "logwood test" proposed by Mrs. E. H. Richards is by far the most valuable.

Boil some logwood chips in a little water for a few minutes and drain off the resulting extract. Repeat the boiling and again discard the extract. Boil for the third time about 15 minutes and keep the extract for use.

Place about 100 c.c. of water in a porcelain dish, add a little of the logwood extract, followed by a little acetic acid. If even a trace of alum be present in the water a violet tinge will be obtained which will not be discharged upon addition of the acetic acid. A "blank" should always be run for comparison. The logwood extract is reliable but for a short time, especially if exposed to air. It is never safe to trust it when more than a day old.

Logwood for this test cannot be readily purchased, that obtainable from the druggists being absolutely worthless. The best method of obtaining it is to personally bore the chips from the centre of the log.

PHOSPHATES.

Phosphates are rarely present in more than minute traces in waters fit for domestic use, although not uncommon in those which are contaminated. Excellent waters do at times contain them, however, in very notable quantities. For instance, the writer found as much as 2 parts per million (calculated as calcium phosphate) in an artesian water on the New Jersey coast.

To determine them Phipson's method is very convenient. He takes a large measure of the water, adds a little alum solution, followed by a few drops of ammonia, and then makes the solution acid with acetic acid. The aluminum phosphate is filtered off, dissolved in nitric acid, and precipitated with ammonium molybdate solution in the usual way.*

MINERAL RESIDUE.

Should an analysis of the mineral residue be demanded, which is not commonly the case, one or more litres of the water are strongly acidulated with hydrochloric acid and evaporated to dryness in platinum. The dry residue is heated in the air-bath at 120–130° C. until acid fumes cease, then cooled, thoroughly moistened with strong hydrochloric acid, digested with water and the separate silica filtered off.

The silica is weighed, ignited with hydrofluoric acid, and determined by difference in the usual manner.

Barium, if present, will be found in the residue after volatilizing the silicon fluoride.

It should be fused, brought into solution, and precipitated by sulphuric acid.

Iron and Aluminum are weighed together as oxides after precipitation by ammonium hydrate followed by ignition.

Calcium is thrown out of the filtrate from the iron and aluminum by ammonium oxalate as is usual, and its filtrate is evaporated to dryness in platinum and ignited to remove excess of ammonium salts before precipitating *magnesium* in the customary manner.

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* *Chem. News*, lvi. 251.

Sulphates are determined by use of barium chloride in a separate evaporation after removal of silica.

DISSOLVED GASES.

Oxygen.—No process with which the author is acquainted for the determination of the dissolved oxygen gives more desirable results than that devised by M. Albert-Levy, of the Montsouris Observatory, Paris. It is as follows:

A pipette of about 100 c.c. capacity is provided with an upper and a lower stopcock, and the capacity of the same between the stopcocks is determined. Above the upper stopcock the tube is expanded into a short, cylindrical funnel. The pipette is completely filled with the water to be examined, and the funnel is emptied. The cocks having been closed, the pipette is wiped off and fixed in a suitable clamp, with its lower point dipping into a little dilute sulphuric acid. Two c.c. of dilute potassic hydrate solution are placed in the funnel and, by careful opening of the cocks, introduced within the pipette without the admission of air. After washing the funnel 4 c.c. of a solution of ammonium ferrous sulphate are placed therein, and, by similar means, also admitted within the pipette. In presence of the alkaline solution the oxygen dissolved in the water will immediately oxidize the ferrous salt to ferric, and a mixture of the two hydrates will shortly settle to the bottom after gentle agitation. After again washing the funnel 2 c.c. of sulphuric acid (equal parts acid and water) are placed therein, and the upper stopcock alone is opened. The higher gravity of the acid will cause it to slowly enter the pipette, where it will acidify the contents and dissolve the hydrates of iron. The contents and washings of the pipette, together with the acid from the beaker into which the lower extremity of the pipette has dipped, are now turned into a flask and titrated with the standard solution of potassic permanganate * already prepared on page 75. A blank is now titrated containing a mixture of 100 c.c. of the

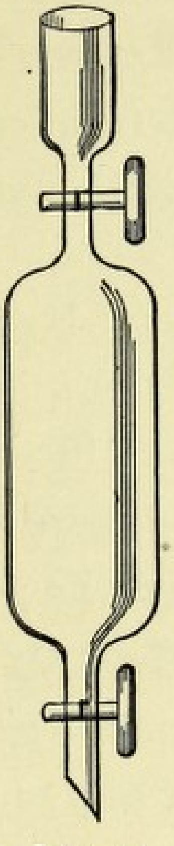

FIG. 3.

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* Should much chloride be present, as in sea-water, M. Albert-Levy suggests the substitution of the bichromate in place of the permanganate of potassium.

water, 2 c.c. of the sulphuric acid, 2 c.c. of the potassic hydrate solution, and 4 c.c. of the ammonium-ferrous sulphate solution. The difference between these two titrations (acid reaction having prevented oxidation in the second instance) will give the amount of ferrous salt oxidized by the oxygen dissolved in the water. The volume of the water operated upon will be the volume of the pipette (*V*) less the volumes of the alkaline and iron solutions, namely :

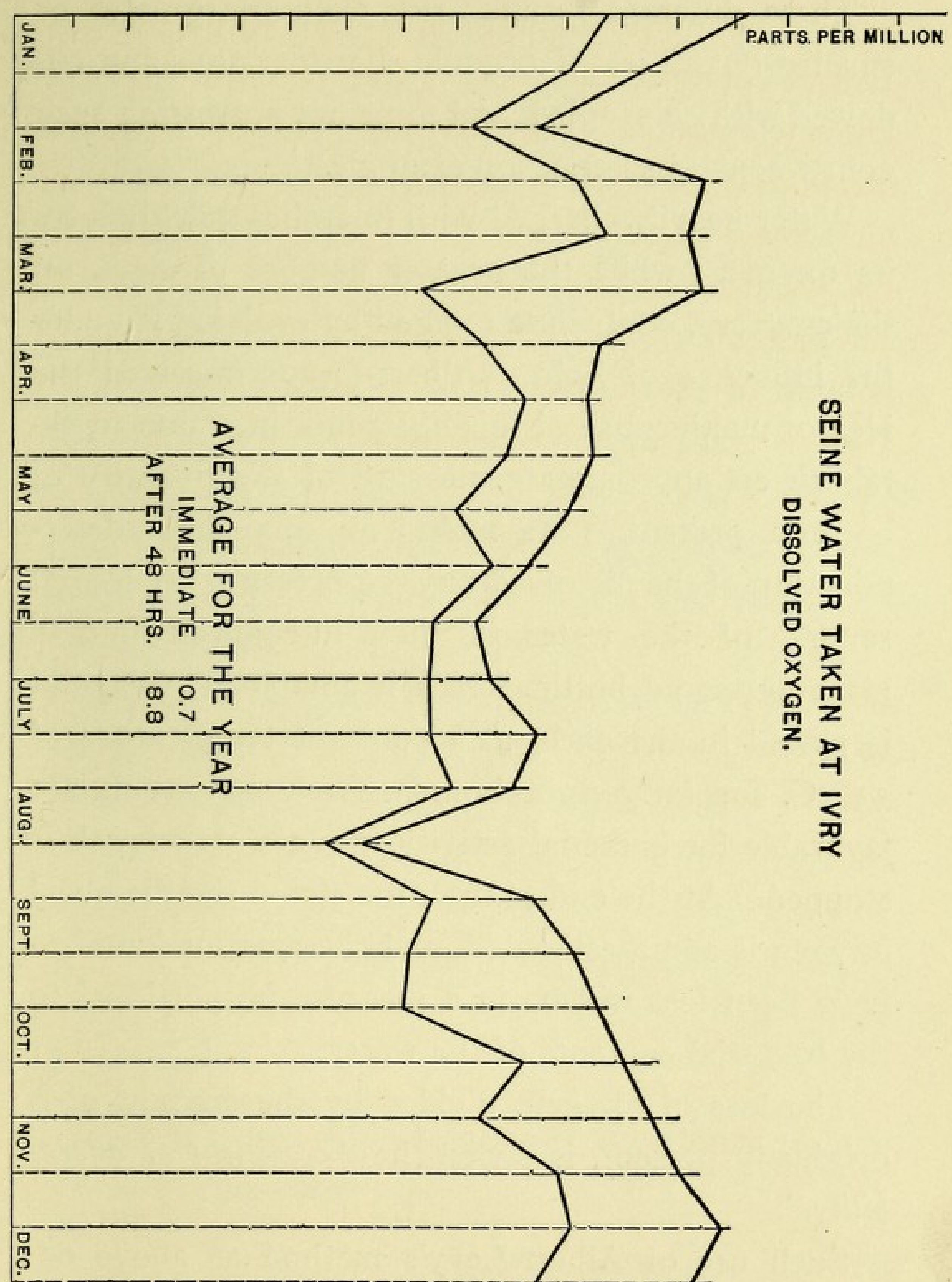
$$V - (2 + 4) \text{ c.c.}$$

M. Albert-Levy makes further and valuable use of this method beyond the mere estimation of dissolved oxygen. Some time since he pointed out that various waters behave very differently when exposed to the light, so far as their contained oxygen is concerned. Some quickly lose their oxygen, thus :

	Mg. Oxygen per Litre.
Seine water	10.6
Same after 8 days	7.2
Same after 15 days	none

Others act in a manner quite the reverse, thus :

Vanne water	11.1
Same after 9 days	20.2
Same after 60 days	39.7


PARTS. PER MILLION
SEINE WATER TAKEN AT IVRY
DISSOLVED OXYGEN.
JAN.
FEB.
MAR.
APR.
MAY
JUNE
JULY
AUG.
SEPT.
OCT.
NOV.
DEC.
AVERAGE FOR THE YEAR
IMMEDIATE 10.7
AFTER 48 HRS. 8.8
IMMEDIATE
AV. 10.7
AFTER 48 HRS.
AV. 8.8

These different results arise from the greater or smaller quantities of organic life present, some endowed with chlorophyll and some not possessing such constituent—bacteria, for example.

Water heavily charged with microbes quickly loses its oxygen ; while the greater number of algæ, on the contrary, contribute oxygen by evolving it under the influence of light. Albert-Levy conceived the idea of making use of this difference in action to establish an approximate measure of the quantity of bacteria present. He makes an immediate determination of the dissolved oxygen present. A second sample of the water is then introduced into a glass-stoppered bottle, which it completely fills, and is placed in the dark, at a constant temperature of 33° C. for forty-eight hours. Such conditions are favorable for bacterial activity, while algæ-growth is stopped. At the end of the time stated the dissolved oxygen is again estimated, and the amount found to have been lost will be in a measure proportional to the bacterial contents of the water.

The loss of oxygen, divided by the amount of it originally present, is styled the "*coefficient of alterability.*"

Such use of Albert-Levy's method as above described is graphically shown on page 87. The distances apart of the two curves for dissolved oxygen indicate the monthly variations in number of bacteria present in the water. The influence of surface-washing in spring and autumn is here well shown in increased bacteria, as is also the comparative bacterial purity in midsummer.

The determination of other gases present in solution is not commonly of sufficient value to repay the expenditure of time required for such work.

Should it be decided upon, however, to include an estimation of carbon dioxide in the analysis, Seyler's method, as given in *Chemical News*, lxx. 104, is to be recommended.

An odor like that of sulphuretted hydrogen must not be taken as proof positive of the presence of that gas in a water, inasmuch as mixtures of sundry hydrocarbons will often greatly mislead the sense of smell.

The author has in mind such a case from Northern New York, and also another from Kansas, which latter water, by the way, contains much iron.

According to Bunsen, one litre of water at 760 mm. pressure may contain :

	At 0° C.	At 10° C.	At 20° C.
Oxygen	41 c.c.	33 c.c.	28 c.c.
Nitrogen	20 "	16 "	14 "
Carbon dioxide......	1797 "	1185 "	901 "

What is generally known as the "Michigan standard of the purity of drinking-water," as specified by the Michigan State Laboratory of Hygiene, is here given :

"1. The total residue should not exceed 500 parts per million.

"2. The inorganic residue may constitute the total residue.

"3. The smaller amount of organic residue the better the water.

"4. The amount of earthy bases should not exceed 200 parts per million.

"5. The amount of sodium chloride should not exceed 20 parts per million (i.e., "chlorine" 12.1 parts per million).

"6. The amount of sulphates should not exceed 100 parts per million.

"7. The organic matter in 1,000,000 parts of the water should not reduce more than 8 parts of potassium permanganate (i.e., "required oxygen" 2.2 parts per million).

"8. The amount of free ammonia should not exceed 0.05 part per million.

"9. The amount of albuminoid ammonia should not exceed 0.15 part per million.

"10. The amount of nitric acid should not exceed 3.5 parts per million (i.e., "N as nitrate" .9 part per million).

"11. The best water contains no nitrous acid, and any water which contains this substance in quantity sufficient to be estimated should not be regarded as a safe drinking-water.

"12. The water must contain no toxicogenic germs as demonstrated by tests upon animals.

"The water must be clear and transparent, free from smell, and without either alkaline or acid taste, and not above 5 French standard of hardness."

Such a standard is much too severe for general use. The specifications for the new supply for Jersey City were based thereon, and it was not surprising that they were declined by the contractors. As has been so often said, a general standard of purity is impossible. Each case should be judged upon its own merits, after a careful study of all the relating facts.

Water results are best reported in "parts per million," as already stated, but at times a demand will be made for a report stated in "grains per U. S. gallon," and to facilitate conversion from one form to the other the table given herewith was prepared :

CONVERSION OF "MILLIGRAMMES PER KILOGRAMME" INTO "GRAINS PER U. S. GALLON" OF 231 CUBIC INCHES.

One U. S. gallon of pure water at 60° F., weighed in air at 60° F., at atmospheric pressure of 30 inches of mercury, weighs 58334.94640743 grains.*

Parts per Million.	Grains per U. S. Gallon.	Parts per Million.	Grains per U. S. Gallon.	Parts per Million.	Grains per U. S. Gallon.
1	0.058335	36	2.100058	71	4.141781
2	0.116670	37	2.158393	72	4.200116
3	0.175005	38	2.216728	73	4.258451
4	0.233340	39	2.275063	74	4.316786
5	0.291675	40	2.333398	75	4.375121
6	0.350010	41	2.391733	76	4.433456
7	0.408344	42	2.450068	77	4.491791
8	0.466679	43	2.508402	78	4.550126
9	0.525014	44	2.566737	79	4.608461
10	0.583349	45	2.625072	80	4.666796
11	0.641684	46	2.683407	81	4.725130
12	0.700019	47	2.741742	82	4.783465
13	0.758354	48	2.800077	83	4.841800
14	0.816689	49	2.858412	84	4.900135
15	0.875024	50	2.916747	85	4.958470
16	0.933359	51	2.975082	86	5.016805
17	0.991694	52	3.033417	87	5.075140
18	1.050029	53	3.091752	88	5.133475
19	1.108364	54	3.150087	89	5.191810
20	1.166699	55	3.208422	90	5.250145
21	1.225034	56	3.266757	91	5.308480
22	1.283369	57	3.325092	92	5.366815
23	1.341704	58	3.383427	93	5.425150
24	1.400039	59	3.441762	94	5.483485
25	1.458373	60	3.500097	95	5.541820
26	1.516708	61	3.558432	96	5.600155
27	1.575043	62	3.616766	97	5.658490
28	1.633378	63	3.675101	98	5.716825
29	1.691713	64	3.733436	99	5.775159
30	1.750048	65	3.791771	100	5.833494
31	1.808383	66	3.850106		
32	1.866718	67	3.908441		
33	1.925053	68	3.966776		
34	1.983388	69	4.025111		
35	2.041723	70	4.083446		

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* See article by the author on "The U. S. Gallon" in *Am. Druggist*, January, 1888.

CHAPTER III.

BACTERIOLOGICAL EXAMINATION OF WATER.

WHILE one cannot but feel that in their enthusiasm over the great triumphs of the new science the people at large have gone slightly "bacteria-mad," and are apt to expect more than can be furnished by the means and information at present available, yet the fact remains that the hostility heretofore manifested by the supporters of the allied sciences is now entirely outgrown, and chemistry and bacteriology stand upon an equal footing as necessary means to the thorough investigation of a water-supply.

The water expert of to-day cannot afford to take the risk of basing his opinion upon either form of inquiry alone, and it behooves him to make himself familiar with all the means of throwing light upon the question at issue.

In consideration of the magnitude of the bacteriological field, it is manifestly out of the question, in a book of this scope, to go very far beyond a simple enumeration of the bacteria present in a cubic centimetre of the water under examination, and a determination of the probable presence or absence of germs derived from an intestinal source, thus leaving the problem of final differentiation to be discussed by writers upon bacteriology.

Such elementary applications of the science are of especial value for the testing of filters and watching any variation in their efficiency.

For such a purpose the simple count of germs per cubic centimetre is most valuable, and differentiation is a secondary matter ; the assumption being a just one that a filter which will remove the harmless bacteria will take out the objectionable ones as well.

As to the value of the "count of bacteria per c.c." in the general cases outside of filter examination, much has been asserted to show its uselessness ; and for "single sample" examinations the objections are doubtless well taken, but for "comparative tests," such as watching the distance to which a stream of sewage is felt in a lake, or observing the relation between sedimentation and river flow, there is no question as to its being of great value.

For instance, it was through such means that Dr. Shuttleworth, of Toronto, was led to the discovery that a section of the conduit, leading from the distant intake, was broken, and that, consequently, the city supply was being taken from within a zone of pollution much nearer shore.*

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* *Jr. N. E. Water-works Asso.*, June, 1896, 211.

For the accomplishing of such determinations as are here proposed the following culture media should be carefully prepared and kept ready at hand.*

Bouillon.—Take one pound of lean beef, chop it fine, and let it soak overnight in 700 c.c. water in a cool place. Strain through a cloth with the aid of gentle pressure and make up to one litre with water. Add 10 grammes of peptone (Witte's) and 5 grammes of common salt. Heat in a double-walled "oatmeal-boiler" until the added ingredients are dissolved. Should the medium not be clear, add the white of an egg beaten up with a little of the medium, bring to a boil and filter while hot.

The proper reaction of the finished medium should be about + 15 (i.e., an acidity equal to what would be produced by the addition of 15 c.c. of normal hydrochloric acid to one litre of the medium made neutral to phenolphthalien).

In order to secure such a reaction place 5 c.c. of the medium in a porcelain dish, add 45 c.c. distilled water and 1 c.c. of a solution of phenolphthalein (.5 gramme phenolphthalien in 100 c.c. of 50 per cent alcohol).

Titrate to the neutral point with N/20 NaOH or with N/20 HCl (the former will be the solution most commonly required), and from these data calculate

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* The following formulæ for culture media are largely based upon the recommendations of the special committee of the American Public Health Association. (*J. Am. Pub. Health Asso.*, January, 1898, page 71.)

the amount of normal HCl required to be added to the bulk of the medium in order to bring it to the desired degree of acidity, namely, + 15.

The said normal acid having been added, the "bouillon" is placed in test-tubes plugged with cotton, or in flasks similarly closed; the cotton plug is then covered with a small sheet of "tin-foil" to prevent evaporation, and sterilization is accomplished by heating in an "Arnold's sterilizer" for fifteen minutes on three successive days.

Sterilization may be accomplished by a single heating in an autoclave to 110° C. (6 pounds pressure) for half an hour.

Keep the "stock bouillon" and all other stock media in a cool, dark place.

Nutrient Gelatin.—Take one pound of lean beef, chop it fine, and let it soak overnight in 700 c.c. distilled water in a cool place. Strain through a cloth and make up to one litre with distilled water. Add

Gelatine (best French)......	100	grammes
Peptone (Witte's).........	10	"
Common salt..............	5	"

Heat in a double-walled "oatmeal-boiler" at a temperature between 35° and 40° C. until all is dissolved. Add the white of one egg, previously shaken up with about its own bulk of water, and stir thoroughly. Heat as rapidly as possible to boiling, stirring occasionally. Cover the mixture and keep the water in the outer vessel boiling during fifty minutes.

Filter, with the use of the hot-water funnel. Place 5 c.c. of the filtered medium in a porcelain dish; dilute the same with 45 c.c. water; titrate with N/20 NaHO solution, as in the case of the preparation of "bouillon," and from the data so obtained calculate what addition of normal HCl should be made to the main bulk in order to carry its reaction to the desired point, namely, + 15.

Pour the finished jelly into "Miquel flasks" (20 c.c. in each) or test-tubes (10 c.c. in each). Plug the vessels with cotton and sterilize them in an Arnold's sterilizer for *fifteen minutes* on three successive days, or in an autoclave at 110° C. for half an hour, once only.

It is best to make but one litre of the jelly at a time, as it does not keep well in stock. Old jelly has a tendency to become decidedly acid in reaction, which is unfavorable to the growth of certain bacteria.

The necessity for carefully maintaining the proper reaction of culture media is well illustrated in the following table prepared by G. W. Fuller: *

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* *J. Am. Pub. Health Asso.*, xx. 393; also Mass. Board Health, 1895, 591.

COMPARISON RESULTS OF GROWTH OF BACTERIA FROM THE SAME WATER, ON GELATIN, OF DIFFERENT DEGREES OF REACTION.

The "reaction" numbers give the amounts in c.c. of normal alkali solution required, per litre of the medium, to change the reaction to the phenolphthalein neutral point.

Reaction.	B. per c.c.	Per cent compared with reaction 20.	Reaction.	B. per c.c.	Per cent compared with reaction 20.
40	100	1	5	6900	46
35	400	3	0	5800	38
30	600	4	− 5	4000	26
25	7800	55	− 10	3200	21
20	15000	100	− 15	1300	9
15	13300	89	− 20	500	3
10	8100	54	− 25	200	1

Sugar Bouillon.—Prepared the same as the ordinary "bouillon" except that 10 grammes of pure glucose are added with the peptone and salt.

Inasmuch as the high temperature of the autoclave might partly decompose the sugar, it is better to sterilize in the Arnold's sterilizer for fifteen minutes on three successive days. The best vessels in which to store "sugar bouillon" are the "Smith's fermentation-tubes" in which it is to be used (see Fig. 4).

Sugar Gelatin.—Prepared like nutrient gelatin except that 10 grammes of pure glucose are added with the other ingredients. Sterilize in the Arnold's sterilizer for fifteen minutes on three successive days. It is best kept in test-tubes plugged with cotton; 10 c.c. in each tube.

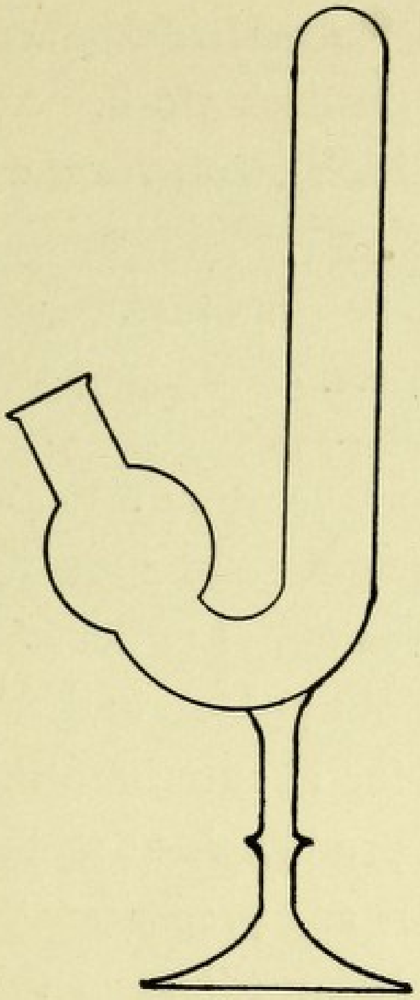

FIG. 4.

Agar-agar.—Although plate cultures for water examination are best made by the use of "nutrient gelatin," it is sometimes convenient to employ a medium with a higher melting-point. It must be noted, however, that "counts" of colonies growing upon agar must not be compared with those upon gelatin; the latter medium being more favorable to an increased growth.

To make one litre of agar-agar take—

A. Chopped meat, 500 grammes.
   Water, 500 c.c.

Mix and place in cool place overnight, then
strain through cloth. Add
Peptone (Witte's), 10 grammes.
Common salt, 5 grammes.

B. Agar-agar, 12 grammes.
Water, 500 c.c.

Place B in octoclave, run up to about 30 pounds of pressure, put out flame, and allow to cool until below 100° C. before opening. Let the solution of agar cool still further to about 75° C., and then mix A and B. Bring to a boil for about three minutes, neutralize and filter. The product is an absolutely clear jelly, which never forms any precipitate.*

Place the finished medium in tubes and "Miquel flasks" and sterilize as usual.

When filling "Miquel flasks" with any solid medium for "stock," it is wise to incline the flasks after sterilizing, so as to permit the jelly to harden in the angle; for if it be distributed over the bottom there is considerable danger of its expanding and breaking the glass while being melted for use.

Empty glassware is best sterilized by a single heating for one hour to 180° C.

By far the best piece of apparatus to use for this

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* The above method for preparing agar-agar is due to Dr. M. P. Ravenel. (*J. Applied Microscopy*, i. 106.)

purpose is the Lautenschlaeger sterilizer illustrated herewith.

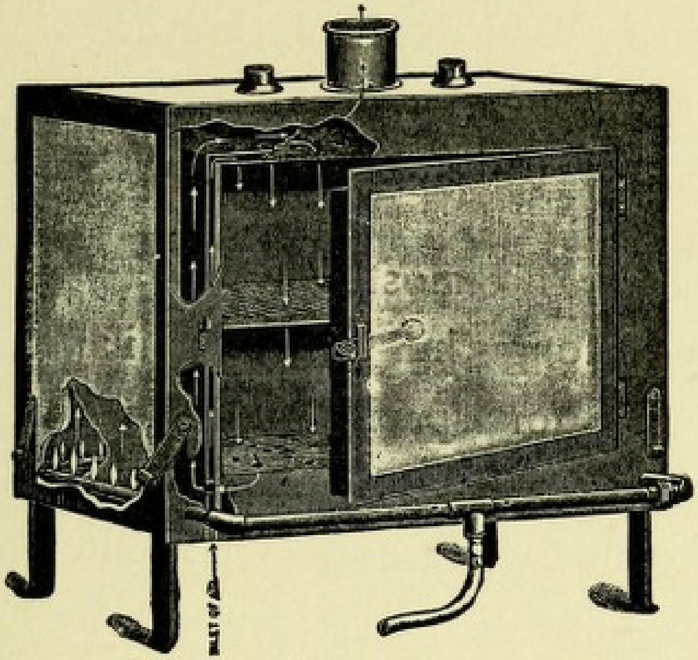


FIG. 5.

The 1-c.c. pipettes used for the measurement of the water should be plugged with cotton near the end which is placed in the mouth; the whole pipette should then be placed in a suitable glass tube containing a cotton plug in its open end.

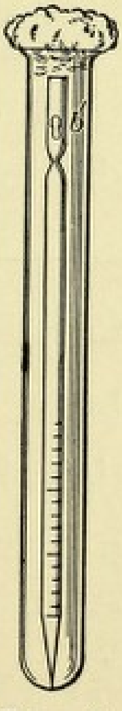


FIG. 6.

Pipettes so prepared may be sterilized, and maintained in stock.

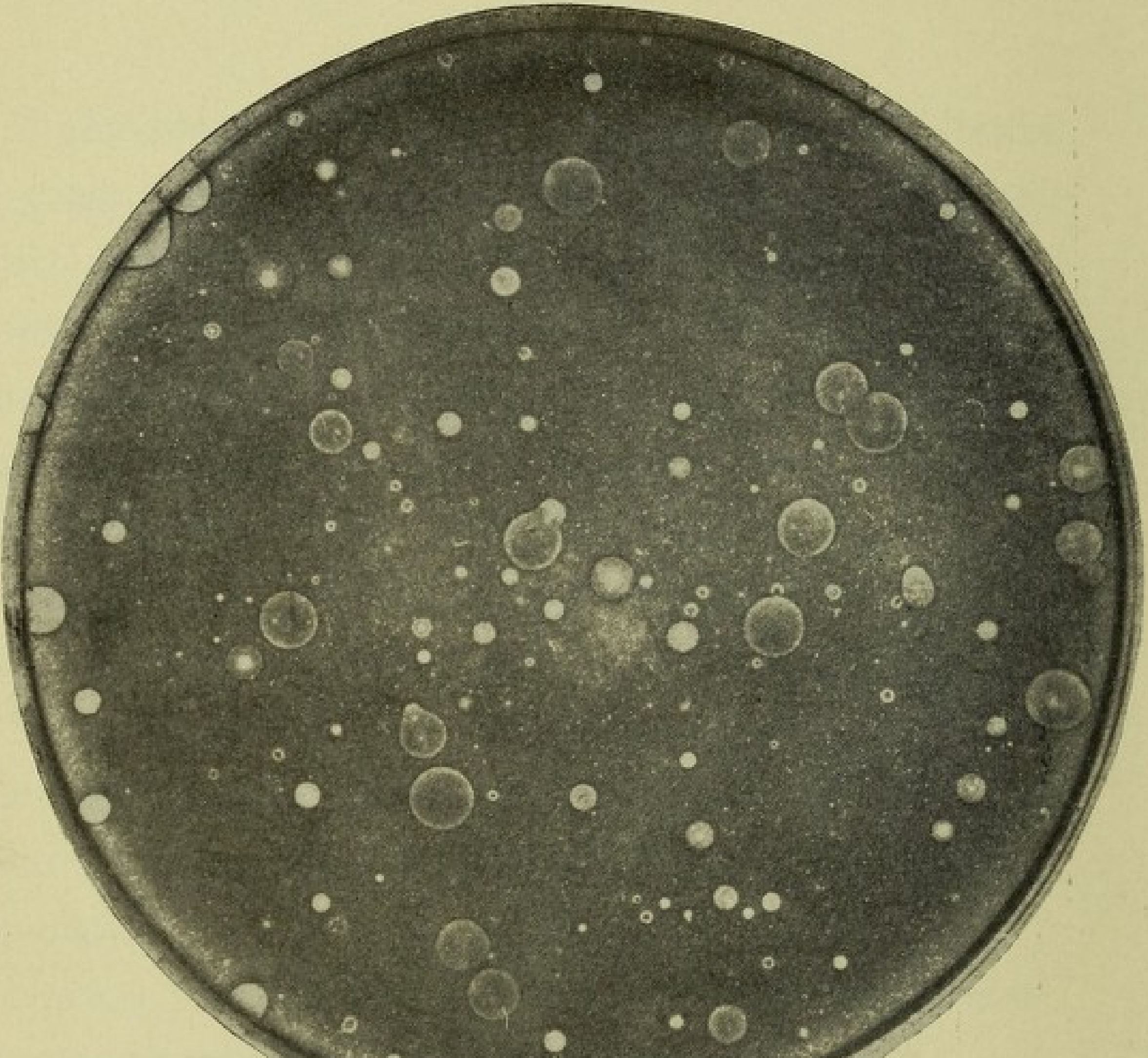
The pipettes used by the author are of the straight form and are graduated to 1/10 c.c.

Water-samples are most conveniently taken in bulbs of glass with long thin stems, similar to the stock article in use for specific gravity determinations. These bulbs can be sterilized by the direct Bunsen flame and sealed while hot. Upon afterwards breaking off the point of the stem under water the water will enter the vessel because of the partial vacuum, and the stem can be at once resealed by using a candle-flame and a blowpipe.

Such bulbs are very convenient for taking deep samples, as the point of the stem can be broken by a separate string while the bulb is held by the sinking-apparatus.

During transportation the bulbs filled with water-samples should be packed in ice.

Upon arrival at the laboratory the base of the stem is cut through with red-hot carbon ; 1 c.c. or less of the water, after agitation, is transferred, by means of a sterilized pipette, to a tube of culture-jelly (the jelly having been previously liquefied by immersing the tube in warm water at 35° C., and the open end



PETRI DISK SHOWING COLONIES OF BACTERIA. (OHLMÜLLER.)

of the tube having been held for a moment in the Bunsen flame); mixing is accomplished by tilting the tube forward and back, and the mixture is then quickly poured into a sterilized Petri dish (3½ inches diameter).* After the jelly has again hardened the dish should be maintained, in the dark, at a temperature of about 20° C.

Each individual bacterium, finding itself imbedded in material supplying abundance of food, proceeds to surround itself by a multitude of its offspring, until at length the "colony" so produced becomes large enough to be seen by the naked eye. These colonies, each of which corresponds to one original bacterium, are of various sizes, as shown in the accompanying illustration. Some of them do, and others do not, liquefy gelatin.

Petri dishes which contain sowings in "agar" should be inverted during development in the incubator to avoid the spreading of surface condensation-water.

The method of sowing water-samples as given above is that in most common use, but the author much prefers to make use of conical "Miquel" flasks, usually 2½ inches in diameter at the bottom, with a tubulated glass cap, ground at the joint. The

---

* When "agar" is employed it is more convenient to melt it at 100° C. and then cool it to about 40° C. before sowing.

tubulation is plugged with cotton. Such flasks receive 20 c.c. each of the culture-jelly when it is first

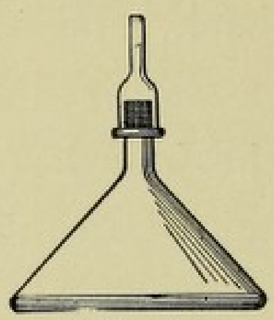


FIG. 8.

made, and are kept in stock like the test-tubes. Taken to the field, they receive, on the spot, the measured amount of water, and the chances of contamination during transfer to the Petri dish, and of multiplication during the journey of the water-sample to the laboratory, are thereby avoided. This method of working has proved very satisfactory, as no transportation of the water-sample is required. In place of sowing a fraction of a c.c. of water, the "dilution method" may be often conveniently employed. One c.c. of the water is mixed with 9 or 99 c.c. of sterilized water and then 1 c.c. of the mixture is sowed in the medium. This is Miquel's favorite method. Of course this dilution must be done with great care, as any error is multiplied.

Counting the colonies of bacteria is undertaken forty-eight hours after the sowing, according to the official German regulations,* but it is well to delay it longer

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* P. Frankland, "Bact. Purification of Water," 29 and 73.

or to count sooner, according as the danger may be less or greater of the colonies growing into each other and thus confusing the count.

Miquel does not usually count inside of two weeks, and at times waits even longer.

Of course what is required is the maximum count, and good judgment must be exercised as to when is the best time to secure it.

For purposes of comparison (and usually comparison examinations are the most important form of water investigations) four days will be found a convenient interval between the sowing and the final counting of the colonies.

As Pearman suggests, much information may be obtained by first plating the water immediately and then keeping a sample of it at blood-heat for 24 hours, and again plating. Such temperature favors the growth of germs derived from intestinal sources, while it is fatal to most water-bacteria.*

When the number of the colonies is large, counting must be done with the aid of a ruled glass plate. The best device for this purpose with which the writer is familiar is the " Miller-McPherson " counting apparatus, which needs but little explanation beyond what is given in the illustration shown in Fig. 9.

The " Wolffhüggle " device which is still so commonly employed has the disadvantage of not firmly

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* *Analyst*, xxi. 144.

fixing the "Petri" dish in place, so that there is no small danger of counting the same colonies more than once. Nor is it possible to make use of a "Miquel" flask as a substitute for the "Petri" dish, if the "Wolffhüggle" counter is to be employed.

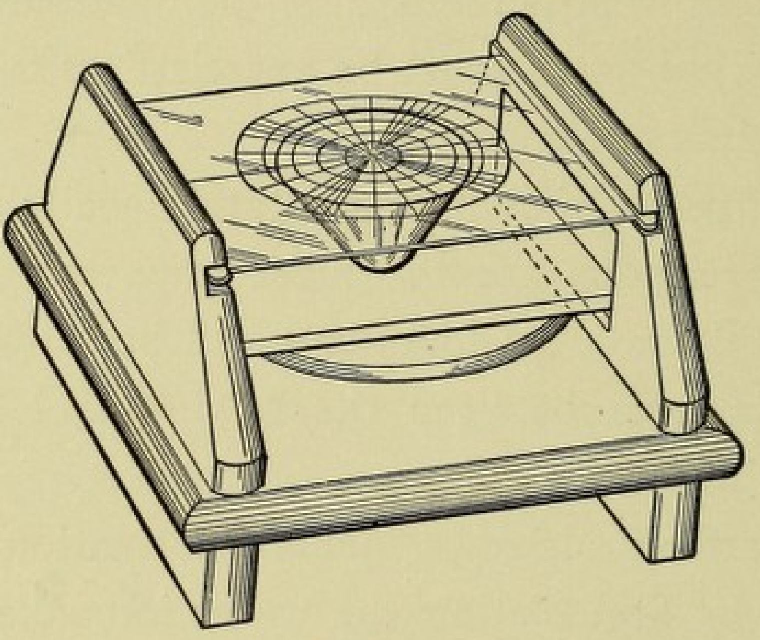

FIG. 9.

The new apparatus is so arranged as to have the ruled glass plate a fixture, while the "Petri" dish rests upon a movable ebonite plate, which is raised or lowered by the wheel beneath it actuating a hollow screw. The dish may be thus always kept firm against the ruled plate, with no chance of slipping, and moreover it will be always in focus no matter what may be its thickness.

Both "Petri" dishes and "Miquel" flasks are counted bottom upwards.

When a "Miquel" flask is used the neck of the inverted flask passes through a hole in the ebonite plate and into the hollow screw, while the wheel beneath raises the bottom of the flask against the ruled plate the same as when a " Petri " dish is employed.*

Sundry bacteria are capable of inducing a fermentative action, with evolution of gas (CO_2 and H), when sown in a medium containing one per cent of glucose. The *Bacillus coli communis* is a prominent member of this group, and the detection of many of these germs in a sample of water is strong evidence of its contamination by intestinal products from man or the higher animals.

Tests for B. coli communis.—1. To each of ten Smith's "fermentation-tubes," charged with sterile "sugar bouillon" (see page 96), add 1 c.c. of the water. Mix by tilting the tube, and place in the incubator at 37.5° C. for three days.

If any gas-forming bacteria be present, gas will collect in the closed limb of the tube, and some knowledge of the numbers of such organisms present may be gained by observing how many of the ten tubes show the reaction.†

2. The amount of gas produced is stated in per-

---

* The counting apparatus and also the " Miquel " flasks may be obtained of Eimer & Amend, No. 211 Third Avenue, New York City.

† See the original article by Dr. Theobald Smith in *The American Journal of the Medical Sciences* for September, 1895.

centages of the length of the closed limb. *B. coli communis* will fill this closed limb about half full of gas, or "50 per cent," by the third day.*

Too little gas (say 25%) or too much (over 75%) would not point towards *B. coli*, especially if the other tests were negative.

In the original experiments reported by Smith the total gas averaged 52 per cent for *B. coli*, and about 23 per cent for certain "transitional forms"; while for *B. lactis aërogenes* and *B. cloacæ* the average ran as high as 76 per cent.

With two exceptions none of the cultures of the latter two gave the "indol" reaction.

3. *B. coli* forms its gas rapidly, evolving most (often all) of it within the first twenty-four hours. Sundry other "gas-formers" act much more slowly. Therefore, note the volume of gas formed during each of the three days.

4. Dr. Theobald Smith holds that *B. coli* forms a gas having the approximate composition of $H : CO_2 : : 2 : 1$, but such has not been the author's experience, although he has always noted that the hydrogen is in excess of the carbon dioxide. With sundry other gas-producers the carbon dioxide is the

---

* As *B. coli* usually ceases to evolve gas by the end of the second day, the total gas will often on the third day be diminished in volume, due to solution of CO_2 in the liquid present.

greater. To determine the CO_2 measure the depth of total gas with a millimetre rule, after filling the bulb to overflowing with solution of KOH. Close the orifice with the thumb and tilt the tube a number of times to cause the KOH to absorb the CO_2. The remaining gas is rated as hydrogen.

5. The liquid in the bulb of the fermentation-tube must be distinctly acid to indicate *B. coli.*

6. Indol is a compound belonging to the aromatic series, which produces a red color when acted upon by nitrous acid. It is formed by the breaking up of peptone by the action of putrefactive bacteria, including *B. coli.*

To test for its presence place 50 c.c. of the water, together with 50 c.c. of sterile bouillon, in a sterilized cotton-plugged flask, and keep the same in the incubator at 37.5° C. for two days. The high temperature will destroy common water-bacteria, but will encourage the growth of the colon group.

Place 2 c.c. strong sulphuric acid and 2 c.c. of the stronger sodium nitrite solution (see page 37) in a 100-c.c. "Nessler" jar.

Dilute with 50 c.c. water and then pour in 25 c.c. of the incubated culture prepared above. A red coloration indicates indol, and is an additional evidence of the presence of *B. coli.*

7. Inoculate "nutrient gelatin" from the incubated culture obtained in 6. Let the plates develop as usual

and observe if any of the *non-liquefying* colonies are *whitish*, with *irregular, leafy* outlines and showing lines more or less radial. Such characteristics point to *B. coli.*

8. Place a quantity of new milk in an Arnold's sterilizer for fifteen minutes, and afterwards allow it to stand overnight in a cool place. Siphon off the lower layer of milk, avoiding the cream. Place it in cotton-plugged test-tubes and sterilize as usual.

Add to several of these tubes 1 c.c. of the incubated culture obtained in 6, and keep in the dark at room temperature.

B. coli coagulates milk in from 1 to 3 days. Other forms usually take more time.

9. If inoculations from the colonies obtained in 7 or from the culture obtained in 6 be examined as "hanging-drop" cultures, the bacilli will be found to be motile if they be *B. coli.*

This motility will be manifested, however, by only a portion of the bacilli present in the field, and its intensity will be far less than that shown by the typhoid bacillus.

Smith's summary of characteristics of *B. coli communis* may very properly be given place here : *

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* *Am. J. Med. Sci.*, Sept., 1895.

Colony: non-liquefying, opaque, whitish with irregular margin.

Motile in hanging-drop culture.

Coagulates milk in 1 to 3 days.

Bouillon culture gives "indol" reaction.

In fermentation-tube total gas = about 50 per cent.

Most of the gas evolved during first 24 hours.

Ratio of H to $CO_2 = 2:1$.

Reaction of liquid in bulb of fermentation-tube, strongly acid.

Differentiation of species, as has already been said, must be left for discussion to writers upon general bacteriology; but a moment can be properly spent here upon the often broached topic of the recognition of the typhoid germ in water, and also a word added with reference to the diagnostic value of the demonstration of the presence of *Bacillus coli communis.*

Laws and Andrewes in their report to the London County Council show that the chance of discovering *B. typhosis* in sewage is exceedingly small. They entirely failed to find it in London sewage.

Finally they examined the sewage flowing (without disinfection) from the Eastern Hospital at Homeston, which same received the dejections of forty typhoid patients.

Out of a whole series of samples examined from this latter source only two colonies of *B. typhosis* were differentiated with certainty.[*]

Similar experience has been recorded by practically all the recent observers, and consequently search for the typhoid germ in water is becoming very unusual.

The present position of this question is tersely summed up by Dr. W. H. Welch :

"We possess no satisfactory method for the determination of the presence of the typhoid bacillus in water. With our present methods the most which can be expected from the biological examination of water as regards this question is the determination, not of the actual presence of the typhoid bacillus, but of the possibility or probability of its presence. Our principal guide at present in drawing conclusions as to the possible presence of the typhoid bacillus in suspected drinking-water is the recognition of fæcal bacteria, and more particularly of members of the colon group." [*]

This brings us down to our second query regarding the diagnostic value of the "colon group."

Speaking of a bacterial index of fæcal pollution, Stoddart says : "Until we are able to single out a form specifically associated with fæcal matter, and at least as easily recognizable as the tubercle or diph-

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[*] See Rafter's "Water of Lake Erie," page 14.

[†] *J. Am. Pub. Health Asso.*, xx. 502.

theria bacillus, we shall do well to avoid such expressions as contain 'index of fæcal pollution.' It is an assumption to say that *B. coli communis* does not occur in abundance in organic matters other than animal excreta." *

It is certainly the author's experience that the "colon group" is widely distributed, he having found it in waters that a "sanitary survey" would unquestionably pronounce pure; but it cannot be denied that its persistent presence in large numbers is an indication of pollution that must not be overlooked; and, moreover, the proof of its absence serves to materially aid in formulating an opinion concerning the purity of a water.

Bacteriologists will, of course, very properly, extend their differentiations to the utmost limit, in the hope of positively establishing the much-needed "index of fæcal pollution," but for routine water-work it is doubtful if it pay to go beyond the tests already given. Moreover, it should be said that to make such tests as have been here outlined of real value, they should be comparative in character; and the interpretation of results should be based upon data furnished by closely related local standards.

Samples for bacteriological work are far more

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* *Analyst*, xxii. 122 and 123.

quickly damaged by keeping than are those intended for chemical analysis ; thus :

SPRING-WATER, TROY, N. Y.

Kept at room temperature.

November	10.....	830 bacteria per c.c.
"	12.....	8,128 " " "
"	13.....	9,433 " " "
"	15.....	12,740 " " "

Much more striking instances are given by Miquel. Thus for the Dhuis water :

	Temperature.	Bacteria per c.c.
12 noon..................	16.6° C.	57
1.30 P.M..................	19.5° "	143
3 P.M....................	20.9° "	456

For Vanne water :

	Temperature.	Bacteria per c.c.
Immediate	17° C.	56
After 24 hours.........	21.2° "	32,140

Also for Vanne water :

Immediate	15.9° C.	48
After 2 hours..........	20.6° "	125
" 1 day	21.0° "	38,000
" 2 days...........	20.5° "	125,000
" 3 days...........	22.3° "	590,000

Deep-well water (Frankland):

Immediate	20°	C.	7
After 1 day	20°	"	21
" 3 days	20°	"	495,000

The last instance shows that multiplication of bacteria is not to be accounted for by a simple increase of temperature.

As has been already stated, it is exceedingly important to keep water-samples at freezing temperature during transportation to the laboratory. Germs do not commonly multiply at such a temperature, but, as has been shown in France, this does not hold good for waters heavily charged with bacterial food. In one instance "sea-water, constantly maintained at 0° C., changed in bacterial contents from 150 to 520 per cubic centimetre in 24 hours."

On the other hand, great cold is not fatal to germ-life. The author submitted cultures of ordinary bacteria to the temperature of solid carbon dioxide during many hours without causing their destruction.*

The number of bacteria per cubic centimetre in water-samples taken from the same source at different times will greatly vary with the season and changes in local conditions. Thus the Hudson River

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* Ravenel has since exposed them for hours to the temperature of liquid air with like result.

water sampled at the Troy intake shows the following variation in bacterial contents during the colder half of the year; similar results for a Rensselaer County spring-water are also given:

	Hudson River.	Spring.
October	1,487	158
November	626 8,128	750
December	1,463	1,620
January	4,022	2,519
February	3,322	166
March	....	8,520
April	1,343 17,665	476

The influence of high water in the river is well shown by the difference between the early and late April samples. Surface-washing is the cause of such an increase. The effect of melting snow, and consequent surface-washing, is also shown in the March sample of spring-water.

In general it may be said that so long as a river is fed by springs, that is, during the hot months, the bacteria tend to remain low in numbers, but with the advent of floods germ-life increases in quantity, because of the washing of the surface of the ground by heavy rainfall and melting snow. During the period when severe frost ties up all surface sources the bacteria again diminish in numbers.

Miquel gives the following averages for bacteria per cubic centimetre of Seine water taken at Ivry, and for the Vanne spring-water supply of Paris :

	Seine.	Vanne.
January	52,670	400
February	43,120	1,625
March	34,710	1,560
April	38,640	860
May	12,930	720
June	28,150	590
July	14,130	865
August	6,780	985
September	20,220	465
October	22,350	495
November	37,720	495
December	78,950	525

or

	Seine.	Vanne.
Spring	26,570	720
Summer	13,710	770
Autumn	46,340	505
Winter	43,500	1,200
Annual mean	32,530	800

The River Seine, unlike the Hudson, is not affected in the spring by the melting of great masses of northern snow.

A well-water will sometimes show great and irregular variations in bacterial contents ; thus Buchner cites the following case from Munich : *

July 1.............	600 bacteria per c.c.			
" 8.............	1,200	"	"	"
" 15.............	4,000	"	"	"
" 21.............	80	"	"	"
" 29.............	10,000	"	"	"
August 3.........	400	"	"	"

As has been said, Miquel permits only a few bacteria to enter the culture-jelly of a single flask, accomplishing this end by his system of dilution, and he then waits at least two weeks before making the count. This method is impracticable for routine water examination, nor is it necessary for the obtaining of satisfactory results, although, of course, it has the advantage of permitting slowly developing colonies which would be otherwise lost in the crowd of liquefying bacteria to grow sufficiently to be recognized, as is very evident from the figures of Miquel given below.

Out of 1000 bacteria in water, sowed in culture-jelly, the following numbers of colonies will appear on the successive days :

---

* " Das Wasser," Fischer, 36.

1st day	20	colonies
2d “	116	“
3d “	118	“
4th “	133	“
5th “	143	“
6th “	107	“
7th “	88	“
8th “	55	“
9th “	41	“
10th “	38	“
11th “	33	“
12th “	29	“
13th “	30	“
14th “	25	“
15th “	24	“

“The complete analysis of a water may require from several weeks to several months of constant and difficult work; I may add that, in the present state of our knowledge, it is often impossible to complete it, for the reason that several of the species of bacteria we meet with are as yet unknown.” (Miquel.)

Sterilizing a water by heat is not so easy as most people imagine. Absolute sterility can be attained in about forty-five minutes by heating the water, under pressure, to 115° C.

Ordinary boiling for half an hour will destroy about

99 per cent of all bacterial life, and fortunately that which remains is entirely harmless. No pathogenic germs are capable of resisting such a temperature for half an hour.

Experimenting with Seine water, which contained, at the ordinary temperature of 22° C., 848 bacteria per cubic centimetre, Miquel found the following decrease in numbers of germs as the temperature was raised :

Water maintained 15 Minutes at	Bacteria per c.c. remaining.
43° C.	640
50° "	132
60° "	40
70° "	27.2
80° "	26.4
90° "	14.4
100° "	5.2

The following is a freely translated extract from Miquel's "Auto-infection of Waters" :

"When samples of various waters, pure and impure, are maintained at constant temperature, say 20° C., they behave very differently in the matter of the increase of their bacterial contents. With pure waters the increase is rapid and temporary, while with impure waters it is slow and lasting. This fact appears to present great hygienic interest, for it shows that many waters are not only incapable of favoring the multiplication of certain organisms, but may be, for them, indifferent or even deadly media.

"The waters which are relatively the most nourishing to the known pathogenic organisms are the

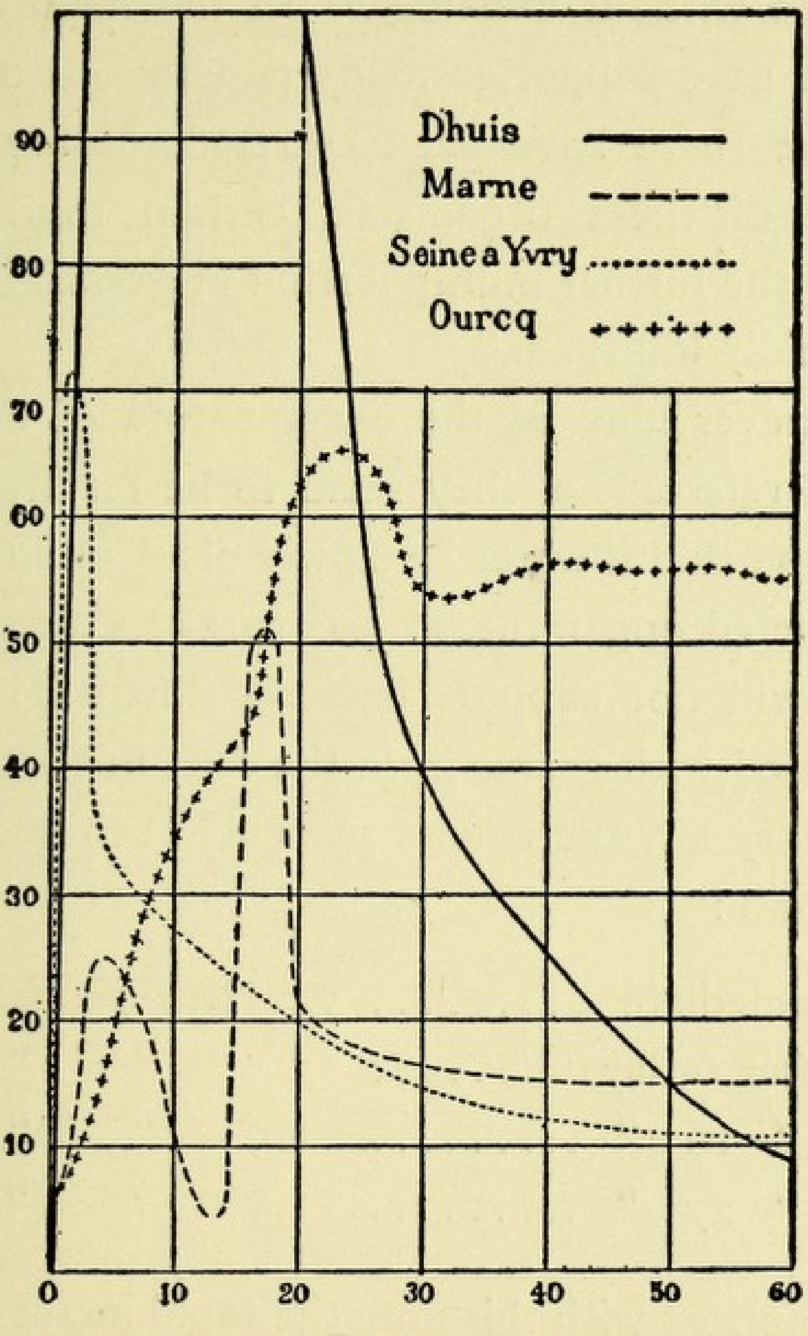

Fig. 10.

'new' waters, that is to say, those waters which are slightly charged with bacteria, and which have never been the seat of large and sudden growths. Epidemics are more intense when the disease is transmitted by waters which are ordinarily of a high degree of bacterial purity, by a ‘new’ water, for instance, which permits the disease-bacilli to multiply in great numbers without difficulty. In the impure waters, such as have supported many generations of various bacteria, there have been secreted, during the existence of these organisms, certain toxins which oppose the further multiplication of germs, if they do not rapidly kill them.”

Whatever may be the exact nature of the toxins Miquel referred to, they seem to be readily volatile. He found that if water be slowly distilled, in specially constructed apparatus, at 30° to 35° C., such a distillate will not support bacterial life. Thus when inoculated with germs from the dust in 1650 litres of laboratory air, the counts per cubic centimetre were as follows :

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Immediate	75	bacteria per c.c.
After 6 days.........	7	“ “ “
“ 16 “	1.5	“ “ “
“ 27 “	1.5	“ “ “

In contrast with this note the rapid increase following the addition of the dust from 20 litres of air to river-water previously sterilized in the ordinary manner.

Immediate....	6.5	bacteria per c.c.
After 7 days..	750,000	" " "
" 10 " ..	900,000	" " "
" 31 " ..	1,675,000	" " "
" 90 " ..	62,500	" " "
" 119 " ..	86,750	" " "
" 272 " ..	48,000	" " "

Note also the decrease in germ-life as the bacterial toxins accumulate.

Cramer's observations upon water from Lake Zurich also show the same decline in bacterial life through long keeping of samples :

Immediate	143	bacteria per c.c.
After 1 day......	12,457	" " "
" 3 days.....	328,543	" " "
" 8 "	233,452	" " "
" 17 "	17,436	" " "
" 70 "	2,500	" " "

For the enumeration of organisms not bacterial, in water, Mr. D. D. Jackson has devised a most valuable modification of the original Sedgwick-Rafter apparatus.

The body of the filter is cylindrical, as shown, and 2 inches in diameter. The distance from the top to the conical base is 9 inches. The small cylindrical prolongation of the cone's apex is 2½ inches long and

½ inch in diameter. A perforated rubber stopper, with its hole covered by a disk of fine bolting-cloth, is fitted to the smaller end of the funnel and about ¾ inch of carefully screened fine sand (between 80 and 100 mesh) is poured into the narrow tube and wet down with distilled water.

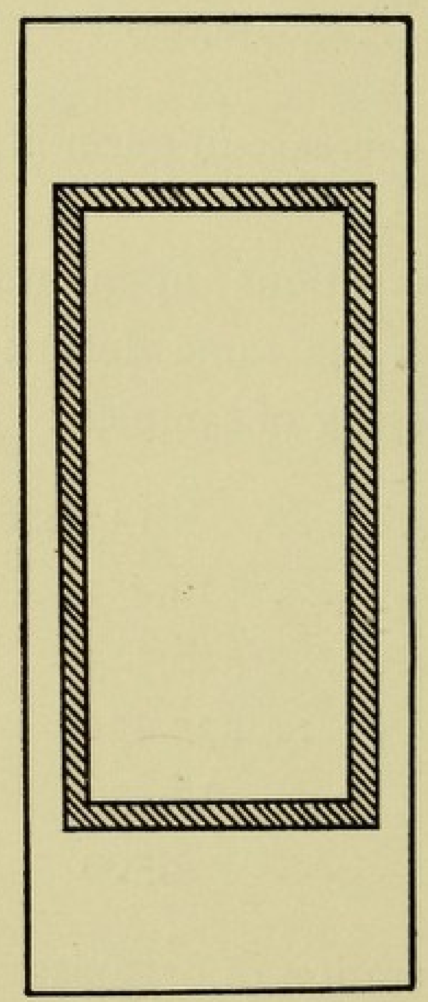

FIG. 11.

From 250 to 500 c.c. of the water under examination are now permitted to filter through the sand. After the water has run through, the sand with the material strained off by it is washed into a test-tube by 5 c.c. of distilled water delivered from a pipette. The organisms, sinking in the test-tube much more slowly than the sand-grains, may be decanted, with the water in which they float, into a second test-tube. From this decanted portion, after agitation, 1 c.c. is delivered by a pipette to the covered " counting-cell," which it completely fills.* See Fig. 11.

This excellent device will be found of great service in recognizing and enumerating the various forms of life commonly met with in waters.

For purposes of general differentiation recourse must be had to the writings of biologists who have made such work a specialty. Particularly valuable is the recent publication by G. C. Whipple, " The Microscopy of Drinking-water," published by John Wiley & Sons.

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* These cells, together with all accessory apparatus, may be obtained of Richards & Co., No. 30 East 18th Street, New York City.

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