and we are forced to resort to certain lines of procedure which experience has taught us differentiate more or less sharply between good and bad waters, even though the actual composition of such waters be not thereby revealed.

Organic matter containing nitrogen, when breaking down through putrefaction, liberates its nitrogen as ammonia, and this in turn changes to nitrites and nitrates of the bases present. The ammonia formed, as above, may be separated from the water by simple distillation and when determined is reported as "free ammonia." Such of the nitrogenous organic matter as yet remains undecomposed may be in great part broken up, and its nitrogen given off as ammonia, by distillation with strongly alkaline potassium permanganate. This second evolution of ammonia is called "albuminoid," for the reason that albumin acts in like manner when similarly treated. Wanklyn's "albuminoid ammonia process" is based upon the foregoing facts.

The reagents necessary are:

"*Nessler's*" *Solution.*—Dissolve 16 gm. mercuric chloride ($HgCl_2$) in about half a litre of pure water. Dissolve 35 gm. 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 gm. solid potassium hydrate (KOH). Dilute to 1 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.

Pure Water.—This must be prepared with great care, in a room free from the usual laboratory fumes. In short, 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 account 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 gm. solid potassic hydrate and 8 gm. crystallized potassic permanganate in 1,250 c.c. of pure water. Boil down to 1 litre and keep for use.

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

Standard Ammonia Solution.—Dissolve 1.5706 gm. 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 0.01 mgm. 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 and through a large-size soft-rubber stopper. This connection must be thoroughly tight. Place 250 c.c. *pure* water in the retort and add 10 c.c. of the sodic carbonate solution. Distil off three 50-c.c. jars of water, and "nesslerize" the third in order to be 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.*, 150 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 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 customary to 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 0.01 mgm. NH_3, the whole amount of "free ammonia" present in the original half-litre of water would be: First, 0.09 mgm.; second, 0.03 mgm.; third, 0.01 mgm.; fourth, 0.00 mgm.—total, 0.13 mgm. Multiplying this by two to obtain the quantity for an entire litre, and remembering that 1 mgm. 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.

Concerning the interpretation of results, Wanklyn 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." This statement is altogether too strong. Waters of high organic purity or those of gross pollution are undoubtedly easy to classify, but with the numerous 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.

As an illustration of variation in the ammonias, the following data are offered:

	Free ammonia.	Albuminoid ammonia.
Average in sundry surface waters known to be pure	0.063	0.066
Average in sundry surface waters known to be polluted	.182	.228
Average in sundry ground waters known to be pure	.009	.007
Average in sundry ground waters known to be polluted	.107	.081

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.

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.

As a further aid to judgment the following analyses are given of sundry waters in different parts of the country, several of them having caused disease. Also a few instances of waters of reliable quality.

No.		Free ammonia.	Albuminoid ammonia.	Chlorine.	N as nitrate.	N as nitrite.	Required oxygen.	Total solids.
	Impure.							
1	Shallow city well	.025	.68	122.0	17.38	Trace.	1.4	554.0
2	City well 30 feet deep (caused typhoid)	.005	.035	146.0	10.0	.0	1.0	769.0
3	Rock-drilled city well 57 feet deep	2.025	.0	69.0	.025	.0	.85	487.0
4	Spring-water (caused repeated cases of dysentery)	.01	.025	6.0	7.0	.0	.8	35.0
5	Well near city	.005	.045	24.0	9.0	.0	1.1	215.0
6	Country well, strong salty taste	.59	.245	2,803.0	.....	.25	.....	5,325.0
7	Town well	.815	.075	36.0	.0	Trace.	.....	421.0
8	City well 250 feet deep	1.59	.395	102.0	.0	Trace.	.....	681.0
9	City well 255 feet deep	.31	.02	58.0	.0	.0	6.45	635.0
10	City well 226 feet deep	1.11	.08	199.0	.0	.0	1.3	779.0
11	Deep well in large stock-yard, Kansas City	1.745	.025	80.0	.0	Trace.	.....	.....
12	Hudson River, at Troy, during freshet	.42	1.0	3.0	.5	Trace.	.....	205.0
13	Deep city well, in "made ground"	Excessive.	Excessive.	47.0	.875	.0	2.5	637.0
	Pure.							
14	Peaty mountain stream (autumn)	.055	.23	2.4	.0	.0	7.4	34.0
15	Same stream in winter	.055	.117	1.9	.08	.0	6.6	47.5
16	Mountain spring	.04	.048	4.0	1.404	Trace.	.3	228.0
17	Town supply, Elizabethtown, N. Y.	.048	.002	1.05	.05	.0	.35	106.0
18	Large well-situated spring	.027	.006	2.2	1.6	.0	.0	90.0
19	High mountain lake (peaty)	.01	.34	2.0	.0	.0	6.6	43.0
20	Lake Erie (middle of lake)	.045	.112	3.5	.08	Trace.	1.25	134.0
21	Lake Superior (forty miles from shore)	.03	.02	1.0	.1	.0	1.15	54.0
22	Flowing wells (New Jersey coast)	.023	.05	9.0	.5	Trace.	.4	30.0
23	Driven wells (Hempstead, N. Y.)	.013	.004	2.5	1.25	.0	.35	22.0
24	Domestic well (Catskill Mountains)	.016	.007	.75	.175	.0	.35	32.0

Oxygen-Consuming Capacity ("Required Oxygen").—This method for estimation of organic matter is Kubel's modification of the old permanganate process of Forschammer. The necessary solutions are.

Standard Potassic Permanganate Solution.—Dissolve 0.3952 gm. of the salt in 1 litre of distilled water. Each cubic centimetre of such solution will contain 0.1 mgm. of oxygen available for oxidation. The available oxygen of the permanganate in presence of sulphuric acid may be represented by the equation

$$K_2Mn_2O_8 + 3H_2SO_4 = K_2SO_4 + 2MnSO_4 + 3HO_2 + 5O.$$

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 gm. of the crystallized acid in 1 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 cubic centimetre for cubic centimetre. In practice, however, this correspondence will be found to be approximate only.

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 cubic centimetres 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 milligrams 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:

Total permanganate solution used	25.0 c.c.
Less that required for the oxalic acid	9.7 c.c.
Hence that required to oxidize organic matter	15.3 c.c.

Corresponding to 1.53 mgm. oxygen.

Therefore "required oxygen" is $1.53 \times 5 = 7.65$ 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 given above.) Average in sundry surface waters known to be pure, 1.58; known to be polluted, 3.00; average in sundry ground waters known to be pure, 0.31; known to be polluted, 1.06.

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 gm. of the salt in 1 litre of distilled

water. Each cubic centimetre will contain 1 mgm. metallic lead.

Place the water in a 100-c.c. "Nessler" jar; add four 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 organisms 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 sulphocyanate 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 sulphocyanate solution.

The *standard iron solution* is prepared by dissolving 0.1 gm. pure iron in a little HCl to which a few drops of HNO_3 have been added, and then diluting to 1 litre.

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" 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 fifteen 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 personally to bore the chips from the centre of the log.

When examining the filtrate from a mechanical plant by the logwood test, it would be well to take the alkalinity also.

If the filtrate be alkaline free alum cannot be present, and the logwood reaction, if then found, is due to particles of $Al_2(OH)_6$ passing the sand bed and becoming dissolved in the reagents employed.

Lacmoid should be used as the indicator, as methyl-orange does not indicate an acidity due to alum.

Bacteriological Examination of Water.—For the accomplishing of the determinations that 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 1 litre with water. Add 10 gm. of peptone (Witte's) and 5 gm. of common salt. Heat in a double-walled "oatmeal-boiler" until the added ingredients are dissolved. Should the medium not be clear, cool somewhat, add the white of an egg, bring to a boil without stirring, 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 1 litre of the medium made neutral to phenolphthalein).

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 (0.5 gm. phenolphthalein in 100 c.c. of fifty-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 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 "tinfoil" to prevent evaporation, and sterilization is accomplished by heating in an "Arnold's sterilizer" for twenty minutes on three successive days.

Sterilization may be accomplished by a single heating in an autoclave to fifteen pounds pressure for fifteen minutes.

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: Gelatin (best French), 120 gm.; peptone (Witte's), 10 gm.; common salt, 5 gm. 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 NaOH 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 or test tubes (10 c.c. in each). Plug the vessels with cotton and sterilize them in an Arnold's sterilizer for *twenty minutes* on three successive days or by a single heating in an autoclave at fifteen pounds pressure for fifteen minutes.

Sugar Bouillon.—Prepared the same as the ordinary "bouillon" except that 10 gm. of pure glucose are added with the peptone and salt. Its reaction should be neutral.

Inasmuch as the high temperature of the autoclave might partly decompose the sugar, it is better to sterilize in the Arnold's sterilizer for twenty 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.

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

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 1 litre of agar-agar take: A. Chopped meat, 500 gm.; water, 500 c.c. Mix and place in cool place overnight, then strain through cloth. Add: Peptone (Witte's), 10 gm.; common salt, 5 gm. B. Agar-agar, 12 gm.; water, 500 c.c.

Place B in autoclave, run up to about thirty 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. (*Ravenel's method.*)

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

Nitrate Solution.—Dissolve 1 gm. of peptone and 0.2 gm. of KNO_3 in a litre of water. Place the solution in cotton-plugged flasks (50 c.c. in each) and sterilize.

Dunham's Solution.—Dissolve 10 gm. of peptone and 5 gm. of NaCl in a litre of water. Place the solution in cotton-plugged flasks (50 c.c. in each) and sterilize.

Empty glassware is best sterilized by a single heating for one hour to 170° C.

The 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.

Water samples for bacteriological purposes 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 afterward 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 1 c.c. or less of the water, after thorough agitation, is transferred, by means of a sterilized pipette, to a sterile Petri dish. 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 of the tube having been held for a moment in the Bunsen flame) is then quickly poured into the Petri dish, and mixing is accomplished by tilting the dish forward and back. After the jelly has again hardened the dish should be maintained, in the dark, at a temperature of about 22° C.

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

Each individual bacterium, finding itself embedded 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 and shapes. Some of them do, and others do not, liquefy gelatin.

The method of sowing water samples as given above is that in common use, but preference is sometimes given to the use of conical "Miquel" flasks, usually two and one-half inches in diameter at the bottom, with a tubulated glass cap, ground at the joint (Fig. 5036). The tubulation is plugged with cotton. Such flasks receive 10 c.c. each of the culture jelly when it is first 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 of 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.

Fig. 5036.—Miquel Flask.

In place of sowing a fraction of a cubic centimetre of water, the "dilution method" conduces to greater accuracy. One cubic centimetre or 10 c.c. of the water are diluted to 100 c.c. with 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 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.

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. When the number of the colonies is large, counting must be done with the aid of a ruled glass plate.

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 persistent presence of many of these germs in a 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," add 1 c.c. of the water. Mix by tilting the tube, and place in the incubator at 38° to 40° 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.

The amount of gas produced is stated in percentages of the length of the closed limb. *B. coli communis* will fill this closed limb about half full of gas, and will do so rapidly, usually ceasing to form any more after the end of the first day. Sundry other "gas-formers" act much more slowly. Therefore note the volume of gas formed during each of the three days.

The total gas will often on the third day be diminished in volume, due to solution of CO_2 in the liquid present.

To determine the CO_2, fill 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, although it contains also a little nitrogen and methane, as shown by Pennington and Kûsel.

Sowings from pure cultures gave the following averages: Total gas, 35.3 per cent.; gas formed during first day (as percentage of total gas), 100 per cent.; ratio of H to CO_2, 71 to 29.

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

3. *Indol* (C_8H_7N) 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 25 c.c. of the water, together with 50 c.c. of sterile Dunham's solution, in a sterilized cotton-plugged flask, and keep the same in the incubator at 38° to 40° C. for three 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 sodium nitrite solution in a 100-c.c. "Nessler" jar. Cool. Dilute with 50 c.c. water and then pour in the previously cooled incubated culture prepared above. A red coloration indicates indol, and is an additional evidence of the presence of *B. coli*.

4. Inoculate "nutrient gelatin" from the incubated culture obtained in 3. 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*.

5. Place a quantity of new milk in an Arnold's sterilizer for fifteen minutes, and afterward 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 3, and keep in the dark at room temperature.

B. coli coagulates milk in from one to three days. Other forms usually take more time.

6. If inoculations from the colonies obtained in 4 be examined as "hanging-drop" cultures, the bacilli will be found to be motile if they be *B. coli*. This motility may 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.

7. Add 25 c.c. of the water to a flask of "nitrate solution" and place in the incubator for three days. Test the contents of the flask at the end of that time for "nitrite."

B. coli reduces the nitrate to nitrite.

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. typhosus* 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. typhosus* were differentiated with certainty.

A 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, of Johns Hopkins University: "The most which can be expected 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."

With regard to the diagnostic value of the colon group as supplying the much-needed "index of fæcal pollution," it must be stated that the group is widely distributed, and is often found 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 materially to aid in formulating an opinion concerning the purity of a water.

It is, of course, possible to proceed much further than has been here outlined in the bacteriological examination of water, but for routine work it is doubtful if it pays to go beyond the tests already given. Moreover, it should be said that to make such tests 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.

"Microscopical" examination of water has for its object the study of such suspended material, whether organized or not, as may be examined directly without the intervention of the "culture" methods required for bacteria. Plants and animals, dead or alive, amorphous organic material and inorganic particles of all kinds, are classified and their numbers estimated by this form of investigation. It is of especial value in determining the cause of those disagreeable odors and tastes which occur in stored water from the abundant growth of certain small aquatic plants. Concentration of the water sample is, of course, necessary in order to make a proper examination possible, and this end is accomplished by a modification of the original Sedgwick-Rafter apparatus.

This is a glass cylinder two inches in diameter, terminating in a conical base. The small cylindrical prolongation of the cone's apex is two and one-half inches long and one-half inch in diameter. A perforated rubber stopper, with its hole covered by a disc of fine bolting-cloth, is fitted to the smaller end of the funnel and about three-fourths inch of carefully screened fine sand (between eighty and one hundred mesh) is poured into the narrow tube and wet down with distilled water. 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 a covered "counting cell," which it completely fills. The objects discovered in this counting cell will, if multiplied by five, give the total number originally existing in the entire volume of water filtered.

It is manifestly impossible, in an article such as this, to dwell at length upon the very extensive subject of microscopic examination; therefore for purposes of general differentiation recourse must be had to the writings of biologists who have made such work a specialty.

William P. Mason.

WATER-GAS, POISONING BY.—Water gas is a mixture of carbon monoxide and hydrogen with some nitrogen and carbon dioxide, obtained by the action of steam on highly heated carbon (*e.g.*, coke or anthracite). It is nearly odorless and burns with non-luminous flame. For use as an illuminant, it is enriched; that is, charged with hydrocarbons from petroleum or bituminous coal. In this form it has come into extensive use. Owing to the highly poisonous character of carbon monoxide as compared with the hydrocarbons that form the bulk of the older "coal gas," a great increase in fatalities from the inhalation of illuminating gas has been noticed in the mortality statistics of late years. The characteristic effect of carbon monoxide is its affinity for hæmoglobin, with which it forms a compound that cannot be broken up by free oxygen. Hence, even a small amount of carbon monoxide in the inspired air will cause a steady accumulation of the toxic material until so much of the hæmoglobin is rendered inactive that death results from a chemical asphyxia. The symptoms are marked muscular weakness, rapid pulse, flushed face, and a strong tendency to sleep. The blood becomes abnormally red, the breathing becomes stertorous, and the patient dies from failure of respiration. If the patient be rescued before the condition is far advanced, the return to consciousness is slow, even under treatment, and a fatal result from brain disease may yet occur. In advanced cases, red spots are noted on the skin. The blood putrefies much more slowly than normal blood. The fatal period is somewhat uncertain, but may be several hours. The treatment is not satisfactory. Artificial respiration does little good. Inhalation of oxygen under pressure has been proposed, but this method does not promise much. Transfusion of blood has been successful in experiments on animals. Good results have been claimed for the administration, by the stomach and hypodermically, of hydrogen dioxide, but this seems doubtful. Hot applications should be used to restore normal temperature. The bright redness and slow putrefaction of the blood are important post-mortem data. Several chemical tests have been proposed, but they are not very satisfactory in actual practice, as ordinary illuminating gas contains several active substances.

Henry Leffmann.

WATSON'S SPRINGS.—Greene County, Georgia.

POST-OFFICE.—Maxey's boarding-houses and cottages.

These springs are located eleven miles north of Greensboro, on the Georgia Railroad, and eight miles west of Maxey's Depot, on the Athens branch of the Georgia Railroad. The group is one-quarter of a mile from the Oconee River, which will be navigable at this point when the government works on the river are completed.

The springs are reached by private conveyance from the points above mentioned. The scenery in the neighborhood of the springs is varied and the climate delightful, the temperature rarely falling below the freezing point in winter or rising above 90° F. in summer (95° F. is the highest temperature known). The springs are four in number, viz.: the "Sulphur" Spring, the "Chalybeate," the "Alum," and the "Ice" Spring. The following partial analysis of two of the springs was made some years ago by Dr. J. R. Duggan:

The Sulphur Spring contains calcium carbonate, potassium carbonate, iron carbonate, potassium sulphate, sodium sulphate, sodium chloride, silicic acid, hydrogen sulphide gas, and carbonic acid gas. The temperature of the water is 59° F.

The Chalybeate Spring contains iron carbonate, magnesium carbonate, sodium chloride, potassium sulphate, calcium sulphate, and silica. The temperature of the water is 61° F.

The "Ice" spring has not been analyzed, but its waters are delightfully cold and refreshing and palatable at all times. The sulphur and chalybeate springs yield about one gallon of water per minute. Their waters are stated to be highly efficacious in rheumatism and dyspepsia, and in renal, cutaneous, and blood diseases.

James K. Crook.

More than forty boarding-houses. In addition, the houses of private citizens are often thrown open to visitors. Among the best-known hotels are the Fountain Spring House, which accommodates 800 guests, the Park, 300; the Spring City, 250; National, 150; Terrace, 100, etc. Most of the hotels maintain bands during the season. Open-air public concerts are also given morning and evening. There is one theatre in the place besides two public halls. The Fox River runs through Waukesha, and is large enough for row boats. This is a region of lakes, there being no less than thirty-six in Waukesha County, the most remote being only eighteen miles distant. The lakes are surrounded by hotels and cottages, and during the summer they constitute a vast picnic ground. The prime attraction of Waukesha, however, is found in the great group of mineral springs located here. The waters of these springs are chiefly alkaline, chalybeate, and calcic. They have become known throughout the United States. Following are analyses of some of the most important:

ONE UNITED STATES GALLON CONTAINS:

Solids.	Bethesda. C. F. Chandler. Grains.	Clysmic. 1. Rathbone. Grains.	Clysmic. 2. R. O. Doremus. Grains.	Clysmic. 3. R. O. Doremus. Grains.	Fountain. Blaney. Grains.	Hygeia. A. Thiel. Grains.	Silurian. W. S. Haines. Grains.	Vesta. G. Bode. Grains.
Sodium bicarbonate	1.26	1.26	4.31	0.80	1.02	2.36	0.30	0.41
Calcium carbonate	....	....	....	....	....	....	9.93	
Calcium bicarbonate	17.02	16.04	16.15	15.90	13.78	16.73	....	13.43
Magnesium carbonate	....	....	....	....	....	....	6.83	
Magnesium bicarbonate	12.39	13.56	9.22	8.54	9.20	13.14	....	10.74
Iron carbonate	....	....	....	....	....	....	.13	
Iron bicarbonate	.04	.04	.57	.69	.05	.58	....	.05
Iron phosphate	....	....	....	....	....	....	Trace	
Sodium sulphate	.54	.56	.69	1.08	.36	.52	.29	.55
Manganese phosphate	....	....	....	....	....	....	Trace	
Potassium sulphate	.46	.46	.50	.20	....	.82		
Sodium phosphate	....	.03	.43	.45	....	.04		
Sodium chloride	1.16	1.17	.35	.55	Trace	1.25	.19	0.30
Aluminum oxide	.12	Trace	....	....	.09	.72	.59	.13
Alumina	....	....	Trace	Trace	....			
Silica	.74	.72	.80	.81	.85	.15	.70	.85
Organic matter	1.98	1.62	Trace	Trace	.31	Trace	Trace	
Total	35.71	35.46	33.02	29.02	25.36	36.21	18.69	26.46

WAUKESHA MINERAL SPRINGS.—Waukesha County, Wisconsin.

POST-OFFICE.—Waukesha. Hotels and boarding-houses.

ACCESS.—Viâ Chicago and Northwestern, Chicago, Milwaukee, and St. Paul, and Wisconsin Central Railroad. An electric line is also being built from Milwaukee.

Waukesha, the county seat of Waukesha County, is located sixteen miles west of Milwaukee, and ninety-eight miles northwest of Chicago. The elevation here is about 800 feet above tide-water. The surrounding country is of a rolling character, well wooded and has a sandy, gravelly soil. The natural advantages of the place have made it a general society centre of the Northwest during the summer season. The usual population, of about sixty-four hundred, is increased during the hot months to more than ten thousand. In the year 1895 there were one hundred and forty-three clear days, one hundred and thirty partially cloudy, and ninety-two cloudy days. The summer weather is usually of a delightful character, and quite free from days of oppressive heat. The average annual rainfall from 1892 to 1895, inclusive, was 28.02 inches. The village contains eleven hotels and

Other well-known springs at Waukesha are the "White Rock," "Glen," "Horeb," "Gibson," "Siloam," "Mineral Rock," and "Vitaqua." It will be observed that the principal ingredients of all these waters is the bicarbonate of magnesium. Their action in the system is antacid, mildly laxative after continuous use, and diuretic. They have a useful application in dyspepsia, abdominal engorgement, Bright's disease, diabetes, and bladder troubles. Some of them are excellently adapted for table use.

James K. Crook.

WAX; BEESWAX.—(*Cera Flava*, U. S. P., B. P., P. G.); A peculiar, concrete substance, prepared by *Apis mellifica* L. (Ord. *Hymenoptera*).

Wax is an animal product secreted by bees under the rings of the abdomen, where it accumulates, when the insects are comb-making, in scales or flakes. These flakes are disengaged by the bees, and, with jaws and legs, moulded into that remarkable structure, the honeycomb. It appears to be an analogue of the sebaceous secretion of the skin. The wax is obtained by depriving the comb of its honey by draining and pressing, and is purified by melting one or more times in boiling water and cooling. It is then melted and cast in large solid cakes.

Wax is too familiar to need description, were it not for its very frequent adulteration, which unfortunately is not always easily detected. The Pharmacopœial description and tests are as follows: "A yellowish or brownish-yellow solid, having an agreeable, honey-like odor, and a faint, balsamic taste. It is brittle when cold, but be-