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Some Bacteriological Examinations of Iowa Waters

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SOME BACTERIOLOGICAL EXAMINATIONS OF IOWA WATERS.

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A good supply of water is of prime importance for every community. We are more and more beginning to appreciate and to insist on the disposal of sewage and a proper and good supply of water. It is astonishing that epidemics of typhoid fever are so common. Especially so when we know that typhoid fever is a preventable disease in large measure. It is astonishing that it is so common a disease in countries where sanitary science has attained its greatest development.

Swithinbank and Newman* in their recent exhaustive treatise on the Bacteriology of Milk give an extensive review of something like forty pages of the milk-borne outbreaks of typhoid fever. The following rather interesting statistics are collected by these authors. Dr. Cooper-Patton of Norwich has presented the following table derived from 656 cases of typhoid fever at Norwich, from the years 1895 to 1897.

Typhoid Fever Patients in Norwich (656).	Percentage of Patients.			Triennial Averages.
	1895	1896	1897	
Who drank no milk	10.0	8.0	2 0	6 6
Who drank milk raw and uncooked	25.3	24.0	29.0	26.0
Who drank milk boiled, cooked, or in tea, etc	65 0	67.5	68.0	66.8
Who used condensed milk	0.0	1.0	1 0	0.6

*SWITHINBANK AND NEWMAN.—Bacteriology of Milk. 315.

And recently Schuder,* who collected statistics of 638 epidemics of typhoid fever in different countries finds that 70.8 per cent of such epidemics are spread by drinking infected water; 17 per cent by drinking infected milk, and 3.5 per cent by other forms of food. The remainder, 9 per cent, are caused by clothes, etc., worn by typhoid patients. 29 per cent of the epidemics spread by infected milk are caused by the use of dairy utensils washed with infected water. Swithinbank and Newman have likewise collected statistics on this subject. Of the 200 epidemics studied by them they arrived at the following conclusions: But in epidemics, it has been possible to obtain the likely channel of infection between 70 and 80 typhoid epidemics.

Milk-borne typhoid epidemic probably started:	Per cent.
(a) By cases of typhoid at the farm or milk-shop	70
(b) By cases of typhoid at the farm	40
(c) By cases of typhoid at the milk-shop	30
(d) By using polluted water for dairy purposes, method of pollution unknown	20
(e) By insanitation at the farm or milk-shop and miscellaneous	10

Sedgwick† in his "Principles of Sanitary Science and Public Health" makes the following interesting comments of the spread of typhoid fever in the village of Marlborough, Mass., through skimmed milk.

"In August and September, 1894, a small epidemic of typhoid fever appeared in the city of Marlborough, Mass. Various 'theories' of the cause of the outbreak were held or suggested, and the local newspapers contained numerous letters on the subject, some alleging that the water supply was infected, some that the sewers were to blame, and some that accumulations of filth, especially dump-heaps, were responsible. The localization of the cases, however, not only disproved these theories but also suggested milk as the probable cause. It soon became evident, nevertheless, that none of the regular milkmen were involved, the cases apparently deriving their milk supplies from a variety of different sources. Eventually, however, it turned out that there existed within the city itself a creamery from which was dispatched daily a wagon loaded with skimmed milk ('separator' milk), and that nearly all of the cases of typhoid fever had been supplied with such skimmed milk either from this wagon or directly from the creamery itself. Further investigation showed that the driver of the skimmed-milk wagon was at the time of the inquiry living on the upper floor of the creamery, and just recovering from a severe attack of typhoid fever. This young man had

* SWITHINBANK AND NEWMAN.—Bacteriology of Milk. 315.
† SEDGWICK.—Principles of Sanitary Science. 275.

not only been the driver of the wagon, but had also worked over the milk, transferring it, filling cans, and otherwise making himself useful about the creamery."

The Springfield epidemic of August, 1892, was likewise attributed to the milk as a source of infection. Other cases are also here recorded.

A severe epidemic of typhoid fever occurred among the college students at Ames in the fall of 1900. A careful investigation of the well water, spring and deep well water supplying the college was made by Dr. Harriman, who had charge of the college hospital, and therefore had a good opportunity for studying all the conditions. He concluded that the milk supplying the college dining room was the source of infection.*

The general conclusions reached by the writer in a paper† were as follows: It may be stated that so far as the analyses show the college water supply may be considered excellent. It is true that in a number of instances more organisms were found than at other times, but an examination made from time to time shows that the number is not unusually large, and on the whole that we may consider our water supply practically pure, and I should also state that the water from the spring supply is unusually good. We should bear in mind that the failure to find the typhoid fever bacillus in the water supply of the Briley well is not at all surprising. It is a well known fact that the saprophytic species grow so readily in the nutrient media that the typhoid fever bacillus has not the same chance to grow. The same may also be said with reference to milk, only here we are dealing with such a large number of species that it would be a mere accident to discover the organism. As stated before, it seems to me to be reasonable that the milk formed a favorable medium for the growth of the typhoid organism, and be it especially remembered that Mr. Briley, from his own testimony, failed to wash the cans with boiling water as should have been done. The milk cans could easily have been

* The Jr. of the Am. Med. Assoc. 38: 511. 1902.
† Proc. Ia. Acad. Sci. Vol. 8. 274.

contaminated, and the failure on his part to wash the cans, it seems to me, made it not only possible but probable that these germs propagated in the milk dippings. A comparison of water of the Briley well and the college effluent shows that the Briley well had a greater amount of contamination than the college effluent from the sewage filter beds.

The general conclusion seems evident that the flies transferred the organisms from the dejecta which were not sufficiently sterilized over to the *milk, and thus the germs* found their way into the milk cans. The pails used to carry the milk were simply rinsed with cold water and dumped out near the well. The typhoid bacilli no doubt developed rapidly in the milk and thus might have found their way into the well.

All bacteriologists recognize the importance of making both bacteriological and chemical analyses of water to determine whether it is suitable for sanitary purposes. Many of our Iowa waters have been examined from a sanitary standpoint. In most of our smaller towns the city water is derived from deep wells, while the larger cities like Des Moines, Cedar Rapids, Waterloo, Burlington, Davenport, Dubuque, Iowa City, and Council Bluffs derive their water from streams.

The sanitary analysis of water from a bacteriological standpoint involves a great many difficulties. Among these we may mention the interpretation of results obtained in these analyses. The earlier bacteriological results seemed to indicate the general presence of the bacillus of typhoid fever in suspected water. No less than half a hundred of these positive determinations are on record until bacteriologists began to suspect that the finding of the bacillus of typhoid fever by these observers was an error. It appears, however, from some very careful studies made by Kübler and Neufeld* and a few others that the *B. typhosus* has been found in water. Sedgwick and Winslow† found that of the typhoid bacillus in ice or cool water over 40 per cent will perish in three hours

* ZEITSCH.-f. Hygiene. 31: 13?.
† Mem. of the Am. Acad. Arts and Sci. 12: 467.

and 98 per cent and upwards in two weeks. It does not seem probable that this organism will retain its vitality for a great while in ordinary water. It certainly does not propagate. Shortly after the discovery of the agglutinating property of *B. typhosus* by Widal renewed efforts were made to detect the typhoid bacillus in water and on the strength of these tests several French investigators found the bacillus everywhere, in water, in soil, and the faeces of healthy individuals.* A German investigator relying on this test found the water in certain cisterns infected and an Englishman, Hankin,† found it common in the soil and other places. Further investigation, however, revealed the fact that the colon bacillus is also agglutinated by the typhoid serum. It, therefore, appears that so far as these tests are concerned that we still need a great deal of information on the subject, that we are still somewhat uncertain on some points.

Dr. Vaughan ‡ says, "I have never found in drinking water a germ that responded to the Wi ·:i test."

For a valuable paper on bacteriological analysis of water the paper by Winslow || should be consulted.

The English have used phenolated media.§ "It was at one time thought that the addition of .2 per cent carbolic acid to the ordinary media inhibited the growth of all bacteria but the typhoid bacillus. It has been found, however, that the growth of the *B. coli* is also unaffected by such a medium, though it prevents the growth of most putrefactive organisms which liquify gelatin." And Hankin, of the British Army Medical Corps in India, uses Parietti solution as follows: Four per cent of hydrochloric five per cent carbolic acid or phenol. His method is as follows: "He adds portions of the water to be tested to tubes containing successively increasing proportions of Parietti solution. The tubes at the bottom of the series, in which the acid is not too strong, become turbid, and

*Ann. de l' Inst. Pasteur. **11**: 55.
† Centr. Bakt. **26**: 554.
‡ The Jr. of the Am. Med. Ass. **42**: 941.
|| WINSLOW, C. E. A.—Bacteriological Analysis of Water and Its Interpretation. New Eng. Water Works Assoc. **15**: 470.
§ MUIR & RITCHIE.—Manual of Bacteriology. 330.

instead of taking the tube with the highest amount of acid in which growth occurs (which would probably contain only *Bacilli coli*), Hankin* takes the one just below for the inoculation of a new series. Finally pure cultures are isolated on the agar plate and tested by various subcultures."

In a bacteriological examination of waters it is essential to carry out the rules laid down by the American Public Health Association and those adopted by the Massachusetts State Board of Health, and the methods used in the laboratory of the Boston Institute of Technology. The methods consist essentially in making quantitative analyses, and first of all it is important that all media should be perfectly sterilized for the least amount of error in this respect will make the results of no value. In spite of the fact that the greatest care is used in the making of media it sometimes happens that some of the tubes become infected in some way. As to the use of media, it is generally admitted that gelatin, agar agar, and litmus lactose agar and the fermentation tube with dextrose are important in obtaining reliable results.

Quite recently Dr. Vaughan† has given the results of an examination of water supplies from various sources under the head of "Some Toxicogenic Germs Found in Drinking Water." He uses for his work agar plates, one grown at the room temperature, the other at 38 degrees C. The toxicogenic bacteria have their optimum growth at 38 degrees C., while many of the saprophytic water bacteria grow at lower temperatures. The pathogenic bacteria crowd out the saprophytic at a higher temperature. The colonies are counted at the expiration of twenty-four, forty-eight and seventy-two hours. Bouillon tubes are inoculated at the same time with like amounts of water. These tubes are kept in the incubator for twenty-four hours at from 38 degrees to 40 degrees C. The temperature is not allowed to fall below 38 degrees C. If bacteria do not develop at this temperature it is regarded as safe. If bacteria are

* HANKIN, E. H.—Centr. f. Bakt. 26: 554.
† Jr. of the American Medical Assoc. 42: 935.

found in the tubes in the incubator from 1 to 2 c. c. of the beef tea bouillon is injected intra-abdominally into white rats or guinea pigs. Two animals are inoculated. If the animals do not die it is regarded as safe. If animals die agar cultures are made from the heart's blood. The plates are incubated at the temperature of blood. The colonies are carefully studied. The *Bacillus venosus* seems to be the germ which was found in wells suspected to have caused the infection of typhoid fever.

The chemical composition of the medium as has been shown by Messrs Winslow, Fuller, Sedgwick and Prescott* is important.

Mr. Winslow especially recommends agar agar, gelatin, glycerin agar, and the litmus lactose agar.

On the importance of the use of this medium Winslow and Nibecker † give the following rather interesting table:

Sources of Water.	Average No. of Colonies per c. c.	
	Gelatin 20°	Wurtz-Agar 37.5°
Well, spring	1,664	28
Reservoirs	153	43
Ponds	296	95
Taps	242	24
Streams	273	101

In a general average of 259 samples of water studied by Winslow and Nibecker they give the following interesting summary of their work:

*Jr New Eng. Water Wks. Assoc. Vol. 15: 462. Also Technological Quarterly 16: 228.

† WINSLOW, C. A. E. and NIBECKER, C. P.—Techn. Quar. Vol. 16: No. 3, Sept. 1903.

TABLE I.

EXAMINATION OF 259 SAMPLES OF WATER FROM APPARENTLY UNPOLLUTED SOURCES.

Source of Samples.	Number of Samples.	Gelatin Plates.				Litmus-Lactose-Agar Plates.					Dextrose-Broth Tubes.			
		Number of plates.	Maximum number of colonies.	Minimum number of colonies.	Average number of colonies.	Number of plates.	Maximum number of colonies.	Minimum number of colonies.	Average number of colonies.	Plates showing red colonies.	Number of tubes.	Number of tubes with gas.	Number of tubes with gas. 1-0.	Number of tubes with gas. 2-1.
Cambridge supply (tap)	5	10	240	20	94	8	15	2	11	0	15	0	0	0
Wakefield and Stoneham supply (tap)	7	14	150	20	59	13	12	2	6	0	21	0	0	0
Lynn supply (tap)	6	12	20	0	16	12	6	1	3	0	18	0	0	0
Brookline supply (tap)	1	2	All	liquefi	ed.	2	21	15	18	2	3	0	0	0
Plymouth supply (tap)	6	12	70	10	35	12	6	0	2	0	18	0	0	0
Peabody supply (tap)	3	6	261	30	141	5	36	1	21	0	9	2	2	2
Dedham supply (tap)	6	12	13,500	0	3,717	11	3	0	1	0	18	0	0	0
Newburyport supply (tap)	6	12	100	10	36	12	14	2	9	0	18	0	0	0
Salem supply (tap)	5	10	267	200	232	10	23	10	14	0	15	0	0	0
Taunton supply (tap)	4	7	41	10	13	8	5	0	2	0	12	0	0	0
Sharon (well) (tap)	3	6	845	630	738	6	74	30	46	2	9	3	0	3
Medford supply (tap)	5	10	1,250	240	524	10	16	3	8	0	15	0	0	0
Milton supply (tap)	2	4	5,000	4,200	4,700	4	0	0	0	0	6	0	0	0
Westerly, R. I. supply (tap)	1	2	All	liquefi	ed.	2	13	12	12	0	3	0	0	0
Brooks	61	122	1,010	30	223	221	83	0	7	0	183	13	13	0
Driven wells	15	30	54	0	18	30	5	0	1	0	45	0	0	0
Springs	32	64	1,270	20	294	64	13	0	2	0	95	13	13	0
Ponds, fed by brooks	15	30	340	100	167	30	24	0	9	0	45	1	1	0
Melted snow	1	2	All	liquefi	ed.	2	5	3	4	0	3	0	0	0
Pools in fields	22	44	760	100	365	44	95	2	31	0	66	2	2	0
Pools in woods	22	44	580	10	181	43	14	0	3	0	65	0	0	0
Roadside pools	10	20	9,000	410	811	20	10	0	4	0	30	2	2	0
Stream, Blue Hill Reservation	1	2	All	liquefi	ed.	2	0	0	0	0	3	0	0	0
Flow from rocks	2	4	60	30	47	4	2	0	0	0	6	0	0	0
Ponds, fed by springs	6	12	420	60	188	12	5	0	2	0	18	0	0	0
Drains from manured pastures	1	2	1,270	1,200	1,235	2	28	26	27	0	3	0	0	0
Swamps	3	6	310	240	269	6	10	0	6	0	9	5	5	0
Rain water, after 12 hours' heavy fall	7	14	All	liquefi	ed.	14	6	0	2	0	21	0	0	0
Shallow well in Lynn woods	1	2	20	10	15	2	1	1	1	0	3	0	0	0
Totals	259	517	....	......	....	511		......	......	4	775	41	38	3

They concluded as follows: "If we consider the results of this examination of the litmus-lactose agar plate it becomes apparent that organisms capable of growth at the body temperature are not numerous in unpolluted waters; acid formers are practically absent."

In addition to these media we have used in our water investigation of the well supplies ordinary litmus agar and litmus gelatin, dextrose agar. None of these media have, however, been very satisfactory. The litmus agar and gelatin, and especially the dextrose contained as a rule less organisms than the gelatin.

In order to get accurate data on water supplies especially from a bacteriological standpoint the bacteriologist should at least insist on collecting the samples himself, and under conditions which will guarantee a perfect sample. They should be plated as soon as possible after the water is collected. We can not insist too strongly on the statement made by C. E. A. Winslow * that "The extreme delicacy of the test renders imperative the utmost care in the technique of bacteriological analysis; for a speck of dust, a delay of a few hours, or a mistake in the preparation of the nutrient gelatin, may introduce an error in excess of the normal difference between the purest and the most polluted drinking-waters. First, in the collection of the sample, it must be certain that the small portion taken represents fairly the whole body of water from which it is drawn. The first water flowing from the tap or a pump must be rejected, as it may have acquired impurities from the mouth of the faucet or of the spout. On the other hand, water which has stood all night in the service pipes of a house will be lower in bacteria than the supply from which it is derived. In a lake or pond the surface scum and bottom sediment must be equally avoided."

Bacteria begin to multiply more after the sample is collected. All of our waters contain sufficient nutrient material to cause such multiplication.

*WINSLOW, C. E. A. Jr. New Eng. Waterworks Assoc. 15: 460.

One of us called attention to this fact some years ago,* but as early as 1879, Miquel,† found that the number of organisms increased very perceptibly after it had been kept a certain length of time. The following quotation from Frankland ‡ indicates a final decline. "Miquel has extended these observations in an interesting manner by keeping a bottle of river Seine water shut up for nine years, and whilst at the time of collection 4,800 bacteria per c.c. were found, at the end of the nine years there were only 220 discoverable. Again, a sample of Vanne water, containing at the time of collection 66 organisms per c.c., at the end of ten years was found to be absolutely sterile."

Cramer § gives the following increase:

Hours and days during which the water was preserved.	Number of micro-organisms in 1 c.c. of water.
0 hours	143
24 hours	12,457
3 days	328,543
8 days	233,452
17 days	17,436
80 days	2,500

The bacteriologist is often asked to examine water that is shipped from long distances. He is asked to make a report on the sanitary conditions of the water. This water is frequently collected in ordinary bottles, simply rinsed. Of course, it goes without saying that such analyses can not be reliable. A case in point of the undoubted multiplication of bacteria may be found in spring water from Canton, northeastern Iowa. The spring water is said to be unusually good, the number of bacteria found in the water was very large, the average in different media was 22,000 per c.c.

Sometimes water is packed in ice and sent for study. This is not always a desirable method for shipping water. Thus, Jordan ‖ found that three samples of river water packed in ice for 48 hours, fell off from 535,000 to 54,500; from 412,-

* PAMMEL, L. H.—Proc. Ia. Acad. Sci. 1: 493. 1893.
† MIQUEL, MANUEL.—Pratique d'analyse bacteriologique des aux. Paris, 1891. p. 12.
‡ FRANKLAND.—Micro-organisms of Water. 219.
§ CRAMER.—Die Wasserversorgung von Zürich und ihr Zusammenhang mit der Typhusepideme des Jahres 1884. Zürich, 1885: 91.
‖ WINSLOW, C. E. A.—Jr. New Eng. Water Works Assoc. XV: 461.

000 to 50,500, and from 329,000 to 73,500, respectively. It is thus evident that the bacteriologist must not only see to the collecting of his own samples, but must be intimately acquainted with their history until they reach his laboratory.

A further precaution that should be taken in making an analysis is the proper plating and the amount of dilution. We have found in ordinary practice of wells that $\frac{1}{10}$ of a c.c. of water is sufficient. The melted agar is poured out on the plate and the water is added to it. Then by gently tilting the plate backward and forward an even distribution of the germs is obtained. The same method is used in gelatin plates. The gelatin plates harden less rapidly and hence a better distribution will be obtained than on agar, care, of course, being used when the plates are poured that there is no dust in the room, that the ends of the tubes shall be run through flames so that no germs will adhere when the agar is poured into the Petri dishes. For river water, especially if strongly polluted, it is necessary to dilute about ten times although this may have to vary. It is not advisable to have the plate covered with colonies as the different colonies are inimical to their development.

The following is the result of our analyses of wells supplying the college boarding houses. The shallow wells are marked S.

TABLE II.

BACTERIOLOGICAL TEST OF WELL WATER. I. S. C.

Owner.	Date.	Depth of well.	Temperature.	1st. Agar.	2nd Pumping. Agar.	Lit. Agr.	Acid.	Gel.	Liquef.	Lit. Gel.	Acid.	Gluc. Ag.	Gas.
Baughman, John.	Jan. 7, 1904	..	8 °C.	20	20 10	110 100	0 0	180 120	60 20	120 50	0 0	20 0	None.
Baughman, John.	March 5, 1904	....	....	....	50 10	20 20	0 0	10 0	0 0	30 -0	10 0	80 30	None.
Briley, O. A. 1 ..	Jan. 15, 1904	110	9¼ °C.	710	40 100	10 20	0 0	0 30	0 0	0 0	0 0	50 60	None.
Briley, O. A. 1 ..	....	....	....	....	....	....	. ..	.... ..	. ..	....	.. ..	... ...	......
Briley, O. A. 2 ..	Jan. 5, 1904	40	8 °C.	30	160 800	120 20	0 0	200 190	40 30	220 180	0 0	40 0	CO_2 22%. H 88%.
Briley, O. A 2 ..	March 11, 1904	....	7¼ °C.	1500	1100 970	410 330	0 0	0 0	0 0	0 50	0 0	20 30	Abundant
Cattell, M. C .	Jan. 7, 1904	60	9 °C.	50	40 30	70 0	0 0	30 10	0 10	20 30	0 0	1000	Slight.
Cattell, M. C	March 5, 1904	....	....	....	0 0	60 50	0 0	0 0	0 0	30 80	0 0	0 0	None.
Caughey	Jan. 5, 1904	60	7¾ °C.	20	20 0	10 5	0 0	40 50	10 25	60 40	0 0	0 20	None.
Caughey	March 11, 1904	....	10 °C.	....	70 80	70 20	0 0	30 0	10 0	50 110	10 0	90 210	None.
Cole, A. W.	Jan. 7, 1904	65	8¾ °C.	....	80 50	10 0	0 0	30 70	0 0	40 30	0 0	40 70	Slight.
Cole, A. W.	March 5, 1904	....	....	..	0 20	30 0	10 0	10 0	10 0	20 0	0 0	20 10	None.

TABLE III.

BACTERIOLOGICAL TEST OF WELL WATER. I. S. C.

Owner.	Date.	Depth of we l	Temperature.	1st. Agar.	2nd Pumping. Agar.	Lit. Agr.	Acid.	Gel.	Liquef.	Lit. Gel.	Acid	Gluc. Ag.	Gas.
*Connell, R. D.	Jan. 3, 1904	114	9½ °C.	500	14350 1680	60 80	0 0	14700 31500	0 0	75 23100	0 0	33600 14750	None.
Connell, R. D..	Mar. 11, 190.	...	13½ °C.	21000	21000 21000	0 28000	0 0	35000 28000	0 0	40	0 0	2100 4200	None.
Dixon, F. N....	Jan. 13, 1904	50	9 °C.	750	36 20	60 0	0 0	100 80	70 30	20 90	0 0	30 0	None.
Dixon, F. N....	Mar. 11, 1904	...	6 °C.	.. .	1000 500	520 0	0 0	2000 200	1000 0	6300 6300	0 0	4200 0	None.
Eldridge, Mrs..	Jan. 6, 1904	...	....	20	10 10	·10 20	0 0	0 0	0 0	30 20	0 0	30 40	None.
Eldridge, Mrs..	Mar. 5, 1904	...	....	.....	20 20	40	0 0	100 20	0 0	0 0	0 0	0 10	None.
Foster, Clay....	Jan. 6, 1904	Shallow.		40	100 10	30 400	0 0	80 0	20 0	20 30	0 0	20 0	None.
Foster, Clay....	Mar. 5, 1904	...	.... ..	.....	20 10	10 20	.0 0	20 10	10 0	10 0	0 0	0 20	None.
Freed, O. D ...	Jan. 7, 1904	50	....	670	110 .	30	0 0	210 150	50 100	150 50	0 2	30 300	None.
Gray	Jan. 7, 1904	...	....	120	50 80	50 250	0 60	All All	liq liq	uifie uifie	d d	10 20	In 3 of 5 tubes.
Gray	....	..	10 °C.	100	120 50	50 90	0 0	0 30	350 500	350 500	0 .	0 90	In 1 of 3 tubes.

* Colonial Club.

TABLE IV.

BACTERIOLOGICAL TEST OF WELL WATER. I. S. C.

AVERAGES OF ALL PLATES.

Owner.	Depth of well.	Distance From		Average Number of Bacteria per C. C.							
				1st Pouring.				2nd Pouring.			
		Barn.	Cess pool.	Date.	Temp.	1st pump.	2nd pump.	Date.	Temp.	1st pump.	2nd pump.
Baughman, John	...	...	40	Jan. 7, 1904	8 °C	20	73.0	Mar. 5, 1904	....	.....	25
Briley, O. A. 1..	110	100	...	Jan. 5, 1904	9¼ °C.	110	31.0	....	....	.....	.....
Briley, O. A. 2..	40	20	300	Jan. 5, 1904	8 °C.	30	193.0	Mar. 11, 1904	7¼ °C.	1500	291
Cattell, M. C....	60	...	...	Jan. 7, 1904	9 °C.	50	123.0	Mar. 5, 1904	....	...	22
Caughey	60	.	100	Jan. 5, 1904	7¾ °C.	20	25.0	Mar. 11, 1904	10 °C.	....	73
Cole, A. W	65	...	100	Jan. 7, 1904	8¾ °C	. .	42.0	Mar. 5, 1904	....	.	11
Connell, R. D. ..	114	...	100	Jan. 3, 1904	9½ °C.	500	13329.5	Mar. 11, 1904	13½ °C.	21000	1493[illegible]
Dixon, F. N.	50	5	80	Jan. 13, 1904	9 °C.	750	43.6	Mar. 11, 1904	6 °C.	.....	2102
Eldridge, Mrs...	...	150	50	Jan. 6, 1904	....	20	17.0	Mar. 5, 1904	....	....	2[illegible]
Foster, Clay.....	...	75	...	Jan. 6, 1904	....	40	69.0	Mar. 5, 1904	......	.....	12
Freed, O. D.	58	...	100	Jan. 7, 1904	....	670	108.0	....	....	.....	.....
Gray	...	25	200	Jan. 7, 1904	....	120	98.0	... .	10 °C.	100	128

TABLE V.

BACTERIOLOGICAL TEST OF WELL WATER. I. S. C.

AVERAGE OF ALL AGAR PLATES.

Name.	Depth.	No.	Name.	Depth.	No.	Name.	Depth.	No.
Baughman, John	.....	23	Hodgdson .	Shallow	347	Minert	94	66
Briley, O. A. 1..	110	87	Holmes, M. L.	Deep	226	Munn, H. J ...	65	53
Briley, O. A. 2..	S. 40	1140	Hook, W. A...	150	56	Otis, Mrs.	80	22
Cattell, M. C....	60	30	Hunt, M.	10[illegible]	67	Overholser, I...	S. 57	340
Caughey	60	47	Johnson	155	10	Parker, east ..	107	37
Cole, A W......	65	38	Kibby..........	Shallow	263	Parker, west...	87	43
Connell, R D. .	114	13288	Long, W.	100	20	Parsons	S. 30	25
Dixon, F. W....	S. 50	564	MacBurney ..	85	152	Poage, Mrs. H.	83	20
Eldridge, Mrs...	50	16	Madson, N ...	S. 57	46	Ross, Mrs.	100	...
Foster, Clay....	Shallow	36	McCune, H	Deep	28	Rubel, E. S.	110	185
Freed, O. D. ...	S. 58	390	McDaniel	118	158	Scroggie, C. R.	Deep	47
Gray	Deep	87	Miller House ..	110	50	Stalker, Dr.	75	172
Greer, W. K ...	74	26	Miller Store...	....	86	Story, N. F	110	50
Hanson	100	185	Miller, R. J. ..	60	860	Tuttle, E. C...	120	182
Hill, L.	50	120	Miller, J B. ..	110	160	Walters, Mrs...	75	37

TABLE VI.

BACTERIOLOGICAL TEST OF WELLS AND WATER SUPPLIES, GRINNELL, AND DES MOINES, IOWA.

Locality.	Source of Water.	1st Pumping.		2nd Pumping.	
		1st Agar.	2nd Agar.	1st Agar.	2nd Agar.
Grinnell ..	Bliss, Mr., private well	50	1400	80	1900
Grinnell ..	Kingston, Mr., private well	160	....	3500	.. ....
Grinnell .	Jenkins, Mr., private well	100	150	3500	3500
Grinnell ..	Tap water, collected 1 min., 5 min. and 10 min.	100	80	60	....
Grinnell ..	Cistern, city supply	10	10	.....	....
Grinnell ..	Effluent of cistern, city supply	100	1000	....	.. ....
Grinnell ..	Soft water for boilers, city main	540	270	....	....
Grinnell ..	Inflow, city cistern	0	0	....	.. ....
Des Moines	Shallow well, Walnut street	400	7500	....	....
Des Moines	Artesian well, 360 feet deep	400	50	....	....

TABLE VII.

BACTERIOLOGICAL TEST OF WATER FROM COLLEGE CREEK, I. S. C.

Date.	Point.	1st Agar.	2nd Agar.	1st Glu. Ag.	2nd Glu. Ag.	1st Lit. Ag.	2nd Lit. Ag.	1st Gelatin.	2nd Gelatin.	1st Lit. Gel.	2nd Lit. Gel.
*Jan. 15, 1904	Above the inflow of Colonial House sewage. I.	1100	3500	140	.....	350	50	70	60	0	0
*Jan. 15, 1904	Below the Colonial House. II	510	1320	670	620	160	110	10	0	10	0
*Jan. 15, 1904	By the President's House. III	80	100	220	3200	55800	70000	10	0	0	0
*Jan. 15, 1904	Below the College sewer. IV	4900	3500	1460	1220	2330	6300	10	0	0	0
†Mar. 30, 1904	Above the inflow of Colonial House sewage. I	46000	....	80500	54000	72000	51000	102000	92000	62000	9000
†Mar. 30, 1904	Below the Colonial House. II	.....	....	30000	52000	24000	91000	84000	90000	105000	86000
†Mar. 30, 1904	By the President's House. III	.....	....	....	.....	70000	80000	90000	80000	81000	70000
†Mar. 30, 1904	Below the College sewer. IV	.....	....	25000	45000	22000	25000	90000	92000	10000	45000

*Ice. † Flood.

TABLE VIII.

BACTERIOLOGICAL TESTS OF VARIOUS IOWA RIVER AND SPRING WATERS.

River.	Locality.	Plated by	Date.		Number Bacteria per C. C. on Agar.					
Skunk	Story City	E. V. Larson	Feb. 27, 1904	Sample each hour for six hours	3200	5000	10320	3080	10030	19200
Skunk	Story City	E. V. Larson	Feb. 27, 1904	Sample each hour for six hours	3240	2040	6200	1560	....	9080
Skunk	Story City	E. V. Larson	March 26, 1904	Sample each hour for three hours	11410	16590	12040	.	....	....
Skunk	Story City	E. V. Larson	March 26, 1904	Sample each hour for three hours	10220	......	6650	.	...	
					Hour.	Temp.	Bacteria 1''.	Bacteria 2''.		
Des Moines	Boone	J. W. Jordan	Feb. 18, 1904	Sample each hour for six hours	10 A. M.	0°C.	40	....		
Des Moines	Boone	J. W. Jordan	Feb. 18, 1904	Sample each hour for six hours	11 A. M.	0°C.	1000	2750		
Des Moines	Boone	J. W. Jordan	Feb. 18, 1904	Sample each hour for six hours	12 M.	0°C.	500	800		
Des Moines	Boone	J. W. Jordan	Feb. 18, 1904	Sample each hour for six hours	1 P. M.	1°C.	250	1000		
Des Moines	Boone	J. W. Jordan	Feb. 18, 1904	Sample each hour for six hours	2 P. M.	1°C.	600	....		
Des Moines	Boone	J. W. Jordan	Feb. 18, 1904	Sample each hour for six hours	3 P. M.	1°C.	250	500		
					Above Sewer 1''.	Above Sewer 2''.	Below Sewer 1''.	Below Sewer 2''.		
Des Moines	Des Moines	H. S. Fawcett	March 19, 1904	....	1400	4200	87500	77700		
					Agar.	Lit. Lac. Agar.				
Iowa	Marshalltown	L. H. Pammel	April 5, 1904	Back of Old Soldiers' Home	3500	500	——			
Mississippi	Davenport	L. H. Pammel	April 13, 1904	Foot of Brady street	18900	5600	Gel.			
Mississippi	Davenport	L. H. Pammel	April 13, 1904	Near waterworks	8400	....				
Mississippi	Davenport	L. H. Pammel	April 13, 1904	Near waterworks	8900	....	——			
Mississippi	Davenport	L. H. Pammel	April 13, 1904	Near waterworks	1 000	5600				
Maple	Carroll	L. H. Pammel	May 28, 1904	....	400	3 0	700			

SPRING WATERS.

Spring.	Locality.	Plated by	Date.	No.	Date.	No.	Date.	No.	Remarks.
Wayne's	Boone	Miss Rowe	Oct. 19, 1903	600	Dec. 30, 1903	440	Jan. 10, 1904	490	....
Williams'	Boone	Miss Rowe	Oct. 10, 1903	1400	Jan. 17, 1903	3300	March 13, 1904	5800	Surrounded by herbage.
College	Ames	Miss Rowe	Oct. 15, 1903	150	Oct. 21, 1903	160	Oct. 29, 1903	140	Brick cased
College	Ames	Miss Rowe	Jan. 5, 1904	120	Feb. 19, 1904	120	March 3, 1904	150	Brick cased.
Farm	Ames	Miss Rowe	Oct. 14, 1903	3100	Feb. 22, 1904	2400	April 4, 1904	3600	Surrounded by herbage.

The conviction has grown on us that none of the Iowa wells are absolutely free from bacteria. Generally speaking, the deep wells contain less organisms than the shallow. Prescott and Winslow* conclude that "it is plain that water absolutely free from bacteria is not ordinarily obtained from any source and that even deep wells contain quite appreciable numbers." They find that the bacteria from deep wells show a slow development at room temperature, "the entire absence of liquefying colonies and the abundance of chromogenic bacteria." In our investigation two types of wells have been examined. (1) The shallow which are usually bricked or tiled. They rest on a blue clay. (2) Tubular wells varying in depth from 60 to over 100 feet. In the vicinity of Ames they have gone through the Wisconsin drift. The plates were not incubated but they were plated in a covered case and counted in 48, 72 and 120 hours. Duplicate plates were poured in each case. The older the well the greater are the number of bacteria. It is also evident that there are many interesting problems in connection with a study of our water supplies.

*Elements of Water Bacteriology. 18.

PLATE XI.

Fig. a. A pond and well in Marshalltown, showing the location of the well close by the pond. Bad surface water.

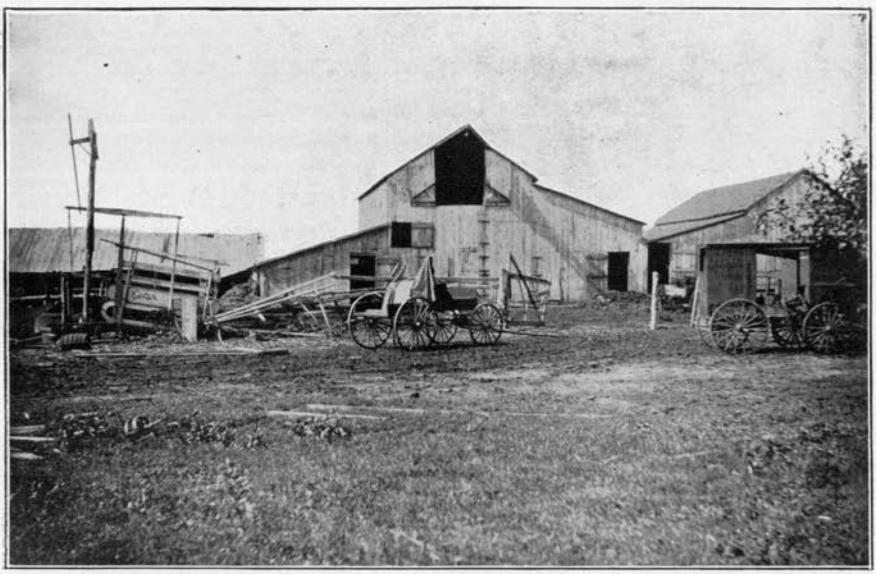

Fig. b. A dairyman's premises in Marshall County, Iowa, with dilapidated barns, poor well and general untidy conditions. Near Marshalltown, Iowa.

Fig. a. A Colonial club-house well. Well with excellent surroundings, neat and clean, on a hill; the well over 100 feet deep and yet containing a large number of organisms. Probably an underground channel occurs, or perhaps there is a leak in the pipes.

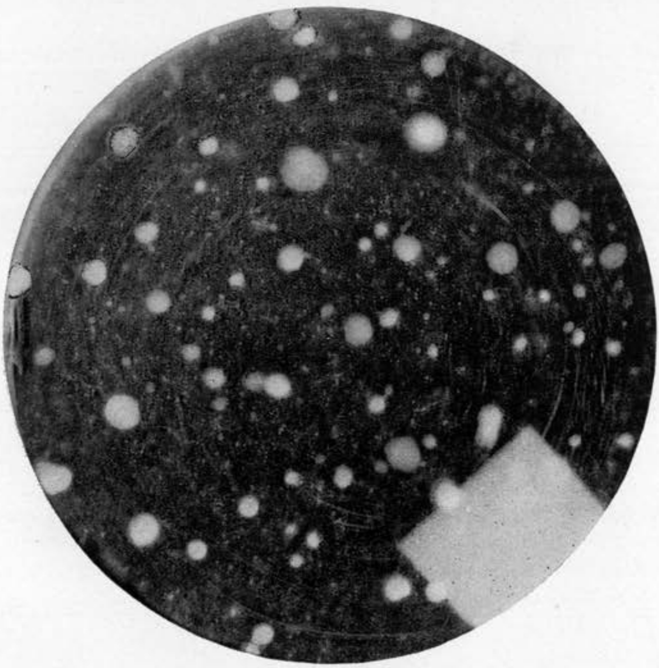

Fig. b. Organisms.

PLATE XIII.

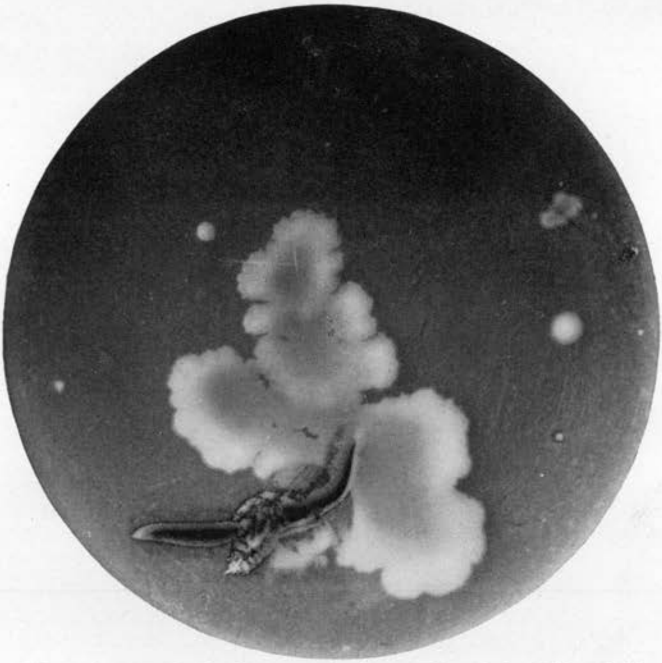

Fig a. Pla:e showing the development of colonies taken from Briley shallow well. Containing but few organisms.

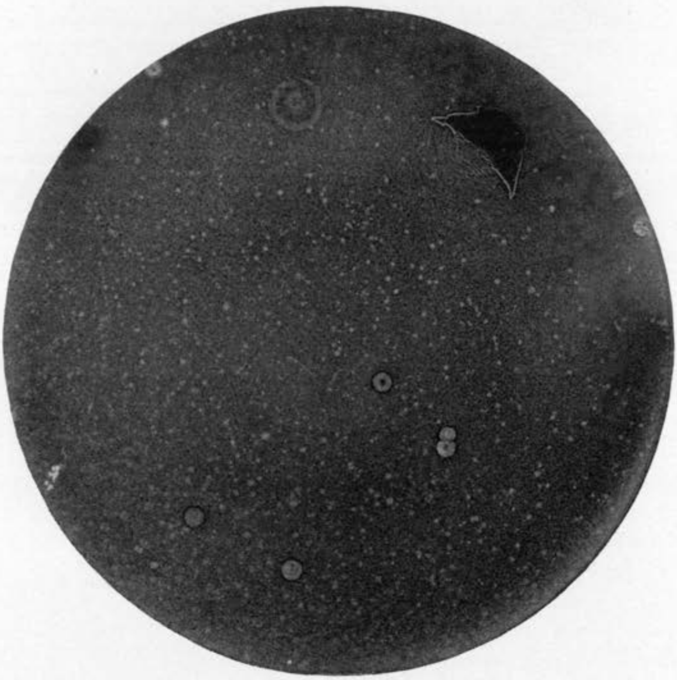

Fig. b. Taken from shallow creek receiving sewage. Three types of colonies are shown, the small colonies very numerous.