OBSERVATIONS ON THE VIABILITY OF THE BACTERIUM COLI GROUP UNDER NATURAL AND ARTIFICIAL CONDITIONS

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THE dry summer of 1916 afforded an opportunity for certain observations in the State of Kansas, which explained many of the apparent anomalies existing between sanitary surveys and the bacteriological examinations of samples of water. At Lawrence, Kansas, there were 72 days without any precipitation. Other localities recorded as many as 93 days without any rainfall. Certain water-supplies that were credited with being polluted by superficial soil washings, whenever it rained, were found to continually return positive results for organisms of the colon group. This single observation was freely and frequently discussed by members of the staff of the Kansas State Board of Health. As a result of this, certain preliminary studies were made which led to a series of experiments. Practical field demonstrations and theoretical laboratory studies were made in an attempt to prove the apparent fact that organisms of the colon group under certain natural conditions would multiply and thereby fail to be an indicator of contamination of recent fecal origin.

During the years after this investigation started, the war made many changes for the individuals working on the problem. Necessarily, much of the numerical data was lost so that this paper cannot be a truly scientific presentation of the problem. It is in reality a series of notes all tending to show that there are certain conditions of temperature, food supply and moisture, which will permit organisms of the colon group not only to live for a long time but grow and multiply in both soil and water.

Observation No. 1. During this period of drouth, cracks were observed to open up in clay soil on the hillsides. These were in some instances four to six inches across the top. A nine-foot "fly rod" was used to measure the depth and at two or three points the cracks would admit the rod to full length. How far beyond this point the hair line cracks extended, we were unable to determine. Undoubtedly earth worms and boring insects would make burrows very much below these points and without doubt carry organisms of the colon group from the surface soil well down into the water strata.

A recent report from Williamston, Michigan, stated that a crayfish burrow left an opening ¾ inch in diameter from the surface of the ground 46 feet to water, the crayfish having burrowed through a layer of shale. This was discovered by a well digger who followed the burrow up to the surface. This statement was investigated and substantiated by one of us.

With the fact in mind that there was ample opportunity for surface soil to be carried down to the water strata that were not thoroughly protected by an impervious shale, sand stone, or other rock layer, Mr. Edman Greenfield proceeded to collect samples of soil from various locations in and about Lawrence, Kansas, which were apparently free from recent animal or human pollution. Only one sample of soil out of 25 failed to return some organisms of the colon group, and that was the freshly stripped shale bed of the Lawrence Brick Company.

Observation No. 2. McCook's Field Well. A point was selected on the campus of the University of Kansas where there was no source of contamination on the watershed. A 2-inch test well, 52 feet deep, was drilled and a pump installed. This well was so constructed with a double casing and concrete apron that if it did rain surface pollution would not be carried down the casing. Naturally the first pumping of this well contained organisms of the colon group. The well was pumped dry and the casing filled with 1 per cent chloride of lime solution and allowed to stand several days. The first water pumped was sterile, containing free chlorine. By the time the well had been pumped free from chlorine, organisms of the coli group reappeared in 1 c.c. portions, and were continually present in the water. This experiment was repeated several times with the same result. The organism recovered was classified, according to Levine,[1] as B. coli of fecal origin.

Observation No. 3. The McNish Well. The city water supply was so depleted during the period of drouth that it was necessary to draw a supply from the Kaw River for fire protection. The water was heavily chlorinated and consequently unfit for domestic use. Mr. McNish, owner of the only distilling plant of the town, could not supply the demand for drinking water. He submitted the proposition to the Department of Health that a well on the high prairie three miles west of the town be used. The bottom of this well was above any source of pollution except the surrounding soil, which could not, by any stretch of the imagination, be dangerous to human beings. This well always showed organisms of the colon group in 1 c.c. samples. The same disinfecting experiment was carried out with this source of supply that was carried out with the McCook field well. In every instance as soon as the water pumped was free from chlorine the organisms of the colon group appeared in 1 c.c. specimens. The organism recovered was Bact. Aerogenes, probably not of fecal origin.

Observation No. 4. Potter Lake. This was a small artificial lake on the campus of the University of Kansas which was sometimes used as a bathing pool. On account of the enormous growth of algae, diatomes and crustaceans, it was not fit for this use during the period of drouth. Periodic examinations of the lake showed that at times it was practically sterile and at other times there were organisms of the colon group present in the water. It could easily be observed that the algae, protozoans and other life developed and died off in cycles. Although we were unable to get any definite correlation between the disappearance of the various types of organisms and the appearance of B. coli, yet it was suggestive that these organisms furnished a pabulum for the growth and development of colon bacilli.

Observation No. 5. Humboldt Filter Plant. One of the duties of the laboratory at this time was to run the official test for efficiency on the new filter plants. The Humboldt plant had been in operation some time but had not been officially approved by the engineer of the State Board of Health. In running this official test it was observed that there was a heavy growth of Daphnia on the beds. It was impossible to get a colon free effluent, although all factors in the filtration were being properly controlled. One of the beds closed down at night showed the presence of the colon group in 10 c.c. samples. The effluent on starting the plant the next morning showed the presence of the colon group in 1/1000 of a c.c. and the surface of the sand was coated with dead crustaceans.

Observation No. 6. Osage River. During this period of drouth the Osage river was pooled, the entire run-off coming from the stored ground water. One collecting reservoir which was observed daily showed that the number of B. coli

1. Max Levine. Jr. Bact., 1916, p. 153.

varied from 1/10 of one organism per 1 c.c. to 10 organisms per c.c.

Observation No. 7. Soil Experiment. A galvanized iron tank 3 feet square by 3 feet deep was equipped with under drains and filled with soil collected from a spot on the campus that was not obviously contaminated. This tank was installed on top of Frasier Hall, the highest building on the campus, protected by a screen cone and an anemometer, so that birds could not possibly roost on the tank. The Weather Bureau anemometer on the same tower recorded only one instance where there was not sufficient wind to keep the anemometer moving. At the time the tank was installed late in the summer of 1916, the soil was very dry. The organisms of the colon group were present. Both the so-called soil and fecal strains were isolated. As our attention was focused on the fecal strains, we retained a record of the cultural characteristics of the organisms recovered: short gram negative rod, methyl red positive, Voges Proskauer reaction negative, gelatine negative, dextrose positive, lactose positive, saccharose negative, adonite negative, dulcite positive, indol positive.

In late September or early October, 1916, there was a period of heavy rainfall. The soil became saturated with water. At least six attempts were made to isolate the fecal strains of colon at that time, only two of which were successful, the organisms being masked by an overgrowth of B. fluorescens liquefaciens. Later in the fall there was another short dry period and we were able to isolate the fecal strains of B. coli in large but varying numbers, the fluorescent group practically disappearing.

The number of colon bacilli per gram of soil diminished during the freezing weather to a point where it was not possible to recover the organism in less than 10 grams of soil. With the early spring rains of 1917 the organism was completely overgrown for a period by the growth of fluorescent bacilli. During the first dry spells of June and July, we were again able to isolate the so-called fecal strains of colon bacilli in large numbers.

Specimens of soil were periodically examined for B. coli of fecal origin until the summer of 1918 when all thought of this work was laid aside. As neither of the authors returned for any length of time to the University of Kansas after discharge from service, no continuation of the study was attempted until recently. When the tank was removed from the top of Frasier Hall in July, 1922, Mr. W. R. Schreiner, Bacteriologist for the Water Survey Laboratories of the Kansas Board of Health, sent a sample of soil to Young at Lansing. Organisms of the colon group subscribing in every detail to the cultural characteristics of the original organism present in the soil were isolated from 1/100 of a gram of soil.*

LABORATORY EXPERIMENTS STARTED SEPTEMBER 1916, ENDED JANUARY 1918

Two sets of salt mouthed bottles were filled with 200 grams of garden soil in each. One set, " A ", was sterilized with dry heat and the other set " B " was air dried. All had cotton stoppers. Each bottle was inoculated with 1 c.c. suspension of B. coli, the suspension made in isotonic salt solution. One-half with a soil strain and the other with a fecal strain. The moisture content was so adjusted that each strain was in soil held at 100, 60, 40, 20, 10 per cent saturation, adjustment being made each month.

SET " A " (STERILIZED)

100% Saturation. B. coli, both soil and fecal strains dropped rapidly at end of two months found only in 2 grams soil. At end of 1 year not found in 10 grams soil.

60% Saturation. B. coli content dropped rapidly, fecal strains more

* On March 9, 1923, organisms of the colon group were isolated from residue of this sample of soil.

rapidly than the soil strain. Both strains isolated from 10 grams soil in January, 1918, seventeen months after the experiment was started.

40, 20, and 10% Saturation. B. coli content dropped slowly with no apparent difference between soil and fecal strains. Both isolated from 1 gram soil January, 1918.

SET "B" (UNSTERILIZED)

100% Saturation. After the first month it was impossible to isolate B. coli that corresponded with the strains originally added. The soil soured and became overgrown with moulds. The organism predominating was B. fluorescens liquefaciens.

60% Saturation. B. coli content dropped rapidly. At the end of two months no organisms conforming to the original strains were isolated from 10 grams of soil but at the end of eight months the original cultures were isolated from 10 grams of soil. They were not isolated again during the time of the experiment.

40, 20, and 10% Saturation. At the end of the first month the B. coli content, both soil and fecal, had dropped. At the end of the third month there were more organisms per gram than when started. The number of the soil strains being the larger. During the entire period the B. coli content varied from month to month, the soil strain tending to run the higher.

In July, 1919, when one of the authors returned to the University of Kansas for a short time, two or three bottles of this set were found, all the rest were lost. The moisture content had not been adjusted so that all were very dry; however, both the soil and fecal strains of the colon group were isolated from 10 grams of the specimen.

SUMMARY

While the authors of this paper in no way believe that conclusions can be drawn from these observations depreciating the value of the presumptive and confirmatory tests for B. coli as an indicator of pollution, yet we do believe that these results point very definitely to conditions which will permit any of the colon group to become saprophitic and that this fact must be given serious consideration in judging the quality of a water-supply. Further, we believe that the differences between soil and fecal strains is of little practical significance to the sanitarian, and will not help him in judging as to the proximity of the source of contamination.

Certainly a ground water to be safe should be free from B. coli just the same as it should be free from great numbers of any soil organisms. But without doubt, unwarranted condemnation of farm water supplies are being made every day based on the presence of lactose fermenting bacteria.

This then is a plea for more efficient, sanitary and biological surveys before judging the potability of a water-supply, or the efficiency of any method of purification.

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DR. F. F. RUSSELL SUCCEEDS DR. WICKLIFFE ROSE

Dr. Wickliffe Rose on March 1 tendered his resignation as General Director of the International Health Board to accept the presidency of the newly incorporated International Education Board and of the General Education Board, which latter office was made vacant by the retirement of Dr. Wallace Buttrick to become chairman of that Board.

Dr. Frederick F. Russell, formerly director of the Board's public health laboratory service, has succeeded Dr. Rose as General Director of the Board.