Streptococcus as an Indicator of Swimming Pool Pollution

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THE behavior of bacteria in swimming pools although studied intensively during the past few years, is in need of further investigation. For many years, there was no well recognized procedure for testing for contamination and each bacteriologist devised his own methods of analysis with his own interpretation of what constituted dangerous water. The methods employed were generally based upon his knowledge of water bacteriology and hence were similar to, if not the same, as those which he employed in testing drinking water. Standards were developed by various individuals and later regulations concerning methods of procedure and standards were adopted by various states.

California, in 1917, was the first state to adopt regulations governing the operation of swimming pools. In 1919,[1] the California State Board of Health devised the following bacterial standard:

> As a tentative standard, a total count of 1,000 colonies per c.c. on agar incubated at 37.5° C. and an Escherich's bacillus count of 1 per c. c. as set for the pool water in any part of the pool examined within 48 hours after sampling. All tests are to be made in accordance with the latest methods of the American Public Health Association.

During the years 1918 and 1919, the writer made daily examinations of the college swimming pool for the *B. coli* content and the total bacterial count to determine their value as indicators of pollution. In the course of this work, it was finally decided that with filtration and a reasonable amount of chloride of lime the *B. coli* content could be kept below 1 *B. coli* in five 1 c.c. portions and that the total bacterial count could be kept below 1,000 bacteria per c.c. This standard was adopted tentatively with the realization that it did not mean that water conforming to this standard was safe and that water not so conforming was dangerous. It was accepted merely because, under existing methods of purification it represented the highest quality of water obtainable from day to day. During the four years that this standard was in force, no cases of disease were traced to the swimming pools.

In 1923 a committee on bathing places,[2] appointed by the American Public Health Association made the following report:

The committee is strongly of the opinion that the methods usually employed for bacterial analysis of potable waters do not show the true sanitary condition of the waters of the swimming pools. We wish at this time to emphasize strongly the need of studies of the bacterial flora of the waters of swimming pools as a basis for special methods for the sanitary analysis of such waters. Until such special methods have been worked out, we recommend that analysis be made according to the *Standard Methods of Water Analysis* of the American Public Health Association and that the following standards of quality be tentatively adopted .....

Sec. 5. Bacterial Count on Agar—2 days—20° C. (this count optional): Not more than 10 per cent of the samples covering any considerable period of time shall exceed 1,000 bacteria per c.c. No single sample shall contain more than 5,000 per c.c.

Sec. 6. Bacterial Count on Agar or Litmus Lactose Agar—24 hrs.—37° C.: Not more than 10 per cent of the samples covering any considerable period shall contain more than 100 bacteria per c.c. No single sample shall contain more than 200 bacteria per c.c.

Sec. 7. B. coli—Partial confirmed test: Not more than two out of five samples collected on the same day, or not more than three out of any ten consecutive samples collected on different dates to show a positive test in 10 c.c. of water.

The adoption of this tentative standard places swimming pool water on the same standard of quality as drinking water. This is justified on the grounds that bathers invariably swallow small amounts of water. As far as we know, swimming pools conforming to such a standard are safe in every respect, but unfortunately many pools fail to reach such a high degree of purity.

In adopting a standard using *B. coli* as an index of pollution it is well first to know that the microörganism indicates dangerous contamination. Is the swimming pool containing *B. coli* necessarily dangerous? Does the presence of *B. coli* indicate fecal contamination?

In drinking water, *B. coli* indicate fecal contamination and water containing this microörganism is considered dangerous. In streams, lakes and wells, *B. coli* of fecal origin tend to decrease in number, due to adverse growing conditions. The amount of organic matter is limited and is not an available food for this organism. Further, the temperature of such water is relatively low and tends to discourage growth. In swimming pools, conditions are entirely different. A large amount of organic matter is present and in an available form for food. The temperature is high and encourages the multiplication of the bacteria present. It is possible under such conditions that *B. coli* would increase. They would thus cease to be indicators of pollution since they would tend to increase in numbers while the pathogens introduced in the fecal matter would tend to decrease and disappear altogether.

Under laboratory conditions, *B. coli* will grow abundantly in swimming pool water. Several years ago, the writer kept a culture of *B. coli*, recently isolated from feces, growing in sterile pool water by transferring to a fresh flask of water each morning from the preceding flask. At the end of 2 weeks, the last flask after 24 hours incubation, had a count of approximately 200,000 *B. coli* per c.c. Would *B. coli* grow in the swimming pool where other bacteria are present, where the pool is constantly receiving fresh pollution, and where a constant inflow of fresh water containing chlorine occurs?

In order to answer this question, a study was made of *B. coli* in the swimming pool. Before studying the behavior of *B. coli* in swimming pool water, the writer studied the flora of polluted swimming pool water, hoping to find other microörganisms indicative of body pollution. The microörganisms besides *B. coli* most commonly found were staphylococcus and streptococcus which were undoubtedly introduced into the pool by the bathers. Streptococcus was found in four pools studied. Practically every sample of water that was polluted with *B. coli* contained streptococcus.

This microörganism is used by the English for detecting pollution in drinking water. The Americans have never used this organism for testing drinking water because a negative test does not mean that the water is potable, although a positive test for streptococcus does indicate to a greater extent than one for *B. coli* that the water is unsafe. Its significance in swimming pool water was worth considering.

Last year (1925), all of the routine examinations made on swimming pool water included a test for streptococcus along with the usual *B. coli* test as recommended by the American Public Health Association. The present paper deals with the occurrence of these two microörganisms and the relation of both to the amount of pollution entering the pool.

QUANTITATIVE PROCEDURE

The method of procedure for measuring the number of streptococci is quite similar to the methods used by the English in the determination of streptococcus in drinking water. Glucose and lactose nutrient broths are used. Quadruple strength broth is used for volumes of water from 50 to 100 c.c., double strength broth for volumes from 5 to 50 c.c. and single strength broth for volumes of 1 to 5 c.c. of water. Ten c.c. of broth is placed in each tube. The following amounts of water are tested: 10 tubes containing from 1 to 10 c.c. varying by 1 c.c., 9 tubes containing from 20 to 100 c.c. varying by 10 c.c. each, 5 tubes containing 10 c.c. each and 5 tubes containing 1 c.c. each. The five 10 c.c. and five 1 c.c. samples are tested with lactose nutrient broth while the remainder

are tested with glucose broth. The broth is made according to the methods recommended by the *Standard Methods for Water Analysis*, 1925.

The tubes are incubated for 48 hours at 37°, readings being made for *B. coli* at the end of 24 hours' and 48 hours' incubation. At the end of 48 hours' incubation at 37°, the tubes are removed and kept at room temperature for 3 days to allow the streptococci to settle to the bottom of the tubes. The tubes may be centrifugalized at the end of 24 to 48 hours' incubation to throw down the streptococci. However, as the former method is more accurate and less difficult, it was adopted.

After the streptococci have settled to the bottom of the tube, the supernatant fluid is removed carefully by suction, leaving the precipitated bacteria in the bottom of the tube in the form of a thick creamy mass. A loopful of this thick mass of bacteria is smeared on glass slides, dried and stained with gentian violet. The smears are then examined under the microscope for streptococci. No attempt is made in routine procedure to isolate the streptococci. When isolations are made, blood agar plates are smeared with the sediment obtained by centrifugalizing tubes incubated for 24 to 48 hours. Pure cultures can be readily picked from these plates after 24 hours' incubation at 37° C.

RESULTS

The data presented represent for each day, the total number of tubes containing *B. coli* or streptococci considering all of the tubes tested, namely five 1 c.c. portions, five 10 c.c. portions, 10 portions ranging from 1 to 10 c.c., each portion varying from the preceding one by 1 c.c., and 9 portions ranging from 20 to 100 c.c., each varying from the preceding one by 10 c.c. amounts, making in all twenty-nine tubes containing collectively 550 c.c. of water. The data represent days when the swimming pool water had a high bacterial content which continued for 2 or more days. These periods were selected because the *B. coli* content was generally high in the evening and thus there was presented an opportunity to study the behavior of the microörganisms during the night when the only means of purification was circulation of the water through the filters. The morning samples were generally taken before the chloride of lime was added.

A. *A Study of the Pool When in Use*—A study of the *B. coli* and streptococci content of the swimming pool was made, taking samples hourly, when the pool was in use. During this period no chloride of lime was added, so the effect of the accumulated pollution could be measured by the *B. coli* and streptococci introduced by the bathers. During the morning, when there were only a few bathers in the pool, a

marked reduction of the streptococci occurred, which was paralleled somewhat by the decrease of the *B. coli.* In the afternoon, the attendance was larger and the number of streptococci increased until in the case of the 10 c.c. portions, all 5 tubes contained streptococci. The *B. coli* content did not increase to any extent; in fact, a slight decrease occurred in the three cases reported. In these cases, the increase of streptococci parallels the increase in pollution. These experiments were repeated several times with similar results.

B. *A Study of the Pool When Not in Use*—In this series of experiments a similar test was made, only over a longer period of time and represents a period when the pool was in use followed by a period when it was not in use. Single samples were collected on a Thursday night, Friday morning and night and then starting Saturday morning, a sample was taken every 3 hours throughout the day, that night and all day Sunday. No chloride of lime was added during the whole period of the test, the only means of purification being filtration which was used continuously. *B. coli* content increased materially during Thursday night and the streptococci content decreased to zero, i.e., no streptococci were found in any of the tubes. During Friday, when the pool was in use, streptococci increased to a greater extent than the *B. coli.* Friday night, both the *B. coli* and streptococci content dropped and continued to fall until 3 o'clock Saturday afternoon, when the pool was again in use. At this time, the streptococci content increased decidedly while only a slight increase of *B. coli* occurred. Shortly after, the number of streptococci decreased rapidly and at 9 o'clock Sunday night, they had practically disappeared. *B. coli,* on the other hand, although the number decreased, were still present in large enough numbers to represent a dangerous condition of the pool as indicated by the standard test. This experiment was repeated several times with similar results.

C. *Behavior of B. coli and Streptococci During the Night*—These data represent the changes occurring in the *B. coli* and streptococci content of the swimming pool during the night. Samples were taken in the morning before chloride of lime was added. In practically every case, the streptococci content dropped from a large number to a relatively small number in the morning. The *B. coli* content was more or less erratic; however, there was little or no decrease during the night, despite the fact that during the night the filters were in continuous operation. The average decrease of streptococci amounted to 66.5 per cent while in the *B. coli,* the average decrease was only 0.6 per cent. This indicates that the *B. coli* must have multiplied in the pool, for the filters remove a large number of microörganisms and would thus have

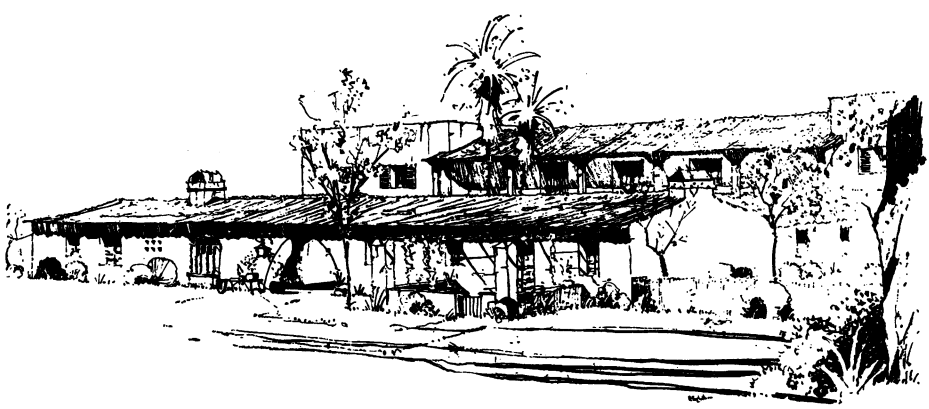
caused a marked reduction in numbers unless the organism actually reproduced in the pool. The reduction of streptococci, on the other hand, indicates that they do not grow. The data do not necessarily indicate that they die out in the pool, since they may be removed by filtration, but the data do prove that the streptococci content parallels the pollution.

CONCLUSIONS

1. *B. coli* content is not a universally reliable indicator of intestinal pollution in swimming pools.
2. Streptococci are constant indicators of intestinal pollution and the number found in the pool parallels the amount of pollution as indicated by the number of bathers.
3. *B. coli* tend to multiply in the swimming pool, while streptococci do not.
4. Streptococci when present indicate an unsafe condition of the swimming pool.
5. *B. coli* do not necessarily indicate pollution or danger, although the absence of *B. coli* is an excellent index of safety.

REFERENCES

1. Gillespie, C. G. (1919) *Sanitation of Swimming Pools*, Special California State Bd. of Health.
2. Report of Committee on Bathing Places. (1923) *A. J. P. H.*, 14, 7:597 (July), 1924.



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