

# THE DESTRUCTION OF ACID-FASTNESS OF THE TUBERCLE BACILLUS BY AN AUTOLYTIC PROCESS<sup>1</sup>

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There are several articles in the literature dealing with the subject of autolysis of the tubercle bacillus. McJunkin (1923) dehydrated living tubercle bacilli in 95 per cent ethyl alcohol for two minutes, suspended the microorganisms in oleic acid which contained a few drops of water, and incubated the mixture at 37°C. After several days, only a limited number of bacilli retained their acid-fast properties. The action was partially inhibited when the microorganisms were killed by heat or toluene. The experiments suggested to the author the "possibility that the solvent action was due to a thermolabile enzyme contained in the tubercle bacillus acting only in the presence of undiluted oleic acid." Boissevain (1926) obtained similar results using a number of different unsaturated fatty acids, but found that preliminary dehydration was unnecessary. Isabolinsky and Gitowitsch (1924, 1927, 1928) confirmed the results using lecithin, olive oil and liver oil. Steenken (1938) observed lysis of old colonies grown on solid egg media. Subsequent growth of secondary colonies within and surrounding the mother colonies showed evidence of lysis in their interior. Corper and Sweany (1918) noted that human and bovine tubercle bacilli, killed by toluene, liberated non-coagulable nitrogen on incubation at 37°C. Long (1921), on the contrary, found that 5 grams of moist organisms killed by toluene and incubated for two weeks liberated only 5 milligrams of soluble nitrogen. The liberation of such small quantities of soluble nitrogen appeared to Wells and Long (1932) to indicate that the microorganism had but weak autolytic powers. However, our observations, here recorded, indicate that there is present in the tubercle bacillus an active autolytic substance.

It is common knowledge that virulent tubercle bacilli may remain in water for an indefinite period and still retain their acid-fastness. However, if, preliminary to their immersion in water, they are treated in the manner to be described, the bacilli not only lose their acid-fastness, but, with few exceptions, the structure of the microorganisms is completely destroyed. Experimental data will also be presented suggesting the probable nature of the agent responsible for the autolytic process.

## PROCEDURE

A six-weeks-old culture of virulent human tubercle bacilli, Strain H37, grown in Long's synthetic medium, is transferred to a Buchner funnel and washed with distilled water until the microorganisms are free from culture medium. Approximately 1 gram of the washed bacilli is transferred to an Erlenmeyer flask and

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suspended in 40 ml. of 95 per cent ethyl alcohol. The flask is gently shaken for one-and-a-half minutes. At this time, 40 ml. of ether are added, and the mixture is allowed to remain at room temperature for a period of two days. The flask is again gently shaken and 10 ml. of the contents removed by pipette, placed in a test-tube and centrifuged at high speed for ten minutes. The supernatant fluid is decanted and the sedimented bacilli are dried on blotting paper. The bacilli are then placed in an agate mortar and mixed with distilled water until a heavy uniform suspension is obtained. One-tenth ml. of this heavy suspension is now transferred to a test-tube containing 1 ml. of distilled water, and incubated at 37°C. Sterile precautions are required in carrying out the entire procedure. Contaminations may alter the pH of the solution and confuse the readings. Stained preparations of the heavy suspension of bacilli reveal that their morphology and acid-fast properties are retained (fig. 1). However, when the

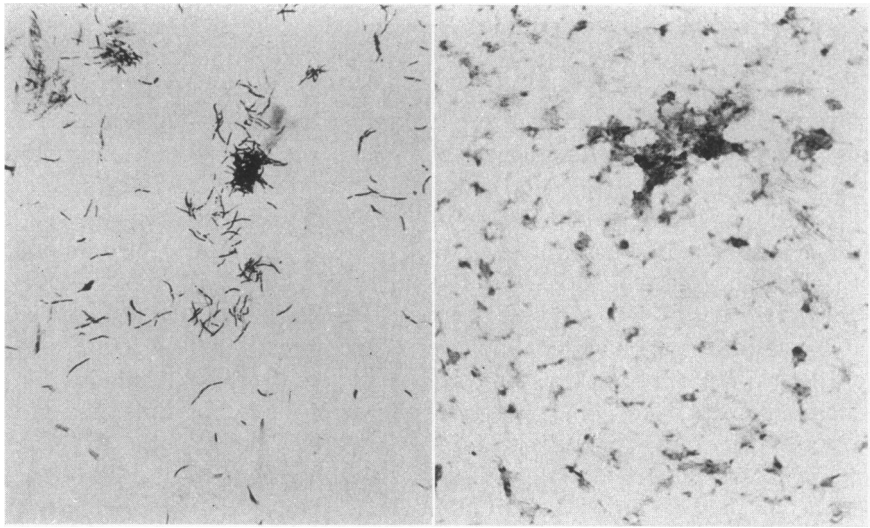


FIG. 1

FIG. 2

microorganisms are transferred to water and incubated for a period of two days at 37°C., there is evidence of marked autolysis with loss of acid-fastness (fig. 2). It now became of interest to examine some of the properties of this autolysis and the agent responsible for it.

#### TEMPERATURE AND RATE OF AUTOLYSIS

Suspensions of extracted bacilli prepared according to the procedure outlined above were incubated at 10°, 25° and 37°C. At varying intervals from 1 to 7 days, the suspensions were examined and the extent of the autolysis was noted. At 10°C. autolysis was markedly delayed, no appreciable change being apparent by the end of 48 hours (table 1). At 37°C., on the other hand, the evidence of autolysis at the end of 48 hours was very pronounced (table 2). The results at 25°C. were approximately intermediate between those obtained at 10° and 37°C. Temperatures above 37°C., while not extensively examined, appear to be inhibi-

tive, since suspensions incubated at 50°C. for 48 hours showed no autolysis. It was noted also that suspensions kept at 10°C. for 2 days, and then transferred to 37°C. assumed the rate of autolysis characteristic of this latter temperature. From these various experiments, it appears that the rate of autolysis is dependent upon the temperature, and that the temperature for optimum activity is probably in the vicinity of 37°C.

TABLE 1  
*Autolysis of tubercle bacilli at 10°C. and various values of pH*

pH	24 HOURS	48 HOURS	4 DAYS	5 DAYS	7 DAYS
3.0	—	—	—	—	+
4.6	—	+	+	+	+
5.2	—	—	+	+	+
6.0	—	+	++	++	++
6.6	—	—	++	++	++
7.0	—	—	+	+	+
8.8	—	—	—	—	+

TABLE 2  
*Autolysis of tubercle bacilli at 37°C. and various values of pH*

pH	24 HOURS	48 HOURS	4 DAYS	5 DAYS	7 DAYS
3.0	—	+	+	++	++
4.6	+	+	++	+++	++++
5.2	+	++	+++	++++	++++
6.0	++	+++	+++	++++	++++
6.6	++	+++	++++	++++	++++
7.0	+	++	++++	++++	++++
8.8	—	—	+	++	++

Note: —, no destruction of acid-fastness; +, 25 per cent destruction of acid-fastness; + + + +, 90 per cent or more of destruction of acid-fastness.

#### HEAT INACTIVATION OF AUTOLYTIC AGENT

In a preliminary experiment extracted bacilli were submitted to a temperature of 90°C. for ten minutes, and then incubated at 37°C. for 1 to 7 days. Examination of the microorganisms showed that no autolysis had occurred, indicating a destruction of the autolytic agent by the short heat-treatment.

#### pH AND RATE OF AUTOLYSIS

The autolytic process was markedly affected by the pH of the solution. In solutions of water buffered at pH 3.0 and 8.8, and incubated at 37°C., autolysis proceeded slowly and was incomplete at the end of seven days. Maximum autolysis, on the other hand, occurred in suspensions having pH values between 5.2 and 7.0 (table 2). Autolysis in suspensions kept at 10°C. showed similar pH relationships (table 1).

## INFLUENCE OF IODINE AND FORMALDEHYDE ON AUTOLYSIS

It was found that the autolytic process was completely inhibited when very dilute solutions of iodine and formaldehyde were added to the water in which the bacilli were suspended at pH 3.0 to 8.8, and incubated at 37°C. for periods of 1 to 7 days.

## LOCATION OF THE AUTOLYTIC AGENT

The autolytic agent apparently is not liberated when the microorganisms undergo autolysis, as disclosed by the following experiment: Heat-killed, extracted, tubercle bacilli, treated in the manner already described were not affected when placed in a suspension of tubercle bacilli that were undergoing autolysis at a maximum rate.

## DISCUSSION

It appears from the experimental data submitted, that the tubercle bacillus contains an autolytic agent. The action of this agent first becomes apparent after the microorganisms have been treated (lipoids extracted) with alcohol and ether, and suspended in water. It is of interest to note in this connection that earlier investigations of Jobling and Petersen (1914) showed the lipid content of bacteria to be functional in the resistance of the bacteria to tryptic digestion.

The response of this autolytic agent to different temperatures, its inactivation by high temperatures, its response to variations in pH and to weak solutions of iodine and formaldehyde, conforms to that of certain well-recognized enzymes. The relatively rapid loss of acid-fastness and destruction of the morphology of the tubercle bacillus suggests that the autolytic agent or enzyme is endowed with considerable activity.

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