THE EFFECT OF HEAT ON FLAGELLAR AND SOMATIC AGGLUTINATION.

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In 1904, Beyer and Reagh[1] differentiated between flagellar and somatic agglutinins by means of heat. They worked with a motile and a non-motile culture of hog-cholera bacilli from different sources. They found that a temperature of 70°C. injured the flagellar agglutinating substance and the somatic agglutinins but did not injure the flagellar agglutinins nor the somatic agglutinating substance. Furthermore they reported that a motile culture heated to 70°C., although it no longer gave a flagellar agglutination reaction, still would cause the production of flagellar agglutinins in the animal body.

These experiments have been repeated in the course of the work here to be reported, but this time the two forms of the one strain hog-cholera Md. were used. This hog-cholera Md. culture has been under cultivation since 1898. It was originally a motile strain but in 1923 a non-motile form was isolated from it and this mutant has been kept as a separate strain since that time. The separation of the flagella from the bodies of the bacilli and the use of the separated flagella as a separate antigenic substance has been reported in a previous paper.[2] In the present experiments the non-motile mutant of the Md. culture was used for the somatic agglutinogen, the separated flagella for the flagellar antigen, and the sera produced by these two forms supplied the flagellar and somatic agglutinins. In general the results agree with those of Beyer and Reagh.

When the serum produced by the separated flagella was heated at 70°C. for 20 minutes (Table I) it still agglutinated the flagella, but the somatic serum pro-

[1] Beyer, H. G., and Reagh, A. L., *J. Med. Research*, 1904, xii, 313.

[2] Orcutt, M. L., *J. Exp. Med.*, 1924, xl, 43.

TABLE I.

Flagellar suspension.	Serum dilutions. 1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	Control.	Remarks.
Flagellar serum (Rabbit 6-40), unheated.											
Unheated.	+++*	++	++	+	+	−	−	−		−	
Heated, 70°C.	+ (fine)	−	−	−	−					−	No fluffy flagellar reaction.
Flagellar serum (Rabbit 6-40), heated, 70°C., 20 min.											
Unheated.	+++	++	+	+	−	−				−	
Heated, 70°C.	−	−	−							−	

*+ = visible clumping; ++ and +++ = stronger reactions; no standard for complete reaction because original suspension is clear.

TABLE II.

Non-motile culture.	Serum dilutions. 1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	Control.
Non-motile culture serum (Rabbit 5-84), unheated.										
Unheated.	C.	C.	C.	C.	C.	++++	++	+	−	−
Heated, 20 min., 70°C.	"	"	"	++++	++++	+++	++	+	−	−
" 20 " 75° "	"	"	+++	+++	++	++	+	±	−	−
" 20 " 100° "	"	"	C.	++++	+++	++	+	+		−
" 20 " 120° " (autoclave).	"	"	"	++++	+++	++	++	+		−
Non-motile culture serum (Rabbit 5-84), heated, 70°C., 20 min.										
Unheated.	+++	++	+	±	−	−	−	−		−
Non-motile culture serum (Rabbit 5-84), heated, 75°C., 20 min.										
Unheated.	−	−	−	−	−	−	−	−		−

duced by the non-motile mutant when heated at 70°C. gave a considerably reduced reaction with that culture (Table II). Again, the flagella heated at 70°C. for 20 minutes and then tested with the unheated serum gave no agglutination reaction (Table I), but the non-motile mutant heated at 70°C. and tested with

the unheated somatic serum gave a final titer equal to that of the unheated culture. In these tests 70°C. did not give as complete a destruction of somatic agglutinin as the results of Beyer and Reagh indicated, although it caused a considerable lowering of the reaction. For instance, in the results of Beyer and Reagh the serum produced by the non-motile strain heated at 70°C. gave no reaction with the unheated bacilli, while in the corresponding experiment repeated at this time the serum produced by the non-motile mutant heated at 70°C. gave a strong reaction at 1:20 and a trace of clumping at 1:160 in contrast with the result with the unheated serum which gave a complete agglutination at 1:20 and a slight reaction at 1:2,560. If a temperature of 75°C. was used the agglutinating power of this serum was completely destroyed (Table II), but this temperature altered the flagellar serum so that a zone of inhibited agglutination occurred in the lower dilutions of 1:20 to 1:40 and the whole reaction took place more slowly than with the unheated serum. This temperature of 75°C., however, had little effect on the agglutinating substance of the non-motile bacilli. In fact, a temperature of 100°C. or even 120°C. did not destroy their agglutinating substance. Tests were carried out with cultures of the non-motile mutant heated in a water bath for 20 minutes at 70°, 75°, 80°, 85°, 95°, 100°C., and heated in the autoclave at 120°C., and an unheated culture as control. They all gave a similar agglutination reaction in the unheated somatic serum. The unheated culture gave a greater degree of completeness but the titer limit was the same for both heated and unheated cultures.

The second experiment by Beyer and Reagh was the production of a flagellar antiserum with a motile culture heated at 70°C. This experiment was repeated with a suspension of separated flagella heated at 70°C. Such a suspension no longer gave an agglutination reaction. However, on injection into a rabbit it gave rise to a serum containing flagellar agglutinins just as did the unheated flagellar suspension.

A microscopic examination of the heated and unheated cultures revealed certain differences.

A preparation of the unheated flagella stained with a flagella stain showed many wavy flagella, some in more or less of a network and some scattered. In a preparation of the same suspension heated at 70°C. and stained with the flagella stain none could be distinguished. A preparation of the unheated motile bacilli showed the organisms with long wavy flagella and this same culture heated at 70°C. and stained with the flagella stain showed the bacilli as distinctly as in the preparation of the unheated culture but the flagella were no longer seen. Sometimes granular lines appeared between groups of bacilli suggesting flagella but quite different in appearance from the flagella of the unheated culture. On the other hand, the non-motile mutant unheated and heated up to 100°C. in a water

bath and 120°C. in the autoclave when stained with flagella stain or with carbol-fuchsin, methylene blue or Stirling's gentian violet showed a similar appearance. The outline of the bacilli was as definite in the preparation of the heated culture as in that of the unheated culture, but sometimes the heated bacilli seemed a little more faintly stained.

These observations indicate that the heat destroys the structure of the flagella but does not cause the disintegration of the bodies of the bacilli. This helps to explain why the heated flagella gave no visible reaction while the heated bacilli, which still kept their form intact, continued to react visibly.

Another experiment was made by absorbing the flagellar serum with heated flagella and the heated motile culture and then centrifuging and retesting with fresh unheated flagellar suspension and motile bacilli. This experiment was performed three times and the last time a very heavy suspension of flagella and a heavy growth of the motile bacillus was used for the absorption. In every case the results were the same. The serum after contact with the heated flagella or the heated motile culture still gave a strong reaction when the fresh unheated culture or flagella were added. This result indicated that the disintegrated flagella not only failed to become clumped but did not even combine with the agglutinins. However, since the heated flagellar suspension produced flagellar agglutinins in the animal the antigenic nature of the flagella was not destroyed by the heat although their form, their ability to clump, and their ability to absorb agglutinins were destroyed. The reactions of each of the four different substances to heat have been summarized in Table III.

Eisenberg and Volk[3] stated that in the case of typhoid bacilli, heat above 60°C. injured the agglutinating ability of the bacilli but did not destroy their power to absorb agglutinins, and they explained this reaction by the presence of two factors in the agglutinating substance, a thermolabile clumping factor and a thermostable binding factor. At the time of their work no distinction had been made between flagellar and somatic agglutination. From previous work[4, 2] we know that a motile culture contains two antigenic substances, a flagellar antigen and a somatic antigen, and the present results show

[3] Eisenberg, P., and Volk, R., *Z. Hyg. u. Infektionskrankh.*, 1902, xl, 155.

[4] Smith, T., and Reagh, A. L., *J. Med. Research*, 1903, x, 89.

that the flagella are destroyed by heat so that they no longer give an agglutination reaction nor do they absorb agglutinins, while the bodies of the bacilli are not destroyed by heat and they still react with agglutinins.

The results of the present observations with the non-motile mutant and the separate flagella show a general agreement with the work of Beyer and Reagh on the differentiation of flagellar and somatic agglutinins by heat. The results indicate that heat destroys the structure

TABLE III.

Effect of Heat on Hog-Cholera Md. Flagellar and Somatic Antigen and Agglutinins.

Flagellar antigen.	+	70°C.	= Destruction of form and of ability to clump and to absorb agglutinins, but no destruction of the power to generate or stimulate agglutinin production.
Somatic "	+	70°C. or heat up to 120° C.	= Little or no destruction of form or of ability to absorb agglutinins and form clumps.
Flagellar agglutinins.	+	70°C.	= Little or no destruction of agglutinating ability.
" "	+	75°C.	= Alteration, with occurrence of inhibition zone in lower dilutions.
Somatic "	+	70°C.	= Considerable injury to agglutinating ability.
" "	+	75°C.	= Destruction of agglutinating ability.

of the flagella but not that of the bodies of the bacilli, and finally that flagella or motile bacilli heated at 70°C. do not absorb flagellar agglutinins.

CONCLUSIONS.

1. Heat at 70°C. destroys the form of the flagella and their ability to combine with flagellar agglutinins but it does not destroy their antigenic nature since they can still generate flagellar agglutinins in the animal body.

2. Heat at 70°C. and even at 120°C. in the autoclave does not destroy the forms of the bacilli themselves nor their ability to become agglutinated and to absorb agglutinins.

3. Somatic agglutinins are destroyed to a considerable extent by heat at 70°C. and completely destroyed by heat at 75°C.

4. Heat at 70°C. causes little or no destruction of flagellar agglutinins but a temperature of 75°C. changes the agglutinins so that they react more slowly and produce a slightly lower reaction with a zone of inhibition in the stronger dilutions.