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Comparative Observations on Bacteriolytic and Hemolytic Titers of Certain Sera.

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The Bordet Gengou reaction was designed to test the hypothesis of the identity of bacteriolytic and hemolytic complement,[1] although Bordet recognized later that the identity could not be regarded as absolute, since he was able to make specific anticomplements for the sera of various animals.[2] Muir and Browning[3] suggested that the bactericidal action of normal serum might be due to some moiety of complement which they termed "bacteriophilic". Gordon and his coworkers[4] have been unable to obtain a serum which is devoid of hemolytic complement and yet possesses bactericidal action, though the reverse might be true of sera absorbed with bacteria, unless absorption were continued too long or the number of bacteria used were overwhelming.

A series of tests was made to determine which bacteria in the School collection could be depended upon to resist the bactericidal action of certain non-immune sera, and which bacteria were sensitive to it. The results with pooled human sera did not vary, but occasionally an organism was killed by some rabbit sera and not by others (*e. g., B. enteritidis, B. typhosus* Rawlins). Table I is a resumé of these tests.

The technique used was as follows: The bacterial suspensions, from 18-hour cultures on agar slants, were made in saline containing 0.1% gelatin. Very few bacteria survived incubation in saline, but the addition of this very small amount of gelatin removed the toxicity of the saline. The first dilution was made to correspond with Tube 1 of the McFarland nephelometer, the final dilution was one ten-thousandth of the first. The number of bacteria on control plates without serum was 1,000 to 2,000 per cc., 1 cc. being the amount of suspension used in the tests. The mixtures of bacteria and serum were incubated for 4 hours in a waterbath at 37.5°C., and the entire mixture was plated by pouring. Complete inhibition

[1] Bordet, J., *Traité de l'immunité*, 1920, 320.

[2] Bordet, J., *Traité de l'immunité*, 1920, 95.

[3] Muir, R., and Browning, C. H., *J. Path. and Bact.*, 1909, **13**, 76.

[4] Gordon, J., and Wormall, A., *J. Path. and Bact.*, 1928, **31**[2], 753.

TABLE I.
Bactericidal Action of Normal Human and Rabbit Serum.

	Human		Rabbit		
	Active	Inactivated 56° C.	Active	Inactivated 56° C.	Controls, no serum
B. typhosus Rawlins	—	++++	+++ / —	++++ / ++++	++++
" F	—	++++	++	++++	++++
B. dysenteriae Flex 2	++	++++	++	++++	++++
Shiga 1			+	++++	++++
Shiga 2	—	++++	+	++++	++++
B. coli 1	—	++++			++++
B. aerogenes			—	++++	++++
B. paratyphosus A	—	++++	++	++++	++++
B	—	++++	—	++++	++++
Proteus X 19	—	+++	++	++++	++++
			++++	++++	
" from rabbit	++++	++++	++++	++++	++++
B. alkaligenes	++	++++	++++	++++	++++
B. morgani	—	++++	++++	++++	++++
B. suipestifer	—	++++	—	++++	++++
B. icteroides	—	++++	—	++++	++++
B. aertrycke (mouse)	—	++++	++++	++++	++++
B. enteritidis (mouse)	+++	++++	++++	++++	++++
" (old laboratory str.)	—	++++	— / ++++	++++ / ++++	++++ / ++++
B. pyocyaneus	—	++++	++	++++	++++
B. bronchisepticus			++++	++++	++++
B. prodigiosus	++++	++++	++++	++++	++++
B. subtilis (old lab. str.)	++++	++++	++++	++++	++++
" (Soule)	+	++++	—	—	++++
B. anthracis	+++	+	—	—	+++
B. friedländeri (old lab. str.)			++++	++++	++++
(non-mucoid)	++	++++	+	++++	++++
(mucoid)	+	++++	—	++++	++++
Staphylococcus aureus (old lab. str.)	++++	++++	++++	++++	++++
" " (empyema)	++++	++++	++++	++++	++++
" " (osteomyelitis)	++++	++++			++++
" " (Brodie's absc.)	++++	++++			++++
" " (ear abscess)	++++	++++			++++
" " (nose-throat)	++++	++++			++++
Streptococcus 20	++++	++++	++++	++++	++++
M. tetragenus	++	++	+	+++	++
Sarcina lutea	—	++	—	+	++
B. diphtheriae	+++	+++			+++
B. abortus	++++	++++	++++	++++	++++
B. melitensis	++++	++++	++++	++++	++++
Pneumococcus	++	++	++	++	++

of growth is recorded as —, a few colonies as +, 50-100 colonies as ++, and abundant growth as +++ or ++++. Titrations of hemolytic complement were carried out by the antisheep system.

The complement (hemolytic) titer of the human serum, which was a mixture of specimens taken for Wassermann tests, was remarkably constant (0.02-0.05 cc.), that of rabbit serum was much lower and varied in different animals from 0.14 to 0.20. The bac-

tericidal titer in each instance was much higher than the hemolytic, being 0.001-0.0005 cc. for the human and 0.01-0.005 cc. for the rabbit. Comparative tests of several rabbit sera of high and low hemolytic titer showed no correlation between bacteriolytic and hemolytic titer.

When dog serum, which had a constant hemolytic titer of 0.05 cc., was tested for bactericidal action, it was found that only 3 bacteria in the entire collection (*B. morgani, Proteus X 19,* and *Sarcina lutea*) were killed by 0.1 cc. of serum. An experiment to test the feasibility of reinforcing the complement content of diluted or heated serum by means of dog serum revealed the fact that the dog serum was antibactericidal when mixed with fresh rabbit or human serum, a property which was not removed by heating. For example, a serum which alone was bactericidal in 0.0005 cc. was no longer bactericidal below 0.05 cc. in the presence of 0.1 cc. of dog serum (2 hemolytic units), though such a mixture showed complete lysis in the hemolytic test. Hence, although dog serum can function as complement in the Wassermann test, as shown by Noguchi and Bronfenbrenner[5] and confirmed in the present work, it not only fails to function as bacteriolytic complement but seriously interferes with bactericidal action. Bacteria submitted to bactericidal tests after treatment with dog serum proved to be only slightly more susceptible to bacteriolysis than bacteria not so treated.

The susceptibility or resistance of the various bacteria, while showing little or no variation when tested against a given animal serum, seems to follow no general rule. In some instances (*B. enteritidis, B. typhosus*) the pathogenic organisms resist bactericidal action, the non-pathogenic not, but in the case of *B. friedländeri* this finding is reversed. Some saprophytes are killed (*Sarcina lutea, B. aerogenes*), others are resistant (*B. prodigiosus*). That the mechanism is different for Gram negative bacteria[6] is not borne out, *Sarcina lutea* being killed under the same conditions as the Gram negative bacteria, instead of falling into the class with *B. subtilis* or *Staphylococcus.*

The failure of dog serum to reactivate heated human serum seems to indicate that hemolytic complement is in this case distinct from bacteriolytic complement, but no explanation offers itself for the interference of dog serum with the bactericidal action of other sera.

[5] Noguchi, H., and Bronfenbrenner, J., *J. Exp. Med.*, 1911, **13**, 78.

[6] Mackie, T. J., and Finkelstein, M. H., *J. Hyg.*, 1932, **32**, 1.