ON THE SPECIFICITY OF THE THERMOSTABLE OPSONINS FOR STREPTOCOCCI.

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Although it is now generally recognized that streptococci comprise a group of possibly very various organisms, it is very doubtful if any very satisfactory method of differentiating one member of the group from another has been evolved up to the present. The well-known sugar and other cultural reactions which were worked out by Gordon, and elaborated later by Andrewes and Horder, have given, in the hands of one of us, the most discordant results. Agglutination phenomena with streptococci are notoriously irregular, whilst it has hitherto been found impossible to demonstrate any bactericidal action of immune sera on streptococci. It appeared, therefore, desirable to attack the question from another standpoint, and it seemed possible that a study of the thermostable opsonins resulting from the injection of streptococci might give some further information on the subject.

A number of streptococci were isolated from normal throats, from sore throats, and from a tonsillar abscess, as well as from a case of acute pyæmia, a case of erysipelas, and from a case of traumatic lymphangitis. A list of these, together with their principal reactions, is given in Table I. Living cultures of a number of these strains were injected into rabbits, each animal receiving one strain of streptococci only; the doses were given at intervals of about ten days, the first dose in each case being given intravenously, the later ones subcutaneously. Although some of the cultures were freshly isolated from suppurative foci in man, in no case did the animal seem to suffer any serious inconvenience beyond, in one or two instances, a small nodule at the point of inoculation. After a suitable interval experiments were made to find out whether the sera of the immunised animals contained thermostable opsonins for the cocci with which the rabbits had been inoculated, and, if they did, whether these opsonins were active for other streptococci. The technique adopted was as follows: The sera were heated to 60° C. for half an hour, after which mixtures of heated serum, washed blood corpuscles (human), and a thick emulsion of a twenty-four-hour agar culture of streptococcus were taken up in capillary

TABLE I.

	Morphology of chains	Broth	Milk	N. red	Sacch.	Lactose	Raffinose	Inulin	Salicin	Coniferin	Mannite	Maltose	Hæmolysis	Origin
J2 ..	Very long chains in pairs	Flocculent deposit	–	–	±	+	–	–	–	–	–	–	0	The same normal throat
J9 ..	Lanceolate diplococci and small chains	Ditto ..	Clot	–	–	–	–	–	–	–	–	–	0	
J11 ..	Mostly round diplococci and short chains	Diffuse ..	–	–	+	+	+	+	+	+	–	+	0	
V7 ..	Very long	Conglomerate	Acid	+	–	–	–	–	–	–	–	–	0	Tonsillar abscess
H ..	Long ..	Flocculent deposit	Clot	+	+	+	–	±	–	±	–	+	0	Traumatic lymphangitis
Hks	Short ..	Diffuse ..	Ditto	–	+	–	–	–	–	–	–	+	+	Scarlet fever throat
G6 ..	Very long	Flocculent deposit	Ditto	–	+	+	±	±	–	+	–	+	+	Normal throat
Br19	Short ..	Diffuse ..	Ditto	–	+	+	–	–	–	Olive-green	–	0	+	Ditto
Br16	Ditto ..	Ditto ..	–	–	+	+	+	–	+	+	–	+	–	Ditto
St13	Ditto ..	Ditto ..	–	–	+	–	+	–	+	+	–	0	0	Ditto
M10	Short to medium	Ditto ..	–	–	+	–	+	+	±	–	–	0	Light green	Ditto
Co18	Ditto ..	Ditto ..	Clot	–	+	+	–	±	–	–	–	0	+	Ditto
Br9 ..	Ditto ..	Ditto ..	Acid	+	+	+	+	+	+	–	–	+	0	Ditto
Br4 ..	Short ..	Ditto ..	–	–	+	+	+	+	–	–	–	–	0	Ditto
G1 ..	Long ..	Heavy flocculi	Acid	+	+	+	±	±	–	–	–	+	+	Ditto
Co10	Ditto ..	Diffuse ..	Clot	–	+	+	+	–	–	–	–	0	–	Ditto
Ca11	Short to medium	Light flocculi	–	–	+	+	+	–	–	–	–	0	0	Ditto
A2 ..	Large diplococci and short chains	Granular ..	Clot	–	+	+	–	–	+	+	–	0	0	"Sore throat"
Co17	Long ..	Heavy flocculi	Acid	–	+	+	–	–	–	–	–	0	+	Normal throat
E2 ..	Medium	Flocculent deposit	Ditto	–	+	–	–	–	–	–	–	+	+	Case of erysipelas
E3 ..	Ditto ..	Ditto ..	Ditto	–	+	–	–	–	–	–	–	+	+	
L1 ..	Long ..	Ditto ..	Ditto	–	–	+	–	–	–	–	–	+	+	Pus from pyæmic abscess

+ = Acid; – = No change; 0 = Not tried.

tubes; these were incubated in a water-bath at 37° C. for fifteen minutes; films were then made and stained by Leishman's stain. In examining the films fifty cells at least were looked over, but no attempt was made to count the number of cocci in each phagocyte, since the only object of the experiment was to determine whether the serum contained thermostable opsonins for the coccus with which it was put in contact or not. This point was very easily settled in all but a couple of instances, since there was either no phagocytosis at all, beyond occasionally a few cocci in one or two of the fifty cells examined, in which case thermostable opsonins were deemed to be absent; or else practically every cell was packed with cocci, when it was assumed that thermostable opsonins for those particular streptococci were present in the serum.

TABLE II.

Cocci	Heated normal serum	Heated Sera of Rabbits Immunised with								
		J2	J9	J11	H	V7	Hks	G6	Br19	Br16
J2	0	+	0	0	0	0	–	–	–	–
J9	0	0	+	0	0	0	–	–	–	–
J11	0	0	0	+	0	0	–	–	–	–
H	0	0	0	0	+	0	–	–	–	–
V7	0	0	0	0	0	+	–	–	–	–
Hks	0	0	0	0	0	0	+	–	–	–
G6	0	0	0	0	0	0	0	+	0	–
Br19	0	0	0	±	–	–	–	0	+	0
Br16	0	0	0	0	–	–	–	–	–	+
St13	0	0	0	0	–	–	–	–	–	–
M10	+	+	+	+	–	–	–	–	–	–
Co18	0	0	0	0	–	–	–	–	–	–
Br9	0	0	0	0	–	–	–	–	–	–
Br4	0	0	+	0	–	–	–	–	–	–
G1	0	0	0	0	–	–	–	–	–	–
Co10	+	+	+	+	–	–	–	–	–	–
Cal1	0	0	0	±	–	–	–	–	–	–
A2	+	+	+	+	–	–	–	–	–	–
Co17	0	–	–	–	0	0	–	–	–	–
E2	0	0	0	0	0	0	–	–	–	–
E3	0	0	0	0	0	0	–	–	–	–
L1	0	0	0	0	0	0	–	–	–	–

+ = Phagocytosis. 0 = No phagocytosis.

The results obtained are given in Table II., which represents the collected results of a very large number of frequently repeated observations. It will be seen that each serum contained thermostable opsonins for the coccus with which the rabbit providing it had been inoculated, and for no other except in cases where the heated

normal serum also produced phagocytosis. The only exception to this was in the case of serum J9, which produced phagocytosis of coccus Br4; unfortunately this strain died out before we were able to carry out the further experiments which the finding suggested. In the case of serum J11 there were two doubtful results, the only ones in the series; they were probably due to the fact that this serum was one which produced extreme agglutination of human blood cells. The rabbit which provided it died at a late stage of the proceedings, and was found to be very intensely infected with coccidiosis.

It appears, then, that the injection of streptococci into rabbits results in the production of thermostable opsonins which are extremely specific, so much so that our earlier hopes that one might perhaps be able to use the principle as a basis for the classification of streptococci have declined considerably; for it is evident that streptococci must form a group of a very large number of organisms differing among themselves as much or more than typhoid bacilli differ from the other members of the coli group. We propose, however, to continue the research from this point of view.

The more immediately practical bearing of the results seems to be that it is very necessary, in the vaccine therapy of streptococcus infections, to use a vaccine prepared from a culture of the germ from whose activities the patient is suffering, a point which has been already recognised, chiefly on clinical grounds. It also seems possible that the indifferent success which has followed the use of anti-streptococcus serum may be due to the fact that it is prepared by the injection of organisms which only by a lucky chance are able to produce opsonins active for the streptococci which are causing the trouble.