Effect of Antiserum and Prolonged Cultivation on the Agglutinative Characteristics of a Type I Meningococcus

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The present communication reports the effect of different methods of cultivation on the agglutinative characteristics of a Type I meningococcus. Whether any change that occurred was to be considered indicative of S to R dissociation or as evidence of the apparent ability of many strains of meningococcus to alter their antigenic characteristics (*2*, *5*, and others) was questionable. However, these experiments were based upon the possibility that the agglutinative characteristics of a strain which had maintained antigenic constancy over a number of years might be altered by cultural conditions known to induce variation in some other organisms and shown also to reduce the virulence of meningococci.

The stock culture was a Type I strain of meningococcus (44F) obtained from the New York City Department of Health. This strain was selected from among several which agglutinated to high titer in polyvalent serum because, in preliminary attempts to produce an agglutinative variant, its substrains showed the greatest change. It produced small, round, slightly yellow colonies with smooth surface and even borders; there was no evidence of secondary colony formation. According to Rake's description (*6*), it was a "stock" strain. After 9 serial subcultures of single colonies, at 48-hr intervals, during which time no morphological change was observed, it was maintained as the stock culture on 1% glucose agar.

Schwartzman (*7*) has reported loss of toxigenicity of meningococci maintained on egg media, and Cohen (*1*) found that both virulence and precipitative activity of "stock" strains were decreased by subculture in semifluid agar. Enders (*3*) has reported S to R dissociation of meningococci which were repeatedly subcultured in defibrinated rabbit blood; however, Rake (*6*), in repeating the work, felt that the observed changes were, rather, analogous to those found when recently isolated strains are maintained on artificial media and that the strains tended to die out after two or three subcultures in this medium, before true R variants appeared. Of the various methods which suggested themselves as possible incitants to agglutinative variation, the following were selected as being most feasible for this work: (1) Dorset's egg medium, sealed with paraffin, transplanted monthly; (2) 1% glucose semisolid agar containing polyvalent antimeningococcus serum, transplanted weekly; and (3) 1% glucose broth containing polyvalent antimeningococcus serum, transplanted on alternate days.

[1] This work was performed in the Department of Bacteriology, New York University College of Medicine. The author wishes to record her sincere appreciation for the guidance of the late Dr. Maurice Brodie.

Agglutination tests were performed with saline suspensions of 18- to 20-hr cultures as antigen. After 2 hrs at 56° C and 18 hrs in the refrigerator, the results were read by macroscopic inspection. Agglutinin absorption tests were carried out under the same temperature conditions, the absorbing antigen being the washed packed cells thrown down from saline suspensions previously maintained at 60° C for 1 hr. Serum against the stock strain was obtained from rabbits which had received intravenously, at 24-hr intervals, 3 injections of heat-killed organisms, followed, after a week's rest, by 3 injections of live organisms from 18-hr cultures. The serum was

TABLE 1

AGGLUTINATION REACTIONS WITH HOMOLOGOUS AND DERIVED STRAINS

Serum	Antigen	Dilutions of serum 1–40*	1–320	1–640	1–1,280	1–2,560	1–5,120	1–10,240	Antigen control
320†	St	4	4	4	4	2	1	..	..
	E_3	4	4	4	4	3	..	..	..
	A_7	4	4	4	3	±	..	..	..
	AA_6	4	4	4	4	4	4	4	2
	B_{44}	4	4	4	3	1	..	..	..
321†	St	4	4	4	3	2	..	..	..
	E_3	4	4	4	4	2	..	..	..
	A_7	4	4	3	2	±	..	..	..
	AA_6	4	4	4	4	4	3	3	2
	B_{44}	4	4	4	4	±	..	..	..

* The numerals under each serum dilution represent degrees of agglutination visible macroscopically in strong light.

† Serum prepared with stock strain as antigen.

Explanation of strain designations: St = stock strain, E = strain cultured in egg medium, A = strain cultured in semisolid agar containing 10% antiserum, AA = strain cultured in semisolid agar containing 20% antiserum, B = strain cultured in broth containing 10% antiserum. The subscripts represent the number of passages in the medium.

TABLE 2

AGGLUTININ TITER OF SERUM PREVIOUSLY ABSORBED WITH HOMOLOGOUS AND SUBSTRAINS

Serum 320	Antigen	Dilutions of serum 1–20	1–40	1–80	1–160	1–320	1–640	Antigen control	Absorption (%)
Unabsorbed	St	4	4	4	4	4	4*	..	
Absorbing strain									
St	St	4	4	3	±	..	..	..	97
E_3	St	4	4	4	±	..	..	..	97
A_7	St	4	4	2	1	..	..	..	97
AA_6	St	4	4	2	±	..	..	..	97
B_{44}	St	4	4	3	2	1	±	..	87.5

* Final titer = 1–5,120.

obtained by heart puncture 6 days after the final injection.

Table 1 gives the agglutinative titer of the substrains in sera prepared against the stock strain, while Table 2 shows the results of agglutinin absorption tests performed with samples of serum, each of which had previously been absorbed with one of the substrains.

During the course of the work there was no observable change in cultural or fermentation reactions.

Of the substrains derived from a stock culture apparently stable in agglutinative potency, only those cultivated in the presence of 10% antiserum gave any evidence of change in agglutinogenic property. While the loss in titer was not great, the fact that the parent strain had been maintained for a number of years without change in antigenic characteristics may give reason to believe that further cultivation in the presence of antiserum might produce greater change. This possibility is indicated by the fact that the strain which had gone through 44 generations showed greater loss than the one which was observed through only 7 generations. Should further changes occur during prolonged cultivation the results would confirm the report of Evans (*4*), who attributed the spontaneous appearance of an R variant of a Type III stock strain to the presence of antibody in the human blood used for the medium on which it was cultivated.

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The Inactivation of Invertase by Tyrosinase

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The problem of the inactivation of an enzyme by the action of oxidases on certain groupings in the enzyme protein was raised by Sizer (*5*), who demonstrated that a fraction of the tyrosyl groups of certain proteins could be oxidized by tyrosinase. In view of the importance of tyrosyl groups for the activity of most biologically active proteins (*4*), it seemed possible that certain enzymes might be oxidatively inactivated by tyrosinase. No effect of tyrosinase on enzyme activity could be demonstrated (*2, 5*), however, for the proteases pepsin, trypsin, and chymotrypsin. Despite these negative results, it seemed wise to investigate further the possible control of one enzyme system by another. In the initiation of this problem we have studied the action of mushroom tyrosinase on yeast invertase.

[1] The author is grateful to Mr. John Fenessey and Miss Janette Robinson for their assistance in this study.

In a typical experiment, 0.5 ml of tyrosinase (Treemond, 3,500 Miller and Dawson units/ml) is incubated with 0.1 ml of diluted invertase[2] (*1*), 0.5 ml of M/20 phosphate buffer, pH 6.0 and 0.5 ml of water plus toluene for 18 hrs at 37° C. A control experiment, run simultaneously, is identical except for the fact that the tyrosinase has been inactivated by boiling. In certain experiments both control and experimental solutions were placed in Cellophane sacs and dialyzed continuously during the reaction against the phosphate buffer. After incubation with tyrosinase the residual activity of the invertase was measured. The samples were diluted to 5 ml with buffer (at 37° C) and at zero time were rapidly mixed with 5 ml of 12% sucrose. One-ml samples were removed at successive time intervals and added to 3 ml of dinitrosalicylic acid reagent for reducing sugars (*6*). The intensity of color developed after heating the solutions in the usual way (*6*) was measured at 575 mμ with the Coleman Universal Spectrophotometer and converted to milligrams of invert sugar. Typical results are presented in Fig. 1, from which it appears that the liberation of invert sugar from sucrose by invertase follows zero-

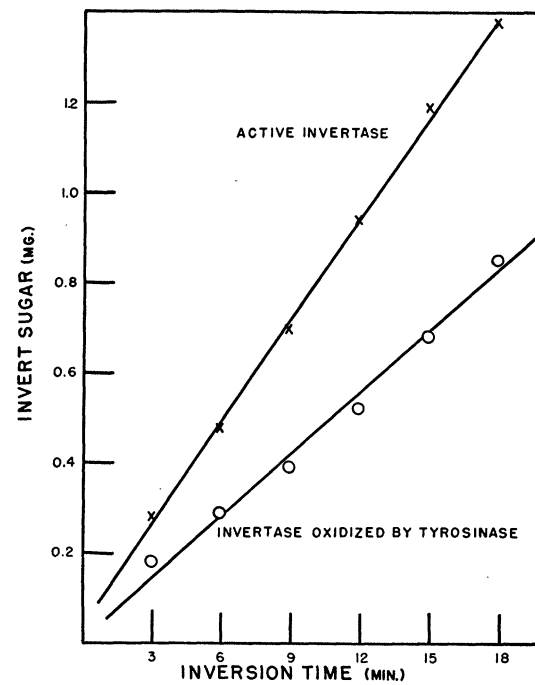

FIG. 1. The formation of invert sugar from 6% sucrose at pH 6.0 at 37° C in the presence of 0.004% Dieu invertase is plotted as a function of the inversion time. The "active invertase" had been treated previously with boiled Treemond tyrosinase for 18 hrs at 37°, while the "invertase oxidized by tyrosinase" had been incubated with active tyrosinase for the same time.

[2] The author is most grateful to Hector Dieu, University of Liège, for a generous sample of high-purity invertase. The invertase solution had a time value of about 0.30 min and contained 0.20 mg of nitrogen and 0.40 mg of carbohydrates/ml. It was diluted 250 times before use.