

strates could also interact with non-enzymic inert proteins.

The number of enzymes with which the enzyme-substrate complex has been demonstrated by the crossing-paper electrophoresis is not yet large, even if the demonstrations with crude preparations are included. The proof of the enzyme-substrate complex, as is well known, has hitherto been regarded as one of the most difficult problems, as the complex is too unstable to be isolated as such. The demonstration of the complex with some oxidases by the change in light absorption⁴ has been regarded as the only possible and sure one. But this is not applicable to other enzymes. However, by the procedure of the crossing-paper electrophoresis, it has now been established that the proof of enzyme-substrate complex is no longer a difficult problem. The complex formation of individual enzymes will be demonstrated sooner or later.

Detailed reports will appear elsewhere.

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¹ Nakamura, S., Takeo, K., Tanaka, K., and Ueta, T., *Z. Physiol. Chem.*, (in the press).

² Nakamura, S., Hosada, T., and Ueta, T., *Proc. Japan Acad.*, **34**, 742 (1958).

³ Thomas, K. (personal communication).

⁴ Chance, B., in "Currents in Biochemical Research", ed. D. E. Green, 308 (Interscience Pub., New York, 1956).

HÆMATOLOGY

Influence of Streptomycin Solutions on the Interaction Between the Agglutinating Sera and the Corresponding Red Blood Cell Receptors

WORK on the influence of different chemical compounds on the reaction between blood group receptors of red cells and the corresponding antibodies has been published; but the action of antibiotics on this reaction, however, has not yet been fully examined except by Neter *et al.*¹, who described the effect of antibiotics on enterobacterial lipopolysaccharides utilizing hæmagglutination and hæmolysis reactions. Our chance discovery of the inhibiting effect of a streptomycin solution on the reaction between anti-*D* antibodies and *D*-positive erythrocytes led us to study the effect of different streptomycin concentrations on the antigen-antibody interaction in blood group systems. For our experiments we used streptomycin of Czechoslovak origin ('Streptomycinum sulphuricum', Penicillin Works, Prague). The different streptomycin concentrations were prepared by diluting 1 gm. of streptomycin in 2, 5, 10, 20, 50, 80 and 100 ml. of saline. Agglutinating sera of the systems *A₁A₂BO*, *MN* and *Rh/Hr* were chosen for the reaction; the red blood cells of the corresponding blood groups were washed three times in saline before use.

In the first group of tests the effect of different streptomycin concentrations was investigated in the following manner: after mixing equal parts of antisera (titrated progressively in twofold dilutions) with the corresponding streptomycin concentration an equal amount of a 4 per cent suspension of type red cells was added. The control tests were carried out in the same way by adding the corresponding

amount of saline instead of the solution of antibiotics. In sera of the *ABO* and *MN* blood group systems the tests were carried out in agglutinating tubes (9×89mm.) and in that of the *Rh/Hr* system in microtubes (5×45 mm.). After suitable incubation at optimal temperature the results in the *ABO* and *MN* systems were read macroscopically and in the *Rh/Hr* system microscopically. The results are shown in Table 1.

TABLE 1

Sera	Cells	Control titre*	ml. saline containing 1 gm. streptomycin solution									
			2	5	10	20	30	50	80	100		
<i>A</i> (anti- <i>B</i>)	<i>B</i>	1:16	4†	8	8	16						
<i>B</i> (anti- <i>A</i>)	<i>A₁</i>	1:64	16	64								
anti- <i>A₁</i>	<i>A₁</i>	1:16	2	8	8	8	8	16				
anti- <i>O</i> (<i>H</i>)	<i>O</i>	1:16	2	8	16							
Lectin anti- <i>H</i>	<i>O</i>	1:64	16	64								
anti- <i>M</i>	<i>M</i>	1:32	2	8	16	32						
anti- <i>N</i>	<i>N</i>	1:8	0	0	2	4	8					
anti- <i>D</i>	<i>CC Dee</i>	1:8	0	0	0	2	2	4	4	4	8	
anti- <i>D</i>	<i>CC Dee</i>	1:128	0	0	4	16	32	32	64	64	64	
anti- <i>D</i> + <i>C</i>	<i>CC Dee</i>	1:64	0	2	4	8	16	16	32	32	32	
anti- <i>C</i>	<i>CC Dee</i>	1:64	2	8	16	32	64					
anti- <i>C</i>	<i>CC Dee</i>	1:512	0	32	64	128	128	256	256	512		
anti- <i>c</i>	<i>ccdee</i>	1:64	0	32	32	32	64					
anti- <i>e</i>	<i>ccdee</i>	1:16	0	2	2	4	4	8	8	16		

*Streptomycin solution was substituted by equal amount of saline. †Figures indicate titres; 0, no agglutination.

It can be seen that in higher streptomycin concentrations the reaction with most sera (mainly in the *Rh/Hr* system) is inhibited. The inhibition declines gradually with the decrease in streptomycin concentration but differs according to the type of antibodies used until it gradually disappears in higher streptomycin dilutions.

The next task was to observe whether the streptomycin solution acts on red blood cell receptors or on the antibodies. After exposure of red blood cells type *D* positive to the action of the streptomycin concentrations at 37° C. and for various lengths of time (1, 2, 4, 8, 16, 24 and 48 hr.) the erythrocytes were washed three times and again titrated with specifically reacting anti-*D* agglutinating antibodies. In the controls we used erythrocytes which had been stored for the same length of time and instead of antibiotics the same amount of saline was added. It was found that the activity of the *D* receptor is not lowered as compared to the controls. The following experiment confirmed our assumption that streptomycin in 1:2 and 1:5 concentration does not act on the blood group receptor *D* of the red cell membrane. Red cells which in the first experiment did not produce a positive reaction in the presence of antibody and the streptomycin solution, were again incubated after a single washing with saline and the addition of the specific antibody. The ensuing positive result showed that the cells had not lost their agglutinating capacity.

If, however, normal erythrocytes were exposed to the action of the supernatant from our first experiment, the results were negative as opposed to the controls. It can be concluded from our experiments that streptomycin acts apparently on the antibody to which it has a greater affinity than to the red blood cells receptors. This is also in keeping with the findings of Neter *et al.*¹

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¹ Neter, E., Gorzynski, E. A., Westphal, O., and Luderitz, O., *J. Immunol.*, **80**, 66 (1958).