THE NEURAMINIDASE OF Corynebacterium diphtheriae AS PATHOGENICITY FACTOR WITH SPREADING FUNCTION

Yu. V. Ezepchuk, Yu. V. Vertiev, and N. N. Kostyukova

UDC 576.852.23.06:577.155.3

By precipitation with zinc chloride and fractionation on a column with Biogel P-60 a preparation of neuraminidase free from contamination with toxin and from hyaluronidase activity was obtained from the nontoxigenic, nonlysogenic strain Corynebacterium diphtheriae C7. The preparation was homogeneous with respect to several immunochemical and electrophoretic tests. A study of the biological role of the C. diphtheriae neuraminidase demonstrated that it possesses diffusion properties.

Corynebacterium diphtheriae is known to produce the enzyme neuraminidase [9, 11, 13, 16], the biological function of which is unknown. In the investigation described below an attempt was therefore made to study the neuraminidase from the point of view of its participation in the pathogenicity of this organism.

EXPERIMENTAL METHOD

To obtain neuraminidase, the nontoxigenic, nonlysogenic strain C7 was grown on Muller-Miller medium [2]. The method of precipitation with $ZnCl_2$ [13] was used for primary purification, after which fractionation was carried out on a column with Biogel P-60, using 0.0075 M NaCl solution, pH 7.0, as the eluent. Fractions were collected by means of the KhKOV-1 chromatographic collector. Homogeneity was studied by double diffusion in gel [1] and radial electrophoresis in polyacrylamide gel [10]; an apparatus of the Tiselius type was used for free electrophoresis. The isolated preparations were tested for the presence of neuraminidase [17] and hyaluronidase [4]. The protein concentration was determined by Lowry's method [12].

TABLE 1. Results Showing how Area of Diffusion Depends on Quantity of Neuraminidase Injected in Rabbits

Concn. of neuraminidase		Area of diffusion (in cm^2) at following times of observation		
as protein (in μg)	as activity (in milliunits)	24 h	48 h	72 h
200	10	2,5	3,1	5,3
400	20	3,7	5,3	7,0
900	45	3,8	8,0	10,0
1500	75	5,3	11,1	13,2
Control	—	0,7	1,1	1,5

The diffusion test was carried out by the modified McClean's method [2]. Neuraminidase was mixed with an equal volume of 0.75% trypan blue solution and the mixture was sterilized by passage through a No. 5 glass filter, and injected intradermally into rabbits in a volume of 0.2 ml. Physiological saline and the enzyme inactivated by heating to 80°C for 30 min were used as the controls.

To study the action of neuraminidase on a culture of human amnion cells the preliminarily sterilized enzyme was added to the cells in different concentrations. Inactivated enzyme was used as the control. After incubation for 1 h at 37°C the cells were washed with physiological saline, then washed off with versene solution and counted in a Goryaev chamber.

N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 75, No. 2, pp. 63-65, February, 1973. Original article submitted January 21, 1972.

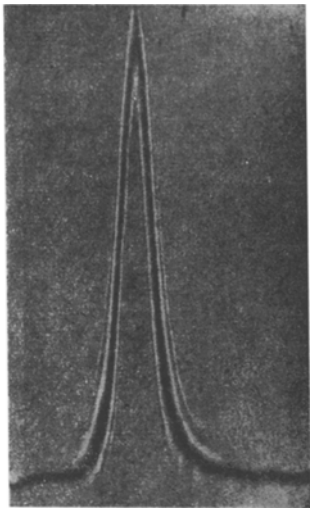

Fig. 1 Fig. 2

Fig. 1. Radial electrophoresis of neuraminidase preparation isolated on column with Biogel P-60 in polyacrylamide gel. Electrophoresis carried out in tris-glycerol buffer, pH 8.4 (current 5 mA applied to gel, temperature 5°C, time 50 min).

Fig. 2. Free electrophoresis of purified neuraminidase (Elektroforez-35 apparatus).

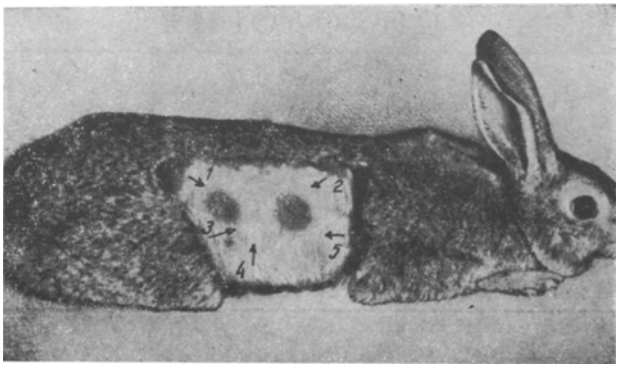

Fig. 3. The McClean diffusion test: 1) 20 neuraminidase milliunits (400 μg) injected together with the dye; 2) 75 neuraminidase milliunits (1500 μg) injected together with the dye; 3) control: dye injected with physiological saline; 4) control: 1500 μg neuraminidase, inactivated at 80°C, injected; 5) 75 neuraminidase milliunits (1500 μg) injected without dye.

EXPERIMENTAL RESULTS

Neuraminidase was eluted as the first peak during fractionation of the preparation precipitated by $ZnCl_2$ on a column with Biogel. The yield of the enzyme was 1-2% as protein and 80-90% as activity. According to the results of immunodiffusion in gel, radial electrophoresis (Fig. 1), and free electrophoresis (Fig. 2), the fraction containing neuraminidase was uniform in composition. Special experiments showed that the resulting homogeneous preparation of neuraminidase was free from contamination with toxin and free from hyaluronidase activity.

Intradermal injection of the neuraminidase showed that the area of diffusion of the dye depended on the concentration of enzyme injected (Table 1).

An inflammatory reaction with local edema was observed at the site of injection of the enzyme (Fig. 3), followed by the formation of a focus of infiltration which disappeared after 10-15 days. After injection of the inactivated enzyme, a swelling formed, disappearing after a few hours.

These results indicate that the neuraminidase of C. diphtheriae is a pathogenicity factor with a spreading function.

After addition of neuraminidase in a dose of 230 μg to a culture of human amnion cells the number of cells capable of attachment to the slide was reduced by 52.7%. After addition of the inactivated enzyme, and also the enzyme in a concentration of 4.6 μg, to the cells the number of fixed cells was not less than in the control. These results indicate that neuraminidase modifies the functional state of the amnion cells.

Investigations [2, 5, 6, 14, 15] have shown that a diffusion factor is present in C. diphtheriae, but the actual substance responsible for this function has not hitherto been isolated and identified. The search for hyaluronidase in this species of bacterium has proved unsuccessful [2, 7, 8]. The investigation described in this paper is the first in which the spreading function of the pathogenic agent has been linked with neuraminidase activity. By obtaining the neuraminidase in a homogeneous state, free from the toxin and hyaluronidase activity, it was possible to establish the nature of the diffusion factor of C. diphtheriae. The biological activity of neuraminidase thus demonstrated shows that this enzyme is one of the pathogenicity factors of C. diphtheriae.

It is to be hoped that these new facts showing that neuraminidase is a pathogenicity factor with spreading function will help to shed light on the biological nature of the pathogenic agent of diphtheria and on the pathogenesis of the disease.

LITERATURE CITED

1. L. A. Zil'ber and G. I. Abelev, in: The Virology and Immunology of Cancer [in Russian], Moscow (1962), p. 198.
2. N. A. Kravchenko, The Permeability Factor of C. diphtheriae. Candidate's Dissertation, Moscow (1947).
3. E. A. Kabat and M. M. Meyer, Experimental Immunochemistry [Russian translation], Moscow (1968), p. 577.
4. M. Sh. Mogilevskii, Vopr. Med. Khimii, No. 3, 288 (1963).
5. B. D. Palant and V. A. Blagodetelova, Transactions of the Ukrainian Mechnikov Institute of Epidemiology and Microbiology [in Russian], Vol. 19, Kiev (1952), p. 183.
6. Yu. V. Solov'eva and A. I. Gindin, Zh. Mikrobiol., No. 2, 39 (1950).
7. K. I. Suchkova and I. N. Blokhina, Zh. Mikrobiol., No. 2, 23 (1950).
8. M. Antolova, G. Hanzel, and A. Novak, G. Mal Infect., 14, 70 (1962).
9. L. Barcsdale, L. Garmise, and K. Horibata, Ann. New York Acad. Sci., 88, 1093 (1960).
10. B. D. Davis, Ann. New York Acad. Sci., 121, 404 (1964).
11. M. Jagielski, Med. Dosw. Mikrobiol., 21, 137 (1969).
12. O. H. Lowry, N. I. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
13. T. Moriyama and L. Barcsdale, J. Bact., 94, 1565 (1967).
14. D. McClean, J. Path. Bact., 42, 447 (1936).
15. R. A. Q. O'Meara, J. Path. Bact., 51, 317 (1940).
16. L. Warren and C. W. Spearing, J. Bact., 86, 950 (1963).
17. L. Warren, J. Biol. Chem., 186, 237 (1959).