MOLECULAR HETEROGENEITY OF α-HEMOLYSIN PRODUCED BY DIFFERENT STRAINS OF STAPHYLOCOCCI

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Pathogenic staphylococci produce α-hemolyzine of different molecular weights. Some strains secrete low- and high-molecular weight α-hemolysine simultaneously, others only α-hemolysins of one type or the other.

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Enzymes, antigens, and toxins secreted by pathogenic strains of staphylococci have recently been carefully investigated [2, 2, 10, 13, 14, 20, 22]. Considerable attention has been paid, in particular, to the study of α-hemolysin by means of the method of molecular filtration through Sephadex gel.

Experiments have shown that the α-hemolysin of strain S-6 is eluted from a Sephadex G-100 column only in the second peak [8, 9]; at the same time, α-hemolysin from strain No. 196E, when fractionated in the same gel, appears not only in the second peak, but also in intermediate fractions between the second and third peaks. α-Hemolysin from strain Walker, phage-type 42D, was eluted from a similar column in the first and second and, to some extent, the third peaks [21], while α-hemolysin from strain Wood-46, according to some data is eluted only in the second peak [7], while according to others it is eluted in the first, second, and also the third peaks [1].

The reason for this discrepancy, in the writers' opinion, is that the α-hemolysin from different strains of staphylococci may consist of molecules of different sizes. This hypothesis is confirmed by the results of sedimentation analysis, according to which the α-hemolysin of strain Wood-46 contains components with a sedimentation coefficient of 1.4 S [16], heavier fragments with a sedimentation coefficient of 2.8-3.1 S [4, 7, 19], and macromolecular fragments with a sedimentation coefficient of 16 S [19]. This heterogeneity of the α-hemolysin secreted by strain Wood-46 is due to the heterogeneity of the bacterial population composing this strain [15].

To test this hypothesis, the molecular structure of α-hemolysin of pathogenic strains of staphylococci circulating among groups of people was investigated.

EXPERIMENTAL METHOD

Four strains of staphylococci (Nos. 117, 126, 180, and 190), isolated from patients with puerperal mastitis in the microbiological laboratory of the Institute of Pediatrics in 1966-1967, were chosen for study. All strains formed a yellow pigment, coagulated plasma, and produced α-hemolysin.

The strains of staphylococci were grown in casein broth for 5 days at 37° in an exsiccator in an atmosphere of CO_2 [18]. By cultivation in this way, the possible appearance of Δ-hemolysin in the filtrate was prevented, because this substance is extremely sensitive to the action of proteolytic enzymes accumulating in the filtrate [11, 12]. On the 6th day the bacterial mass was separated under sterile conditions on a centrifuge, and the α-hemolysin titer in the transparent supernatent was determined on a suspension of rabbit erythrocytes. The titer of toxin was taken as the highest dilution at which complete hemolysis took place.

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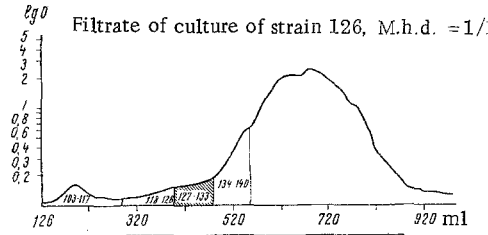
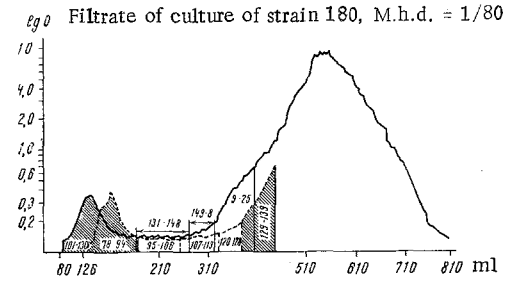


Fractions isolated	103-117	118-126	127-133	134-140
Optical density	3,0	3,5	4,0	2,8
Hemolytic activity	0	0	1:40	0



Fractions isolated	101-130	78-94	131-148	95-106	149-8	107-113	9-25	120-128	129-139
Optical density	3,0	4,5	3,0	3,0	3,2	4,0	not investigated	3,2	3,0
Hemolytic activity	1:10	1:20	0	0	0	0		0	1:80

Fig. 1. Study of hemolytic activity of fractions isolated on Sephadex G-100 column from filtrates of cultures of staphylococci of strains Nos. 126 and 180. Shaded areas on graphs denote fractions contained α-hemolysin.

The filtrate, in a volume of 40-60 ml, was applied to a Sephadex G-100 column measuring 4×60 cm, equilibrated with acetate buffer as recommended by Sorensen (pH 5.0; g/2 = 0.1). This system was chosen because of observations showing that the isoelectric point of α-hemolysin lies at pH 6.4 [5], and also because α-hemolysin is stable in the zone pH 3.6-9.0 [16], the pH zone 4.5-5.6 being optical for exhibition of α-hemolytic activity [5, 17, 23]. Elution of protein from the columns was carried out with the same buffer, and fractions were selected on the KhKKV-1 chromatograph. The protein concentration in the selected fractions was measured on the SF-D-2 spectrophotometer at 280 mμ. Depending on the character of the protein elution curve, the selected samples were pooled to form fractions, these being identified by the serial numbers of the combined tubes, and concentrated by dialysis against a 50% solution of gum arabic in the same buffer.

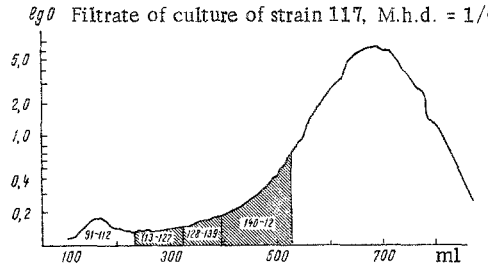
The concentration of the fractions was considered satisfactory for subsequent analysis if their optical density was about 2 units, taking as a guide the statement [10] that 6.6 units of optical density is characteristic of 1% solution of α-hemolysin.

The titer of α-hemolysin in the fractions was determined by the method described above. In some experiments, the content of antigens of bacterial origin in these same fractions was determined by the complement fixation test (CFT) in the cold. A serum against the bacterial precipitinogens was prepared by immunizing rabbits with filtrate of a culture of strain Wood-46 heated to 65°. Two antisera were used in the experiments, Nos. 905 and 956, containing 7 and 4 antibody units/ml, respectively, and giving the same titer in the CFT (1:640).

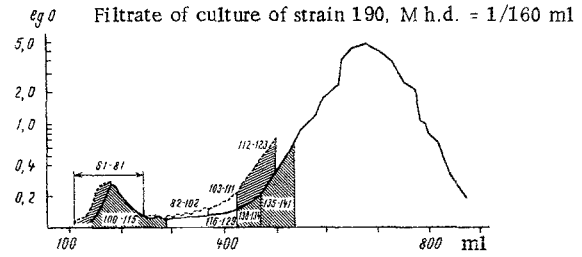
EXPERIMENTAL RESULTS

The results of a study of the hemolytic activity of the separate fractions isolated from filtrate of cultures of strains Nos. 126 and 180 on a Sephadex G-100 column are shown in Fig. 1. The experiment with the filtrate of strain No. 180 was repeated twice. The results of this section of the experiments show that the two strains investigated differ in the structure of the α-hemolysin formed by them. Strain No. 180 secretes hemolysin of two types: 1) passing freely through the column, and 2) retained by Sephadex G-100 gel. Strain No. 126 secretes one type of α-hemolysin, eluted from the column at the boundary of the free capacity.

In the next series of experiments filtrates from cultures of strains Nos. 117 and 190 were investigated. The fractions obtained from these filtrates were titrated in addition in the CFT with rabbit antisera Nos. 905 and 956. The results of this experiment are given in Fig. 2. The experiment with the filtrate of strain No. 190 was repeated twice. The results show that filtrates of strains Nos. 117 and 190 also contain α-hemolysins of different molecular structures. The hemolysin of strain No. 190, like that of strain No. 180, is of two types. The filtrate of strain No. 117 has no macromolecular α-hemolysin, and in this respect it resembles strain No. 126. Meanwhile, a serologically active macromolecular component was present in the filtrate of strain No. 117, and in the CFT it gave a titer of 1:100. Several fractions in the filtrate of strain No. 117 possessed α-hemolytic activity, indicating the heterogeneity of the α-hemolysin in this filtrate. These fractions also were highly active in the CFT. Meanwhile, in the filtrate of strain No. 190, fractions giving a very weak reaction in the CFT contained low-molecular weight hemolysin.



Fractions isolated		91-112	113-127	128-139	140-12
Optical density		3,0	2,5	1,9	2,6
Hemolytic activity		0	1:40	1:320	1:640
Filtrate of culture of strain 180, M.h.d. = 1/80 ml	ser-um 905	1:100	1:200	1:200	1:400
	ser-um 956	1:100	1:200	1:200	1:200



Fractions isolated		61-81	100-115	82-102	103-111	116-129	130-134	135-141	112-123
Optical density		8,0	3,6	5,0	3,0	2,4	1,9	3,6	6,0
Hemolytic activity		1:160	1:40	0	0	0	1:10	1:40	1:80
Filtrate of culture of strain 180, M.h.d. = 1/80 ml	ser-um 905	—	—	—	—	—	—	—	—
	ser-um 956	1:800	1:200	1:100	0	1:100	0	0	1:50

Fig. 2. Study of hemolytic and complement-fixing activity of fractions isolated on Sephadex G-100 column from filtrates of cultures of staphylococci strains Nos. 117 and 190. Shaded areas on graphs denote fractions containing α-hemolysin.

In a special section of the investigation the stability of the different α-hemolysins in the filtrate was tested during prolonged storage at 4°. Part of the previously studied filtrate of strain No. 190 was kept under sterile conditions in a refrigerator for 10 weeks, and then subjected to gel-filtration on the same Sephadex G-100 column. It was found that hemolytic activity ceased to be recorded in fractions eluted from the column in the free capacity. Activity could be found only in the fraction retained by Sephadex G-100 gel, and in the same titer (1:40).

It can be concluded from the results described above that different strains of staphylococci secrete α-hemolysins of different molecular structures. Some strains produce α-hemolysins of high and low molecular weight simultaneously, others an α-hemolysin of only one type possessing some degree of homogeneity. The macromolecular hemolysin is less stable on keeping than its low-molecular weight derivative.

The results confirm the writers' hypothesis of the possible heterogeneity of staphylococcal α-hemolysin depending on the strain producing it. Some strains of staphylococci are evidently capable of secreting two types of α-hemolysins simultaneously, like, for example, the two types of enterotoxin [6]. However, the possibility is not ruled out that the heterogeneity of the molecular structure of α-hemolysin is due to the presence of genetically determined different enzyme systems in different strains of staphylococci, and that these systems can hydrolyze the original macromolecular α-hemolysin present in the culture filtrate into smaller fragments.

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