PROLIFERATIVE ACTIVITY OF STAPHYLOCOCCI ISOLATED FROM DIFFERENT POPULATION FRACTIONS

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A previous investigation showed that after centrifugation of an infectious exudate at 170 g staphylococci proliferating more slowly than the bacterial cells remaining in the supernatant were found in the residue [1].

The object of the present investigation was to study whether sedimentation of the slowly proliferating microorganisms takes place only because they are inside the leukocytes thrown down at 170 g, or whether it is associated with changes in the properties of the bacterial cells themselves.

EXPERIMENTAL METHOD

Albino rats weighing 120-140 g were inoculated subcutaneously with 2 billion cells of a suspension of a culture of Staphylococcus aureus (strain Zhaev). On the 7th day after infection, the purulent exudate was removed. The large floccules were first removed from the exudate by allowing it to stand, and it was then divided into fractions by centrifugation. In the experiments of series I the fractions were isolated from native exudate, and the leukocytes they contained were then destroyed, while in series II the fractions were isolated from an exudate in which the cells had first been broken up by grinding with quartz sand. In the experiments of series III, a broth culture was fractionated by centrifugation.

The first fraction was obtained by centrifuging the sample at 170 g for 5 min, and the second by centrifuging the supernatant from the first centrifugation at 17,000 g for 15 min. To remove bactericidal substances which may be liberated into the medium from the destroyed leukocytes, the samples were centrifuged after grinding and the supernatant was replaced with fresh Hottinger's broth.

Since staphylococci are capable of forming conglomerates, it could be assumed that during centrifugation at a slow speed large conglomerates would be sedimented. For this reason, before incubation, films were taken from each sample, stained with Loeffler's methylene blue, and the number of microorganisms per conglomerate was counted under the microscope. In each sample 100 conglomerates were examined.

EXPERIMENTAL RESULTS

The experiments showed that during centrifugation of a broth culture at 170 g the larger conglomerates of staphylococci are sedimented. However, centrifugation of the ground exudata at 170 g leads to sedimentation mainly of single bacterial cells both in fraction No. 1 and in fraction No. 2. Since staphylococci present in microphages and macrophages are sedimented from the unground exudate at 170 g, this fraction was evaluated under the microscope after grinding. This gave an idea of the size of the conglomerates in the original sample. In this case, single microorganisms also were found in fractions Nos. 1 and 2.

The rate of propagation of the population was determined by periodic subcultures, assessing the increase in number of microorganisms after incubation for 3 h at 37°. It was found that the staphylococci isolated from the different population fractions developing in the animal organism propagate at different rates (Table 1). Microorganisms isolated from this population by centrifugation at a low speed propagated more slowly than cells sedimented later at 17,000 g. The slowly propagating fraction of microorganisms was isolated both from the native exudate and also from the exudate whose cells had first been broken up by grinding. No such pattern was observed during fractionation of the population grown on artificial nutrient

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TABLE 1. Rate of Propagation of Staphylococci Isolated from Different Fractions of the Population

Source from which microorganisms obtained	Fraction no.	No. of microorganisms (geometric mean)		Coefficient of increase (arithmetic mean)	P
		initial	after incubation for 3 h		
Broth culture	1	$5,1\times10^4$	$2,0\times10^6$	38,2	>0,05
	2	$6,2\times10^4$	$1,9\times10^6$	31,1	
Unground exudate	1	$8,6\times10^5$	$9,8\times10^5$	1,1	0,0001
	2	$8,2\times10^4$	$5,0\times10^5$	6,1	
Ground exudate	1	$6,3\times10^4$	$1,3\times10^5$	2,0	0,001
	2	$8,5\times10^4$	$3,5\times10^5$	4,1	

medium. The rates of propagation of staphylococci obtained from it at 170 g, and of those sedimented later at 17,000 g were identical.

Assessment of the rate of propagation of the staphylococci by counting colonies after seeding from the samples cannot be regarded as reliable because of the property of the staphylococci of agglutinating during division. It was impossible to count the true number of microorganisms by the method of Hart and Rees [2] for the initial number of microorganisms in samples obtained from the exudate was too small for detection under the microscope. Accordingly only the number of bacterial cells in the conglomerates after incubation for 3 h was counted. The results given in Table 2 show that the mean number of microorganisms per conglomerate in fraction No. 2 of the population isolated from the animal's body was higher than the number per conglomerate of fraction No. 1. Consequently, the conglomerates were bigger in those samples in which a higher rate of propagation was found by the method of counting colonies.

Hence, during development of a staphylococcal population in the animal body, it acquires properties distinguishing it qualitatively from a population grown on an artificial nutrient medium. This difference is seen in the fact that the population isolated from a purulent exudate may be separated by differential centrifugation into individuals with different proliferative activity, which cannot be done in experiments with a bacterial population grown in vitro.

The cause of sedimentation of the slowly dividing staphylococci from the exudate by centrifugation at 170 g is insufficiently clear. It may perhaps be associated with the larger size of these bacterial cells, for if a ground exudate is passed through an S_1 glass filter, microorganisms dividing more slowly themselves found in the filtrate are left behind on the filter.

TABLE 2. Size of Conglomerates in a Population of Staphylococci After Incubation for 3 h at 37°

Test sample	Fraction No.	No. of microorganisms in conglomerate: number of conglomerates												Mean no. of microorganisms per conglomerate
		1	2	3	4	5	6	7	8	9	10	10—20	20—50	
Broth culture	1	31	37	6	19	1	3		2			1		2,6
	2	21	46	2	22	1	4		2		1	1		2,8
Unground exudate	1	50	35	6	6	2	1					1		1,9
	2	49	26	1,5	7	1,6	3	1	2	0,5	2	1,5	5	7,1
Ground exudate	1	41	24	4,3	6		2,3		0,3		1	1	0,3	1,9
	2	35	32,5	6	10	2,3	1,7	2,3	2	0,7	2,3	2,3	0,7	3,5

LITERATURE CITED

1. V. S. Zueva and V. N. Solov'ev, Antibiotiki, No. 5, 438 (1965).
2. P. D. Hart and R. J. Rees, Brit. J. Exp. Path., Vol. 41 (1960), p. 414.