USE OF DOMESTIC MEMBRANE FILTERS FOR THE DETERMINATION OF MICROBIAL CONTAMINATION OF NONINJECTION DRUGS SUCH AS SULFAGUANIDINE

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The method of concentrating microorganisms by filtration proposed by N. G. Kholodii has been carried out by A. S. Razumov using Zigmond–Backman filters for the quantitative determination of bacteria [1]. Filters of nitrate film have been developed [2] because of the deficiencies of the original filters (structural heterogeneity, etc.).

Subsequently, membrane filters were used during a bacterial investigation of water by putting them onto solid nutrient media [3]. The portability of the apparatus, the simplicity of executing operations, the high precision compared with other methods of estimating microorganisms, particularly in bulk investigations, have permitted the successful use of membrane filters in a series of bacteriological investigations in our country [4, 5].

Latterly, as a result of the weight of a series of advantages of membrane filters (they do not change the pH, color, concentration, ionic composition of the filtrate, or the volume of the solution being filtered; they possess a high throughput; they may be subjected to a significant pressure, etc.) they have begun to be applied widely abroad and in our country for the purification of liquids and gases from various types of particles including microorganisms. In recent years membrane filters have been used successfully in several foreign countries for the control of sterility and microbial contamination of drugs [6, 7]. The use of membrane filters makes more effective the microbiological control of drugs especially those pertaining to antibacterial preparations and also to preparations with an insignificant microbial contamination.

The point of our investigation was the elucidation of the possibility of applying domestic membrane filters for the determination of microbial contamination of noninjection drugs, tableted preparations in particular.

Microbial contamination of sulfaguanidine has been investigated in tablets, a suspension of which in a dilution 1:10 was infected with microorganisms. *Escherichia coli* 2 (All-Union Scientific-Research Institute for Antibiotics) and *Staphylococcus aureus* 209 P (L. A. Tarasevich Institute of Standardization and Control of Medicinal Biological Preparations) were used as test microbes.

A suspension was prepared from a day culture of microorganisms and had optical density 0.4 determined on a FÉK photoelectrocolorimeter at a wavelength λ = 530 nm (the cuvette working length was 10 mm). From this suspension a working dilution of 2×10^{-7} was prepared. Tablets of the preparation were ground in a sterile mortar, transferred to a flask, diluted 1:10 with a suspension of test culture in 0.1 M phosphate buffer, pH 7.0, and kept at room temperature (20-22°) for 15-20 min.

Filtration was carried out through membrane filters from the Millipore company (white, smooth) of pore diameter 0.22 μ and domestic filters Nos. 1 and 6 from the Mytishchenski Experimental Factory having pore diameter 0.3 and 3-5 μ respectively. The Millipore filters were sterilized in paper parcels in an autoclave for 20 min at 121°. Domestic ultra-

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TABLE 1. Number of Colonies of *Staphylococcus aureus* and *Escherichia coli* on Membrane Filters after Infecting a Sulfaguanidine Suspension with These Microorganisms

	Test culture	Number of colonies after 24 h		Number of colonies after 48 h	
		experiment	control	experiment	control
Membrane filter: Mytishchinski experimental factory (0.3 μ)	Staph. aureus	12,5± 1,7 (8)	>200 (8)	>200 (8)	>200 (8)
Millipore (0.22 μ)	Staph. aureus	12,1± 1,7 (8)	>200 (8)	>200 (8)	>200 (8)
Mytishchinski experimental factory (0.3 μ)	E. coli	75,5± 12,2 (10)	>200 (10)	75,5± 12,2 (10)	>200 (10)
Millipore (0.22 μ)	E. coli	74,0± 8,9 (10)	>200 (10)	74,0± 8,9 (10)	>200 (10)

Note. Here and in Table 2 the number of experiments is given in parentheses.

TABLE 2. The Effect of Washing the Filter on the Growth of *E. coli* after Infecting a Sulfaguanidine Suspension with this Microorganism

Washing solution	Number of colonies	
	experiment	control
0.9 % sodium chloride solution	68,0± 4,4 (10)	>200 (10)
Peptone water	76,4± 4,9 (10)	>200 (10)
No washing	57,0± 4,8 (10)	>200 (10)

filters were sterilized by boiling according to the appropriate instructions, previously marking with an ordinary pencil the dull side of the filter which must be turned upwards during filtration. Millipore company filtration equipment was used for carrying out the investigations. All filters and filter-paper disks were the same diameter at 47 mm (domestic filters were obtained as sheets from which 47 mm disks were cut).

For the removal of suspended solid the preparation was first filtered through a prefilter (sterile filter paper or filter No. 6) and the solid on the filter was washed several times with phosphate buffer solution. The clear filtrate, in portions corresponding to 1 g preparation, was passed through a moistened membrane filter which then was transferred to a Petri dish onto the surface of an appropriate nutrient medium (Éndo medium for *E. coli* and mannitol–salt agar for *Staph. aureus*). Media with a lower agar–agar content (1-1.2%) were used since under these conditions the nutrient substances readily diffuse through the filter surface. The inoculations were incubated at 37° for 48 h. A suspension of microorganisms without the preparation served as control, and was filtered first through the prefilter and then through the membrane filter. After 24 and 48 h the number of colonies of microorganisms was counted on each filter. If the colonies numbered more than 200 the results was described as follows: >200.

The obtained data were treated statistically. Arithmetic mean values and standard errors of better than 95% probability are given in the tables.

As is seen from Table 1 the use of domestic filter No. 1 and a Millipore filter of pore diameter 0.22 μ gave extremely close results both on infection of the sulfaguanidine suspension with *E. coli* and with *Staph. aureus*. The difference in the number of colonies on the filters being compared is statistically insignificant. After 24 h incubation at 37° on Éndo medium the colonies of *E. coli*, which appeared on domestic and Millipore filters, were smooth, shiny, with an even edge, and an intense coloration crimson, and with a metallic sheen which was more marked on the domestic filters. After 48 h incubation the number of colonies of *E. coli* on the filters was unchanged, only the sizes of the colonies were increased. On mannitol–salt agar after 24 h incubation at 37° discrete, finely divided, unpigmented colonies of staphylococci appeared. After 48 h all the surface of the filter was covered with colonies having intense orange pigmentation. The filter was surrounded by a yellow zone clearly distinct on the red background of mannitol–salt agar, the reverse side of the filter was also colored yellow. It must be recorded that on seeding on meat–peptone

agar colonies of golden staphylococci were colored with an orange pigment on the filters even after 24 h, while on direct seeding it was necessary to use special media in order that pigment should appear.

Results presented in Table 2 indicated the antimicrobial action of sulfaguanidine against the cultures taken. It must, however, be recorded that the depressing action of this preparation on the growth of golden staphylococcus was marked only in the first 24 h of incubation, after 48 h it was no longer apparent, while the reduction in the number of *E. coli* colonies on the filters remained constant for 48 h in comparison with control.

Assuming that the antimicrobial action of sulfaguanidine in our experiments is a result of its absorption onto the filters we carried out sixfold washing of filters with 50 ml portions of 0.9% sodium chloride solution and 0.1% peptone water [7]. It is evident from Table 2 that the difference in numbers of *E. coli* on the filters subjected to washing and those unwashed was insignificant. Washing did not lead to the expected effect. On preliminary passage through the filter of a suspension of uncontaminated preparation and subsequent filtration through it of a microbial suspension neither the absence or suppression of growth of *E. coli* and staphylococci in comparison with control (without the preparation) was recorded. The data permit the conclusion that sulfaguanidine is either not absorbed on the membrane filters or the amount of absorbed preparation does not inhibit the growth of the test cultures taken for the experiment.

On sequential application onto the surface of agar—agar first of the sulfaguanidine suspension and then of a suspension of microorganisms the bacteriostatic action of the preparation was not as a rule detected on incubation at 37° for 24-48 h. On the other hand, on keeping a suspension of sulfaguanidine contaminated with microorganisms for 15 min at 20-22° and subsequently seeding onto the same nutrient medium, an absence or setback of growth of the test culture was observed. These results provide a basis for the conclusion that the antimicrobial activity of sulfaguanidine appearing on the filters is a consequence of the brief contact of the preparation with the test cultures prior to filtration.

LITERATURE CITED

1. V. I. Tovarnitskii and G. P. Glukharev, in: Ultrafilters and Ultrafiltration [in Russian], Moscow (1951), p. 184.
2. E. Dianova and A. Voroshilova, Mikrobiologiya, 1, No. 3, 271-279 (1932).
3. K. K. Barsov and A. A. Sochilova, Mikrobiologiya, 2, No. 3, 292-295 (1933).
4. L. E. Rakhlina, Zh. Mikrobiol., No. 5, 126-131 (1940).
5. V. S. Rossovskaya, Zh. Mikrobiol., No. 5, 121-126 (1940).
6. Ceskoslovensky Lekopis, Prague (1970), p. 1278.
7. U. S. Pharmacopoeia, 18th Edition, New York, p. 1115.