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USE OF CARBOCYANINE DYES IN THE ANALYSIS OF BACTERIAL ENDOTOXINS.

I. SPECTRAL CHANGES OF THE DYE-ENDOTOXIN COMPLEX

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Carbocyanines have found use in the determination of bacterial endotoxins — lipopolysaccharides (LPSs) of Gram-negative microorganisms. The determination is based on the capacity of the dye for forming aggregates with the LPSs the spectral maxima of which are shifted into a shorter-wave region (470–480 nm) as compared with the maximum of the dye itself (530 nm) [2]. The reaction is characterized by specificity, since on interaction with other polyanionic compounds the absorption maximum is shifted into the longer-wave region — 650 nm [1, 3].

On the basis of the properties of the carbocyanines that have been described, methods have been developed for the colorimetric determination of LPSs isolated from the following microorganisms: the colon bacillus *Escherichia coli* OB 0111B₄, the salmonella *Salmonella minnesota* S51 [2], and the spirochete *Treponema pallidum* [4].

In our work, we used a cationic carbocyanine dye described in the literature: 1-ethyl-2-[3-(1-ethylnaphthol[1,2-d]thiazolin-2-ylidene)-2-methylpropenyl]naphtho[1,2-d]thiazolium bromide.

Procedure. A 100-ml measuring flask was charged with 10 mg of the dye, and then 10 ml of 50% aqueous dioxane and 10 ml of acetate buffer (pH 4.05) were added. After the solution had been kept in the ice bath for 30 min, it was made up to the mark with the same buffer (solution A). For the determinations, solution B with the following composition was used: 25 ml of solution A and 0.5 ml of a 0.01 M solution of ascorbic acid. The solution containing the LPS (1 ml) was treated with 0.4 ml of acetate buffer and 0.6 ml of solution B. The comparison solution was a mixture of 1 ml of apyrogenic water, 0.4 ml of buffer, and 0.6 ml of solution B.

Under the conditions described, the absorption maximum of the dye itself is at 530 nm, and the maxima of the complexes of the dye with LPSs isolated from the abdominal typhoid bacillus *Salmonella typhi* (Pyrogenal) and the "miraculous bacillus" *Bacillus prodigiosum* (Prodigiousan) shift into the shorter-wave region — 478–480 nm and 438–440 nm, respectively. Under the same conditions, the absorption maxima of the complexes of the dye and the LPSs contained in solutions of cultures of the colon bacillus *Escherichia coli* 675, the blue puss bacillus *Pseudomonas aeruginosa* 21, and the hay bacillus *Bacillus subtilis* 720 (killed by autoclaving at 120°C for 15 min) also have a similar shift to 456–460 nm.

Thus, the possibility has been shown of using carbocyanine dyes for determining bacterial endotoxins.

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