Thus, the increase in the yield of WSPs when European goldenrod plants were treated with chlorocholine chloride took place at the expense of the fraction of pectin substances. The MEs increased the yield of PSs somewhat more feebly, their increase taking place at the expense of the galactose. The combined treatment of the plants with both preparations showed an intermediate effect on the yield of WSPs. Their accumulation took place through an increase in the proportion of pectic substances, as in the experiments with the retardant.

LITERATURE CITED

1. A. I. Yakovlev, and P. A. Pychenkova, Abstracts of Lectures on Chemistry and Chemical Technology [in Russian], Tobol'sk (1977), p. 225.
2. A. I. Yakovlev and P. A. Pychenkova, Khim. Prir. Soedin., 790 (1981).
3. E. G. Martynov and D. D. Peskov, Khim. Prir. Soedin., 524 (1983).
4. E. A. Stroev and E. G. Martynov, Khim. Prir. Soedin., 601 (1979).

THE POSSIBILITY OF DETECTING BACTERIAL ENDOTOXINS BY THIN-LAYER CHROMATOGRAPHY

M. N. Mikheeva and L. I. Brutko

UDC 615.4:543.3

In their chemical structure, bacterial endotoxins (or pyrogenic substances) are intricate complexes of lipopolysaccharides, phospholipids, and proteins of high molecular weight. At the present time, it is considered established that endotoxins are lipopolysacchrides [1]. The physicochemical properties of bacterial endotoxins are due, on the one hand, to a hydrophobic lipid A, and on the other hand, to highly polar polysaccharide radicals, and therefore the effective separation of such compounds requires polar mobile phases. It has been reported that the desorption of pyrogens from various agents sorbing them takes place most effectively with a phosphate buffer solution having pH 9-11 [2]. In the course of an experiment, the literature information has been confirmed completely, but the clearest round zones were obtained for a mobile phase with a pH of 11.05.

To detect bacterial endotoxins on chromatograms, the use of such reagents as Rhodamine 6G, molybdophosphoric acid, and iodine vapor [3], and Bromocresol Green [4] has been described. On comparing the sensitivity of detection of bacterial endotoxins by the reagents mentioned, it was established that the most sensitive was a 1% solution of molybdophosphoric acid (0.4 μg), and then a 0.1% solution of Bromocresol Green (0.8 μg), a 0.02% solution of Rhodamine 6G, and a 0.2% solution of fluorescein (1 μg). On this basis, as the detecting reagent we selected a 1% solution of molybdophosphoric acid.

We have investigated the lipopolysaccharides secreted by the typhus bacterium *Salmonella typhi* and used in medicine in the form of solutions with different biological activities under the name of Pyrogenal. As the sorbent we used Silufol prepared plates (Czechoslovakia).

Procedure for Detecting Pyrogenal. With the aid of a MSh-1 microsyringe, 0.3 μl of a solution of the polysaccharide (Pyrogenal) was deposited at the starting line of a Silufol plate with dimensions of 6 × 4 cm. After being dried with a stream of hot air, the plate was placed in a chamber with the mobile phase — a phosphate buffer solution having pH 11.05. After the solvent front had run a distance of 5 cm, the plate was taken out and dried in the air. Then the chromatogram so obtained was treated with a 1% solution of molybdophosphoric acid and it was kept at 160-170°C for 1-2 min. Grey zones of the polysaccharide with a R_f value of 0.75-0.78 appeared against a light yellow background. The sensitivity of detection was 0.4 μg of substance in the zone.

Thus, the basic possibility has been shown of using thin-layer chromatography for detecting bacterial endotoxins.

Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 369-370, May-June, 1986. Original article submitted October 9, 1985.

LITERATURE CITED

1. E. A. Ruban, Microbial Lipids and Lipases [in Russian], Moscow (1977). p. 216.
2. J. P. Todd, J. Pharm. Pharmacol., 6, 303 (1954).
3. W. W. Fullerton, Surfactant Assay, US Patent No. 4,292,041. Cl. 23-230.3; G 01 N 31/08, 3p. (1979).
4. Fang Shaobo and Yan Liying, Chem. Abstr., 98, No. 78199 g (1983).

USE OF CARBOCYANINE DYES IN THE ANALYSIS OF BACTERIAL ENDOTOXINS.

I. SPECTRAL CHANGES OF THE DYE—ENDOTOXIN COMPLEX

M. N. Mikheeva, S. A. Shmel'kova,
and L. I. Brutko

UDC 615.07:543.422

Carbocyanines have found use in the determination of bactrial 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 0111B_4, 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* (Prodigiosan) 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.

LITERATURE CITED

1. R. D. Edstrom, An. Biochem., 29, No. 3, 421 (1969).
2. J. Janda and E. Work, FEBS Lett., 16, No. 4, 343 (1971).
3. R. E. Kay, E. R. Wallwick, and C. K. Gifford, J. Phys. Chem., 68, No. 7, 1896 (1964).
4. P. Zey and S. Jackson, Appl. Microbiol., 26, 129 (1973).

Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 370, May-June, 1986. Original article submitted October 28, 1985.

0009-3130/86/2203-0344$12.50 © 1986 Plenum Publishing Corporation