USE OF CROSSED PREPARATIVE ELECTROPHORESIS AND GEL FILTRATION TO STUDY INTERACTION BETWEEN TETRACYCLINES AND SERUM PROTEINS

G. Ya. Kivman and I. Ya. Geitman

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Chlortetracycline, oxytetracycline, and tetracycline were found to interact with albumin of prepared human blood serum. Only oxytetracycline and tetraycline interact with γ-globulin.

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The study of interaction between therapeutic, and especially chemotherapeutic substances and the serum proteins includes the quantitative aspect of this process and also identification of the protein fractions binding the particular substance studied. The latter often presents technical difficulties. Although in the reports of some investigations [5-7, 13-16] closely similar figures are given for the decrease in activity of antibiotics of the tetracycline group in the presence of serum, there is little information on the actual protein fractions with which they interact, and even that is conflicting [3, 8, 9, 11, 12]. The contradictions are caused, to some extent, by the fact that the horizontal method of electrophoresis, including the crossed variant, which these workers used did not ensure adequate mobility of the tetracyclines, which are very inert in an electric field. The complex of tetracyclines with proteins, on the other hand, is labile [4, 6, 7].

In the present investigation the method of vertical crossed preparative electrophoresis was used, together with gel filtration, to study interaction between tetracyclines and serum proteins.

EXPERIMENTAL METHOD

Experiments were carried out with blood serum from healthy blood donors and with human albumin and γ-globulin. Solutions of the tetracyclines (chlortetracycline, oxytetracycline, tetracycline) and proteins were made up in 0.067 M phosphate buffer, pH 7.4. The concentrations of the antibiotics were determined by the agar diffusion method. The test organism was Bacillus subtilis, strain L_2 (spores).

Crossed preparative electrophoresis was carried out by the method described previously [2]. The starting point for the blood serum on the chromatographic paper was located above the 20th notch (from anode to cathode), and that of the tetracyclines for crossing the albumin of the prepared serum above the 16th or 17th notch. To cross the γ-globulin, the antibiotics were applied above the 22nd notch. The reservoirs contained 2 ml serum and 2 ml of solutions of the tetracyclines (20 μg/ml). Control electrophoresis of the tetracyclines was carried out simultaneously from two points (the 16th or 17th and the 22nd), and crossed electrophoresis (with serum) from three points (16th or 17th, 20th, and 22nd). After electrophoresis, the concentrations of the preparations in the receiving tubes were determined. The chromatographic paper was stained with amido black 10B. Before the crossed electrophoresis, experiments were carried out to determine the rate of movement of serum proteins and tetracyclines over the chromatographic paper during preparative electrophoresis. The time taken by the proteins to pass was determined from their detection by means of 20% sulfosalicylic acid in the buffered solution from the receiving tubes, and the rate of movement of the tetracyclines was estimated with the aid of disks of filter paper soaked in the same buffer. The disks were placed on infected agar.

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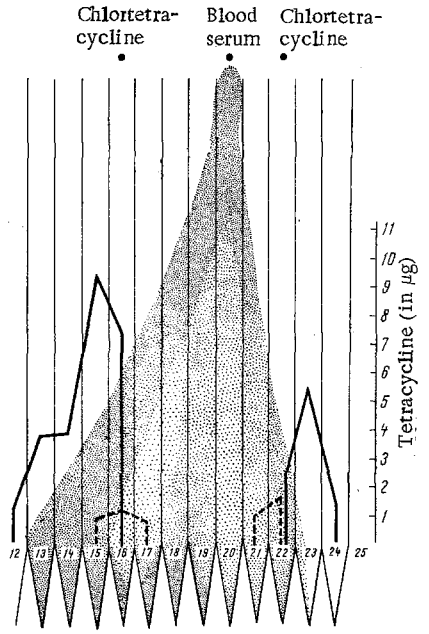

Fig. 1. Electrophoresis of blood serum after crossed preparative electrophoresis with tetracycline. Broken line indicates content of tetracycline in receiving tubes in control; continuous line gives content of tetracycline in receiving tubes after crossing of albumin and γ-globulin fractions.

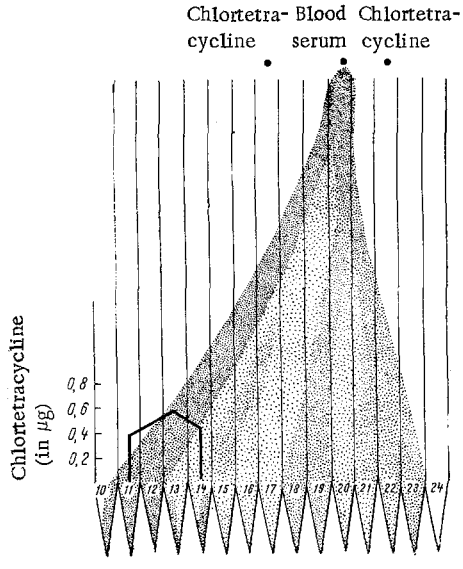

Fig. 2. Electrophoresis of blood serum after crossed preparative electrophoresis with chlortetracycline. Continuous line indicates content of chlortetracycline in receiving tubes after crossing of albumin fraction.

Gel filtration was carried out through Sephadex G-50, suspended in phosphate buffer, pH 7.4; this buffer was also used as the eluting solution. The gel was prepared and the column filled as recommended for working with Sephadex [10]. To a column measuring 2 · 10 cm was applied 1 ml of a solution containing 0.9 ml of blood serum, or of 4.2% albumin solution or 2% γ-globulin solution, and 10 μg of one of the tetracyclines in 0.1 ml phosphate buffer. Before gel filtration, the mixture was incubated for 30 min at 37°. Fractions of 1 ml were collected. The zones of localization of the control solutions of tetracyclines and proteins were determined by fractional gel filtration. The protein content in the fractions was determined quantitatively by a microbiuret method on a spectrophotometer [1].

EXPERIMENTAL RESULTS

The study of the rate of movement of proteins and tetracyclines over the chromatographic paper during electrophoresis showed that proteins of prepared blood serum pass the whole way from the starting point to their outlet into the receiving chamber in 6 h, oxytetracycline in 7 h, tetracycline in 10 h, and chlortetracycline in 21 h. Since the rate of movement of the serum proteins and oxytetracycline was almost identical, so that the front of the protein fractions should be crossed, electrophoresis of the antibiotic began 3 h after that of the serum, and electrophoresis of tetracycline and chlortetracycline began at the same time as that of the serum. Control tests (without serum) showed that during preparative electrophoresis of oxytetracycline and tetracycline solutions, the highest concentration of the preparations was found in the tube immediately beneath the starting point: the content of antibiotic was 10-15% of the original. During cross electrophoresis of oxytetracycline and tetracycline with albumin of the prepared serum, displacement of the peak of concentration of the antibiotics to correspond to the position of the albumin and an increase in yield of the preparations to 60% were observed. This is evidence of interaction of the oxytetracycline and tetracycline with albumin. Similar, but less definite results were obtained by crossing the zone of γ-globulin with these antibiotics (yield up to 25%). The results of electrophoresis in which albumin and γ-globulin of the prepared blood serum were crossed by tetracycline are shown in Fig. 1. In the case of chlortetracycline, this could not be detected in the tubes in the receiving chamber after 20 h of electrophoresis in the control. During crossed electrophoresis with albumin of the prepared serum, the antibiotic was found in the albumin zone (3% of the original). When chloretracycline and γ-globulin were crossed this effect was not observed (Fig. 2). The γ-globulin evidently did not accelerate movement of chlortetracycline over the chromatographic paper.

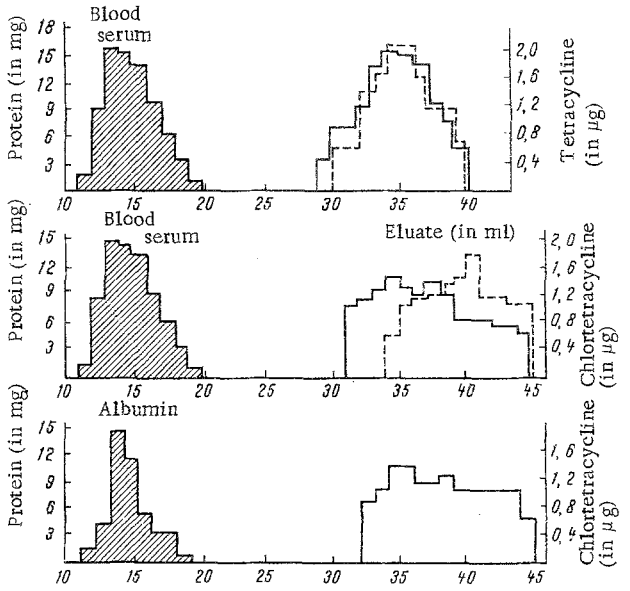

Fig. 3. Gel filtration of blood serum with tetracycline and chlortetracycline, and of albumin with chlortetracycline. Broken line indicates content of tetracycline in eluate (control); continuous line shows content of tetracyclines in eluate after gel filtration with blood serum and albumin.

The results obtained by crossed preparative electrophoresis of tetracyclines with albumin of prepared human blood serum demonstrate that all three antibiotics interact with this protein fraction. In cases of crossing of γ-globulin of prepared human serum with tetracyclines, interaction with this protein was established for oxytetracycline and tetracycline.

During gel filtration of control solutions the serum proteins were localized in the 10-20 ml fraction, tetracycline and oxytetracycline in the 30-40 ml fraction, and chlortetracycline in the 36-45 ml fraction of eluting solution. During gel filtration of a mixture of oxytetracycline and tetracycline with solutions of albumin and γ-globulin from blood serum, a very slight displacement of the zone of the antibiotic toward the protein zone was observed (compared with the control). During gel filtration of chlortetracycline mixed with serum or albumin, displacement of the zone of the antibiotic toward the protein zone was more marked (Fig. 3). The results with γ-globulin coincided with the controls. The higher velocity of filtration of chlortetracycline, when mixed with serum and albumin, than of oxytetracycline and tetracycline may be evidence of the greater strength and the somewhat different character of the binding of this antibiotic by the blood proteins, and in particular, by the albumin fraction.

Comparison of the results obtained by the methods of crossed preparative electrophoresis and gel filtration thus suggests that albumin always participates in the binding of tetracyclines by the blood serum proteins. Interaction with γ-globulin was detected only during crossed electrophoresis for oxytetracycline and tetracycline.

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