We believe types 35 and 49 to be the same, and we also think that our "T/14" strains belong to type 49. There is evidence that they may be nephritogenic, like the original type 39 strains (Maxted *et al.* 1967).

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APPENDIX 2

The Investigation of Some Extracellular Products of Streptococci Group A

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The antigenic components revealed in the preparation of erythrogenic toxin of different stages of purity and in the preparation of crystalline proteinase, obtained by Elliot's method (1950), were investigated.

For this purpose the following methods were used: analytical immunoelectrophoresis in the agar gel, analytical and preparative high volt electrophoresis, and Uriel's method (1960). The activity of the toxin was estimated by titration on the rabbit's skin, the activity of proteinase—with the Kunitz method (1947).

In the preparations of Toxin A with the lower stage of purity (4 ml of skin doses/1 mg of protein) by the reaction with the antitoxic sera, four antigenic components were revealed. The first antigen was identified as erythrogenic toxin, the second as proteinase. The third antigen was attributed to the extracellular components, since the antibodies to it failed to be removed by the absorption with the

microbial cells. This substance reacted not only with the sera obtained by immunization with toxin A, but also with sera towards the erythrotoxic toxin B (Stock 1961). The fourth component reacted with sera of animals immunized with the microbial cells of streptococci group A. The reaction disappeared by the absorption of these sera with the microbial cells of streptococci group A.

Therefore we consider this component as an antigen of the microbial cells. The component possesses a higher electrophoretic mobility compared with that of serum albumin, and it was designated as the "far" fraction. In the preparation of erythrotoxic toxin with a higher level of purity (40 ml skin doses/1 mg protein) three components were revealed: erythrotoxic toxin, proteinase, and the "far" fraction.

From an investigation of the preparation by the method of analytical and preparative high volt electrophoresis in the agar gel, it was shown that the fraction containing proteinase consists of three protein components differing in their electrophoretic mobilities. Three analogous components possessing the same electrophoretic mobility were obtained in the preparations of crystalline proteinase. Using Uriel's method it was shown that all these components possess proteolytic activity.

In the experiments of preparative electrophoresis and subsequent testing of the revealed fractions in the reaction of precipitation in the gel, it was shown that these components are antigenically identical.

From an investigation of the biological action of erythrotoxic toxin on the animals (the toxin was revealed by the method of preparative electrophoresis), the following was shown: the animals which were sensitive in skin testing to this toxin dies when injected with great doses of toxin. An increase of the lymphatic nodules and hemorrhages in them were observed in the dead animals. Similar phenomena had been earlier described with animals injected with preparations of erythrotoxic toxin of lower grade of purity (Lyampert 1956). From the investigation of preparations of proteinase, it was shown that this enzyme removed the metachromatic staining obtained with toluidin blue from the tissue structures containing chondroitin sulphates. Similar action was discovered earlier by testing the broth decanted from cultures of group A streptococci containing proteinase (Beletzkaja and Burshtein 1966). This action has no connection with the presence of the enzymes depolymerizing chondroitin

sulphates. The activation of proteinase with cystein or its inhibition with monoiodoacetic acid results in an increase or in the abolition of the proteinase action, respectively, on the tissues.

By the intravenous injection of proteinase in the rabbits, an increase of the acid mucopolysaccharide level in the blood serum of the animals in the first hours after the injection was revealed.

The determination of the acid mucopolysaccharide level in the blood serum was made by Weisman's (1959) method, in our modification using hexamine cobaltic chloride, which has the ability selectively to precipitate the sulphated mucopolysaccharides (Mathews and Dorfman 1958). In histological investigations of animals injected intravenously with the proteinase, local necroses in the myocardium were revealed similar to the necroses in myocardium produced by the injection of proteinase by Kellner and Robertson (1954).

The described action of streptococcal proteinase (the removal of metachromatic staining of tissue sections and the elevation of the acid mucopolysaccharide fever in the blood) is connected, apparently, with its capacity to split out acid mucopolysaccharides from the mucopolysaccharide-protein complexes, in vitro as well as in vivo. Increasing the acid mucopolysaccharide level in the blood under the action of streptococci proteinase is similar to the occurrence described by the injection of papain in the animals (Thomas 1958, McCluskey and Thomas 1958, Tsaltas 1958, et al.).

Speculation was made about the possible role of streptococcal proteinase in the pathogenesis of rheumatic fever because for this disease the changing of the localization and liberation of acid mucopolysaccharides from the connective tissue structures is characteristic.

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APPENDIX 3

The Bacitracin Test for Recognition of Group A Streptococci

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The bacitracin test introduced by Maxted (1) is a useful procedure for the recognition of group A streptococci in routine diagnostic work; however, the author of the test himself pointed out a certain limitation in its specificity for Streptococcus pyogenes. In the course of time, further evidence has been brought of the existence of group A strains displaying relatively low sensitivity in the bacitracin test. At the same time, if the concentration of bacitracin is increased to the point of detecting almost all the group A strains, the test becomes positive also for some strains of certain other groups, in particular C and G (2, 3, 4, 5, 6).

For the bacitracin test, as performed at the Streptococcus Reference Laboratory in Prague, discs 6 mm in diameter containing 0.7 units bacitracin are employed. This concentration has been selected to make the test clearly positive in all group A strains, i.e. to give an inhibition ring of at least 5 mm. The test was used under standard conditions in samples of group A, B, C, G and L strains currently in circulation among people and animals in Czechoslovakia. Figure 1 shows that among the group C and G strains there were some whose sensitivity was comparable to that of the group A strains of low sensitivity. The strains of these groups were examined in quantitative sensitivity tests by means of the serial tube dilution method. Table 1 confirms the finding in Figure 1. The minimal concentration of bacitracin inhibiting the growth of the less sensitive group A strains equalled 0.075 units per ml; for some group C and G strains, this concentration was either identical or even lower.