QUANTITATIVE ANALYSIS OF BLOOD SERUM IMMUNOGLOBULINS OBTAINED BY ION-EXCHANGE CHROMATOGRAPHY ON DEAE-CELLULOSE

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Individual classes of immunoglobulins were determined quantitatively by Mancini's simple radial immunodiffusion method in native blood sera and serum fractions obtained by ion-exchange chromatography on DEAE-cellulose. In this way the possibility of using ion-exchange chromatography on DEAE-cellulose for the differential isolation of immunoglobulins IgG, IgA, and IgM from blood serum could be evaluated more accurately. Simple radial immunodiffusion can be used as one of the most sensitive methods of testing the purity of fractionation and the percentage yield of immunoglobulins in each fraction of the eluate.

The method of ion-exchange chromatography on DEAE-cellulose is nowadays regarded as the best way of isolating serum antibodies of the IgG type in the pure form [3, 4, 6]. It is considered that IgG can always be found as an impurity in the corresponding chromatographic fractions containing chiefly immunoglobulins IgA or IgM [1].

The object of this investigation was to make a qualitative analysis of the fractions of the eluate after ion-exchange chromatography on DEAE-cellulose and to determine the percentage yield of the individual immunoglobulins in each fraction relative to their content in the original sera tested.

EXPERIMENTAL METHOD

The blood sera from patients with typhoid fever and typhoid carriers were fractionated by stepwise gradient elution as described by Adinolfi et al. [2]. For quantitative analysis of the immunoglobulins in these sera and their fractions, Mancini's method of simple radial immunodiffusion [5] was used. Monovalent antisera against immunoglobulins IgA, IgM, and IgG and the corresponding standards from the 1DR set (Sevac, Prague, Czechoslovakia) were used.

To compare the quantitative levels of the individual immunoglobulins with the native serum as its fractions the mean percentage yield of IgG, IgA, and IgM in each of the three fractions of this serum was calculated.

EXPERIMENTAL RESULTS

The first fraction of the eluate consisted of pure IgG. No contamination by immunoglobulins IgM and IgA was found although the method used is not highly sensitive – its lower limit of sensitivity is 0.003 mg/ml [1]. The mean percentage yield of IgG in fraction I of the eluate was 55% of its content in the native sera. When fraction II of the eluate was obtained, in each case it was found to consist chiefly of IgA, in a considerable quantity. Despite the unavoidable losses of this protein, the mean percentage yield of IgA in fraction II was 61%. However, besides IgA, fraction II also contained immunoglobulins IgM and IgG. The mean percentage of IgM as an impurity in this fraction was 1. IgG was found as traces in only three cases.

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Fraction III after chromatography of all the sera on DEAE-cellulose consisted chiefly of IgM. The mean percentage yield of IgM in fraction III was 38. The results of these tests showed that this chromatographic protein fraction was the most contaminated. Besides the principal component (IgM), it also contained immunoglobulins IgA and IgG. Their mean percentage as impurities in fraction III of the eluate was 9.2 and 1.4, respectively.

The results of this investigation thus confirm that the method of ion-exchange chromatography on DEAE-cellulose, based on the scheme of Adinolfi et al. [2], can be used to isolate the chief classes of immunoglobulins (IgG, IgA, and IgM) from human blood serum, and especially for obtaining IgG in a pure form. Nevertheless, in this method of fractionation of the immunoglobulins, constant monitoring of the purity of the resulting fractions is very desirable. Mancini's simple radial immunodiffusion method can be used as one of the most sensitive monitoring techniques.

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