SERUM MYOGLOBIN OCCURS MAINLY IN THE FORM OF CIRCULATING IMMUNOCOMPLEXES

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The normal blood myoglobin (MG) concentration does not exceed 90 mg/ml [4]. In pathological states accompanied by muscle tissue destruction, this level may rise significantly [8]. The nephrotoxicity of MG has been noted. It occludes the renal tubules and leads to the development of acute renal failure [2]. Mortality among patients with acute renal failure resulting from a myorenal syndrome, arising from prolonged postural compression, amounts to 40-45% [5]. The nephrotoxicity of MG may also be manifested in cases in which there has been no direct destruction of muscle tissues. For example, hypermyoglobinemia has been observed during the first hours of life of newborn infants subjected to asphyxia [9]. The duration of oliguria has been shown to depend on the MG concentration in the blood. Circulation of autoantibodies to MG (aAB) in the blood [6] suggests the presence of a special pathway of MG elimination from the bloodstream, besides renal filtration, namely the binding of MG into immune complexes (IC) and removal of the latter with the aid of the reticuloendothelial system.

In the investigation described below, the importance of this suggested pathway for MG metabolism was determined.

EXPERIMENTAL METHOD

Cold sera from blood donors were subjected to gel-filtration through Sephadex G-200 ("Pharmacia") under conditions of dissociation of IC (the gel was equilibrated with 0.1 M sodium-acetate buffer, pH 4.05), and also at neutral (7.2) pH (0.2 M sodium phosphate buffer, 0.05 M sodium chloride). The elution profile consisted of three peaks, the first of which consisted of high-molecular-weight proteins including IgM, the second of IgG, and the third of low-molecular-weight proteins, including MG. Fractions of the eluate corresponding to a particular peak were pooled and concentrated down to the volume of the original pool, applied to the get. aAB and MB were determined in concentrated fractions by the competitive version of ELISA (Fig. 1). Sheep antiserum to human MG was provided by the staff of the Research Institute of Epidemiology and Microbiology, Ministry of Health of the RSFSR, Gor'kii. Human MG was obtained as described previously [1]. Affinity antibodies to MG were isolated by the standard method on immunosorbent with immobilized MG. A conjugate of horseradish peroxidase (HRP; from "Calbiochem") with human MG was obtained by periodate oxidation [7]. Adsorption of antibodies to MG was carried out on the surface of micropanels (Research Institute of Medical Engineering, Ministry of Health of the USSR) from a solution of antibodies with a concentration fo 5 μg/ml in 0.05 M sodium-carbonate buffer, pH 9.6, for 18-20 h at 37°c. The free surface was blocked with a 1% solution of bovine serum albumin (BSA) in the same buffer. The test samples and conjugate were diluted with 0.01 M sodium-phosphate buffer, pH 7.2, containing 0.15 M sodium chloride, 0.05% Triton X-100, and 0.1% BSA. o-Phenylenediamine was used as the substrate. Photometry was carried out at 492 nm after the enzyme reaction had been stopped by addition of 1 M sulfuric acid solution. Before determination of MG by ELISA, the HRP–MG conjugate was added to the test sample in its working dilution, and the mixture was introduced into prepared wells of the micropanel and incubated for 1 h at 37°C. The level of aAB was determined by ELISA in the same way, but before introduction of the mixture into the prepared wells, it was kept for 1 h at room temperature. Standard curves for determination of MG and of antimyoglobin antibodies are shown in Fig. 2.

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TABLE 1. Determination of MG and aAB in Fractions of Pooled Serum after Gel-Filtration through Sephadex G-200, with Elution at Different pH Values

pH of elution	aAB concentration, mg/ml		Concentration of MG, mg/ml (fraction 3)
	class of IgM	class of Ig	
	fraction 1	fraction 2	
7,2	29	21	152
4,05	3200	168	1125

Legend. aAB concentration determined by standard curve for detection of specific antibodies to MG; content of MG in original pool of blood sera 67 mg/ml.

TABLE 2. Blood Levels of Antimyoglobin aAB and MG, Freely Circulating and in the Composition of IC

State of aAB and MG in blood	Concentration of aAB, %		MG concentration, %
	IgM class	IgG class	
Freely circulating	0,9	12,5	5,9
In composition of IC	99,1	87,5	94,1

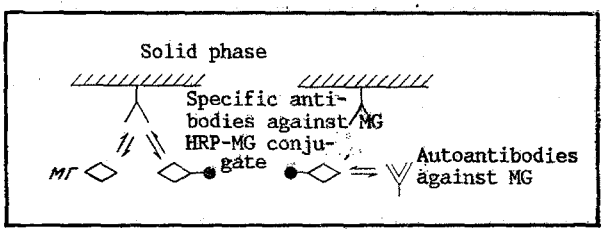

Fig. 1. Diagram showing ELISA for determining myoglobin (left) and antimyoglobin antibodies (right).

EXPERIMENTAL RESULTS

The results of determination of MG and aAB in fractions of pooled serum after gel-filtration through Sephadex G-200 are given in Table 1.

Fractionation of the pool of blood sera on Sephadex G-200 at neutral pH enabled the freely circulating MG to be separated from the aAB to it. No MG was found in fractions 1 and 2, which evidently contained both freely circulating aAB and IC.

Table 1 shows that fractionation of serum proteins by molecular weight with elution at pH 4.05 leads to a marked increase in the quantity of antimyoglobin aAB and MG itself, accessible for detection, most probably on account of dissociation of the IC under these conditions. For instance, the MG concentration rose to 1125 mg/ml as a result of elution at pH 4.05 (conditions for essential dissociation of IC) compared with 152 mg/ml in the case of gel-filtration at neutral pH values (only slight dissociation of IC) compared with 67 mg/ml in the original serum pool. The results also are evidence of absence of interaction between MG, in the composition of IC, with specific antibodies to it adsorbed on the micropanel and, consequently, of predominant detection of freely circulating aAB by the ELISA method.

The relations betweeen free-circulating antimyoglobin aAB, freely circulating and in the composition of IC, and also between freely circulating MG and those in the composition of IC are illustrated in Table 2. The calculations are based on the fact that the free MG concentration corresponds to its concentration in the initial serum pool, whereas the total (free

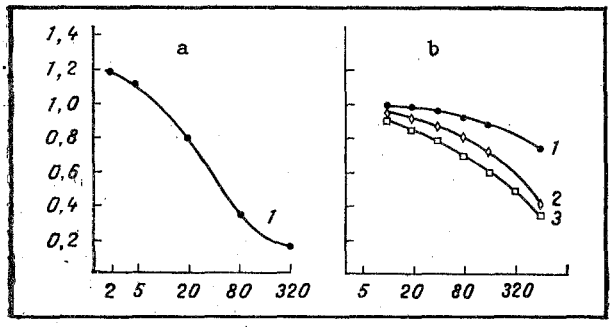

Fig. 2. Calibration curves for determination of myoglobin (a) and antimyoglobin antibodies (b) in ELISA, using different times of preincubation of the test sample with the HRP–MG conjugate. Abscissa, myoglobin concentration, mg/ml (a); concentration of specific antimyoglobin antibodies, mg/ml (b). Ordinate, optical density at 492 nm. 1) 0 min; 2) 30 min; 3) 60 min.

and in the composition of IC) MG concentration was equivalent to its concentration in fraction 3 of the elution profile after fractionation relative to molecular weight at pH 4.05. The blood level of freely circulating aAB of the IgM class corresponded to their level in fraction 1, and those of the IgG class in fraction 2, after fractionation of serum proteins on Sephadex G-200 at pH 7.2. Dissociation of IC after elution at pH 4.05 enabled the total concentration of aAB of the IgM and IgG classes to be detrermined in fractions 1 and 2 respectively.

First, therefore, the concentration of MG in the blood was found to be significnatly greater than is shown by known methods of analysis [4, 8], but it is present in the blood mainly in the bound state; second, the blood contains aAB against MG, but they also circulate mainly in the form of IC. The results are evidence also that aAB can perform a protective function, i.e., can prevent the nephrotoxic action of MG.

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