results that stimulation of the immune response after blockade of the serotonin receptors is dopamine-dependent and takes place through the participation of the thymus [2, 6].

It has been shown that the modulating action of serotonin is effected by central mechanisms with the participation of the hypothalamo-hypophyseal complex [2, 5, 10]. Meanwhile some investigations have shown that serotonin can be bound directly with lymphocytes [3] and that serotonin can inhibit the immune response in experiments in vitro [12]. The workers cited accordingly concluded that serotonin has a peripheral action on immunogenesis. Our own investigations, in which serotonin receptors were blocked in animals with a divided pituitary stalk, showed that in the absence of connections between hypothalamus and pituitary, Cyp does not exhibit its effect. The number of RFC in these animals was the same as in the control (Fig. 3).

It can accordingly be postulated that the C_2 receptors of the brain itself participate in the mechanism of the inhibitory action of serotonin on immunogenesis.

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IMMUNOCHEMICAL STUDY OF HETEROGENEITY OF TROPHOBLASTIC β_1-GLYCOPROTEIN

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The blood serum of pregnant women has been shown to contain a trophoblastic β_1-glycoprotein (TBG) [4], which has been widely used for the diagnosis of pregnancy and of trophoblastic tumors [6].

It has been shown that TBG forms complexes with mucopolysaccharides [2, 7], but the reactions of its components with these polysaccharides have not been studied. TBG also exists in the plasma of pregnant women in several forms, which differ in their affinity for monospecific

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antisera and in their electrophoretic mobility during zonal electrophoresis [12-14]. However, reactions with antibodies have been studied mainly in an alkaline medium (pH 8.6), although it is known that the conditions of the medium largely determine interaction in the antigen–antibody system [11].

The aim of this investigation was to study the effect of pH of buffer solutions and heparin on the electrophoretic mobility of different forms of TBG.

EXPERIMENTAL METHOD

A mixture of blood sera from women in the second half of pregnancy was used as the antigen. An α_2-glycoprotein (α_2-GP) associated with pregnancy, which is a critical-phase protein and whose active biosynthesis is observed in pregnancy and in patients with malignant neoplasms [5], was studied as the control. Hyperimmune antisera against TBG and α_2-GP were obtained by immunizing rabbits with blood serum from pregnant women, followed by absorption with lyophilized plasma [1]. To study the effect of pH of the buffer medium on the antigen–antibody reaction, low-voltage crossed immunoelectrophoresis [15] and a system of buffer solutions with a pH range from 6.6 to 10.0 [11] were used. The effect of heparin on the electrophoretic mobility of TBG and α_2-GP was analyzed by means of a slightly modified technique of crossed immunoelectrophoresis. The modifications consisted of incorporation of heparin (from Spofa, Czechoslovakia) into the gel for electrophoresis in the first direction (from 0.001 to 10 mg/ml). Heparin is known to form complexes with glycoproteins and to endow them with a negative charge [2].

EXPERIMENTAL RESULTS

In a weakly acid (pH 6.6) and weakly alkaline (pH 7.7) medium both pregnancy proteins formed complete precipitates. In more alkaline buffer solutions the precipitates of α_2-GP preserved their compact form, whereas with TBG three immunochemically identical components with different electrophoretic mobility were discovered. The dominant component had mobility of β-globulins, whereas the minor fragments were α- and γ-components respectively. All forms of TBG were clearly detectable at pH 8.6. In a more alkaline medium (pH 10.0) the clarity of the precipitates was sharply reduced. This was possibly due to denaturation of the TBG and α_2-GP, for a medium of such alkalinity is known to prevent the reaction of many antigens with the corresponding antibodies [11]. The increase in areas of the above-mentioned antigens proportional to the increase in pH of the buffer solutions was due to a change in the mobility of these proteins. Depending on the electroendosmotic properties of the agarose and the pH of the buffer medium, they not only could be static, but could also migrate toward the anode or cathode [11].

These results suggest that the α_2-GP precipitates were homogeneous in the various buffer media except in the medium with pH 10.0, where melting of the precipitate takes place, although we know that the structure of this antigen consists of two identical subunits [9]. The presence of two TBG fragments with different electrophoretic mobility was demonstrated previously by the use of monospecific antisera and the introduction of polyethylene-glycol with mol. wt. of 6 kilodaltons into the reaction [9, 12, 14]. We found that in an alkaline medium these forms can also be detected without the use of polyethylene-glycol. However, in weakly acid and weakly alkaline media only one form of this protein could be found, namely the β-component. It was shown that the basic structural unit of TBG is a fragment with mol. wt. of 42.3 kilodaltons. It has been suggested that all components of TBG are formed by the combination of such fragments through the intervention of glycoside bonds [10]. It has been shown that TBG and the α_2-GP associated with pregnancy are bound with antibodies against all chains of immunoglobulins [3]. The inhibitory effect is most marked when blood serum from pregnant women is absorbed with antisera against immunoglobulin heavy chains. These proteins may perhaps form complexes with certain other components of the blood plasma, leading to the appearance of new forms of this antigen with different electrophoretic mobility [8]. Incidentally, additional fragments of TBG can be found only at pH 8.6, i.e., under standard conditions of quantitative immunoelectrophoresis. In near-physiological conditions this antigen is present in a single form. This is confirmed by the fact that the isolated TBG preparation with the corresponding antiserum reveals one component with an electrophoretic mobility of β_1-globulins. This suggests that in an alkaline medium TBG forms complexes with components of the blood plasma, with the result that new fragments arise: these fragments have a different charge and acquire increased electrophoretic mobility, either anodal or cathodal. To test this hypothesis, it was decided to carry out electrophoresis of the proteins in the presence

of heparin-glucosaminoglycan, which increases the anodal electrophoretic mobility of glycoproteins [2].

Introduction of heparin into the gel for the first direction of electrophoresis increased the anodal electrophoretic mobility of both proteins when the polysaccharide concentration was not less than 0.1 mg/ml. Higher heparin concentrations, introduced into the reaction, caused an increase in area of the precipitate of the TBG α-fragment and reduced the area of the precipitate of the principal form with mobility of β-globulins. Moreover, the mobility of the α-component was increased proportionally to the heparin concentration. Incidentally, there was no change in the area and form of the α_2-GP precipitates under these conditions. Thus, compounds with marked cationic properties (heparin), introduced into the reaction, create conditions for such a compound to form a complex with TBG with enhanced anodal electrophoretic mobility. Possibly the introduction of an anionic preparation, reacting with TBG, into the reaction may cause the formation of a complex with high cathodal electrophoretic mobility.

Under standard conditions of quantitative immunoelectrophoresis heparin forms complexes with glycoproteins and endows them with a negative charge [2, 7]. It can also displace glycoproteins (including TBG and α_2-GP) from complexes with hyaluronate. Disappearance of the γ-form of TBG when heparin is used in the reaction is evidently similar in nature.

It has been shown on the basis of the results of gel chromatography and a combination of zonal and quantitative immunoelectrophoresis that the α-fragment of TBG has an mol. wt. of about 200 kilodaltons, and the β-and γ-forms mol. wt. of about 80 kilodaltons [13]. An α_2-globulin immunochemically identical with TBG and with mol. wt. of about 30 kilodaltons also has been found [12]. In the light of our own data the α-unit of TBG can be regarded as a complex of a TBG dimer with a high-molecular-weight compound or as binding of a TBG trimer or tetramer with a relatively low-molecular-weight antigen. In view of the virtually equal molecular weights of the β- and γ-forms of TBG it can be tentatively suggested that the positive charge of the TBG γ-fragment is provided by a compound with very low molecular weight. These results suggest that native blood serum from pregnant women contains TBG which is in the form of identical components, possessing the electrophoretic mobility of β_1-globulins.

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