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FRACTIONATION OF IMMUNE RABBIT SERUM GAMMA-GLOBULIN

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It has been recently reported that it is possible to separate electrophoretically several subfractions of human serum albumin by using buffers of low ionic strength at pH values close to the isoelectric point[1]. Human γ-globulin (Cohn fraction III) has also been fractionated in three boundaries by this method[2]. SAIFER AND COREY[3] suggest that these results are due to a "microheterogeneity" of the protein molecules present in albumin and in γ-globulin, *i.e.* to the existence in them of patterns of molecules having similar but not identical physical and chemical properties.

The principle described by SAIFER AND COREY has been used by HUMPHREY AND PORTER[4] for studying by partitional column chromatography the immune globulin of rabbits at different stages of immunization with various antigens. The detection of three subfractions was possible with rabbit serum globulin also, but the antibodies were not present in all subfractions. The specific antibody was found either in the slow migrating boundary or in the middle one, depending upon the time after the immunization and the type of the antigen. The authors suggest that different types of cells may produce several types of γ-globulins differing slightly one from another.

In the present paper an account is given of an attempt to separate different subfractions of serum γ-globulin of both normal and immunized rabbits by means of paper electrophoresis, and to detect the specific antibody in these subfractions.

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METHODS

Rabbits weighing 1500–1700 g were used. The immunizing treatment consisted of injecting into the ear vein of each animal 3 ml of a 20 % suspension of guinea pig erythrocytes in physiological saline. These erythrocytes were taken from the heart of guinea pigs and washed three times with physiological saline containing a small amount of sodium oxalate. Some rabbits received only 1 injection; the agglutinating power of the serum of these animals was 1:50 a week after the injection of the antigen. Other rabbits received one injection every week for the period of 6 months. At the end of the treatment, the agglutinating power of the sera of these animals was 1:400. Other rabbits did not receive any treatment and were retained as normal controls.

Gamma-globulin was isolated from the rabbit serum by the method of KEKWICK[5].

Paper electrophoresis was performed in a Elphor apparatus (AESSE, Milano), 110 V and 0.1 A being applied at the electrodes. Whatman No. 1 paper was used. Two buffer solutions were employed: (1) sodium barbiturate–acetate buffer, pH 8.6, ionic strength 0.15. (2) acetic acid–acetate buffer, pH 4.5, ionic strength 0.04. Then 0.01 ml of the 12 % solution in 0.067 *M* phosphate buffer, pH 8, of the isolated γ-globulin was placed on the paper, two parallel strips being run each time. After 12 hours, the strips were removed, dried at room temperature, and the first stained with Amido Schwarz 10 B (Bender and Hobein, C.M.B.H.) to localize the spots of the proteins. The regions in the second strip that corresponded to the spots in the first one, were removed, carefully cut in small pieces with scissors, and immersed in 2 ml of 0.067 *M* phosphate buffer, pH 7.4, at room temperature for 1 hour. The eluates were then tested for the detection of the specific antibody against guinea pig erythrocytes, after convenient dilution in physiological saline.

RESULTS

Fig. 1 represents the paper electrophoretical pattern of the γ-globulin of normal rabbit serum, at pH 4.5. It is clear that by this method it was impossible to distinguish any subfractions in γ-globulin of normal animals. Only one spot was obtained, independently of the pH used.

Gamma-globulin from the serum of rabbits receiving only 1 injection of guinea pig red cells also showed one spot, at both pH values tested. Gamma-globulin from the serum of rabbits submitted to repeated injections with the antigen, however, showed three, and in some instances four, spots at pH 4.5, (Fig. 2 and 3), while they continued to show only one spot at pH 8.6. The agglutinating antibody was eluted from the unique spot in the electropherograms run at pH 8.6. In electropherograms run at pH

Fig. 1. Electropherogram of γ-globulin of normal rabbit serum. Acetate buffer, pH 4.5, ionic strength 0.04.

Fig. 2. Electropherogram of γ-globulin of the serum of a rabbit treated with guinea pig erythrocytes for 6 months. Acetate buffer, pH 4.5, ionic strength 0.04. Three subfractions are evident, but the antibody was present only in the top fraction (darkest spot).

Fig. 3. Electropherogram of γ-globulin of the serum of a rabbit treated with guinea pig erythrocytes for 6 months. Experimental conditions as in Fig. 2. Four subfractions are evident, but the antibody is present only in the top fraction.

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4.5, however, the antibody was shown to be present only in the most rapidly migrating subfraction (top fraction), while it was practically lacking in the others. These results agree substantially with those of SAIFER AND COREY and of HUMPHREY AND PORTER. The impossibility of separating γ-globulin subfractions from normal rabbit serum by the method used here is perhaps due to the fact that our method is relatively less sensitive compared with those used by the above authors. The fact that the separation was possible in the case of sera from rabbits submitted to long-time immunization probably shows that the "microheterogeneity" is more marked under these conditions. The hypothesis of HUMPHREY AND PORTER that in the immunization process different types of cells produce different types of γ-globulin is not in disagreement with the results obtained in these experiments, but the presence of the antibody in only one subfraction suggests that each cell produces antibodies with relatively identical properties.

SUMMARY

Rabbit serum γ-globulin was submitted to paper electrophoresis in two different buffers of pH 8.6 and 4.5, and of ionic strength 0.15 and 0.04, respectively. Only one spot was detected for the γ-globulin of normal animals, but the γ-globulin of animals treated for many months with guinea pig erythrocytes was separated into 3–4 subfractions. The specific antibody was present only in the most rapidly migrating subfraction and was completely absent in the others.

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BORATE AND PHOSPHOGLUCOSE ISOMERASE IN THE ASSAY OF PHOSPHOMANNOSE ISOMERASE

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Phosphomannose isomerase (PMI) and phosphoglucose isomerase (PGI) are widely distributed, but in every case so far examined the activity of PGI greatly exceeds that of PMI[1,2,3]. Moreover, PMI is usually far less stable than PGI. Consequently, fractionation procedures applied to several tissues have been successful in giving preparations of PGI free of PMI but not vice versa.

In the assay of PMI by the direct method of estimation of ketose formation (fructose-6-phosphate, F-6-P) from mannose-6-phosphate (M-6-P), the presence of

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