Interaction of Fragment III of Rabbit Gamma Globulin and Guinea Pig Complement.* (26963)

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Porter(1) has reported that rabbit antibody, when treated with crystalline papain, is degraded into 3 fragments. Fragments I and II are univalent and capable of combining specifically with antigen, while fragment III retains the antigenic properties of gamma globulin. Recently we examined the immunochemical properties of the fragments of rabbit γ_2 hemolytic and agglutinating antibodies resulting from digestion with papain (2). We found that all 3 fragments inhibited immune hemolysis. The inhibitory property of III is nonspecific, since III derived from rabbit antipertussis gamma globulin also inhibited immune hemolysis.

In view of the earlier assumptions of Heidelberger and co-workers(3) that complement (C′) yields easily dissociable combinations with dissolved antibody and firm combinations with certain immune aggregates, the possible role of fragments of gamma globulin in both combinations was investigated. Rabbit antibody can be degraded also with pepsin producing a bivalent fragment which is converted to univalent fragments when a disulfide splitting reagent is added(4). This work is, therefore, concerned with the study of additional properties of fragment III and of the fragments obtained by degradation of normal and immune rabbit gamma globulins with pepsin.

Material and methods. Antisera to sheep erythrocytes were prepared by intravenous injection of rabbits with 1.0 ml of a suspension of boiled sheep red cell stromata in saline (1 mg N/ml) thrice weekly for 4 weeks. Rabbits were bled 7 days after the last injection and sera were pooled. Rabbit antipertussis serum was kindly supplied by Miss Jessie L. Hendry of this Division.

Gamma globulin fractions were obtained by fractionation of normal or immune rabbit sera by the Na_2SO_4 precipitation technic of Kekwick(5) and the modified procedure(6) of anion exchange chromatography(7). Gamma globulins were degraded with crystalline papain (Lot 5503, Worthington Biochemical Corp., Freehold, N. J.) and the fragments were isolated by the methods outlined by Porter(1). Digestion of gamma globulin with pepsin (Lot 643, Worthington Biochemical Corp.) was carried out according to the method of Nisonoff and coworkers(4).

Sheep erythrocyte suspensions (E) were prepared as described by Mayer *et al.*(8), except that the concentration of cells was 2×10^9 cells/ml. Veronal-saline buffer(8) containing 1.5×10^{-4} M Ca++ and 5.0×10^{-4} M Mg++ was used for all dilutions and washings. Pooled guinea pig sera were the source of complement (gp C′) and were stored in small portions at −30°C.

Titrations of hemolysin activity of rabbit antisheep erythrocyte serum or antiserum fractions were carried out essentially as outlined by Kabat and Mayer(9). Each reaction tube contained 0.2 ml of E, 0.4 ml of gp C′ (at a concentration which contained four 50% hemolytic units when added to a comparable number of optimally sensitized cells), and 0.2 or 0.4 ml of antiserum or fraction at varying dilutions. Buffer was added to give a final volume of 1.6 ml. The tubes were incubated for 60 minutes at 37°C, centrifuged, and the supernatant fluid, after being diluted 1:4 with distilled water, was read at 541 mμ in Beckman DU spectrophotometer. Hemolytic activity is expressed as number of 50% hemolytic units (H_{50})/ml undiluted fraction. Agglutinin titers were determined in the manner described by Kabat and Mayer(9). Aliquots (0.2 ml) of antiserum or fractions at various dilutions were added to 0.1 ml of E in a final volume of 0.8

* This study was supported by research grant E-3757 from the National Institute of Allergy and Infectious Diseases.

ml. Tubes were incubated for 60 minutes at 37°C and read. Agglutinin unit is expressed as the highest dilution of serum giving definite agglutination. Agglutinin activity is the number of units/ml undiluted fraction. Inhibition of immune hemolysis by fragments of gamma globulin was tested by the same procedure except that fragments were allowed to incubate with E and buffer for 30 minutes at 0°C prior to addition of γ_2 hemolytic antibody and gp C′.

The method of quantitative complement fixation by immune aggregates is that outlined by Kabat and Mayer(9).

Results. Preincubation of fragment III with reactants of immune hemolysis. Varying amounts of normal rabbit gamma globulin and fragment III, derived from the former by papain digestion, were preincubated with either E or gp C′ for 30 minutes at 0°C. The remaining reactants (gp C′ or E and γ_2 hemolytic antibody) were added and tubes were incubated at 37°C. In addition, tubes containing varying amounts of gamma globulin or fragment III were allowed to react with E, γ_2 hemolytic antibody, and gp C′ with no preincubation period. The results are shown in Fig. 1. Preincubation of gamma globulin or fragment III with gp C′ results in greatest inhibition of immune hemolysis. There is little or no change in inhibitory property of gamma globulin or fragment III when preincubated with E as compared with no preincubation.

Interaction of fragment III with E. Varying amounts of fragment III derived from a gamma globulin fraction of rabbit antisheep erythrocyte serum were preincubated with E for 30 minutes at 0°C. Tubes were centrifuged and the residual cells were washed twice with 1.0 ml buffer and reacted with γ_2 hemolytic antibody and gp C′. Each supernatant fluid was added to fresh E. γ_2 hemolytic antibody and gp C′ were then added, and tubes were incubated at 37°C. Similar amounts of fragment III were reacted with E, γ_2 hemolytic antibody, and gp C′. Though most of inhibitory property of fragment III resides in the supernatant fluid, there is some interaction of fragment III, at high concentrations, with E (Fig. 2).

Effect of pepsin-digested immune and normal gamma globulins on immune hemolysis. Normal and immune (antipertussis and antisheep erythrocyte) rabbit gamma globulins were degraded with pepsin and resulting fragments were examined for inhibitory properties in immune hemolysis. In addition, fragment III derived from papain digest of rabbit antipertussis gamma globulin was tested. Fig. 3 indicates that fragments of peptic digestion of normal and antipertussis gamma globulins do not affect the immune hemolytic reaction, while comparable concentrations of a similar fragment derived from antisheep erythrocyte gamma globulin and fragment III of antipertussis gamma globulin are inhibitory.

TABLE I. Effect of Pepsin on Activities of Rabbit Antisheep Erythrocyte Gamma Globulin.

	Agglutinin activity	Hemolytic activity
Rabbit antisheep erythrocyte gamma globulin	17	12,000
Pepsin digest	17*	<100

* After 16 hr at room temperature. No activity after 60 minutes at 37°C.

The pepsin digest of rabbit antisheep erythrocyte gamma globulin was tested for agglutinin and hemolytic activity. The results are shown in Table I. It is of interest to note that though hemolytic activity is lost, agglutinating ability is retained, albeit at a slower rate.

In view of these results, the ability of the pepsin digest of rabbit antisheep erythrocyte gamma globulin to fix complement was examined. One milliliter of a solution of rabbit antisheep erythrocyte gamma globulin (26 μg N/ml) and 1.0 ml of pepsin digest (18 μg N/ml) were reacted with varying amounts of boiled sheep erythrocyte stromata in the presence of 110 $C'H_{50}$. The fixation of gp C′ by these systems is depicted in Fig. 4. Treatment of rabbit antisheep erythrocyte gamma globulin with pepsin results in about 70% less fixation of gp C′.

Discussion. It was reported earlier(2) that nonspecific inhibition of immune hemolysis by fragment III of rabbit gamma globulin might be due to a site on the fragment which combines with the red cell similar to the skin attachment site(10) and mem-

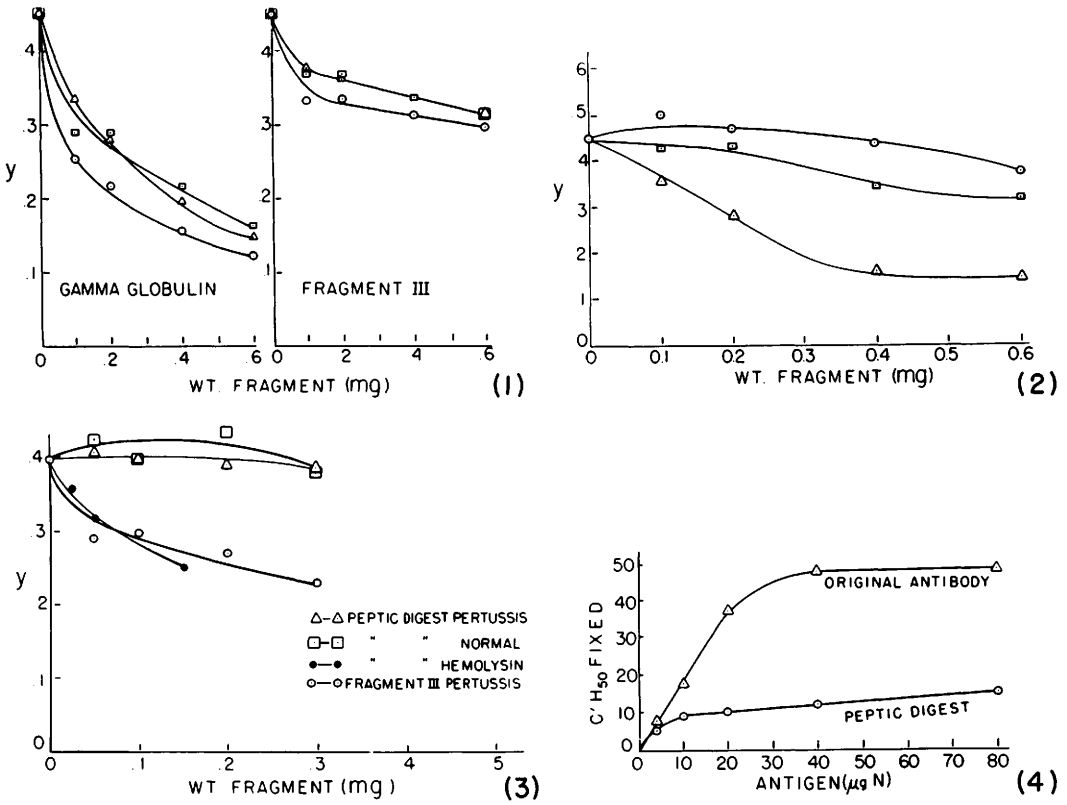

FIG. 1. Effect of preincubation of gamma globulin or fragment III with reactants of immune hemolysis
⊙—⊙ Preincubation with gp C′
□—□ Preincubation with E
△—△ No preincubation

FIG. 2. Interaction of fragment III of rabbit anti-sheep erythrocyte gamma globulin with E
△—△ Inhibition of immune hemolysis by fragment III
⊙—⊙ Inhibition of immune hemolysis by pretreatment of E with fragment III
□—□ Inhibition of immune hemolysis by residual fragment III

FIG. 3. Effect of peptic digests of normal and immune rabbit gamma globulin on immune hemolysis

FIG. 4. Fixation of gp C′ by rabbit antisheep erythrocyte gamma globulin and its peptic digest
Intact gamma globulin, 26 μg N
Peptic digest, 18 μg N

brane transmission site(11) associated with this fragment. There appears to be some interaction of the fragment with the red cell (Fig. 2). Inhibition, however, is due primarily to the ability of the fragment to associate with gp C′. Evidence for this is the increased inhibition upon preincubation of fragment III (or gamma globulin) with gp C′ (Fig. 1) and the loss of inhibitory properties of normal and antipertussis gamma globulins upon treatment with pepsin (Fig. 3). Peptic digestion of rabbit gamma globulin decreases the sedimentation constant by removing an inert portion of the molecule and leaves a bivalent fragment that appears to be composed of Porter's fragments I and II(4). The inhibition of immune hemolysis by the pepsin digest of rabbit antisheep erythrocyte gamma globulin is specific since this digest is capable of agglutinating sheep erythrocytes but it is not capable of lysing these erythrocytes in the presence of gp C′. This inability to lyse sheep red cells is probably due to loss in ability to fix gp C′. It appears

therefore that the portion of rabbit gamma globulin that combines with gp C′(3) resides in fragment III. The decrease in rate of agglutination by treatment of rabbit antisheep erythrocyte gamma globulin with pepsin implicates to some extent the role of fragment III in another immune phenomenon.

Summary. Fragment III derived from papain digestion of rabbit gamma globulin inhibits immune hemolysis primarily by its ability to interact with gp C′ and to some extent by interacting with sheep erythrocytes. Peptic digests of normal and antipertussis rabbit gamma globulins do not inhibit immune hemolysis. Peptic digests of rabbit antisheep erythrocyte gamma globulins are capable of agglutinating sheep erythrocytes at a slower rate when compared to that of the intact antibodies. The latter digests will not lyse sheep erythrocytes in the presence of gp C′, because they fix less complement than intact antibody.

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Received Aug. 1, 1961. P.S.E.B.M., 1961, v108.

Effects of Psychotropic Compounds on Enzyme Systems, I. Inhibition of Human Plasma and Erythrocyte Cholinesterases.* (26964)

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Although there is some literature on enzyme inhibition by psychotropic compounds, good quantitative data, particularly relating *in vivo* with *in vitro* effects in the same species are relatively rare. Some enzyme systems, for example choline acetylase, have been largely ignored, probably because of difficulty of assay rather than lack of significance of this enzyme. We propose to report investigations on *in vivo* and *in vitro* quantitative inhibitory effects of a large number of compounds with psychotropic activity on various enzyme systems and to attempt to correlate psychotropic action with effects on enzyme systems.

* Supported in part by grants from New Mexico Heart Assn. and the Nat. Inst. of Mental Health.

† Some of this work carried out in partial fulfillment of requirements for M. S. degree, New Mexico Highlands Univ.

Most of the published inhibition studies have been either in qualitative terms ("inhibits", "enhances") or in semi-quantitative terms (50% inhibition = I_{50}). The I_{50} value, however, is dependent upon substrate concentration, so that it is frequently difficult to compare numerical values obtained by different assay methods. Moreover, determination of I_{50} values does not necessarily entail an analysis of the kinetics of inhibition. It has therefore been found desirable to determine K_i values (K_i = inhibitor constant), as defined by Dixon and Webb(1).

Tranquilizers of the phenothiazine type have been found to be *in vitro* inhibitors of cholinesterases(2,3). It has also been suggested(2) that the effectiveness of phenothiazine tranquilizers might be related to their cholinesterase inhibitory activity.

Material and methods. The tranquilizers