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Reaction of Rheumatoid Sera with Fragments of Papain-Digested Rabbit Gamma Globulin.* (26488)

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Sera from a large proportion of patients suffering from rheumatoid arthritis react with rabbit gamma globulin as demonstrated by hemagglutination(1,2) and quantitative precipitin(3,4) technics. The reactant material in these sera has been termed "rheumatoid factor" and has the physicochemical properties of a gamma macroglobulin(5).

Porter(6) chromatographed a papain digest of rabbit gamma globulin on carboxymethylcellulose and obtained 3 fractions. These fragments (designated Fractions I, II, and III in the order of chromatographic elution) all had sedimentation constants of about 3.5S and molecular weights of about 50,000, 50,000, and 80,000, respectively(7). Fractions I and II were similar in chemical and biological properties. When antibody globulin was digested, each had the power to combine with, but not to precipitate, the homologous antigen. Fraction III showed no antibody activity but appeared to carry most of the antigenic determinants of the native molecule as determined by its ability to precipitate with antisera to rabbit gamma globulin.

In view of this localization of the antigenic determinants of rabbit gamma globulin on a fragment comprising about 40% of the intact molecule, it was of interest to determine which fragment or fragments of the papain digest were involved in reaction with rheumatoid factor. This would serve as a first step in comparing the structural units of the globulin molecule involved in reaction with rheumatoid factor and with specific antibody to rabbit gamma globulin.

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Materials and methods. γ-Globulin. Rabbit gamma globulin (RGG) was prepared by sodium sulfate precipitation according to the method of Kekwick(8). Paper electrophoresis of the preparation demonstrated a single component with the mobility of γ-globulin.

Crystalline papain† was prepared from crude enzyme powder and crystallized as the inactive mercury dimer(9). It was lyophilized and stored as the dimer.

Sera. A pool of sera from rheumatoid arthritis patients was kindly provided by Dr. Wallace V. Epstein, Dept. of Medicine, Univ. of California, San Francisco. The titer of this rheumatoid pool (RP) for tanned sheep erythrocytes coated with human γ-globulin (Cohn Fraction II) was 1:7000. In 3 experiments using tanned sheep RBC coated with RGG, the titer was found to be 1:896 to 1:1792.

Mice were immunized‡ with Fraction I of papain-digested RGG. The antigen was incorporated into a medium consisting of equal volumes of saline and Freund's adjuvant. Mice received 3 injections, each containing 1.0 mg of Fraction I. They were bled 10 days after last injection and the sera pooled.

Prior to use in experiments, all sera were incubated at 56°C for 30 minutes to inactivate complement and absorbed with an equal volume of washed packed sheep RBC for 18 hours at 4°C to remove heterophile antibody activity.

† Mercuripapain was prepared while the author was at the laboratory of Dr. R. R. Porter, Nat. Inst. for Medical Research, London, England.

‡ Mice were immunized and bled by Dr. Leon S. Kind, Dept. of Microbiology, Univ. of California, San Francisco.

Digestion and chromatography. RGG was digested with one percent mercuripapain and fractionated on carboxymethylcellulose (CM-cellulose) (10,11) as described by Porter(6). Three chromatographic peaks were obtained, corresponding to Porter's Fractions I, II, and III (FI, FII, and FIII). For one experiment, FII was rechromatographed on CM-cellulose using 0.01 M sodium phosphate buffer, pH 6.0. It was found that FII was eluted from the column with this buffer whereas FIII was retained, thus effecting a complete separation of these 2 components. FIII could be eluted with 0.2 M phosphate buffer, pH 7.0. Protein concentrations were determined by reading the absorption at 280 and 260 mμ in a spectrophotometer.

Hemagglutination tests. The capacity of RP and the mouse anti-FI pool to agglutinate sheep RBC coated with RGG and the fractions was determined. Erythrocytes were treated with tannic acid and coated with RGG or one of the fractions according to Heller *et al.*(12). Protein solutions were absorbed with washed sheep RBC prior to coating tanned cells in order to remove heterophile antibody activity. One-half ml of serial 2-fold dilutions of serum and 0.5 ml of a 0.25% suspension of coated erythrocytes were mixed in the tests. Dilutions were made with buffered saline, pH 8.0, in assays with RP. It was found that the mouse pool did not give stable endpoints in saline so in assays with this serum the 0.25% suspension of RBC was made up with buffered saline containing 2% bovine serum albumin(13). Hemagglutination endpoints were stable under these conditions. Degree of hemagglutination was read as 4+ (very strong) to— (absent) from the settling pattern of the cells following overnight incubation at 4°C. Control tubes containing tanned, uncoated RBC plus serum were included in each experiment. These never showed detectable agglutination.

Hemagglutination-inhibition tests. The capacity of RGG and of the fractions to inhibit agglutination of RGG-coated cells by RP was determined. A dilution of RP giving 4+ agglutination was used in these assays. Varying amounts of the inhibitors were added to tubes containing 0.5 ml of RP. One-half ml of a 0.25% suspension of coated cells was then added to each tube, giving a total volume of 1.5 ml. Control tubes containing the following were included in each experiment: 1) coated RBC plus RP; 2) tanned, uncoated RBC plus RP; 3) coated RBC plus inhibitor. The latter two always failed to show detectable hemagglutination. The rest of the procedure was as described above.

TABLE I. Hemagglutination of Coated Cells by Rheumatoid Pool.

Coated RBC	Titer
γ-Globulin	1:896 1:896
Fraction I	<1:28 <1:28
" II	<1:28 <1:28
" III	1:448 1:448

Results. Table I gives the results of hemagglutination tests of RP with cells coated with RGG and the fractions. Titers are shown for 2 independent determinations. RGG-coated cells and FIII-coated cells were agglutinated by serum dilutions of 1:896 and 1:448, respectively. The titers for cells coated with FI and FII were less than 1:28, this being the lowest dilution of RP tested.

To demonstrate that the negative results obtained in hemagglutination tests of RP with FI and FII-coated cells were not due to inability of these fractions to coat tanned RBC, hemagglutination tests using coated cells and the mouse anti-FI pool were performed. The titers obtained in 2 experiments are given in Table II. Cells coated

TABLE II. Hemagglutination of Coated Cells by Mouse Anti-FI Serum.

Coated RBC	Titer
γ-Globulin	1:3584 1:3584
Fraction I	1:3584 1:3584
" II	1:3584 1:3584
" III	1:896 1:896

TABLE III. Inhibition by RGG and Fractions of HA of RGG-Coated Cells by Rheumatoid Pool.

Amt of inhibitor (mg)	Inhibitor				
	γ-Glob.	FI	FII	FII*	FIII
	Hemagglutination readings				
.001	+4,+4	+4,+4	+4,+4	+4	+4,+3
.002					+4
.004					+3
.008					+
.01	+2,+2	+4,+4	+4,+4	+4	—,—,—
.05	—,—	+4,+4	+3,+3	+3	—,—,—
.10	—,—	+4,+4	+2,+2	+2	—,—
.50	—,—	+4,+4	—,+	±	—,—

* Fraction II rechromatographed on CM-cellulose column, pH 6.0.

with FI and FII were as strongly agglutinated as cells coated with RGG, the titer being 1:3584 in each case. Cells coated with FIII were agglutinated to a titer of 1:896. It had previously been shown that a rat antiserum to FII cross-reacted very strongly with FI but only weakly with FIII(6,7), indicating that FI and FII are very similar antigenically and almost but not completely distinct from FIII.

The results of experiments to determine the effectiveness of RGG and the fractions in inhibiting agglutination of RGG-coated cells by RP are given in Table III. FIII was a more effective inhibitor than RGG on a weight basis, 0.01 mg of the former causing complete inhibition while this amount of the latter produced only partial inhibition. On a molar basis, they might prove very similar in inhibiting capacity. It is not possible, however, to make critical quantitative comparisons on the basis of hemagglutination readings. The inability of FI to cause detectable inhibition and the low inhibiting capacity of FII over the concentration range tested indicates that the former is not at all involved and the latter only weakly involved in reaction with rheumatoid factor. The possibility existed that the weak inhibiting activity of FII was due to contamination with a small amount of FIII as separation of these 2 chromatographic peaks was incomplete under the conditions employed(6). Although precaution was taken to minimize cross-contamination by discarding the effluent in the overlap area of the chromatogram, in order to make certain that contamination was not responsible, FII from a CM-cellulose column developed at pH 6.0 was used in one experiment. Table III shows that weak inhibiting capacity was still present and appears, therefore, to be an inherent property of FII.

Discussion. The results show that most of the reactivity of RGG with RP is associated with FIII of the papain-digested molecule. Erythrocytes coated with FI and FII were not agglutinated by RP whereas cells coated with RGG and FIII were strongly agglutinated. FI was unable to inhibit agglutination of RGG-coated cells by RP. FII was able to inhibit agglutination at higher concentrations but was a much less effective inhibitor than either FIII or RGG. The results suggest that FIII and RGG are about equally effective inhibitors on a molar basis, which would be expected if almost all the reactive groups are located on the FIII segment of the RGG molecule. Since the inhibition tests indicate that FII does react weakly with rheumatoid factor, the absence of agglutination of FII coated RBC by RP might possibly be due to physical blockage of the structural unit involved by virtue of attachment of the molecule to the surface of the red cell. Should this be the case, it would mean that different structural units are involved in reaction with RP and with mouse anti-FI serum since the latter was capable of agglutinating FII coated cells. An alternate explanation might be that the cells were insufficiently coated to give detectable agglutination, since the reaction with RP is weak at best.

FIII has been found to be the carrier of several properties characteristic of RGG. In addition to possessing most of the reactivity of the molecule with specific antisera(6,7) and, as shown here, with rheumatoid factor, of the 3 fragments it most closely resembles the parent molecule with respect to transmission across the foetal yolk-sac membrane of the rabbit(14). The order of transmission across the membrane was found to be FIII> FII>FI, identical to the order of reactivity with RP. The configurations on the γ-globulin molecule comprising the antigenic determinants, the units involved in reaction with rheumatoid factor and those recognized by

rabbit cells as homologous γ-globulin are therefore largely located on a segment comprising about 40 percent of the parent molecule. It will be necessary to obtain much smaller active fragments of FIII in order to establish the relationships of the chemical groupings responsible for these properties.

Summary. Most of the reactivity of rabbit γ-globulin with a pool of sera from rheumatoid arthritics was found to be associated with Fraction III of the papain-digested globulin molecule. Fraction II showed a low degree of reactivity while Fraction I appeared to be completely inactive.

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Rat Virus (RV) Infections in Hamsters. (26489)

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Rat virus (RV) was first recognized in rat embryo tissue cultures inoculated with materials from tumor-bearing rats. In such cultures the production of specific cytopathogenic effects (CPE) and of hemagglutinins indicated activity of the virus(1). Preliminary tests in animals failed to reveal pathogenic properties of RV. The present report describes (a) a fatal disease induced by RV in hamsters of one to 4 days of age, (b) occurrence of virus and viral hemagglutinins in the tissues and fluids of these animals and (c) spread of virus to uninoculated hamsters. Sucklings which happen to survive the acute disease may develop a stunted growth resembling mongolism. This dwarfism has been reported previously(2).

Materials and methods. Modifications and extensions of procedures not previously described(1) are given below.

Virus. The strain of virus used throughout current work was RV-13, which was carried through approximately 12 rat embryo tissue cultures and 4 passages in suckling hamsters prior to a final passage in rat embryo tissue culture. This tissue culture seed virus lot which was stored at –40°C, had an HA titer of 1:512 and an LD_{50} of 10^{-5} as measured by deaths induced in 1-day old suckling hamsters within a period of 10 days. As described below, a 10^{-6} dilution of the seed induced dwarfism but no acute fatal disease.

Inoculation and harvesting of hamsters. Syrian hamsters from the Animal Production Center of the NIH were inoculated as sucklings by the intracerebral route. Uninoculated control animals were retained in each litter tested; controls were identified by clipping a portion of their tails. Animals harvested at various stages of infection were anaesthetized, decapitated and exsanguinated into a dish moistened with heparin. Bladders were then clamped and incised in order