THE INDUCTION OF A RHEUMATOID FACTOR-LIKE SUBSTANCE IN RABBITS*

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Serum factors possessing some of the properties of the human rheumatoid factor(s) (RF)[1] have been produced in experimental animals. The RF and the experimentally induced factors react with various gamma globulins. The anti-gamma globulin specificities of experimentally induced serum factors can be attributed to one or more of the following mechanisms:

(*a*) The production of antibodies against heterologous gamma globulin as in Adler's study (1) where sensitized sheep cell agglutinins developed in the serum of guinea pigs immunized with heterologous (rabbit) immune precipitates.

(*b*) The production of iso-antibodies against rabbit gamma globulin of an allotype other than that of the host. (Studies of rabbit gamma globulin allotypes, references (9–12). This was probably operative in the studies of Milgrom and Dubiski (2, 3) who demonstrated sensitized sheep cell agglutinins in rabbits immunized with isologous antigen-antibody complexes (*Escherichia coli* and *Proteus* OX_{19} agglutinated by their respective rabbit antiserum). The hemagglutinating factor in each instance could be absorbed by some rabbit immune complexes but not by all.

(*c*) Immunization against gamma globulin or gamma globulin products which were adsorbed or assimilated by bacteria, utilized for immunization, from either culture media or recent passage through animals. This was clearly

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[1] The following abbreviations, listed in order of appearance, are used throughout the paper.
RF, rheumatoid factor(s)
RFLS, rheumatoid factor-like substance
AHGG, aggregated human gamma globulin
7S-HGG, non-aggregated human gamma globulin
7S-RGG, non-aggregated rabbit gamma globulin
ARGG, aggregated rabbit gamma globulin

evident in the study of Lerner *et al.* (4) in which a serum factor resulting from immunization of rabbits with *Streptobacillus moniliformis* was dependent on the growth of the microorganisms on media containing human serum protein. It may possibly be operative in other studies in which rabbits were immunized with killed microorganisms (5, 6).

(*d*) The production of anti-antibodies against the host's own gamma globulin "denatured" in the process of combining with its respective antigen. In a study similar to the present one, Aho and Wager noted that 3 of 12 rabbits immunized with ovalbumin alone developed a serum factor which agglutinated sensitized sheep cells (7). This factor was absorbed with rabbit immune precipitates (autologous and isologous) but not with horse immune precipitate. Milgrom and Witebsky (8) demonstrated a serum factor reactive with human gamma globulin in rabbits immunized with their own (autologous) gamma globulin prepared by salt fractionation. The authors suggested that denaturation by physico-chemical means may have occurred during preparation of the gamma globulin. They also considered the possibility of immunization against contaminating heterologous (human) gamma globulin in view of the stronger reactivity of the factor with human gamma globulin than with rabbit gamma globulin but could not establish such a relationship.

The present report concerns the induction of a rheumatoid factor-like substance (RFLS) in 2 groups of rabbits immunized with either formalin-killed *E. coli* or *B. subtilis* that were grown in synthetic media free from any source of contamination with animal protein.

Materials and Methods

1. Bacteria for animal inoculation were cultured in media containing only inorganic salts and glucose.[2] The organisms (*E. coli* and *B. subtilis*) had been maintained in this media, with frequent transfer, for a period in excess of 2 years. The organisms were killed by the addition of formalin (0.4 per cent), and stock suspensions made with normal saline such that a 1:16 dilution had an optical density of 0.32 at 650 millimicrons in a model 14 Coleman spectrophotometer.

In the studies to be described, 1 unit of bacteria represented 0.5 ml of the stock preparation.

2. Rabbits injected intravenously with a suspension of killed bacteria. After the first 2 weeks of immunization, each animal received 0.6 unit of bacteria three times weekly. Smaller doses were used during the first 2 weeks to minimize endotoxin toxicity. Blood was obtained at approximately 1 month intervals *via* cardiac or ear artery puncture. Sera were frozen and stored at −20°C.

3. Serologic methods. All serological procedures utilized sera which had been heated to 56°C for 30 minutes. Sera for hemagglutination tests were absorbed with 1 volume of washed, packed sheep erythrocytes per 2 volumes of serum. Unless otherwise stated, all dilutions were made in phosphate buffered normal saline (pH 8.0).

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[2] 0.2 per cent ammonium sulfate, 1.4 per cent dibasic potassium phosphate, 0.6 per cent monobasic potassium phosphate, 0.1 per cent sodium citrate, 0.02 per cent magnesium sulfate, 0.5 per cent glucose. pH adjusted to 7.0.

The latex fixation tests were performed according to the method of Singer and Plotz (13).

The sensitized sheep cell agglutination utilized 0.25 per cent cells which had been sensitized with ½ B.A.T. (basic agglutination titer) of rabbit hemolysin.

Tanned sheep cell agglutination tests were performed according to the method of Heller *et al.* (14). Materials for adsorption onto tanned sheep erythrocytes included: aggregated human gamma globulin (AHGG) which represented that part of human Cohn FII (heated 60°C for 10 minutes) which was insoluble in 0.62 molar sodium sulfate, non-aggregated human gamma globulin (7S-HGG)—the supernatant of heated FII after removal of AHGG, non-aggregated rabbit gamma globulin (7S-RGG), prepared by precipitation of pooled rabbit serum with 1.35 molar ammonium sulfate or ⅓ saturated sodium sulfate and aggregated rabbit gamma globulin (ARGG) which was prepared by heating 7S-RGG at 70°C for 10 minutes. All materials were dissolved in buffered saline (pH 8.0). Concentrations of these materials used per 2 ml of 33⅓ per cent tanned sheep cells were: AHGG 1.7 mg N, 7S-HGG 2.2 mg N, 7S-RGG 3.5 mg N, and ARGG 3.5 mg N. In some experiments the quantities of ARGG and AHGG used in "coating cells" were varied as indicated in the text. Saline controls and suitable negative and positive serum controls were included in each determination.

Bacterial agglutination tests were performed by adding 1 drop of a 1:2 dilution of stock bacteria to 1 drop of a serially diluted serum on a Kline agglutination slide. Macroscopic agglutination was recorded as 0 to 4+ after incubation at 37°C for 30 minutes.

4. Absorption experiments utilized rabbit anti-ovalbumin-ovalbumin immune precipitate, human antidextran–dextran immune precipitate,[3] *E. coli*, *B. subtilis*, and *Salmonella* suspensions. The quantities of absorbants are indicated in the text. After overnight incubation in the case of bacterial absorption, or 72 hours incubation at 0 to 4°C for absorptions with immune precipitates, the absorbants were removed by centrifugation and serologic studies were conducted on the supernatants.

5. Inhibition studies. A serum from an *E. coli*-immunized rabbit (No. 9) which demonstrated RFLS was used in determining the capacity of AHGG, 7S-HGG, 7S-RGG, and ARGG to inhibit agglutination. 0.25 ml of the test material was serially diluted in 0.25 ml of buffered saline and 0.25 ml of diluted No. 9 rabbit serum added to each tube. (Dilution of No. 9 serum was ¼ of the hemagglutinating titer.) After incubation at room temperature for 30 minutes, "coated" erythrocyte reagents were added to each tube and agglutination tests interpreted in the usual manner.

6. Precipitation studies. Various quantities of ARGG and AHGG were added to aliquots of a normal rabbit serum and a serum with RFLS (No. 9). (Sera were heated at 56°C for 30 minutes.) The tubes were incubated 20 hours at 4°C. The N content of the precipitates was then determined and tanned cell agglutination tests were performed on the supernatants.

7. Density gradient centrifugation. A density gradient was prepared, using 10, 20, 30, and 40 per cent concentrations of sucrose. 1 ml aliquots were carefully layered in a centrifuge tube, with the more dense solutions at the bottom. After gentle stirring of the sucrose layers, an aliquot serum was carefully layered on top of the sucrose gradient and centrifuged for 16 hours at 30,000 RPM (SW39L rotor in Spinco model L ultracentrifuge). Top, middle, and bottom fractions were removed by means of a stabilized needle and syringe and N contents, *E. coli* agglutinin titers, and FII latex titers were determined on each fraction. Aliquots of each fraction were also absorbed with *E. coli*, as described previously.

8. Mercaptoethanol treatment. Mercaptoethanol in various concentrations was added to aliquots of a serum having known hemagglutinating activity. After incubation at 4°C for 24 hours and dialysis against buffered saline, hemagglutination and *E. coli* agglutination tests were performed.

[3] Serum kindly supplied by Dr. Elvin A. Kabat.

9. Starch block electrophoresis was performed according to the method of Kunkel (15). 5 ml of serum were placed on a starch block (pH 8.6) and subjected to 20 ma of current for 45 hours. The block was cut into 1 cm strips and fractions eluted from each strip with 10 ml of normal saline. The N content was determined and tanned cell and *E. coli* agglutination tests were performed on each fraction.

10. Nitrogen determined by the Folin-Ciocalteu method (16), gamma globulin standards (nitrogen determined by Kjeldahl) being included in each series of determinations.

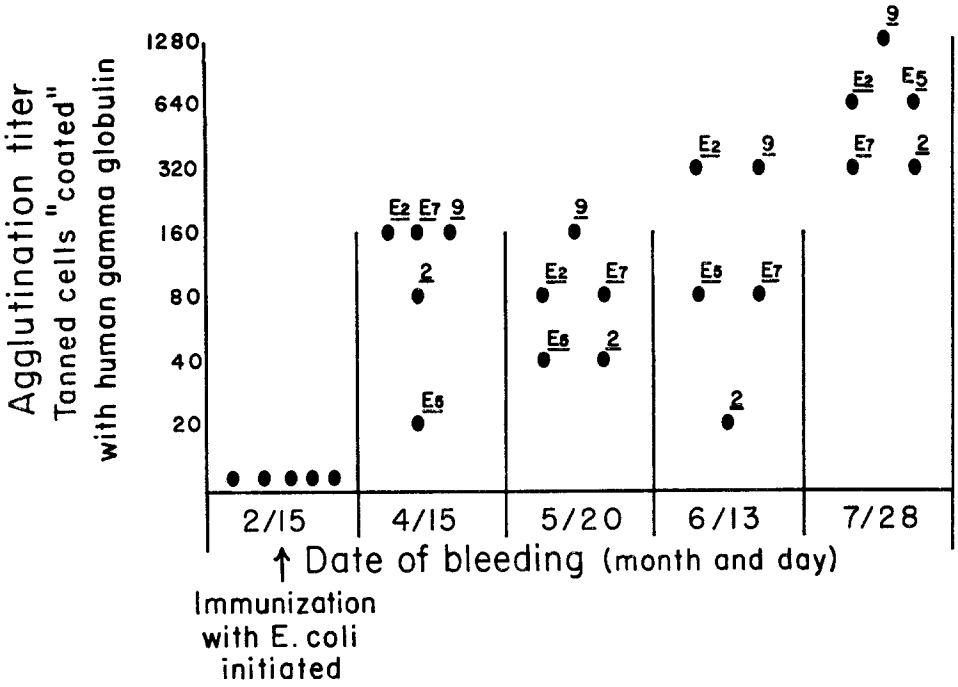

FIG. 1. Development of the rheumatoid factor-like substance (RFLS); monthly determinations of serum titers with aggregated human gamma globulin (AHGG) "coated" tanned sheep cells.

RESULTS

Fig. 1 depicts the development of the RFLS in a group of 5 rabbits immunized with *E. coli*. After 2 months of immunization, the factor could be demonstrated in all animals. This contrasts somewhat with a second group of 4 rabbits immunized with *B. subtilis* (Table I). After 5 months of immunization, only 2 rabbits had detectable RFLS. In general, the agglutination titers increased as immunization progressed. The titers in the various serological systems after 7 months of immunization with *E. coli* are illustrated in Table II. Significant agglutination titers were observed with tanned sheep cells coated with human FII, AHGG, ARGG, the FII latex fixation test, and with sensitized sheep

cells. Tanned cells coated with 7S-RGG were not agglutinated at the lowest dilution (1:20). Absorption studies are summarized in Table III. Absorption of 2 sera (E2 and No. 9) with rabbit anti-ovalbumin immune precipitate resulted in a marked reduction of the tanned cell agglutination titers but no change in the *E. coli* agglutinin titer. The addition of 1 unit of *E. coli* markedly

TABLE I

B. subtilis and rheumatoid factor-like substance (RFLS) agglutination titers in rabbits immunized with B. subtilis for 5 months

Aggregated rabbit gamma globulin (ARGG)-tanned sheep cells used for "RFLS" agglutination.

Rabbit serum	Agglutination titer	
	B. subtilis	"RFLS"
B_1	1:10,240	1:320
B_3	1:1,280	<1:10
B_4	1:20,480	1:2,560
B_5	1:1,280	<1:10

TABLE II

Demonstration of RFLS in Serum of Rabbits Immunized with E. coli Using Various Agglutination Tests

Rabbit serum, 7 mos. immunization	Tanned cell agglutinations					FII latex fixation	Sensitized sheep cell agglutinations
	"Coating materials"						
	Human Cohn FII	Non-aggregated human GG	Aggregated human GG	Non-aggregated rabbit GG	Aggregated rabbit GG		
E_2	1:80	1:20	1:160	<1:20	1:1280	1:256	1:256
E_7	1:20	<1:20	1:40	<1:20	1:40	1:8	1:32
No. 2	1:20	<1:20	1:80	<1:20	1:160	1:2048	1:128
No. 9	1:320	1:20	1:640	<1:20	1:1280	1:2048	1:512

reduced the titer of RFLS but an insignificant reduction (1 tube dilution) occurred after absorption with a comparable quantity (1 unit) of *Salmonella*. 12 units of *E. coli* lowered the *E. coli* agglutinin titer as well as the tanned cell titers. Twelve units of *Salmonella* still produced only a slight reduction in the tanned cell titers and did not affect *E. coli* agglutinin titers. Additional absorption data is presented in Table IV. 1 unit of *E. coli* again effectively removed the RFLS from an *E. coli*–immunized rabbit (E_{14}) but not from a *B. subtilis*—immunized rabbit (B_4) whereas the opposite occurred with *B. subtilis* absorption.

Absorption by a rabbit immune precipitate (ovalbumin, anti-ovalbumin) resulted in a marked reduction of both the AHGG tanned cell titer and the ARGG tanned cell titer (see Tables III and V). The addition of a human

TABLE III

Absorption Study, Demonstrating Removal of "RFLS" from 0.3 Ml Serum by an Immune Complex and E. coli

Rabbits were immunized with *E. coli*.

Rabbit serum	Agglutination system	Agglutination titers					
		Unabsorbed	Absorbed with immune precipitate*	Absorbed with *E. coli*		Absorbed with *Salmonella*	
				1 unit	12 units	1 unit	12 units
E_2	*E. coli*-anti-*E. coli*	1:10,000	1:10,000	1:10,000	1:160	1:10,000	1:10,000
No. 9		1:20,000	1:20,000	1:20,000	1:320	1:20,000	1:20,000
E_2	Cohn FII-tanned sheep cells	1:80	1:20	<1:20	<1:20	1:40	1:40
No. 9		1:320	<1:20	<1:20	1:20	1:320	1:80
E_2	Aggregated human GG-	1:320	1:20	1:20	1:20	1:160	1:160
No. 9	tanned sheep cells	1:1280	1:20	<1:20	1:40	1:640	1:320
E_2	Aggregated rabbit GG-	1:1280	<1:20	1:20	<1:20	1:640	1:160
No. 9	tanned sheep cells	1:1280	1:20	1:20	<1:20	1:1280	1:640

* Immune precipitate = ovalbumin-anti-ovalbumin (0.4 mg nitrogen).

TABLE IV

Absorption Study

Effect of absorption of sera of *E. coli* and *B. subtilis* immunized animals with respective bacteria.

Serum*	ARGG-tanned sheep cell agglutination		
	Unabsorbed	Absorbed with *E. coli*, 1 unit	Absorbed with *B. subtilis* 1 unit
E_{14} (*E. coli*–immunized)	1:1280	1:80	1:1280
B_4 (*B. subtilis*–immunized)	1:2560	1:2560	1:40

* 3 ml of 1:10 dilution of sera.

immune precipitate (dextran—anti-dextran) resulted in a marked reduction of the AHGG tanned cell titer but only a twofold reduction of the ARGG tanned cell titer (Table V).

Inhibition Studies.—Table VI indicates the relative capacities of rabbit and human gamma globulin reactants to inhibit the agglutinating property of a serum with RFLS (No. 9). Although minimal amounts of AHGG (0.03 micro-

grams N) inhibited agglutination of AHGG tanned cells, 64,000 times as much AHGG (1.922 mg) did not inhibit agglutination of ARGG tanned cells.

ARGG inhibited hemagglutinating activity in both test systems. ARGG containing 0.88 micrograms N inhibited the ARGG tanned cell reaction and

TABLE V

Absorption Study Showing "Species" Effect on Removal of RFLS with Immune Complexes

Tanned sheep cell agglutination system	Agglutination titers		
	E_2 unabsorbed	E_2 absorbed with human immune precipitate*	E_2 absorbed with rabbit immune precipitate‡
Aggregated rabbit gamma globulin–"coated"	1:1280	1:640	1:20
Aggregated human gamma globulin–"coated"	1:640	1:20	1:10

* Dextran-antidextran precipitate (0.36 mg nitrogen).

‡ Ovalbumin-anti-ovalbumin precipitate (0.36 mg nitrogen).

TABLE VI

Inhibition of RFLS with Human and Rabbit GG, Demonstrating the Effect of Aggregation and Species Differences

Agglutination system	Minimum quantity (micrograms N_2) required to inhibit 4 units of rabbit hemagglutinating factor (No. 9)			
	Human gamma globulin		Rabbit gamma globulin	
	Nonaggregated	Aggregated	Nonaggregated	Aggregated
Aggregated human GG–"coated" tanned cells	17.5	0.03	No inhibition at 1,800.0	14.1
Aggregated rabbit GG–"coated" tanned cells	No inhibition at 1,115.0	No inhibition at 1,922.0	No inhibition at 1,800.0	0.88

ARGG containing 14.1 micrograms N blocked AHGG tanned cell agglutination.

Inhibition of agglutination of tanned cells coated with either AHGG or ARGG was not observed with 7S-RGG. 7S-HGG failed to inhibit ARGG-coated tanned cells, but in relatively high concentration inhibited agglutination of AHGG-coated cells.

Precipitation Studies.—Fig. 2 illustrates precipitin studies in which AHGG and ARGG were added to No. 9 rabbit serum with RFLS and a serum from a non-immunized rabbit. The quantity of precipitate formed in the immunized

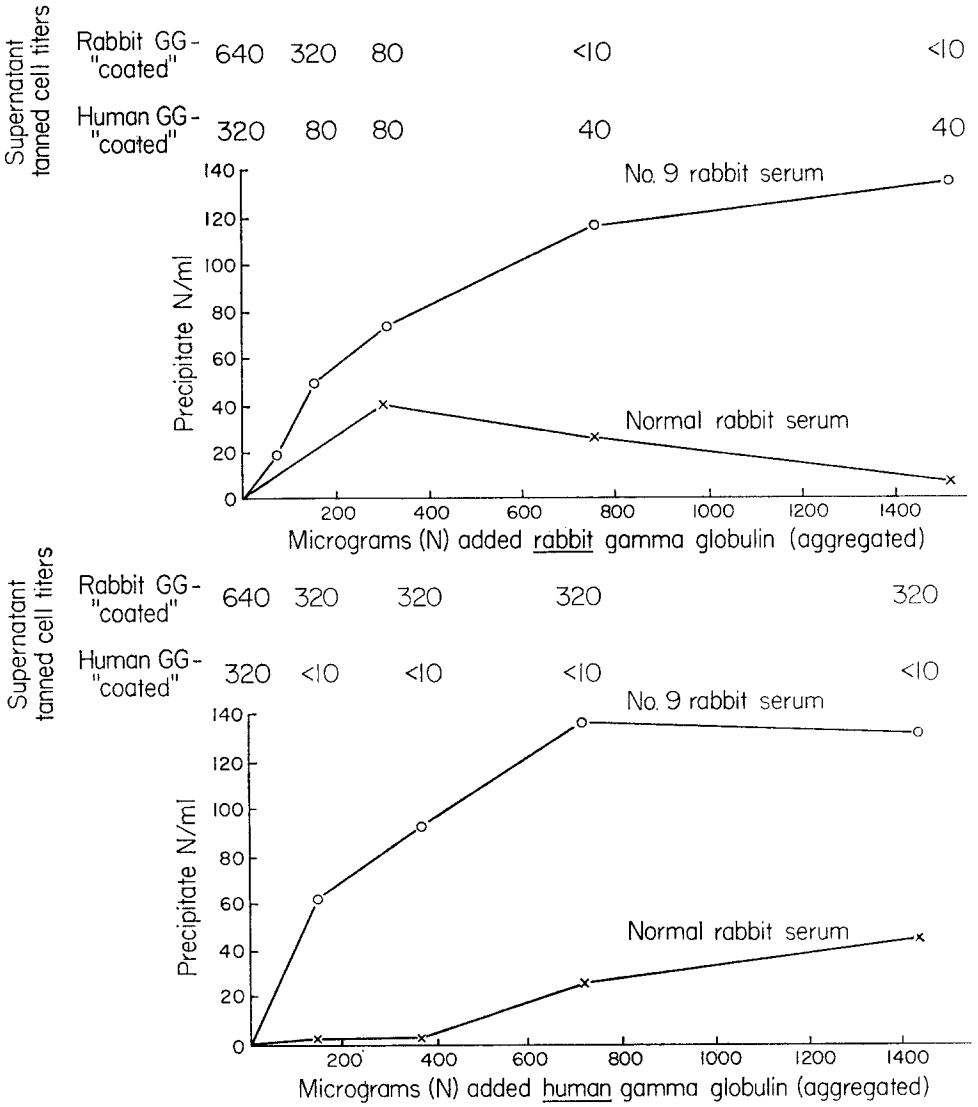

FIG. 2. Precipitin studies. Effect of aggregated gamma globulins (human and rabbit) added to normal rabbit serum and a serum with RFLS (No. 9). Figures at the top of each graph represent supernatant titer.

rabbit was greater than that formed in the non-immunized (control) rabbits at every concentration of added aggregated gamma globulin. Serological studies of the supernatants resulting from the addition of ARGG demonstrated a substantial reduction of titers in both systems (ARGG- and AHGG-coated

tanned cells). In contrast, supernatants of the serum after precipitation with AHGG retained agglutinating activity with ARGG-coated cells (1 tube drop in titer—1:640 to 1:320) but failed to agglutinate AHGG-coated cells.

Density Gradient Centrifugation Studies.—Table VII summarizes an experiment in which rabbit serum 9 was subjected to density gradient centrifugation and separated into 3 fractions (top, middle, and bottom). The *E. coli* agglutinins were concentrated in the top and middle fractions while the RFLS (FII latex agglutination) was contained in the bottom fraction. When agglutination tests were repeated after absorption with 1 unit of *E. coli*, the bottom fraction, which contained minimal *coli* agglutinins, demonstrated a reduction of FII latex titer from 1:640 to 1:320. Comparable absorption of whole serum resulted in removal of the FII latex agglutinating property.

TABLE VII

Separation of RFLS from E. coli Agglutinins by Preparative Centrifugation

Density gradient fraction	Nitrogen mgm/ml	Unabsorbed		Absorbed with *E. coli*	
		E. coli titer	FII latex titer	*E. coli* titer	FII latex titer
Top	1.79	1:2560	<1:20	—	—
Middle	1.76	1:5120	<1:20	1:80	<1:20
Bottom	0.48	1:320	1:640	<1:20	1:320
Whole serum (Rabbit 9)	—	1:20,480	1:160	1:5120	<1:20

Mercaptoethanol Studies.—Table VIII illustrates the results obtained when various concentrations of mercaptoethanol were added to aliquots of serum (E_2) containing the RFLS. The ARGG and the AHGG tanned cell titers both decreased after the addition of 0.01 M mercaptoethanol (the lowest concentration used). The *E. coli* agglutinin titer remained unchanged.

When the concentration of mercaptoethanol in the serum was increased to 0.2 M, the ARGG and AHGG titers were both < 1:20, while the *E. coli* titer decreased from 1:10,240 to 1:5,120.

Starch Block Electrophoresis.—Analysis of the eluted fractions of No. 9 serum provided the data shown in Fig. 3. The peak titers of *E. coli* agglutinins were present in fractions −1 to −7 (cathodal migration). The highest titers of ARGG tanned cells and AHGG tanned cells were both contained in fraction −1 to +4, corresponding to the more rapidly anodal migrating gamma globulin and beta globulin range. Nitrogen concentrations of the eluants are plotted in the top graph of the figure.

DISCUSSION

Rabbits immunized for several weeks with formalin-killed bacteria developed a serum factor which, in properties so far studied, resembled the human rheu-

matoid factor (RF). The rabbit rheumatoid factor-like substance (RFLS) was a *heat-stable* (56°C)[4] protein which migrated electrophoretically in the gamma globulin to beta globulin range (Fig. 3). In density gradient centrifugation studies, the RFLS sedimented more rapidly than antibacterial antibodies (Table VII). Other indirect evidence for the macroglobulin nature of the RFLS derived from observations that mercaptoethanol, in concentrations that did

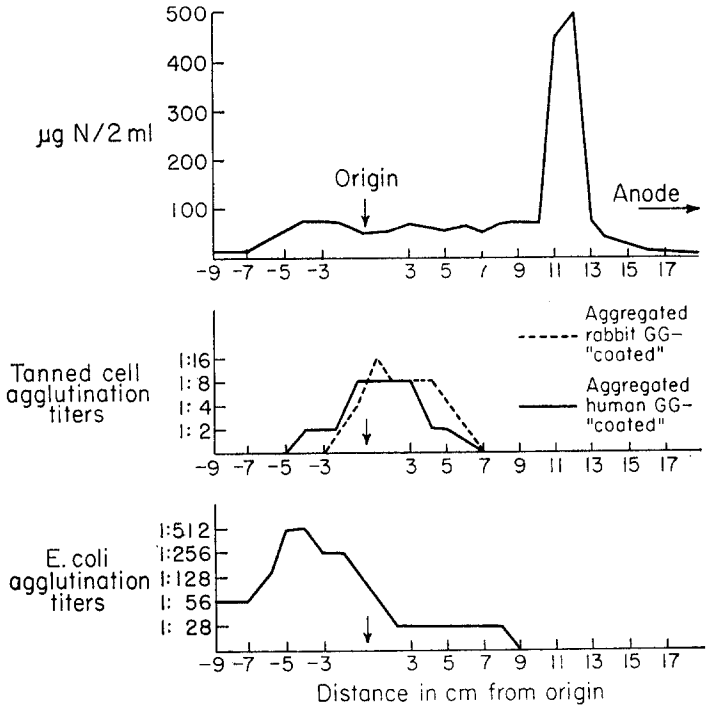

FIG. 3. Starch block electrophoresis showing migration of the RFLS (center illustration) as a beta or gamma globulin. Migrations of the *E. coli* antibodies are shown in the bottom illustration. (Anode to the right).

not affect agglutination of bacteria by antibacterial antibodies, destroyed the RFLS (Table VIII). The RF, like other human macroglobulins of the 19S class of globulins, is reduced to smaller fragments, with resultant loss of serological activity, by a variety of sulfhydryl reagents (17, 18). The RFLS agglutinated FII-"coated" latex, FII-"coated" tanned sheep cells, and sensitized sheep cells; and, like the human RF, precipitated with aggregates of human or rabbit gamma globulin.

[4] A heat-labile factor in normal sera, probably related to the complement system, shares some serological properties with the human RF (19–21).

The RFLS was removed by absorption with immune complexes formed from human and rabbit antiserum (immune complexes included: dextran–human antidextran, bovine serum albumin–rabbit anti-BSA, ovalbumin–rabbit antiovalbumin). Immune complex formation between bacterial and antibacterial antibodies appeared to mediate the removal of RFLS in bacterial absorption studies. *E. coli* removed the RFLS from the serum of an *E. coli*–immunized rabbit but did not affect the titer of serum from a *B. subtilis*–immunized animal, and the reverse was noted with absorption of the sera with *B. subtilis* (Table IV). The RFLS in a preparation with a low titer of *E. coli* agglutinins was incompletely removed by the addition of a quantity of *E. coli* which effectively absorbed the RFLS from whole serum (Table VII).

TABLE VIII

Effect of Various Concentrations of Mercaptoethanol on the RFLS and E. coli Agglutinins

Concentration of mercaptoethanol in treated serum (E_1)	Hemagglutination titer "tanned cell system"		*E. coli* agglutination titer
	Aggregated human GG–"Coated"	Aggregated rabbit GG–"Coated"	
None	1:320	1:1,280	1:10,240
0.01 Molar	1:80	1:640	1:10,240
0.025 Molar	1:40	1:160	1:10,240
0.05 Molar	<1:20	1:40	1:10,240
0.2 Molar	<1:20	<1:20	1:5,120

Studies of (*a*) hemagglutination inhibition by ARGG and AHGG (Table VI), (*b*) precipitation induced by ARGG and AHGG and serological analysis of supernatants (Fig. 3), and (*c*) differential absorption with rabbit and human immune precipitates (Table V) all demonstrate a consistent pattern with reference to species specificity of the RFLS. Aggregates of rabbit GG (either ARGG or rabbit immune complexes) absorbed completely the agglutinating activity for cells "coated" with ARGG or AHGG. Absorption with human gamma globulin aggregates, however, removed only agglutinating activity for AHGG-"coated" cells, leaving in the supernatant agglutinins for ARGG-coated cells. These data suggest that the RFLS has specificity directed primarily against rabbit gamma globulin with cross-reactivity for human gamma globulin. The reverse relationship applies to the human RF and its reactions with human and rabbit gamma globulins. Absorption of rheumatoid sera with rabbit immune complexes (immune precipitates or sensitized sheep cells) removes sensitized sheep cell agglutinins but leaves activity for serological systems utilizing human reactant gamma globulin (22–24).

The development of the RFLS in the present study is clearly not a consequence of immunization against gamma globulin of heterologous or homologous

origin, nor could it be attributed to antibody production specifically against intrinsic antigens of the bacteria utilized for immunization. Rather, it is suggested that, during the course of prolonged immunization the continued formation and elimination of immune complexes stimulates the production of a factor (RFLS) whose specificity is directed against the host's own gamma globulin in the aggregated state (either induced by immune reaction or by physical treatment). The concept of specificity against "immunologically denatured" gamma globulin was suggested by Milgrom (25) for certain human and rabbit sera that react with sensitized cells and by Coombs (26) for complement reactants in the immunoconglutinin system. It is possible that immune aggregation exposes "new" antigenic determinants on the gamma globulin molecule. In the present studies, the development of the RFLS paralleled the duration of immunization and the intensity of the immune response to bacterial antigens. All animals that received *E. coli* injections for 8 weeks or more developed the factor. *B. subtilis*, a poor antigen in contrast to *E. coli*, induced detectable RFLS only in animals that demonstrated bacterial agglutinins in high titer (Table I).

The RFLS in the present study closely resembles that studied by Aho[5] and possibly by Eyquem but its properties differ uniquely from other experimentally induced serum factors. Rabbits immunized with streptococci or the host's own gamma globulin in Milgrom and Witebsky's studies, developed a serum factor with primary specificity for human gamma globulin which reacted poorly with rabbit gamma globulin (5). Furthermore, the requirement for aggregation of reactant gamma globulin was not apparent in their studies. Precipitin bands, with gel diffusion techniques, were obtained with native human gamma globulin.[6] When rabbits were immunized with bacteria agglutinated by homologous antisera, the induced serum factor, which agglutinated sensitized sheep cells could be absorbed by the sensitized bacteria utilized for immunization but not by a different immune complex (2). The possibility that these observations related to homologous immunization with different allotypic gamma globulins was subsequently suggested by one of the authors (27). In the present studies, the RFLS was absorbed by any rabbit immune complex, regardless of the antigen or the source of rabbit antibody.

The presence of the RFLS in hyperimmune sera of any type would be expected to participate in quantitative precipitin analyses, unless the antisera were first absorbed with immune precipitate from an unrelated immune system.

[5] A possible difference concerns reactivity of the RFLS in the sensitized Rh cell system. We were unable to evaluate this in our study because the sera containing RFLS agglutinated unsensitized as well as sensitized Rh"+" cells. After absorption of the sera with Rh"+" cells, there was no agglutination of either sensitized or unsensitized cells.

[6] In our studies, rabbit sera with the RFLS sometimes formed hazy diffuse zones of precipitate with aggregated gamma globulin reactants but did not visibly react with native gamma globulins.

Thus, the RFLS may represent in part the co-precipitating factors in immune sera.

The similarity of the RFLS to RF is apparent. Both factors are heat-stable macroglobulins whose serological specificities are directed against gamma globulin in an aggregated state. Studies of species specificity are consistent: the rabbit RFLS is incompletely absorbed by human immune aggregates, and the human RF is incompletely absorbed by rabbit immune aggregates. The rabbit RFLS appears to result from prolonged immunization; the genesis of the human RF is not known. In this respect, however, it is interesting that the human RF has been associated with a variety of non-rheumatoid states such as leprosy (28), kala azar (29), syphilis (30), pulmonary tuberculosis (31), chronic liver disease (32), and sarcoidosis (29). In at least some of these states, a sustained immunologic response to the antigens of micro-organisms is likely.[7] Thus, the old question of an infectious etiology for rheumatoid arthritis, though lacking support from microbiological studies, might gain some emphasis from a clearer understanding of the RF. The possibility that the RF is a host response to sustained contact with an environmental antigen, living agent or otherwise, deserves consideration.

Consideration should be given to possible genetic factors which might operate in the development of the RF. Two studies have indicated that a higher than normal incidence of the RF occurs in relatives of rheumatoid subjects (33, 34). Patients with rheumatoid arthritis might possess a defect in some, as yet uncharacterized facet of host resistance which impedes elimination of antigenic substances, favoring a sustained antigenic stimulus. (In agammaglobulinemia a diminished or absent capacity to form serum antibodies exists, in the face of seemingly normal mechanisms for the cellular immune response; *i.e.*, delayed hypersensitivity (35).) In this respect, it is of interest that some interrelationship may exist between agammaglobulinemia, rheumatoid arthritis, and the RF. Fudenberg and German (36) and Rodnan (37) have noted a higher than expected incidence of rheumatoid arthritis and the RF in relatives of patients with agammaglobulinemia, and a chronic seronegative arthritis has been observed in as many as one-third of subjects with agammaglobulinemia (38–40).

There has been no discussion of the questions: (a) is the induced RFLS antibody in the classical sense, and (b) if it is auto-antibody against the host's own gamma globulin, does it (or its human counterpart, the RF) in any way mediate disease processes. Answers to these questions are equivocal. One could consider the RFLS as complement-like rather than antibody-like. Rabbits with high titers of RFLS have not demonstrated gross or pathologic

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[7] One patient has been encountered by the authors with subacute bacterial endocarditis (*Streptococcus viridans*) who demonstrated positive rheumatoid serological reactions.

features of arthritis. (Lesions so far identified are compatible with chronic serum sickness.)

SUMMARY

Hyperimmunization of two groups of rabbits with killed *Escherichia coli* and *Bacillus subtilis* has resulted in the formation of a serum factor (RFLS) which resembled the human rheumatoid factor. It was a heat-stable protein that migrated electrophoretically in the gamma-beta globulin range and sedimented rapidly with ultracentrifugation. The serologic properties of the RFLS were destroyed by mercaptoethanol. It reacted primarily with rabbit gamma globulin in an aggregated state (immune complexes or physically aggregated gamma globulin) and demonstrated cross-reactivity with human gamma globulin.

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