many mammals this is a low value and indicates the ability of chinchillas to survive at high altitudes or in other hypoxic surroundings. Some animals appear to be limited to narrow ranges of environmental oxygen tensions, while others survive and successfully adjust to wider ranges. Chinchillas are in the latter group.

One cannot say whether animals have acquired these characteristics as a consequence of residence for hundreds of generations at high altitude or whether they found high altitude tolerable and advantageous because of these characteristics. It seems plausible to suppose that natural selection has been operating to the end that animals may acquire blood with an optimal position of the oxygen dissociation curve, one that yields an adequate supply of oxygen to the tissues under the limitations imposed by their external environment.

Summary. The respiratory functions of the blood of chinchillas *(Chinchilla laniger)* has been studied. Measurements of hematocrit, number of erythrocytes, and hemoglobin concentration have been made. The position of the oxygen dissociation curve has been established. This is to the left of most other rodents of similar size indicating an adaptation to high altitudes. The Bohr effect and the Haldane effect are similar to other closely related mammals.

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Reverse Passive Cutaneous Anaphylaxis with Guinea Pig 7S Antibodies.* (30380)

JOEL S. SPALTER AND ZOLTAN OVARY†

Department of Pathology, New York University School of Medicine, New York City

Guinea pigs can be sensitized for reverse passive cutaneous anaphylaxis (RPCA) reactions with rabbit or human gamma globulins (1,2,3).

The challenging antibody can be from any species, including those which do not sensitize guinea pigs for direct anaphylactic reactions, provided that the antigen is able to sensitize (to "fix" to guinea pig tissues).

Recently, it was shown that guinea pigs can produce 2 types of 7S antibodies(4,5,6); a faster moving gamma 1 capable of sensitizing guinea pigs for PCA reactions but unable to fix complement *in vitro,* and a slower moving gamma 2 antibody unable to sensitize guinea pigs for anaphylactic reactions but capable of fixing complement.

It seemed of interest to investigate these two 7S antibodies from the same species in RPCA reactions. Antibodies specific for the 2,4-dinitrophenyl determinant (DNP) were used for these experiments.

Materials and methods. Passive cutaneous anaphylaxis (PCA) and reverse passive cutaneous anaphylaxis (RPCA). PCA and RPCA were carried out as previously described(7).

Passive lysis. Passive lysis was carried out as described in(6). In brief, sheep erythrocytes were tanned(8) and coated with DNP-bovine serum albumin (DNP-BSA). Antibody preparations were diluted in veronal buffer according to(9). Lyophilized guinea pig complement (Certified Blood Donor Serv-

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† Health Research Council Career Scientist.

ice, Inc., Jamaica, N. Y.) twice absorbed with normal sheep erythrocytes was diluted 1/15 in veronal buffer. Experimental tubes contained 0.5 ml of antibody dilution, 0.1 ml of a 1% suspension of cells in veronal buffer, and 0.1 ml of diluted complement. Results were estimated by direct inspection and recorded as follows: 4+ complete lysis, no turbidity; 3+ slight turbidity; 2+ obvious lysis but definite turbidity; 1+ small reduction in turbidity; 0 no lysis. All lysis experiments were done in duplicate. The titer of a preparation was considered to be the greatest dilution in which both tubes gave lysis of 2+ or more. Results were read after 30 minutes' incubation of tubes in a 37°C water bath.

Immunoelectrophoresis. Immunoelectrophoresis was carried out as previously described(4,10).

Antigens. Gamma globulins from normal rabbit serum were precipitated in 33% saturated ammonium sulfate(11). The precipitate was dissolved in distilled water and extensively dialyzed against 0.1 M phosphate buffer, pH 7.5. The gamma globulins were digested with papain by the method of Porter (12). Crystalline fragment III (Fc) was obtained, free of contaminant by immunoelectrophoretic analysis. This preparation of fragment III was coupled as previously described(13) to dinitrophenyl sulfonic acid (DNPNS) and a preparation with 2 DNP groups per molecule (G/M) of protein was made. Bovine gamma globulin (BGG, Armour, Lot X30604), rabbit gamma globulin (RGG, Pentex R211), bovine serum albumin (BSA, Armour, Lot W69312), and bovine fibrinogen (Armour, Lot no. W5904), were similarly coupled with DNPNS. The following preparations were made: DNP-BGG, 29 G/M; DNP-RGG, 9 G/M; DNP-BSA, 29 G/M; DNP-bovine fibrinogen, 97 G/M.

Antibodies. Albino Hartley strain guinea pigs were immunized with the DNP-BGG (29 G/M). Each animal received a total volume of 0.5 ml of emulsion made from equal volumes of Freund's complete adjuvant and DNP-BGG, 2 mg/ml dissolved in saline (500 γ of antigen/animal). The immunizing material was divided among the 4 footpads. On the 16th and 24th days following the initial immunization, each animal was boosted with 250 γ of DNP-BGG in 0.5 ml of saline. The boosting material was divided into 4 intradermal injections. On the seventh day following the second boosting, the animals were exsanguinated.

The antibody content of these sera was estimated by both passive cutaneous anaphylaxis (PCA) with DNP-BSA as challenging antigen and by passive lysis of DNP-BSA coated tanned sheep erythrocytes. Two sera which showed both PCA and passive lytic activities at dilutions of greater than 1/5000 were pooled and used as sources of gamma 1 (γ_1) and gamma 2 (γ_2) anti-DNP antibodies.

Separation of γ_1 and γ_2 antibodies. To 12 ml of this guinea pig anti-DNP-BGG pool were added 8 ml of saturated ammonium sulfate to give a final ammonium sulfate concentration of 40% of saturation. The gamma globulins so precipitated were dissolved in 5 ml of distilled water and extensively dialyzed against .005 M phosphate buffer, pH 8. The dialysis bath was changed twice daily until the pH and conductivity of the dialysis bath, after equilibration for 12 hours, were equal to the pH and conductivity of the original buffer.

A diethylaminoethyl (DEAE, Schleicher & Schuell Co., Lot 1346) column 2 cm in diameter and 10 cm in height was packed and flushed with .005 M phosphate buffer, pH 8, until the pH and conductivity of the affluent and effluent buffer were equal. The dialyzed antibody preparation was applied to the column and eluted first with .005 M phosphate buffer, pH 8. Five ml aliquots were collected at a rate of 20 minutes per aliquot until the optical density (OD_{280}) of the aliquot was less than .20. The column was then flushed with a rapid flow of about 2000 ml of .005 M phosphate buffer, pH 8. Those aliquots which had passive lysis activity at a dilution of 1/100 and no PCA activity at a dilution of 1/5 were pooled and considered to be pure gamma 2.

Following flushing, 5 ml aliquots were similarly eluted with .15 M phosphate buffer, pH 8. Those aliquots which had PCA activity at a dilution of 1/100 but no passive lysis

TABLE I. PCA Reactions with Gamma 1 and Gamma 2 Globulins.

Sensitizing antibody preparation*	Dilution	Reactions of 4 guinea pigs† 1	2	3	4
γ1	1/25	12	16	20	18
γ1	1/50	6	12	15	10
γ1	1/100	Tr‡	10	15	10
γ1	1/200	0	Tr	10	10
γ1	1/500	0	0	6	Tr
γ2	1/5	0	0	0	0

* 0.1 ml injected into each skin site.
† Challenging antigen was 200 γ/guinea pig of DNP-RGG, 9 G/M. The results tabulated are the reaction diameters in mm.
‡ Tr = trace.

TABLE II. Passive Hemolysis with Gamma 1 and Gamma 2 Globulins.

Antibody preparation	Dilution	Lysis*
γ1	1/2	0
γ2	1/100	4+
γ2	1/200	2+
γ2	1/400	0

* The meaning of numbers is given in text.

activity when diluted 1/2 were pooled and considered to be pure gamma 1.

The purity of the gamma 1 and gamma 2 globulin preparations was confirmed by immunoelectrophoresis.

A second specimen of the pooled guinea pig anti DNP-BGG serum with passive lysis and PCA activities at dilutions of 1/5000 was similarly precipitated with 40% ammonium sulfate, dialyzed, and applied to a DEAE column. The gamma 2 globulins were eluted as from the first column and those aliquots with passive lysis titer of 1/400 and no PCA activity at a dilution of 1/4 were pooled and considered to be pure gamma 2.

This pool was concentrated by pressure dialysis and titrated by the quantitative microprecipitin reaction of Heidelberger and Kendall(14) with DNP-bovine fibrinogen, 97 G/M, as antigen. The concentrated material was also titrated for passive lysis against DNP-BSA coated tanned sheep erythrocytes.

Results. The results of a typical experiment to elicit direct PCA with the pure gamma 1 and pure gamma 2 globulins are shown in Table I.

Table II shows the results of a typical passive lysis experiment with these preparations. No lysis was obtained in the absence of antibody nor when a 1/10 dilution of the gamma 2 preparation was used with tanned sheep erythrocytes which had not been coated with DNP-BSA.

Table III shows the results of a typical experiment to elicit RPCA with a challenge of the pure gamma 1 or gamma 2 globulin preparations.

Table IV shows results of a typical experiment to elicit RPCA with a challenge of a pure gamma 2 preparation. This preparation gave 4+ lysis at a dilution of 1/400 and no lysis at a dilution of 1/800. The skin sensitizing antigens in these experiments were DNP-fragment III (DNP-Fc, 2 G/M) and DNP-RGG (9 G/M).

The gamma 2 preparation concentrated by pressure dialysis gave 4+ lysis of DNP-BSA coated tanned sheep erythrocytes at a dilution of 1/3200 and no lysis at a dilution of 1/4000. The antibody content of this con-

TABLE III. RPCA Reactions with Gamma 1 and Gamma 2 Globulins

Sensitizing material*	Concentration	Guinea pigs†									
DNP-RGG	200 γ/ml	25[1]	25[1]	25[1]	25[2]	0[2]	0[2]	22[3]	25[3]	30[3]	22[3]
"	100 γ/ml	20	25	25	20	0	0	20	17	22	18
"	50 γ/ml	15	20	25	15	0	0	20	12	18	17
"	20 γ/ml	16	15	20	10	0	0	20	14	20	18
"	10 γ/ml	15	15	15	10	0	0	18	14	15	18
RGG	200 γ/ml	0	0	0	0	0	0	0	0	0	0
RGG	100 γ/ml	0	0	0	0	0	0	0	0	0	0
Saline		0	0	0	0	0	0	0	0	0	0

* 0.1 ml injected into each skin site. † The tabulated results are reaction diameters in mm.
[1] Challenged with 1.0 ml of gamma 1 preparation. [2] Challenged with 1.0 ml of gamma 2 preparation (1/200 passive lysis titer). [3] Challenged with 2.0 ml of gamma 2 preparation (1/200 passive lysis titer).

TABLE IV. RPCA Reactions with Gamma 2 Globulins.

Sensitizing material*	Concentration	Guinea pigs†			
DNP-Fc	250 γ/ml	20	25	20	25
"	125 γ/ml	18	25	17	25
"	50 γ/ml	17	22	15	22
"	25 γ/ml	16	20	16	20
"	10 γ/ml	14	20	Tr‡	18
"	5 γ/ml	8	15	0	16
DNP-RGG	50 γ/ml	18	22	18	22
"	20 γ/ml	12	16	10	20
"	10 γ/ml	10	17	0	18
Fc	500 γ/ml	0	0	0	0
"	250 γ/ml	0	0	0	0

* 0.1 ml injected into each skin site.
† Challenge is with 1.0 ml of gamma 2 preparation (1/400 passive lysis titer). Tabulated results are reaction diameters in mm.
‡ Tr = trace.

centrated material by the quantitative microprecipitin reaction was 750 γ antibody protein/ml.

Discussion. A preparation of pure gamma 1 globulins from guinea pig anti-DNP-BGG sera having no lytic activity at a dilution of 1/2 against DNP-BSA coated tanned sheep erythrocytes and preparations of pure gamma 2 globulins from the same guinea pig sera, having no PCA activity at dilutions of 1/4 and 1/5, have been shown capable of eliciting RPCA in guinea pigs whose skin has been sensitized with from one to 20 gamma of DNP-RGG and from 0.5 to 25 gamma of DNP-Fc. No reactions were elicited when sensitization was with non-conjugated RGG (10 or 20 γ per site) or with non-conjugated Fc (25 or 50 γ per site).

For elicitation of RPCA with a challenge of pure gamma 2 globulins, 2 ml of material with a passive lysis titer of 1/200, or one ml of material with a passive lysis titer of 1/400, are required to give positive results in a high percentage of animals (Tables III and IV).

The gamma 2 preparation concentrated by pressure dialysis had a lysis titer of 1/3200. The quantitative precipitin reaction showed this concentrated material to contain 750 γ of antibody per ml.

Thus approximately .23 γ of antibody per ml $\frac{750\,\gamma}{3200}$ suffice to elicit passive lysis of DNP-BSA coated tanned erythrocytes with the conditions used.

RPCA reactions were obtained with 1 ml of a preparation with 1/400 lysis titer, therefore approximately 94 γ of gamma 2 anti-DNP antibody suffices to elicit RPCA with the sensitization method and the preparations employed.

Summary. Dinitrophenylated rabbit gamma globulin, 9 groups per molecule of protein, and dinitrophenylated Fc, 2 groups per molecule of protein, are capable of sensitizing guinea pig skin for reverse passive cutaneous anaphylaxis. Gamma 2 anti-dinitrophenyl antibodies can elicit reverse passive cutaneous anaphylaxis in guinea pigs sensitized with as little as 0.5-1.0 γ of antigen per skin site. Approximately 90-100 γ of gamma 2 antibody suffices to give positive results in most animals. Gamma 1 anti-DNP antibodies can not only sensitize guinea pigs for PCA but are able to elicit RPCA reactions.

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