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Preparation of Rhinovirus Antisera in the South American Hystricomorph Rodent, *Octodon degus*[1] (38507)

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(Introduced by Warren Stinebring)

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Rhinovirus antisera have been prepared in many animals, including guinea pigs, rabbits, dogs, goats, baboons and cattle (1). The guinea pig has been chosen by the Research Resources Branch, NIAID, NIH, as the animal of choice for preparing rhinovirus reference antisera because of its ability to produce high titered homologous antisera with few detectable heterotypic reactions, and because of the relative ease with which these animals can be obtained and maintained in the laboratory. They have proved, for the most part, to be highly acceptable. There have been instances, however, when immunization with certain antigens produced low-titered antisera. For this reason, and because of the availability in our laboratory of a new experimental rodent, the South American hystricomorph *Octodon degus* (degu), we undertook to explore this new source for the production of rhinovirus antisera.

The degu is a rodent found in abundance in the lower altitudes of the Andes Mountains of Central Chile. Its nonaggressive behavior, size (approximately that of the rat), adaptability to animal care facilities and acceptable breeding capability led to the establishment of a colony of approximately 500 animals at the University of Vermont. The most interesting characteristic of this animal, and the feature which initially led us to try to produce rhinovirus antisera in it, was the discovery that it contains two distinct thymus glands, one in the mediastinum and one in the neck region (cervical thymus) (Boraker and Taylor, in preparation). While the mediastinal thymus undergoes the usual atrophy and infiltration by fatty tissue commonly seen in the older adult human and in murine rodents commonly employed in thymus investigations, the degu's cervical thymus retains its size and structural architecture throughout life.

Antisera to several rhinovirus serotypes, including one for which high titered sera have been particularly difficult to prepare, were prepared simultaneously in guinea pigs and degus. The results of this study follow.

Materials and Methods. Animals. A pedigreed colony of approximately 500 degus has been maintained at the Animal Care Facility, University of Vermont, since 1970. The animals are housed in rat-sized pens, and are fed Purina lab chow and water *ad libitum*. Offsprings are weaned at approximately 8 wk of age; the animals used in this study were approximately 6–12 mo old.

Antigens. Seed antigens for rhinoviruses 7, 9, 26, 32 and 67 were obtained from Research Resources Branch, NIAID, NIH. Dr. J. H. Schieble of the California Department of Public Health kindly supplied the RV87 seed antigen. Antigen pools were prepared in our laboratory in WI-38 cells using purified seed antigens. Antigen pool titers are noted in Table I.

Preparation of antisera. Antisera for each rhinovirus were prepared simultaneously in guinea pigs and degus using the same antigen pool and the same method. Animals were given two intramuscular inoculations of a mixture of 1 ml of purified antigen plus 1 ml of Freund's complete adjuvant 6 wk apart. They were bled out 1 wk following the second inoculation. Guinea pigs were obtained from Canadian Breeding Farm, St. Constant, Quebec, while degus were obtained from our colony.

Serum antibody assay. Neutralization test methods used to assay sera for antibody have been described (2). All testing was done in tube cultures of WI-38 cells rolled at 33°.

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Antigens used in homologous and heterologous testing were supplied by Research Resources Branch, NIAID, NIH. All guinea pig and degu sera were collected individually, kept separated, and assayed individually.

Results. Results are summarized in Table II. Initially two rhinoviruses were randomly chosen (RV7 and RV26) and sera prepared. There was no apparent difference between the titers of the RV7 antisera prepared in guinea pigs and degus. The RV26 antisera prepared in degus, however, were of higher titer than those prepared in guinea pigs. No heterologous antibodies were detected in any of the sera using the 89 classified rhinovirus serotypes (3, 4).

Four additional antigens were then selected, RV9, RV32, RV67 and RV87, and antisera prepared in guinea pigs and degus. Results are summarized in Table II. It can be seen that RV9 and RV32 antisera prepared in degus have somewhat higher titers than those prepared in guinea pigs. The RV67 and RV87 prepared in the degu are clearly of much higher titer than those prepared in the guinea pig. The only heterologous antibodies detected using the 89 classified rhinovirus serotypes were between RV9 and RV32 and these were seen in both degu and guinea pig antisera (Table III). This cross relationship has been reported previously (5).

TABLE I. ANTIGENS USED TO IMMUNIZE GUINEA PIGS AND DEGUS.

Rhinovirus	Titer/ml[a]
7	5.5
9	5.0
26	5.5
32	5.0
67	5.0
87	3.5

[a] $TCID_{50}$/ml in WI-38 cells.

Discussion. Preparation of rhinovirus reference antisera in guinea pigs has proved to be quite satisfactory. The antisera are usually of high titer and free of heterologous antibody. There are instances, however, when it is difficult to make high titered serum to certain antigens. One such antigen is RV87.

The degu, a South American rodent with two distinct thumus glands, one of which retains its size and structural architecture throughout life, presented itself as a potentially superior animal in which to prepare high titered antisera. In comparing antisera prepared in guinea pigs and degus using the same method and antigen pool, it has been shown that degu antisera are at least as potent and in several instances clearly superior to the same antisera prepared in guinea pigs. No differences in specificity have been found.

There are at least two drawbacks to using the degu as a source of antisera production. One is the relative lack of availability of the animal compared to that of the guinea pig. This could certainly be remedied if the animals proved useful. The second is that the amount of blood obtained when the degu is exsanguinated (7–10 cc) is considerably less than that obtained from the guinea pig

TABLE II. RHINOVIRUS ANTISERA PREPARED IN GUINEA PIGS AND DEGUS.

	Sera	
Virus[a]	Guinea pig	Degu
RV 7	240, 440, 640[b, c]	120, 120, 640, 960
RV 9	60, 160	160, 160, 240, 640
RV 26	<20, 120, 160	240, 960, 1920, 1920
RV 32	160, 240, ≧640, ≧640	≧640, ≧640, ≧640
RV 67	120, 160, 160	480, ≧640, ≧640, ≧640
RV 87	<10, 30, 40, 80	320, 480, 1280, 2560, 2560, 5120, 5120

[a] 50–100 $TCID_{50}$ used in test.
[b] Reciprocal of the neutralization end point.
[c] Each titer represents a single animal.

TABLE III. NEUTRALIZATION CROSS REACTIONS BETWEEN RHINOVIRUS TYPES 9 AND 32.

	Antisera prepared in vermont			
Virus[a]	RV 9		RV 32	
	Guinea pig	Degu	Guinea pig	Degu
RV 9	160[b, c]	640	15	10
RV 32	<10	10	≧640	≧640

[a] 50–100 $TCID_{50}$ used in test.
[b] Reciprocal of the neutralization end point.
[c] Sera from single animal.

(20–30 cc). Nevertheless, the animal should prove useful in preparing high titered antisera in special instances, such as RV87, when it is difficult to make potent antisera in other laboratory animals.

Summary. Rhinovirus antisera have been prepared for rhinoviruses (RV) 7, 9, 26, 32, 67 and 87 in guinea pigs and in degus. Titers achieved were either similar in the 2 animals (RV7), somewhat higher in the degu (RV9 and RV32) or clearly higher in the degu (RV26, RV67 and RV87). Specificity of the antisera was similar in both animals. In special instances where it is difficult to prepare high-titered rhinovirus antisera in the guinea pig, the degu offers an attractive alternative source.

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