TABLE III. Incidence of Neutralizing Antibodies in Adult Bovine Sera to Bovine Virus #19.

No. tested	Serum dilution *vs* 100 TCD		Total positive
	1:16+	1:4	
40	Pos 35 (88%)	Pos 5 (12%)	40 (100%)

vine #10 has. Dr. James Gillespie, Veterinary Virus Research Inst., Cornell Univ., using our fourth bovine kidney tissue culture passage, inoculated intranasally 10 ml of a 1:10 dilution of virus into 2 susceptible animals. Neither animal showed any evidence of clinical illness, though both animals were infected as indicated by a rise in titer in the neutralization test from less than 1:4 to 1:32 and 1:128 respectively.

Discussion. In their original observations on presence of neutralizing substances to human adenoviruses in bovine sera, Gold and Ginsberg(2) refrained from interpreting the nature or origin of these neutralizing substances, though studies on the nature of the inhibitor were consistent with its being an antibody. In a discussion of the nature of neutralizing substances in animal sera(5), we concluded that inhibitors such as the reported adenovirus inhibitors are true antibodies resulting from viral infection. Isolation of 2 bovine adenoviruses related to human adenoviruses allows one to conclude that the neutralizing substances to the many human adenovirus types in bovine sera are antibodies to bovine adenoviruses antigenically related to human adenoviruses. As noted above, neither of our bovine adenoviruses have been typed, though they are apparently not related to adenoviruses types 1-7. It is of interest to note that neutralizing antibodies to the important human types 4 and 7 are commonly present in bovine sera and the viruses responsible for these antibodies should ultimately be found in cattle.

Summary. A second bovine adenovirus has been isolated. The virus is antigenically similar to a human virus as determined by 1) neutralization with human gamma globulin and human adult sera, and 2) possession of a complement fixing antigen similar to that of the human adenovirus. The related type of human adenovirus remains unknown.

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Immunologic Mechanism of Anaphylaxis. III. Inhibition Phenomenon in Passive Cutaneous Anaphylaxis in the Mouse.* (26105)

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It has been shown that mouse skin can be passively sensitized to an immediate type of hypersensitive reaction(1,2,3). This type of reaction, which consists of an increase in permeability of the small vessels of the skin when antigen reacts with antibody, has been termed "passive cutaneous anaphylaxis" (PCA)(4,5). In the guinea pig as in the mouse, a latent period is required between intradermal injection of antibody and intravenous challenge with antigen(6,1). Benacerraf and Kabat have shown that a latent period is also required for passive systemic anaphylaxis(7). This requisite latent period is evidence that a fixation of the antibody to the tissues occurs before the reaction is elicited.

Further evidence that fixation of antibody

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occurs was furnished by the demonstration that normal rabbit serum competitively inhibits passive cutaneous anaphylaxis in the guinea pig by competing with the antibody for fixation sites(8). Prochazka-Fisher and Cooke confirmed this observation and showed that the globulin fraction was responsible for this inhibition(9). Biozzi, Halpern and Binaghi confirmed and, by quantitative data, extended these observations(10).

The latent period in the mouse, however, was shown to be very short(1,2). Therefore, the question arises as to whether fixation in the mouse does, in fact, exist. A very good way to investigate this query is to see whether rabbit gamma globulin competitively inhibits passive cutaneous anaphylaxis in the mouse, as was demonstrated in the guinea pig. The present study investigates this possibility.

Materials and methods. Animals. Male, adult albino mice weighing approximately 20-25 g were used. *Antibody.* A pool of rabbit anti-egg albumin sera (anti-EA) (505 μg antibody N/ml) was employed. *Rabbit gamma globulin.* A purified rabbit gamma globulin (RGG) (Pentex, Lot 63 FO 5) was used and was diluted in 0.15 M NaCl to a concentration of 10 mg protein/ml and was kept frozen until used. *Antigens.* Twice recrystallized egg albumin (Armour) (EA) was used as antigen. *Histamine Dichlorhydrate.* A stock solution of 250 μg/ml (Fisher, Lot 542851) was prepared in 0.15 M NaCl and diluted 1/50 (2.5 μg/0.05 ml) just prior to use. *Diluent.* 0.15 M NaCl was used for all dilutions, except when the RGG solution was used. *Dye.* Evans blue (Eastman Organic Chemicals), 0.5% was prepared and stored at room temperature until used. The serum was diluted in 0.15 NaCl or in the RGG solution. The volume used for all intradermal injections was 0.05 ml. All intradermal injections were performed on the freshly shaved dorsal surface of each mouse using a short bevel, 26-gauge needle. Care was taken to inject the inoculum only into dermal layers of the skin with as little irritation as possible, to avoid non-specific traumatic reactions. Four intradermal injections were made on the back of each mouse.

In preliminary experiments, it was seen that minimal amount of antibody necessary to produce a reaction graded 3+ or more, in the mouse, was approximately 0.15-0.30 μg antibody N with this system.

Simultaneously with intradermal injection of antibody, 2 other sites were infiltrated, one with saline and the other with RGG. These injections sites were marked so that immediately after challenge with antigen, histamine could be injected in the same sites.

A latent period of one hour was allowed, after which time the specific antigen (EA) was injected intravenously with the dye. Intravenous injections were executed with a sharp, 27-gauge needle through the dorsal vein of the penis, after light ether anesthesia. The volume used in all injections was 0.25 ml. The antigen was mixed with an equal volume of dye before injection and either 0.3, 0.6, or 1.25 mg EA/0.25 ml was injected. Various antigen and antibody concentrations were used because it was found difficult to produce inhibition in this system.

The animals were killed one hour after challenge with antigen. They were skinned and the reactions (*i.e.*, accumulation of dye at site of intradermal injection) were read according to the diameter of the blue spots as follows: 0 = no reaction; ± = less than 4 mm; 1 = 4 mm; 2 = 4-6 mm; 3 = 6-10 mm; 4= more than 10 mm.

Results. It is known that there is an inverse relation between amount of antibody and antigen used; that is, minimal amount of antigen which gives the maximal reaction is less when higher amounts of antibody are used(6). Moreover, it has already been pointed out by Osler, Hawrisiak, Ovary, Siqueira, and Bier, in a study on the mechanisms of hypersensitivity phenomena, that inhibition is much easier to obtain with threshold amounts of antigen(11). In preliminary experiments it was seen that scarcely any inhibition was produced when the animals were injected with 0.3 μg antibody nitrogen and challenged with 1.25 mg EA. On the other hand, the animals injected with 0.15 μg antibody nitrogen and challenged with 0.3 or 0.6 mg Ea reacted very poorly.

Therefore, inhibition produced by RGG

TABLE I. Competitive Inhibition Produced by RGG in Passive Cutaneous Anaphylaxis Elicited by Anti-EA in the Mouse.

Injections	Degree of reaction in diameters*											
	Challenge dose of antigen											
	.3 mg EA						.6 mg EA					
Part I												
.3 μg N anti-EA in saline	0	4	3	3	4	3	4	4	4	4	4	4
Idem in RGG (10 mg/ml)	0	0	3	0	0	0	4	3	0	0	0	0
Histamine (2.5 μg) + saline	4	4	4	4	4	4	4	4	4	4	4	4
Idem + RGG (10 mg/ml)	4	4	4	4	4	4	4	4	4	4	4	4
Part II	1.25 mg EA											
.5 μg N anti-EA in saline	3	0	3	3	3	4						
Idem in RGG (10 mg/ml)	0	3	0	0	0	0						
Histamine (2.5 μg) + saline	4	4	4	4	4	4						
Idem + RGG (10 mg/ml)	4	4	4	4	4	4						

* See *Materials and methods* for key to diameters as represented by numbers in the Table. Vertical columns contain values for reactions in 4 inj. sites on a single mouse.

was investigated in 2 series of animals, one injected with 0.3, the other with 0.15 μg antibody nitrogen. The first series was challenged with 2 concentrations of antigen (0.3 and 0.6 mg EA protein/0.25 ml) and the second with only one concentration (1.25 mg EA protein/0.25 ml).

Five out of 6 mice receiving 0.15 μg antibody N/0.05 ml anti-EA and 1.25 mg EA showed complete inhibition by RGG (Table I, Part II).

Using 0.3 μg antibody N anti-EA, 9 of the 11 mice showed complete inhibition by RGG (Table I, Part I) with 0.3 or 0.6 mg EA. No inhibition of the reaction produced by histamine was observed.

Therefore, inhibition of passive cutaneous anaphylaxis in the mouse by RGG may be produced. However, the competition by RGG is not very strong, as was shown by the fact that adequate amounts of antibody and antigen had to be used to show good inhibition. The fact that competition by RGG in the mouse is very weak is not surprising, since rabbit antibody persists only for a short time at injected site(1,2).

Similar observations were made using another antigen-antibody system, *i.e.*, BGG and rabbit anti-BGG.

A possible explanation for this short persistence of antibody and poor inhibition by RGG might be that other factors play an important role in passive cutaneous anaphylaxis in the mouse. In systemic anaphylaxis in the mouse, for example, Burdon and Treadwell have shown that circulating complexes play an important role(12,3) whereas they do not in systemic anaphylaxis in the guinea pig.

Summary. 1) Competitive inhibition by RGG in passive cutaneous anaphylaxis in the mouse has been demonstrated. 2) Inhibition of PCA was much weaker than in the guinea pig. 3) No inhibition of reactions produced by intradermal injections of histamine was demonstrated. 4) The possible meaning of results was discussed.

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