A New Bovine Adenovirus Related to Human Adenovirus.* (26104)

MORTON KLEIN, JOSEPH ZELLAT, THOMAS C. MICHAELSON
(Introduced by M. J. Oppenheimer)
Dept. of Microbiology, Temple University School of Medicine, Philadelphia, Pa.

We have previously reported on isolation of a bovine adenovirus, type unknown, that is antigenically related to a human adenovirus (1). The initial demonstration that the Bovine virus #10 was related to some human virus was based upon neutralization of the agent by human gamma globulin and individual human sera. Identification of the agent as an adenovirus was based upon its growth properties and presence of the group specific adenovirus complement fixing antigen. The bovine virus was not neutralized by antisera to adenoviruses types 1-7 and we do not know whether it is related to some other known type or an unidentified human adenovirus. The demonstration by Gold and Ginsberg(2) that neutralizing substances to many human adenoviruses could be found in cattle suggested that other adenoviruses would be isolated from cattle. This paper reports a second bovine adenovirus that is related to an unidentified type of human adenovirus.

Materials and methods. The Bovine virus #19 was isolated in June, 1956 from the feces of an apparently well calf maintained as part of the dairy herd at Glen Mills School in Pennsylvania. Procedure for collecting, processing, and isolating these viruses has been previously described(1). Briefly, trypsinized calf kidney cells were used for isolation of the agent. Neutralization tests to determine the possible relationship of the bovine virus to some human agent were carried out with human adult sera and several lots of human gamma globulin. Complement fixation tests confirming Bovine #19 as an adenovirus were kindly carried out by Dr. Hummeler of the Children's Hospital.

Results. The virus on initial isolation gave a slight cytopathogenic effect (CPE) after 13 days incubation. After several passages the CPE was observed in 5-6 days though total destruction of the cells was rarely observed. The titer has remained low and 0.1 ml of virus was never infectious for calf kidney cells at dilutions greater than 10^{-3}. The CPE is shown in Fig. 1. The virus gives the characteristic range of adenovirus intranuclear inclusion bodies. The nucleus is often enlarged and there are a variety of patterns of nuclear changes similar to those described for human adenoviruses(3). We have obtained growth of this virus only in trypsinized calf kidney cells and no CPE has been observed either in HeLa cells or monkey kidney cells which distinguishes this bovine agent from a human adenovirus. No detectable growth of the virus was observed in adult or suckling mice, chick embryos (all routes), guinea pigs, hamsters, or rabbits. No hemagglutination was observed with red cells from the guinea pig, sheep, cow, chicken, or human. Rat and mouse red blood cells were agglutinated by Bovine #19. One-half ml of a 1:64 dilution of virus agglutinated 0.5 ml of a 0.5% suspension of rat and mouse blood cells after 4 hours incubation at 4°C. Nonspecific hemagglutination was not observed with the maintenance medium in which the cells were grown. By contrast the Bovine adenovirus #10 agglutinates rat but not mouse red blood cells. This specificity in agglutination of rat and mouse red blood cells by strains of human adenoviruses has

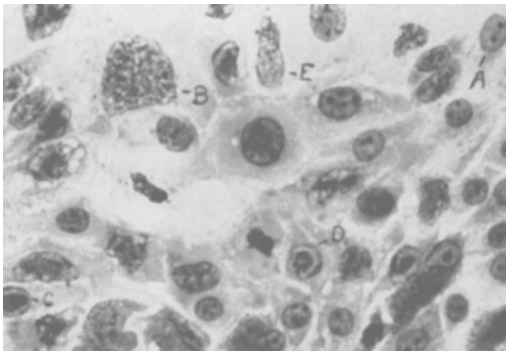

FIG. 1. Bovine #19 grown in calf kidney cells, 4 days, May-Greenwald-Giemsa. A. Normal nucleus. B, C, D, E. Enlarged nuclei with varying patterns of chromatin clumping.

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TABLE I. Neutralization of Bovine Virus #19 by Sera of Adults and Infants.

	No. tested	Neg (1:4)	Positive 1:4	1:8	1:16	1:32	1:64	% positive
Adult sera *vs* 100 TCD	50	41 (82%)	2	1	1	2	3	18%
Infants' sera *vs* 100 TCD	25	25						0%

Age of infants ranged from 4-15 mo. All sera heated to 56°C for 30 min.

been described by Rosen(4). Identifying properties of Bovine #19 are, then: 1) characteristic intranuclear inclusion bodies, 2) growth in trypsinized calf kidney cells to low titer, and 3) hemagglutination of rat and mouse blood cells.

The initial Bovine adenovirus isolate #10 and the present Bovine #19, though very similar in growth properties, differ not only in hemagglutination of mouse red blood cells but antigenically. There is no antigenic cross reaction between these agents. A 1:320 dilution of sera from rabbits immunized with Bovine #10 neutralized 100 TCD of the homologous virus but did not neutralize 10 TCD of Bovine #19 at a dilution of 1:20. Under similar conditions antisera prepared against Bovine #19, and also active at a 1:320 dilution, failed to neutralize 10 TCD of Bovine #10 at a 1:20 dilution.

Identification of Bovine #19 as a virus related to a human adenovirus. Bovine #19 was recognized as being related to some human virus because 100 TCD of virus were neutralized by a 1:40 dilution of human gamma globulin. This human relationship was confirmed by showing that 18% of human adult sera and no infant sera neutralized the agent (Table I). Bovine #19 was specifically identified as an adenovirus by the complement fixation test. The virus grown in calf kidney cells was used as a live preparation to immunize guinea pigs and hamsters. Their antisera were tested against a human adenovirus grown in HeLa cells and the results are shown in Table II. Sera from both guinea pigs and hamsters immunized with Bovine #19 gave positive complement fixation titers with human adenovirus antigens. We were not able to prepare a satisfactory complement fixing antigen of Bovine #19 that could be used against human sera. Whether Bovine #19 is antigenically related to a known type of human adenovirus or an as yet unidentified human adenovirus remains to be determined. Our agent was not neutralized by antisera against types 1-7 adenoviruses.

TABLE II. Reaction of Antisera to Bovine #19 with Complement Fixing Antigen of Human Adenovirus.

Bovine #19 antisera	C.F. antigen	C.F. titer
Guinea pig		
Pre-immunization sera	Human adenovirus	<1:8
	Tissue control*	<1:8
Post- " "	Human adenovirus	1:8
	Tissue control	<1:8
Hamster		
Pre-immunization sera	Human adenovirus	<1:8
	Tissue control	<1:8
Post- " "	Human adenovirus	1:16
	Tissue control	<1:8

* Guinea pigs and hamsters immunized with virus grown in calf kidney. C.F. adenovirus antigen grown in HeLa cells. Tissue control = HeLa cells alone used as antigen.

Confirmation of bovine origin of adenovirus. Since Bovine #19 is antigenically related to a human adenovirus additional studies were carried out to confirm that this was not a human laboratory contaminant but a bovine virus antigenically related to a human adenovirus. As noted above, the virus did not grow in HeLa cells or monkey kidney cells, but grew in continuous passage in freshly trypsinized calf kidney cells, a property which distinguishes it from a human adenovirus. Further, the agent was reisolated from the original fecal specimen. Sera obtained from the cow before viral isolation failed to neutralize the agent, while sera obtained 9 weeks after isolation of the agent neutralized 100 TCD of the virus at a dilution of 1:20. The agent is a common bovine virus, for all of 40 cows had neutralizing antibodies to the virus (Table III). The results confirm the status of Bovine #19 as a widely distributed bovine adenovirus antigenically related to or identical with an unknown type of human adenovirus. Though Bovine #19 has not yet been inoculated into cattle, Bo-

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.

1. Klein, M., Earley, E., Zellat, J., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 1.

2. Gold, E., Ginsberg, H. E., *Fed. Proc.*, 1957, v16, 414.

3. Boyer, G. S., Leuchtenberger, C., Ginsberg, H. S., *J. Exp. Med.*, 1957, v105, 192.

4. Rosen, L., *Virology*, 1958, v5, 574.

5. Klein, M., *Ann. N. Y. Acad. Sci.*, 1958, v70, 362.

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

RICHARD C. GARDNER AND ZOLTAN OVARY
Dept. of Pathology, New York University School of Medicine, New York City

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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