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Isolation from Cattle of a Virus Related to Human Adenovirus.* (25123)

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(Introduced by M. J. Oppenheimer)
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Recent studies of inhibitors in bovine sera to certain human viruses—polioviruses, Coxsackie, and adenoviruses(1-5) have raised the question of the nature and origin of these inhibitors. We concluded, on the basis of previous studies on the nature of these neutralizing substances to types 1, 2, and 3 polioviruses, that they were indeed true antibodies. This observation indicated that cattle were perhaps infected either with these viruses or their antigenic relatives. We accordingly undertook a search for these presumed agents, and the present report describes identification of one virus among some 70 viral isolates obtained from feces of apparently normal cattle. This bovine virus, which we originally detected by screening procedure with human gamma globulin(3), has been identified as an adenovirus related to some as yet unidentified human adenovirus type.

Materials and methods. Fecal swabs were obtained from cattle in the Philadelphia area and immediately placed in tubes of broth containing 500 units of penicillin, 500 units of streptomycin, and 1,000 units of nystatin. Tubes were centrifuged at 3,000 rpm for 20 minutes. The supernatant fluid was removed and incubated at 37°C for 45 minutes to further reduce contaminants. Contamination was only infrequently encountered. The tubes were either stored at −70°C or inoculated directly into tissue culture tubes. These were prepared from freshly trypsinized calf kidney. Growth medium initially used was 80% Eagle's medium in Hanks' BSS plus 20% horse serum. Lactalbumin hydrolysate (0.5%) plus 10% horse serum was subsequently used as growth medium. Maintenance medium was 78% Eagle's medium in Earle's BSS, 20% trypticase soy broth plus 2% chick serum. Calf serum was not used since we wished to avoid any inhibitors to bovine viruses. Several commercial lots of Merck, Sharp & Dohme human gamma globulin were used. Since there was some concern about the inhibitory action of preservatives in the material, special lots of gamma globulin were prepared without preservative from material kindly sent us by Dr. Singher of Ortho Research Foundation. Wassermann negative sera obtained from the hospital serology laboratory. Sera were obtained from children in

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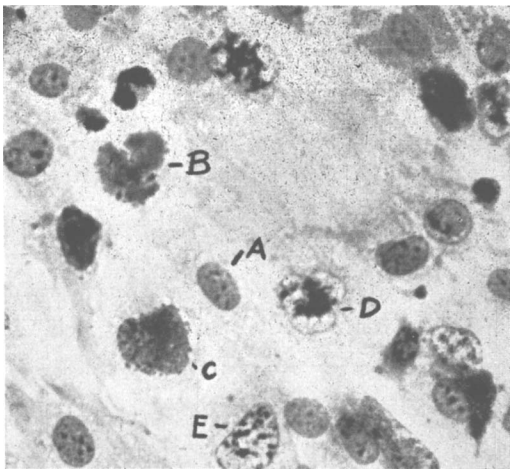

FIG. 1. Bovine #10 grown in calf testes, 3 days, May-Greenwald-Giemsa. A. Normal nucleus. B, C, D, E, enlarged nuclei with varying patterns of chromatin clumping.

age group 7-15 months. Antisera to adenoviruses types 1-7 were kindly given us by Dr. Bartell of Wyeth Institute. Antisera to ECHO viruses types 1-19; Coxsackie virus B types 1-5; antisera to viruses of herpes simplex, measles, mumps, B virus; influenza A, B, swine, Asian; hemadsorption virus types 1 and 2 were kindly supplied by Drs. Hummeler and Lief of Children's Hospital, Philadelphia. Antisera to types 1, 2, and 3 polioviruses were obtained from National Foundation. Complement fixation tests confirming Bovine #10 as an adenovirus were kindly carried out by Dr. Hummeler of Children's Hospital.

Results. The virus Bovine #10 was isolated from feces of apparently normal adult cow in May, 1956. The original cytopathogenic effect in freshly trypsinized calf tissue culture was slight and after 7 passages in calf kidney tissue culture the titer has remained low, never higher than 10^{-3} (0.1 ml) of inoculum. The virus gives a characteristic intranuclear inclusion body when stained with May-Greenwald-Giemsa (Fig. 1). The nucleus is enlarged, there is clumping of chromatin either in large sheets or irregular masses, and the entire sequence of events is similar to that described for human adenoviruses types 3, 4, and 7(6). The growth properties of Bovine #10 are as follows: No detectable activity in: 1. Mice (adult and suckling); 2. Chick embryos (chorio-allantoic membrane, allantoic cavity, amniotic cavity); 3. Guinea pigs, rabbits, hamsters; 4. Hemagglutination and hemadsorption tests (red cells from guinea pig, sheep, cow, chicken, human 'O'); 5. HeLa cells. The only identifying properties of Bovine #10 were a characteristic intranuclear inclusion body and growth at a low titer, 10^{-2} to 10^{-3} in cell cultures of calf kidney and calf testes. There was only a slight cytopathogenic effect in monkey kidney tissue culture and we have had considerable difficulty in passaging the agent in monkey kidney tissue culture. We did not obtain growth in HeLa cells, though 3 passages were carried out.

We concluded that Bovine #10 was related to a human agent because (a) it was neutralized by human gamma globulin; and (b) neutralized by significant percentage of individual adult sera and (c) it was not neutralized by sera of infants. In Table I results of neutralization tests with human gamma globulin are recorded. A 1:160 dilution of human gamma globulin neutralized 100 tissue culture doses of Bovine #10, though there is marked variation with the challenge dose. This level of neutralization is highly significant. In Table II are recorded results of neutralization tests of Bovine #10 with sera of children and adults. Thirty-eight % of 50 adult sera neu-

TABLE I. Neutralization of Bovine Virus #10 by Human Gamma Globulin.

Dilution of commercial globulin	Virus challenge TCD 10	100	1000
1:20	Neut.	Neut.	Neut.
1:40	"	"	Neg.
1:80	"	"	"
1:160	"	"	"
1:320	"	Neg.	"

TABLE II. Neutralization of Bovine Virus #10 by Sera of Adults and Infants.

No. tested	Neg.(1:8)	Positive 1:8	1:16	1:32	1:64	% positive
		Adult sera *vs* 100 TCD				
50	31 (62%)	9	7	2	1	38%
		Infants' sera *vs* 10 TCD				
25	25		—			0%

Age of infants ranged 7-15 mo.
All sera heated to 56°C for 30 min.

TABLE III. Incidence of Neutralizing Antibodies in Adult Bovine Sera to Bovine Virus #10.

No. tested	Serum dilution 1:32+	Serum dilution 1:8		Total positive
40				35(87.5%)
	Pos.	Pos.	Neg.	
	21(52.5%)	14(35%)	5(12.5%)	

tralized the virus in dilutions ranging 1:8 to 1:64 while none of the 25 infant sera neutralized the agent. On the basis of this pattern of neutralization by (a) human gamma globulin (b) individual adult sera, but not (c) infant sera, we may conclude that Bovine #10 is related to a human infectious agent.

Bovine #10 is a bovine virus and not a human laboratory contaminant for the following reasons. (a) It was reisolated from the original fecal specimen. (b) It grows in continuous passage in calf kidney and calf testes but not in HeLa cells. (c) The cow from which the agent was isolated showed a rise in neutralizing antibody titer from zero to 1:16 when sera were collected 6 weeks after isolation of the agent. As shown in Table III, the agent is neutralized by 87% of adult bovine sera. We may conclude that this is a bovine virus, a newly recognized virus, since it is not related to any bovine virus that has yet been described.

In an attempt to identify the specific human relationship, neutralization tests were carried out with antisera against the following viruses, all of which tests were negative. ECHO viruses, types 1-19; Coxsackie B viruses Types 1-5; polioviruses, types 1, 2, and 3; viruses of herpes simplex, measles, mumps, B virus; influenza A, B, swine, Asian; hemadsorption viruses types 1 and 2.

The following experiment established Bovine #10 as an adenovirus. Hamsters and guinea pigs were immunized with Bovine #10 virus grown in calf kidney tissue culture and their antisera tested by the complement fixation test using human adenovirus antigen grown in HeLa cells. There was a certain irregularity in response to the complement fixing antigen of Bovine #10 by the animals. Occasionally the results were obscured by a reaction of the serum with the HeLa cell tissue controls; one of 4 groups of animals immunized with Bovine #10 showed no rise in complement fixing titer against the human adenovirus in spite of a significant neutralizing titer against Bovine #10; typically however the response was clear and the results are shown in Table IV. Neither guinea pigs nor hamsters used in the test had normal inhibitors to adenoviruses before immunization and there was no cross reaction between control tissue in which adenovirus antigen was grown and our immune sera. The results show an unequivocal rise to adenovirus antigen both in antisera prepared in guinea pig and hamster, which identifies Bovine #10 as an adenovirus. Since Bovine #10 is neutralized by human sera and is an adenovirus, it must be an adenovirus belonging either to one of the 18 known human types or to an as yet unidentified human type. We have not yet been able to identify the type of adenovirus, though it is apparently not a type 1-7 based on neutralization tests with antisera against these viruses.

Discussion. Presence of inhibitors in bovine sera to types 1-7 adenoviruses was reported by Gold and Ginsburg(5), though they refrained from interpreting the nature of these inhibitors. We believe our data strongly suggest that if cows are infected with one adenovirus related to human adenoviruses the inhibitors in bovine sera may be considered both as antibodies and indicators of yet other adenoviruses. We have data indicating presence of yet another adenovirus type in cattle.

TABLE IV. Reaction of Antisera to Bovine #10 with Complement Fixing Antigen of Human Adenovirus.

Bovine #10 antisera	C.F. antigen	Complement fixing titer
Guinea pig		
Pre-immunization sera	Human adenovirus	<1:8
	Tissue control*	"
Post-immunization	Human adenovirus	*1:32*
	Tissue control	<1:8
Hamster		
Pre-immunization	Human adenovirus	<1:8
	Tissue control	"
Post- "	Human adenovirus	*1:32*
	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).

Whether Bovine #10 is identical with a human adenovirus or an antigenic relative remains to be determined. If it is identical with a human virus we may have identified an important reservoir of these agents, for the very high incidence of infection in cattle indicates the agents are endemic in these aninals. We are inclined to believe, however, that Bovine #10 is antigenically related to a human virus, in the sense of a cowpox-smallpox relationship, since we have not been able to achieve one characteristic of human adenoviruses, namely growth in HeLa cells. There is of course a vaccine potential if this latter relationship is the proper one.

We believe that isolation of an adenovirus from cattle gives added weight to the implication, based on neutralizing antibodies to polioviruses in calf sera, that cattle may harbor polioviruses or their antigenic relatives. We may note in passing that 45% of bacterial, fungal, and parasitic infections of animals are shared by man, and if we accept this figure as one that will be valid for viruses we must conclude that a large number of human viruses or their antigenic relatives will ultimately be isolated from animals.

Summary. A virus has been isolated from feces of an apparently normal cow that has been identified as an adenovirus related to or identical with an as yet unidentified type of human adenovirus.

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Effect of Compound 48/80 on Toxicity of Histamine and of Serotonin in Mice.* (25124)

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Tissue mast cells rapidly respond to various types of injurious stimuli by shedding their cytoplasmic contents (*i.e.*, granules) into the surrounding ground substance of the connective tissue. Since these cells contain relatively large concentrations of both histamine and heparin and, in the mouse and rat, also serotonin (5-hydroxytryptamine)(1,2,3), release of these biologically-active substances undoubtedly influences the nature of subsequent responses of the local tissue to the injurious stimulus. Degranulation of mast cells can be readily initiated by a synthetic phenyl alkylamine substance called compound 48/80 (4). This drug is a potent releaser of histamine(5), has a protamine-like effect on heparin(4) and, in mouse and rat, will also release serotonin(6,7). In addition, it produces a lethal intoxication in the mouse and various other laboratory animals(8). Although Riley (9), and others(5,10), suggested that pharmacologic or injurious effects of compound 48/80 are mediated by the histamine and/or 5-hydroxytryptamine released from mast cells, it would seem unlikely that such a mechanism could account for *lethal* effects of this drug in the mouse. In the first place, tissue levels of histamine and serotonin in the mouse(11) are many times less than those required for lethal effects of these amines in this species (12,13). Secondly, the interaction of compound 48/80 with mast cells should result in

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