fever, it is of interest to note that the behavior of this strain in guinea pigs is not unlike that of some strains of *R. rickettsii* recovered from human cases of the disease. The implications are obvious. With reference to cottontail rabbits, Jellison(5) noted the highly significant degree of correlation between the geographic distribution of species of *Sylvilagus* and the occurrence of human cases of spotted fever in the United States. Furthermore, *Sylvilagus* spp. are frequently hosts for the immature stages of *Dermacentor variabilis* and *D. andersoni*, which, as adults, may transmit spotted fever to man.

In reporting their recovery of *R. rickettsii* from a naturally infected meadow mouse, *Microtus pennsylvanicus*, taken in Virginia, Gould and Miesse(1) discussed the possible significance of their finding and reviewed pertinent prior studies of previous workers in the United States and Latin America.

In the course of the present study, rickettsial strains have also been recovered from 1 of 330 *Peromyscus leucopus* and 1 of 2 *Pitymys pinetorum*. Although their behavior in complement fixation classifies these strains to be members of the spotted fever group, neither has yet been sufficiently characterized to permit precise species identification.

Serologic studies revealed the presence of complement-fixing antibodies for the spotted fever group in 4 of the 10 wild rabbits (Table I). In addition, a limited survey of the sera of certain other wild mammals revealed similarly reacting antibodies in the sera of 1 or more specimens of red fox, gray fox, opossum, raccoon, white-footed wood mouse, and deer. These findings are interpreted tentatively as evidence of past spotted fever infection.

Further studies are necessary in order to evaluate more precisely the role of other mammals as well as rabbits in the ecology of *R. rickettsii*. Current field and laboratory studies are thus aimed at answering such questions as the following: What is the most sensitive method for detecting present or past infection with *R. rickettsii* in a particular species of mammal? Can one recover viable *R. rickettsii* from mammals whose serum contains complement-fixing antibody? For how long a period of time can an individual mammal serve as a donor of *R. rickettsii* to non-infected ticks that feed upon it? Is there a relationship between age of a rabbit and time that it usually becomes infected?

Summary. From a naturally infected wild cottontail rabbit, *S. floridanus*, trapped in Virginia, an organism identified as *R. rickettsii* has been recovered and characterized. The role of cottontail rabbits and certain other native wild mammals in the ecology of Rocky Mountain spotted fever is discussed briefly in the light of this and other related findings.

1. Gould, D. J., Miesse, M. L., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 558.

2. Jackson, E. B., Smadel, J. E., *Am. J. Hyg.*, 1951, v53, 326.

3. Smadel, J. E., *Diagnostic Procedures for Virus and Rickettsial Diseases*, Am. Public Health Assn., 2nd Ed., 1956, 513.

4. Bell, E. J., Stoenner, H. G., *J. Immunol.*, 1960, v84, 171.

5. Jellison, W. L., *Public Health Rep.*, 1945, v60, 958.

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Serological Comparisons Between Infectious Canine Hepatitis Virus and Human Adenovirus Types. (26582)

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The adenovirus group is a large family of infectious agents which possesses certain common biological properties, including a soluble group-reactive complement-fixing antigen(1,2). Recent studies have disclosed biological similarities between infectious canine hepatitis virus (ICHV) and certain members of the human adenovirus group(3,

4,5). Also, adenovirus types 2, 3, 4 and 7 have been reported to produce an asymptomatic infection in experimentally exposed dogs(6). Additional studies were made and this paper presents further evidence of the antigenic relationships between ICHV and certain human adenoviruses as shown by complement-fixation, indirect hemagglutination, virus neutralization, and gel-diffusion precipitin methods.

Methods. Paired canine sera were obtained from purebred beagles that had been given a single inoculation of adenovirus type 4 or Cornell strain 1 ICHV subcutaneously. Sera were taken at time of inoculation and again approximately 30 days afterwards. Other antisera for adenovirus were: (1) human type 2 antiserum supplied by Dr. W. P. Rowe (Nat. Inst. Health, Bethesda, Md.) (2), acute and convalescent human adenovirus type 4 antiserum obtained from Dr. R. R. Gutekunst (Great Lakes Naval Biological Research Unit, Great Lakes, Ill.), and (3) guinea pig adenovirus antiserum prepared in this laboratory by inoculating 0.2 ml of an infective tissue culture suspension of type 4 intranasally, followed 2 weeks later by an inoculum of 1 ml intraperitoneally. The guinea pigs were bled for serum 21 days after final inoculation.

Complement-fixation (CF) tests. ICHV antigens for CF tests were prepared from 7-day cultures of dog kidney cells infected with Cornell strain 1 ICHV. Cultures were frozen and thawed 6 times, clarified by centrifugation at 2000 rpm for 10 minutes and pooled in 2 ml portions which were then stored at −20°C until used. Adenovirus type 4 CF antigen was prepared from infected HeLa cell cultures. After complete cell degeneration had occurred, cultures were frozen and thawed, clarified by light centrifugation, pooled, then stored at −20°C in 5 ml portions.

In all tests, paired sera were tested simultaneously. Serial 2-fold dilutions of heat-inactivated serum in 0.25 ml amounts were incubated for 2 hours at 36°C with 4 units of either ICHV or adenovirus antigen in 0.25 ml and 2 full units of complement contained in 0.5 ml of Kolmer saline. A unit of antigen had been previously determined by titration. After 2 hours incubation at 36°C, 0.5 ml of a prewarmed mixture of 2 units of hemolytic amboceptor and 2% sheep erythrocytes was added. The test was read after further incubation for 30 minutes and endpoints were then recorded as the highest serum dilution showing at least 50% fixation by visual observation. Appropriate controls were included.

Indirect hemagglutination tests. Hemagglutination procedures have been previously reported(7). Serial 5-fold serum dilutions were heat-inactivated and mixed in equal volumes of 0.2 ml with 1% tanned sheep erythrocytes sensitized with ICHV or adenovirus type 4 antigens. After 2 hours incubation at room temperature (approximately 26°C) the endpoints were recorded as the highest serum dilution giving a 2-plus hemagglutination pattern.

Virus neutralization tests. For ICHV neutralization tests, serial 5-fold dilutions of heat-inactivated serum were mixed with an equal volume of virus suspension containing 100 to 300 $TCID_{50}$ per 0.1 ml. After incubation for 1 hour at 36°C, virus-serum mixtures were inoculated into tubes of dog kidney cells using an inoculum of 0.2 ml per tube and 4 tubes for each dilution. Assay for adenovirus antibody in certain canine sera was kindly performed by Dr. W. P. Rowe. Inoculated cell cultures were maintained at 35°C in stationary racks. Tests were finally read when at least 50% degeneration was evident in the virus control cultures showing 100 to 300 $TCID_{50}$.

Agar-gel precipitin tests. Ouchterlony gel-precipitin tests were carried out according to a modification of the procedure described by Brown and Crick for antigenic analysis of foot-and-mouth disease virus(8). Antigen was prepared from 7-day cultures of infected cells which had been frozen and thawed 6 times and then centrifuged at 2000 rpm for 10 minutes. Clearest precipitin bands were obtained with virus preparations which had been concentrated 10 times by dialysis against 20% polyvinylpyrrolidone (Armour) at 4°C. Noble agar (Difco) at a concentration of 1.5% in buffered saline (pH 7.4), con-

TABLE I. Serological Comparisons of Infectious Canine Hepatitis and Human Adenoviruses.

Paired sera*		Serum antibody titers (reciprocal of dilution)					
		Complement-fixation		Hemagglutination		Neutralization	
		Adeno.†	ICHV	Adeno.	ICHV	Adeno.	ICHV
Human adeno. 4	(A)	4	4	125	15,625	<5	30
	(C)	256	128	625	78,125	>625	30
Human adeno. 2	(A)	<4	<4	<5	<5	NT	<5
	(C)	128	128	125	15,625	"	<5
Guinea pig adeno. 4	(A)	<4	<4	<5	<5	<5	<5
	(C)	128	32	125	78,125	>25	<5
Canine adeno. 4	(A)	<4	<4	<5	<5	<4	<5
	(C)	64	16	25	625	>4	<5
Idem	(A)	<4	<4	NT	NT	<4	<5
	(C)	32	4	"	"	>4	<5
"	(A)	<4	<4	"	"	4	<5
	(C)	32	8	"	"	>4	<5
"	(A)	<4	<4	"	"	4	<5
	(C)	8	4	"	"	>4	<5
"	(A)	<4	<4	"	"	<4	<5
	(C)	64	16	"	"	>4	<5
Canine ICHV	(A)	<4	<4	<5	<5	<5	<5
	(C)	<4	128	<5	390,625	<5	10,000
Idem	(A)	<4	<4	NT	NT	<5	<5
	(C)	<4	128	"	"	<5	300,000
"	(A)	<4	<4	<5	<5	<5	<5
	(C)	<4	16	<5	15,625	<5	25,000
"	(A)	<4	<4	NT	NT	<5	<5
	(C)	<4	64	"	"	<5	300,000
"	(A)	<4	<4	"	"	<5	<5
	(C)	<4	128	"	"	<5	700,000

* Serums taken during acute phase or preinoculation (A) and convalescent or postinoculation (C).

† Antigen used.

taining 0.01% thiomersalate (final concentration) and 1:300,000 methyl orange, was poured in 15 ml portions into 9 cm Petri plates and held at 4°C. Plates were placed at room temperature for 8 to 12 hours before use. Cups 7 mm in diameter and 7 mm apart were cut from the agar with the aid of a cutting template. These held approximately 0.05 ml and were filled 2 or 3 times within a 2-hour period. After filling, plates were kept at 35°C in a humidified chamber and precipitin bands were usually evident within 12 to 24 hours, however, the test was finally read after 72 hours when clearest lines were observed.

Results. Results of serological comparisons of Cornell strain 1 ICHV with human adenovirus types 2 and 4 are shown in Table I. Human adenovirus serum CF titers were nearly the same when ICHV antigen was used in place of the homologous antigen in the test. Adenovirus antisera produced in dogs and guinea pigs, however, exhibited lower titers to the heterologous (ICHV) antigen. In contrast, adenovirus CF antigens failed to fix complement with immune canine hepatitis sera, indicating type-specificity for ICHV antibody.

Antigens participating in the indirect hemagglutination test, which are virtually the same as those responsible for complement-fixation(9), gave results similar to those obtained in the CF test, for adenovirus immune sera agglutinated erythrocytes sensitized with both adenovirus and ICHV antigens, while ICHV immune sera reacted only with the homologous test antigen. Higher adenovirus antibody titers were observed when ICHV-

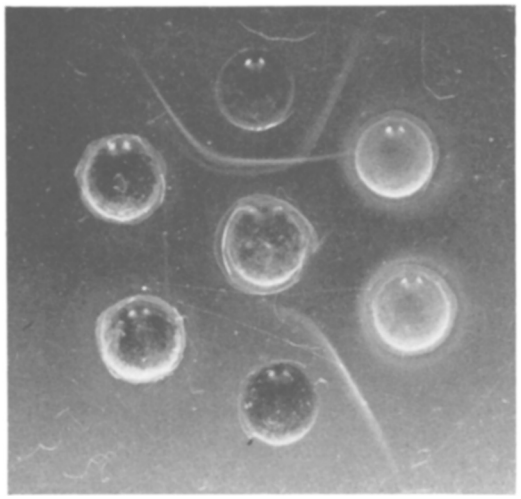

FIG. 1. Gel precipitin test showing unilateral serological relationship between ICHV and adenovirus type 4. ICHV (top well), adenovirus type 4 (bottom well), immune ICH serum (center and left wells), immune adenovirus type 4 serum, human origin (right wells).

sensitized cells were employed. These probably resulted from larger amounts of cell-adsorbed antigen since serum titers are related to the amount of antigen adsorbed onto erythrocytes, provided that all receptors are not saturated(5). The CF antigen titer of the adenovirus preparation used for sorption was 8-times less than the ICHV titer.

ICHV and adenovirus types 2 and 4 were specifically neutralized only by homologous antisera. Dogs inoculated with adenovirus type 4 developed significant neutralizing antibodies only against the homologous virus, for sera from such dogs failed to neutralize ICHV. An interesting finding was the presence of adenovirus antibodies in some dogs, preinoculation sera.

The unilateral serologic relationship between ICHV and adenoviruses was readily demonstrated by employing Ouchterlony precipitin tests. Fig. 1 shows a typical result when immune adenovirus type 4 serum and ICHV-immune serum were tested against adenovirus type 4 and ICHV. At least 3 precipitin lines were formed between adenovirus type 4 immune serum and ICHV, while none appeared between ICHV immune serum and the adenovirus. Insufficient resolution was achieved to determine the precise number of cross-reacting antigens; however, adenovirus type 2 antiserum formed at least 3 and probably 4 bands with ICHV.

Discussion. Many of the basic criteria for placing ICHV taxonomically within the adenovirus group seem to have been adequately met. Since Kapsenberg's report of the serological relationship between ICHV and certain human adenoviruses(3), further studies have shown that the relationship is unilateral, for adenovirus antiserum reacts with ICHV in complement-fixation and in gel-diffusion precipitin tests but canine hepatitis antiserum does not react with adenovirus antigens(4). The serological findings reported here confirm the antigenic relationships and have further demonstrated the multiplicity of shared antigens between the 2 groups. In our experience, human sera containing adenovirus antibodies fixes complement with ICHV and adenovirus antigens to nearly the same degree. In contrast, canine sera containing adenovirus antibodies consistently showed higher complement-fixation titers when the homologous antigen was used, demonstrating a degree of type specificity. Similar findings have been reported for various human adenovirus sera produced in rabbits(1) and for a newly described mouse virus related to the adenovirus group(10).

Since viruses that share group antigens with the adenoviruses have been isolated from monkeys and chimpanzees(1,2), cattle(11), fowl(12), rodents(10), and now apparently the dog, biologic comparisons become all the more important, for the sharing of antigenic determinants does not in itself allow taxonomic grouping. The well-known Weil-Felix reaction depends upon antigens shared by such biologically distinct groups as proteus and rickettsiae. Also, unilateral antigenic relationships based upon immunologic studies have been demonstrated between certain staphylococci and *Listeria monocytogenes* (13). Additional evidence which supports the inclusion of ICHV into the adenovirus group is shown in Table II.

Occasional reports that man and monkeys may become either asymptomatically or manifestly infected with ICHV(17,18) have been based, primarily, upon retrospective

TABLE II. Biological Characteristics Shared by Human Adenoviruses and Infectious Canine Hepatitis Virus.

Characteristics	Adenovirus*	ICHV†
Soluble group-reacting CF antigen	+	+
Ether resistance	+	+
Cultural: Cytopathogenicity—intranuclear inclusions, primarily DNA	+	+
Acid production by infected cell	+	+
Intranuclear "crystals"—some strains	+	+
Latent period 14 to 21 hr	+	+
Greater cell-association virus	+	+
Cell-detachment factor, soluble "toxin"	+	
Stability: Prolonged at 4°C, rapid inactivation 56°C	+	+
pH 3 to pH 9 (30 min., room temperature)	+	+
Lack of pathogenicity for small laboratory animals	+	+
Size: 50-65 mμ (intracellular)	+	+
Hemagglutinin for erythrocytes for various animal species	+	+
Isolation from tonsils and urine of asymptomatic carriers	+	+

* (1,2) † (3,5,14,15,16)

methods which employed the complement-fixation test. In view of the antigenic relationships between ICHV and human adenoviruses, it would seem necessary to re-evaluate these findings, especially since increases in specific neutralizing antibody or virus isolations have not been reported.

Summary. Antigenic relationships between infectious canine hepatitis virus (ICHV) and certain human adenoviruses have been confirmed by complement-fixation, indirect hemagglutination and gel-diffusion precipitin tests. The serological relationship was unilateral, since antisera against human adenoviruses react in various tests with ICHV antigen, while specific antisera to ICHV failed to react with group-reactive human adenovirus antigens (types 2 and 4). There was no specific cross-neutralization between ICHV and the adenovirus types examined. Certain biologic properties shared by ICHV and the human adenoviruses have been compared, and these suggest that ICHV should be included taxonomically within the adenovirus group.

1. Heubner, R. J., Rowe, W. P., Chanock, R. M., *Ann. Rev. Microbiol.*, 1958, v12, 49.

2. Ward, T. G., *Prog. Med. Virol.*, 1959, v2, 203.

3. Kapsenberg, J. C., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 611.

4. Heller, L. A., Salenstedt, C. R., *Virology*, 1960, v11, 640.

5. Carmichael, L. E., Doctoral Thesis, Cornell, 1959.

6. Sinha, S. K., Fleming, L. W., Scholes, S., *J. Am. Vet. Med. Assn.*, 1960, v136, 481.

7. Carmichael, L. E., Sarkar, S., *Cornell Vet.*, 1958, v49, 504.

8. Brown, F., Crick, J., *Virology*, 1958, v5, 133.

9. Ross, E., Ginsberg, H., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 501.

10. Hartley, J. W., Rowe, W. P., *Virology*, 1960, v11, 645.

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

12. Burmester, B. R., Sharpless, G. R., Fontes, A. K., *J. Nat. Cancer Inst.*, 1960, v24, 1443.

13. Welsheimer, H. J., *J. Bact.*, 1960, v79, 456.

14. Fastier, L. B., *Nature*, 1957, v179, 420.

15. Hagan, W. A., Bruner, D. W., *The Infectious Diseases of Domestic Animals*, 4th ed., Comstock, Ithaca, N. Y., 1961.

16. Leader, R. W., Pomerat, C. M., Lefeber, C. G., *Virology*, 1960, v10, 268.

17. Martin, L. H., Delange, B., *Arch. Inst. Past. Maroc.*, 1956, v5, 56.

18. Schindler, R., Mohr, W., *Dtsch. med. Wschr.*, 1959, v84, 2080.

Received March 28, 1961. P.S.E.B.M., 1961, v107.