THE SIMULTANEOUS OCCURRENCE OF THE VIRUSES OF CANINE DISTEMPER AND LYMPHOCYTIC CHORIOMENINGITIS*

A CORRECTION OF "CANINE DISTEMPER IN THE RHESUS MONKEY"

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The injection of suspensions of dog and ferret spleens from various generations of a particular serial passage of canine distemper was found to regularly produce a disease in *rhesus* monkeys which we believed to be distemper (1). Further work has shown this identification to be incorrect. The response in monkeys is not due to Carré's virus but to lymphocytic choriomeningitis which has been simultaneously present with canine distemper virus in all of the samples of the source material tested. In a preliminary correction of the original report it was shown that each of two generations of the source material was capable of producing the clinical responses and lesions of lymphocytic choriomeningitis in mice and guinea pigs. It was also shown that mice which had received suspensions of the material intravenously and recovered were subsequently resistant to a known strain of lymphocytic choriomeningitis virus injected intracerebrally, and conversely that monkeys convalescent from infection with a known strain of virus were resistant to the same dog spleen suspensions which were pathogenic for normal monkeys (2). Having failed at that time to identify the second virus serologically and because it seemed of particular importance that its presence in the source material be thoroughly established, two other samples were examined. The study of these forms the substance of the present report. Certain observations which seem to explain some of the circumstances which led to the original error are also included.

The Source Material

The source material consisted of pooled dog spleens harvested from animals infected by serial passages of distemper virus originally secured from Dunkin and Laidlaw. The strain has been maintained for many years by

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a biological laboratory engaged in the manufacture of distemper antisera and vaccines. Throughout that time it has produced typical responses in both dogs and ferrets as well as antisera capable of controlling canine distemper in the laboratory and in the field. Of the thirteen generations of passage virus studied by us seven have been tested in ferrets and three in dogs. All have produced the symptoms and lesions of distemper.

In brief, canine distemper in the ferret is an acute infectious disease with an incubation period of 4 to 10 days, a duration of 5 to 6 days and a mortality of 90 to 100 per cent. The temperature curves are usually biphasic. The earliest symptoms are a watery discharge from the eyes and nose followed by reddening and swelling of the footpads. As the disease progresses the discharges become purulent, the eyelids are puffed until the eyes are quite closed and the animals become comatose and die.

While distemper is more variable in the dog, a uniform disease occurs in carefully selected, healthy animals. The incubation, under these circumstances, is said to be quite constant, averaging about 4 days. Onset is marked by abrupt fever with watery discharge from eyes and nose, symptoms which are frequently very mild and may easily be overlooked. The discharges later become purulent. These signs may persist but generally disappear after the first week. The dog then refuses food, becomes very weak and difficult to arouse. The cases of distemper which appear in the present report all showed such a course.

The most characteristic histological criterion of distemper is the appearance of cytoplasmic inclusion bodies of variable size and striking appearance, most constant and numerous in the bile ducts, the epithelium of kidney pelvis and urinary bladder and the trachea and bronchi. Our reliance on these inclusions is based on the regularity with which they have occurred in all ferrets showing the classical signs of distemper and their equally regular appearance in dogs examined late in the disease. Such inclusions were noted by Dunkin and Laidlaw (3) and Spooner (4).

A portion of our 12th sample of the source material was ground in a mortar with 9 parts of physiological salt solution, centrifuged for 10 minutes at 2000 R.P.M. and the supernatant fluid pipetted off and shown to be bacteriologically sterile. Portions of this suspension were injected into mice, guinea pigs, monkeys and a ferret.

Mice.—Six mice were injected intracerebrally with 0.03 cc. of the original suspension. On the 7th day two were found dead in the position illustrated by Traub (5) and three others developed convulsions when suspended by the tail and spun. The remaining animals were sacrificed and their brains were harvested. A 10 per cent suspension of this tissue injected into a normal ferret produced no clinical response within 14 days when it was sacrificed. A suspension of this ferret's spleen was injected into six more mice all of which sickened and died. These results and evidence that our mouse colony was free of lymphocytic choriomeningitis are shown in Table I.

Guinea Pigs.—Three guinea pigs were injected subcutaneously with 0.5 cc. of the same suspension. One died the following day. The second developed fever on the 4th day

and thereafter failed rapidly. Death occurred on the 9th day accompanied by emaciation and dyspnea. The third pig was also febrile by the 4th day. Temperature remained elevated until the 11th day when death occurred. Emaciation was present, a considerable area of lung was consolidated by a serous pneumonia. The virus present in the brain of this animal has consistently produced the same clinical response in subsequent generations of guinea pigs.

Ferret.—One ferret was injected subcutaneously with 0.5 cc. of the suspension. Temperature rose to 104.5°F. on the 4th day. On the 5th day the temperature was 105.4°. On the 7th day it returned to normal to rise again on the 10th day. The animal remained febrile thereafter, soon becoming lethargic and developing severe rhinitis, conjunctivitis, reddened footpads and swollen eyelids. This animal was sacrificed on the 13th day. Conspicuous acidophilic inclusion bodies were present in the bile duct epithelium.

TABLE I

Pathogenicity of Source Material for Mice

Inoculum	Response of mice
12th sample of source material...................	$\underline{7}, \underline{7}$, (7) (7) (7), H
12th sample after ferret passage (spleen)...........	2, 3, 4, $\underline{5}, \underline{7}$, 11
12th sample after monkey passage (spinal cord).....	$\underline{7}, \underline{10}$, (10) (10) (11) (12)
Normal mouse brain.............................	S, S, S, S, S, S, S, S, S
Normal monkey cord............................	S, S, S, S, S, S, S, S

0.03 cc. of 10 per cent suspensions used. Numerals indicate day of death. Underlining indicates position in death was typical; parentheses that convulsions were observed.

Monkeys.—Five monkeys were injected subcutaneously with 0.5 cc. of the original suspension. All developed the symptoms we have described (1); approximately 2 weeks of fever followed by several days of subnormal temperature, extreme weakness, diarrhea, emaciation, rhinitis, conjunctivitis and irritability. Two of these monkeys were bled before injection and two others on the 20th day following. None of the sera contained neutralizing antibodies for a known strain of lymphocytic choriomeningitis. One animal was retested 35 days later and its serum found to possess some neutralizing capacity. Another was bled 7 weeks after inoculation and the serum found to completely neutralize the same strain of lymphocytic choriomeningitis virus (Table II).

A third monkey was sacrificed on the 17th day following inoculation. Postmortem examination revealed serous pericarditis, emaciation, purulent rhinitis and conjunctivitis and intranuclear inclusion bodies in the suprarenal cortex. A 10 per cent suspension of the spinal cord of this animal produced the symptoms of choriomeningitis in mice. One mouse was found dead on the 7th day, another on the 10th. Both had the extended legs and arched back often seen in mice dead of choriomeningitis. Two others died during typical convulsions on the 10th day and the remaining two mice on the 11th and 12th days (Table I).

A number of observations which had previously been made indicated that the lymphocytic choriomeningitis virus in the source material was present in considerable amounts.

A 13th sample of the pooled dog spleen was therefore secured and prepared in the usual 10 per cent suspension. Tenfold dilutions of this were made and injected subcutaneously into guinea pigs in 0.25 cc. amounts. Equal amounts of each dilution were

TABLE II

Tests for the Presence of Neutralizing Antibodies in the Sera of Monkeys Injected with Source Material

Monkey No.	Time serum was collected	Effect in guinea pigs of the injection of virus-serum mixtures		
		Test serum	Immune serum	Normal serum
1	Before injection of source material	12, 10, 20	S, S, S,	9, 9, 11
2		10, 10, 19	" " "	" " "
3	20 days after injection	12, 16, 16	12, S, S,	8, 13, 11
4		17, 13, 15	" " "	" " "
3	35 days after injection	9, 11, S	10, S, S,	6, 10, 18
4	48 days after injection	S, S, S,	S, S, S,	9, 10, 10

The numerals, in order, indicate the day of death of guinea pigs injected with 10^{-1}, 10^{-2} and 10^{-3} dilutions of virus.

TABLE III

Titre of Source Material Virus Pathogenic for Guinea Pigs and Neutralization of It by Serum of a Monkey Convalescent from Lymphocytic Choriomeningitis

Animal No.	Dilution of source material	Serum used	Temperature			Day of death
			5th day	10th day	12th day	
			°F.	°F.	°F.	
1	10^{-1}	None	102.0	103.0	102.5	14
2	10^{-2}	"	102.3	104.6	103.3	20
3	10^{-3}	"	101.3	102.8	102.3	S
4	10^{-1}	Normal	104.7	104.8	101.4	13
5	10^{-2}	"	103.2	104.0	103.9	17
6	10^{-3}	"	101.7	102.8	101.6	S
7	10^{-1}	Immune	101.8	103.6	101.9	S
8	10^{-2}	"	102.5	102.3	102.8	S
9	10^{-3}	"	102.2	102.9	102.6	S

The immune serum was from a monkey which had lymphocytic choriomeningitis 3 months earlier. Serum had been demonstrated to contain neutralizing antibodies. 0.25 cc. of virus suspension and 0.25 cc. of serum were mixed and incubated at room temperature for 5 hours and then injected subcutaneously.

mixed with 0.25 cc. of dog serum known to possess no neutralizing capacity for Rivers' strain of choriomeningitis virus and with the serum of a monkey convalescent from lymphocytic choriomeningitis produced by Rivers' strain of virus 3 months previously. This animal had been shown to be immune and its serum to neutralize virus. The serum-suspension mixtures were incubated for 5 hours at room temperature and injected subcutaneously into young guinea pigs. The results are shown in Table III.

Injection of Dogs with Lymphocytic Choriomeningitis Virus

Since no clinical evidence of the choriomeningitis had been recognized in the passage dogs, a number of experiments were planned in which dogs were injected with choriomeningitis virus alone. Two of these experiments are pertinent.

TABLE IV

Tests for the Presence of Neutralizing Antibodies in the Sera of Puppies One of Which Was Injected with Lymphocytic Choriomeningitis

Dog No.	Time serum was drawn	Effect in guinea pigs of the injection of virus-serum mixtures		
		Test serum	Immune serum	Normal serum
1	Previous to inoculation	8, 9, 20	S, S, S	7, 10, 19
2		8, 10, 11	" " "	" " "
1	35 days after inoculation	16, S, S	S, S, S,	2, 12, 12
2	35 days after inoculation of dog 1	11, S, S	" " "	" " "

Dog 2 was not injected but shared a common pen with No. 1.

Five 3 to 4 months old, dogs, raised in isolated quarters, have been injected subcutaneously with lymphocytic choriomeningitis virus. The first experiment consisted of a litter of six cocker spaniel puppies four of which were injected on three occasions, at intervals of 2 weeks, twice with 0.5 cc. of a 20 per cent suspension of virus recovered from dog spleen by monkey passage and once with 1 cc. of blood drawn from a monkey sick with lymphocytic choriomeningitis. None showed clinical response. 30 days after the last injection all six were given 1 cc. of a 20 per cent suspension of source material. All developed the classical symptoms of canine distemper and died or were sacrificed when moribund. In each animal the cytoplasmic inclusions already referred to were found.

Two mongrel puppies, born and raised in the laboratory, were bled 6 weeks after birth. Their sera, tested by the method already described, showed no neutralizing capacity for lymphocytic choriomeningitis virus. 2 weeks after bleeding one of the puppies was injected subcutaneously with 1 cc. of a 10 per cent suspension of guinea pig brain known to contain lymphocytic choriomeningitis virus. The two dogs were then placed in a common pen and observed for 10 weeks. The injected dog had a rectal temperature of 104°F. on the 14th day. No other evidence of reaction to the injection was

noted. The clinical course of the litter mate was uneventful. On the 6th, 15th and 33rd days samples of urine were collected from each dog and injected intracerebrally into mice all of which survived. The survivors on two occasions were subsequently reinjected with suspensions known to contain lymphocytic choriomeningitis virus and all but one proved susceptible. On the 21st day urine was collected and injected subcutaneously into four guinea pigs. None developed clinical symptoms or fever and all survived. However blood serum taken on the 35th day after inoculation showed the presence of neutralizing antibodies in the sera of both the injected and non-injected dog (Table IV). That this is not due to age is shown by neutralization tests on the sera of eight adult dogs all of which were free of neutralizing antibodies.

It appears from the foregoing two experiments that lymphocytic choriomeningitis virus, injected subcutaneously in dogs, produces no recognizable clinical responses although specific antibodies appear in the blood sera 35 days following injection. It is also evident and of considerable interest that an injected dog may rapidly transmit the infection to a cage mate.

DISCUSSION

The results appear to support two conclusions. The presence of two viruses in the source material is indicated by the simultaneous production of distemper in the ferret and a fatal disease in mice and guinea pigs since the latter are not susceptible to distemper (3, 6). The second virus is demonstrated also by passage of the source material in monkeys. Thus while distemper may be recovered from the first passage in monkeys (1) later passages are inactive. A single mouse passage will produce the same effect. The anatomical evidence is also clear since source material is capable of producing both cytoplasmic and intranuclear inclusion bodies in the dog and ferret while passage virus produces only the latter (Table V).

The identification of the second virus as that of lymphocytic choriomeningitis is supported by the clinical and anatomical responses in mice (7), guinea pigs (8), dogs and monkeys (9) and by the demonstration of the appearance, at the proper interval after injection (10) of source material of antibodies capable of neutralizing a known strain of lymphocytic choriomeningitis virus. The identity is also shown by the neutralization of source material virus pathogenic for guinea pigs by known immune serum, the demonstration of cross immunity and the occurrence in all the species studied of certain intranuclear inclusion bodies in the suprarenal gland as a consequence both of the injection of source material and known strains of lymphocytic choriomeningitis virus.

Thirteen samples of source material have been used during the past $2\frac{1}{2}$ years. A brief, chronological summary of the evidence we have indicating that both the virus of canine distemper and that of lymphocytic

TABLE V

Chronological Summary of Evidence of the Continued Presence of the Virus of Lymphocytic Choriomeningitis in the Spleens of Dogs in Which Canine Distemper Was Propagated Serially

Sample		Animals inoculated	Evidence of presence of canine distemper		Evidence of presence of lymphocytic choriomeningitis	
No.	Date		Symp-toms	Inclu-sions	Inclu-sions	Other evidence
	1936					
1	Sept. 19	2 ferrets	Typical	Present	Present	
2	Nov. 12	4 ferrets	"			
		↓				
		Monkey			Present	Clinical course
	1937					
3	Feb. 10	Monkey			"	Clinical course. Lympho-cytic chorioiditis
4	Mar. 4	Monkey				Clinical course
5	Sept. 4	Ferret	Typical	Present	Present	
		↓				
		Monkey			"	
		↓				
		Ferret	Typical	Present	"	
		↓				
		Monkey			"	
		↓				
		Monkey			"	Passed through 24 genera-tions. Virus demon-strated in 5 generations tested
6	Sept. 21	Ferret	Typical	Present	Present	
7	Dec. 8	Monkeys			"	
	1938					
8	Jan. 21	Monkey				Recovered. Immune to known virus given intra-cerebrally
		↓				
		Ferrets	Typical	Present	Present	
9	May 6	Dogs	"	"		Virus demonstrated in 1 of 2 tested
		↓				
		Monkey			Present	Virus demonstrated in spleen
10	May 25	Mice				Symptoms and lesions typi-cal
		Guinea pigs			Present	Typical clinically
11	June 7	Same as 10th sample				Lymphocytic chorioiditis. Same as 10th sample
12	Oct. 11	Mice, ferrets, guinea pigs and monkeys				Evidence covered in pres-ent report
	1939					
13	Jan. 9	Guinea pigs			Present	Neutralization by immune serum. Typical symp-toms

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choriomeningitis were present throughout that period has been compiled in Table V. Samples 12 and 13 have been described in the present report. Samples 9, 10 and 11 have also been discussed in some detail (2). The evidence of the presence of lymphocytic choriomeningitis virus in the earlier samples rests on the lesions, which have been constant throughout, the demonstration of the virus in passage monkeys by mouse inoculation and cross protection. The evidence for the presence of distemper is entirely anatomical and clinical. After reviewing the evidence in detail, however, the constancy of these criteria and various minor observations strongly support the conclusion that both viruses have been present throughout the entire period.

How lymphocytic choriomeningitis virus was introduced into the passage virus is not known. The dogs and ferrets are not believed to have been exposed to mice or guinea pigs and choriomeningitis virus was not recognized in the laboratories during the period in question. The circumstances responsible for the contamination evidently continue to exist, for another strain of distemper first demonstrated by us to be free of choriomeningitis virus and maintained under similar conditions later was found, in two samples tested, to be similarly contaminated.

It is evident that mixtures of these two viruses may be carried on indefinitely in dogs, for during the period of the present work the material has been passed much more frequently than our sampling would indicate.

One unusual circumstance associated with the source material may be of importance in explaining the continued presence of both viruses. Passage was regularly made with pooled dog spleen suspensions to groups of dogs. Thus the likelihood of losing one of the viruses by the injection of a naturally immune animal was largely avoided and the conditions were unusually favorable to the continued propagation of both.

The demonstration of infective amounts of lymphocytic choriomeningitis virus in the 10^{-2} dilution of dog spleen suggests that an active form of infection was present. This is supported by the presence of· inclusion bodies in infected dogs and ferrets, since these are evidently related only to the active phase of infection.

The only clue to the presence of lymphocytic choriomeningitis in the behavior and appearance of the dogs and ferrets injected with the source material has been the presence of intranuclear inclusion bodies in the suprarenal cortex. These have been found in mice, guinea pigs and monkeys as well. Their appearance and occurrence will be described elsewhere.

The absence of clinical manifestations of lymphocytic choriomeningitis

in the dogs and ferrets injected with both viruses is in harmony with the asymptomatic response of these animals to lymphocytic choriomeningitis virus alone. Findlay has reported a similar experience (11). "Of the other species that failed to succumb to infection, neither the dog, ferret or pig showed any noteworthy rise in temperature though immune bodies were subsequently detected in their blood."

The rôle which canine distemper played in our original experiments in which the source material produced a modifying effect on experimental poliomyelitis is unknown. Since lymphocytic choriomeningitis alone produces the same effect it seems probable that the distemper was unimportant. Several efforts have been made to determine whether distemper alone modifies the course of poliomyelitis without success. The failures have been due to the lack of a satisfactory distemper virus.

CONCLUSIONS

A particular strain of canine distemper, long maintained by serial passage in dogs and ferrets was found to contain the virus of lymphocytic choriomeningitis in addition to that of distemper.

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