STUDIES ON THE AGENT OF CANINE VIRUS HEPATITIS (RUBARTH'S DISEASE)

II. THE PATHOLOGY AND PATHOGENESIS OF THE EXPERIMENTAL DISEASE PRODUCED BY FOUR STRAINS OF VIRUS

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(With 1 Figure in the Text)

CONTENTS

I. INTRODUCTION

Virus hepatitis of dogs has been described in Sweden by Rubarth (1947), in Australia by Whittem & Blood (1949) and in England by Parry (1950). We have obtained virus from these countries and have compared the experimental disease produced in dogs and kittens following the inoculation by various routes of the different strains of virus. The main clinical and pathological features of the naturally occurring disease have been reproduced in the experimental disease and in approximately similar percentages of cases. Ferrets show no signs of illness after subcutaneous inoculation of hepatitis virus, and the injection of inocula into them offers a convenient means of excluding viruses of the canine distemper group (Dunkin & Laidlaw, 1926; MacIntyre, Trevan & Montgomery, 1948).

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II. MATERIALS AND METHODS

Source of animals. The dogs were born and reared in isolation in our stud kennel unit. They were fed on a mash, consisting of cooked whale meat and horseflesh, which was used to steep a finely ground cereal mixture to which small quantities of blood fibrinogen, whey concentrate, bone-meal and cod-liver oil were added. Their food intake was about 100–120 cal./kg./day and they were of normal growth. No ectoparasites have been observed, but some puppies had a moderate infestation of alimentary roundworms (*Toxascaris leonina*, *Toxocara* spp.).

The ferrets were born and reared in isolation in our stud ferret unit. The kittens came from country homes at weaning and were then kept in isolation. Ferrets and kittens were fed on raw meat and pasteurized milk.

Strains of virus. The four strains of canine hepatitis virus, two English (KEN and BECK), one Swedish (STOCK) and one Australian (SYD), described by Larin (1951*b*) have been used. They have been stored frozen or freeze-dried at −30° C. The inocula used for the first passage of these strains in dogs were also injected at the same time into ferrets in doses of 1 ml. subcutaneously. No illness was observed in the ferrets, which were inoculated 2–6 months later with virulent distemper-type virus (KENT strain of para-distemper), and to which they were fully susceptible.

Preparation of inocula. The virus material was ground up finely and suspended in normal saline to give a final concentration of 1:5, 1:10 or 1:100. In a few instances the suspension was used within 1 hr. of preparation ('crude' suspension), but more frequently a 'clarified' suspension was prepared from the 'crude' suspension by centrifuging at 3500–4000 r.p.m. for 20 min., decanting the supernatant and adding to it 500 units of penicillin and of streptomycin per ml. The 'clarified' suspension was usually stored at −30° C. for 4–18 hr. before use.

Routes of inoculation. Injections were given intraperitoneally following Rubarth's method (1947), into the anterior chamber of the eye under thiopentone or pentobarbital sodium anaesthesia (Evans, Dowell & Green, 1950), or intracerebrally under similar general anaesthesia.

Clinical appraisal. Rectal temperatures were recorded twice daily. General health, food intake and faecal evacuations were noted, and the eyes were observed closely for signs of the kerato-conjunctivitis described by Evans *et al.* (1950) and Parry (1950).

Pathological examination. The autopsy and histopathological findings have been compared with those reported in the natural disease (Parry, 1950; Platt, 1951).

Serological tests. Specimens were examined for the specific antigen and for antibodies, using the complement-fixation and precipitin tests described previously (Larin, 1951*a*,*b*).

III. RESULTS

A. *Reproduction of the disease in dogs*

(1) *The syndrome of kerato-conjunctivitis*

Intraocular injections were given because Evans *et al.* (1950) had reported that intraocular injections of fox encephalitis virus, which is probably identical with

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canine hepatitis virus (Rubarth, 1947; Siedentopf & Carlson, 1949), will produce clinical illness using smaller concentrations than are required when injections are given by other routes. After inoculation into the anterior chamber at the corneal-scleral junction, there was a cloudiness around the locus of the needle track, which disappeared after 24–48 hr. Thereafter, at various periods, usually the 3rd to 7th day after inoculation, kerato-conjunctivitis of an unusual type developed. The cornea showed a light diffuse opacity which appeared under a × 8 hand-lens as a very fine white opaque network just beneath the anterior surface of the substantia propria. The opacity was often uneven in density, sometimes the central and sometimes the perilimbal cornea being affected more severely, but in severe cases it proceeded to a tota ldense homogeneous opacity. Usually coinciding with, but sometimes preceding, the corneal lesion, there was photophobia, prolapse of the third eyelid and vasodilatation of the conjunctival blood vessels with oedema of the conjunctival sac, which was more marked dorsally. Occasionally the light reactions of the pupils were sluggish in an affected eye and the average pupil size in bright daylight increased from 6 to 10 mm. (diameter of iris 14 mm.) and the eyelids became swollen. These signs subsided occasionally in 24 hr., usually over several days, the corneal opacity disappeared without any ingrowth of blood vessels from the limbus and there were no residual opacities. In microscopic preparations at the height of the clinical reaction the blood vessels of the iris and ciliary bodies were markedly congested and the cells of the substantia propria of the cornea were separated as if by oedema fluid. No signs of rickettsiae were found in scrappings of conjunctival epithelium stained by Coles's (1936) method.

This type of kerato-conjunctivitis, which we have also observed in dogs inoculated by the intraperitoneal route only and in natural cases of the disease, is unlike the types of kerato-conjunctivitis usually seen in the dog; and we consider it to be very suggestive of virus hepatitis infection.

(2) *Inoculation of KEN strain*

First passage of virus. A 7-week-old greyhound pup, DE32, and a 9-week-old mongrel, DE33, both bitches, were injected intraocularly with 0·05 ml. of 1:5 'clarified' suspension from the pooled organs (liver, spleen and lung) of four dogs dying of the original epidemic. In the greyhound the eye reaction began in the left eye on the 4th day and in the right eye on the 6th day. These signs had regressed on the 7th day in the right eye, but not in the left eye. The general health of the dog was normal except on the 6th day, when the animal was listless but without pyrexia. The dog was killed on the 7th day in early convalescence. The autopsy revealed no gross abnormalities, except in the left eye, in which there was congestion of the iris and ciliary body and oedema of the cornea. No inclusion bodies were found on histological examination, either in the hepatic cells, which showed vacuolation of the cytoplasm, or in the eye.

The pup DE33 showed no clinical abnormalities until 4 weeks after inoculation when the cornea of the left eye showed a mild kerato-conjunctivitis, which increased in severity slowly over the next 2 weeks, when the pupillary light reactions were sluggish, the eyelids oedematous and there was photophobia. Thereafter, these

signs regressed during the next 14 days. The right eye remained unaffected throughout. During the height of the eye reaction there was a pyrexia of 1–2° F., with slight listlessness. Specific antibodies were detected in the serum by the complement-fixation and ring-precipitin tests 12 days after the height of the eye reaction.

The observations on these two dogs show that the disease may follow intraocular injection of the virus. However, the delayed response in one dog, the variability in response between the two eyes of the same dog and our failure to demonstrate inclusion bodies in corneal cells, even when the eye showed clinical signs, indicated that with KEN strain of virus the intraocular route was not as reliable as Evans *et al.* (1950) had reported for their strains of fox encephalitis virus. We therefore decided to use other routes in conjunction with the intraocular.

The second passage. To confirm that the syndrome observed in the greyhound, DE 32, was virus hepatitis, a 1:10 'clarified' suspension of its liver was inoculated into a 3-month-old greyhound, DE 34, injecting 0·05 ml. into each eye and 10 ml. intraperitoneally.

The dog showed morning pyrexia to 104° F. from the 8th to 10th days, with some listlessness, reduction of food intake and increase of pulse frequency with reduced amplitude of the pulse wave. Whole blood appeared in the faeces on the 8th and 9th days, and there was a mild kerato-conjunctivitis affecting both eyes on the 8th day, which had disappeared on the 12 day. Specific antibodies were demonstrated in the blood from the 20th day onwards and specific antigen on the 28th and 37th days, but not at 30 days and 10 weeks.

The disease in this dog was similar to the milder forms of the naturally occurring canine virus hepatitis, and the results confirmed that the greyhound, DE 32, was affected with virus hepatitis.

(3) *Inoculation of BECK strain*

The first passage. Four 12-week-old greyhounds, litter-mates of dog DE 32, were used. One, DE 31, ♂, was killed as a control; its tissues were normal on examination and an extract of its liver gave a negative result when tested for specific antigen. The remaining three puppies were inoculated with a 1:10 'clarified' suspension of the original sample of infected liver; one bitch, DE 35, received 1 ml. intracerebrally, one dog, DE 37, 10 ml. intraperitoneally and one dog, DE 38, 0·05 ml. into each eye and 10 ml. intraperitoneally. All three dogs had temperatures of 103·5° F. on the morning of the third day without other signs; on the 4th day the temperatures were lower. On the 5th morning the temperatures were 105° F. and dogs DE 37 and DE 38 were very ill with severe malaise, blood-stained stools and signs of collapse; dog DE 38 had bilateral kerato-conjunctivitis; at noon DE 37 and DE 38 were comatose with temperatures of less than 95° F.; DE 38 was found dead in the afternoon and DE 37 was killed in the evening. Dog DE 35 continued to feed on the 5th and 6th days, when its temperature dropped to 103° F. from 105° F.; it was found dead on the 7th morning. Morbid anatomical findings on the three carcasses were very similar to each other and to those of peracute fatal cases of the natural disease. There was general pallor, but only dog DE 37 showed signs of icterus. In the peritoneal cavity there was serous fluid. The liver, covered with

a thin fibrinous deposit, was slightly enlarged with pin-point necrotic foci. The gall-bladder wall was greatly thickened and oedematous. The wall of the intestines was congested and thickened with the lumen filled with blood. The spleen was enlarged. The lymph nodes were oedematous, especially those in the abdomen. In the lungs there were numerous small circular subpleural haemorrhages which were 2–3 mm. in diameter. The brain appeared normal in dog DE38, but there were a few petechial haemorrhages on the surface of the brains of dogs DE37 and DE35. After fixation the substance of the brains appeared normal in coronal sections.

Microscopic preparations showed marked congestion of the liver, distension of Disse's spaces, and a moderately severe centrilobular necrosis with some leucocytic infiltration. Inclusion bodies in hepatic cell nuclei were numerous, being commoner in dogs DE37 and DE35; they were also common in the endothelial cells of the glomerular tufts of the kidney. The lymph nodes were oedematous with many erythrocytes in the medullary sinusoids. The spleen was engorged. There were small discrete areas of haemorrhage and pneumonitis in the lungs, but the mass of the organ was normal. The right eyes of dogs DE38 and DE37 were examined; in the eye of DE38 nearly every cell of the corneal endothelium contained an intranuclear inclusion body. Intranuclear inclusion bodies were also present in cells of the anterior surface of the iris and ciliary body; no inclusion bodies were found in dog DE37.

This strain of virus, which is indistinguishable serologically from KEN strain (Larin, 1951*b*), caused a fatal illness, very similar in its course, clinical signs and pathological findings with the severe forms of the naturally occurring disease caused by KEN strain. The disease following inoculations by different routes was also very similar. It is of interest that after intra-cerebral inoculation no clinical signs of encephalitis were observed and that the pathological findings in the nervous system were essentially similar to those seen in dogs inoculated by other routes.

(4) *Inoculation of SYD strain*

Two 18-week-old Afghan × red Irish setter dogs, DO62, ♂, and DO64, ♀, were inoculated with 0·05 ml. into each eye and 2 ml. intraperitoneally of a 1:100 'clarified' suspension of virus. Both dogs showed pyrexias of 104° F. from the 3rd to 7th days, with slight malaise and some reduction of food intake on the 4th and 5th days. Dog DO62 had a mild kerato-conjunctivitis in both eyes from the 4th to 7th days; the left eye cleared but the right eye developed a suppurative ophthalmitis, which left a permanent dense opacity of the cornea. Dog DO64 showed a mild kerato-conjunctivitis in both eyes from the 8th to 10th days; this reappeared for 2–3 days in the left eye at the 55th day, but when observations ceased on the 64th day, with clinical recovery of both dogs, both eyes were normal.

The complement-fixation test for antigen in the blood gave a positive reaction on the 6th day and was negative in the 4th week. Antibodies were detected from the 21st and 22nd days onwards. Details are given in Table 3.

(5) *Inoculation of STOCK strain*

Two 18-week-old Afghan × red Irish setter dogs, DO 65, ♂, and DO 67, ♀, littermates of dogs DO 62 and DO 64, were inoculated with 0·05 ml. into each eye and 4 ml. intraperitoneally of a 1:10 'clarified' suspension of virus. Dog DO 67 showed no response. Dog DO 65 had slight photophobia and corneal opacity from the 6th to 14th days in both eyes, and the corneal opacity continued in the right eye until the 30th day. On the 38th day there was a mild hysterical psychomotor seizure, followed by a pyrexia of 104·8° F., but listlessness did not develop till the 44th day when there was also a mild kerato-conjunctivitis of the right eye, which subsided by the 48th day. Complement-fixation tests for antigen in the blood were negative during the first and fourth weeks as well as on the 38th day. Blood from dog DO 67 also gave negative complement-fixation tests for antigen. Antibodies were detected from the 41st and 42nd days onwards. Details are given in Table 3.

The illness produced with the SYD and STOCK strains was comparatively mild, but the kerato-conjunctivitis in one dog and subsequent demonstration of specific antibodies in both is strong evidence that these strains produced the same disease as the English strains, KEN and BECK.

B. *Production of the disease in kittens*

To determine whether the presence of canine hepatitis virus might be demonstrated more readily by inoculation of cats, kittens were inoculated with virus by various routes. A 1:5 'clarified' suspension of virus material (KEN strain) isolated from dogs dying in the original epidemic was inoculated in a dose of 0·05 ml. into each eye of two 6-week-old kittens. Clinical signs were observed in one kitten only, which on the 12th to 14th days developed a mild kerato-conjunctivitis in the right eye only; this condition was similar to that seen in the dogs described above. This animal was killed on the 15th day when the right eye was still affected. The pathological examination revealed no macroscopic evidence of disease apart from the lesion in the right eye. Microscopically the liver showed areas in which the liver cell columns were swollen, the cell cytoplasm vacuolated and the nuclei undergoing karyorrhexis. The cornea of the right eye showed much intercellular oedema without any cellular infiltration; similar but only very slight lesions were also present in the left eye. No inclusion bodies were found in any situation.

Liver from this kitten, stored at −30° C. for 3 months, when extracted gave a positive complement-fixation test for specific antigen, and was inoculated into a 7-week-old mongrel puppy, ♂, DE 48, by the intraocular and intraperitoneal routes using 0·05 and 10 ml. respectively of a 1:10 'clarified' suspension. The typical kerato-conjunctivitis appeared in both eyes of the dog from the 4th to 7th days with slight pyrexia. The complement-fixation test for antigen in the blood was negative at this stage but was positive at the 8th week, while antibodies were detected after 23 days.

Another kitten, 5 months old, was inoculated intracerebrally with 0·5 ml. of a 1:10 'clarified' suspension of the first passage BECK strain virus material; no clinical abnormalities appeared for 1 month, when observations were discontinued.

These results indicate that young kittens are susceptible to the virus, but that their susceptibility is probably less than that of dogs. The recovery of viable virus from the convalescent kitten by inoculation into a dog suggests that the virus had multiplied in the kitten.

C. *Persistence of virus in clinically recovered dogs*

(1) *In a dog dying suddenly three months after complete recovery*

A border collie, DO 50, ♂, 21 months' old, which had been severely ill during the original epidemic of virus hepatitis (Parry, 1950) but had apparently recovered completely, and which had developed a good titre of specific antibodies 3 months after the original illness, suddenly collapsed and died within 2 min. The autopsy suggested acute respiratory arrest as the immediate cause of death; microscopically there was inter-alveolar fibrosis of the lungs, periportal fibrosis of the liver with distension of the peri-sinusoidal tissues without any intranuclear inclusion bodies. No bacteria or Protozoa were found in the heart blood, liver and spleen. The complement-fixation reaction for canine hepatitis virus antigen was strongly positive with this liver.

To confirm the presence of virus in this dog, a 1:5 'clarified' suspension of the liver was inoculated into a 2-month-old mongrel, DE 44, ♀, sera from which had been negative for antigen and antibodies on two occasions during the previous week; 0·05 ml. was injected into both eyes and 10 ml. intraperitoneally. On the 9th day this dog showed mild pyrexia with reduced food intake and whole blood in the faeces; these signs disappeared in 24 hr. On the 14th day there was a mild kerato-conjunctivitis of both eyes which disappeared on the 16th day. The results of serological examinations of the blood are shown in Table 1; antigen was detected in the blood at the 5th week and antibodies from the 7th week onwards.

These results indicate that this dog DO 50 was still harbouring viable hepatitis virus in its liver 3 months after clinical recovery. The role of virus infection in the death of this dog is not clear.

(2) *In the peripheral blood of recovered dogs*

Following the demonstration of hepatitis virus in dog DO 50, 3 months after clinical illness, we attempted to demonstrate virus in the blood of three other dogs, affected in the original epidemic, 6 months after recovery. These dogs, a greyhound dog, DS 10, affected severely, a greyhound bitch, DS 11, affected mildly, and an Irish setter bitch, DO 30, affected mildly, had developed specific antibodies, but no antigen had been detected in their peripheral bloods; 5 ml. of blood was collected in citrate from each dog. The pooled blood was inoculated within 2 hr. into a 2-month-old mongrel, DE 43, ♂, in which no antigen or antibodies had been detected in two different blood samples collected during the previous week; 0·05 ml. was injected into each eye and 10 ml. intraperitoneally. No illness was observed in this dog, but the results of serological examinations of its blood showed the presence of antigen during the 4th week and of antibodies from the 10th week after inoculation (see Table 1).

These results indicate that the inoculated dog developed an inapparent hepatitis

Table 1. *Detection of canine hepatitis virus antigen and antibodies in the blood of inoculated dogs using the complement-fixation reaction*

Dog no.	Source of inoculum		Weeks since inoculation: ½	1st	2nd	3rd	4th	5th	7th	10th	12th
DE44	Recovered dog liver	Antigen	—	—	±	—	—	+	—	—	—
		Antibody	—	—	—	—	—	—	±	+ + + +	+ + + +
DE43	Recovered dog blood	Antigen	—	—	—	—	+	—	—	—	—
		Antibody	—	—	—	—	—	—	—	+ + +	+ + + +
DE40	Lice	Antigen	—	+	—	—	±	+	—	—	—
		Antibody	—	—	—	—	—	—	—	+ + + +	+ + + +

Table 2. *Response of dogs recovered from experimental and natural canine virus hepatitis to a challenge inoculation with virulent canine hepatitis virus*

Dog no.	Breed	Age	Previous infection: Kind	Strain	Time elapsing	Severity	Clinical response to challenge inoculation
DO62	Afghan × Setter	7 months	Experimental	SYD	3 months	M. 1	None
DO64	Afghan × Setter	7 months	Experimental	SYD	3 months	M. 1	None
DO65	Afghan × Setter	7 months	Experimental	STOCK	3 months	M. 1	Slight kerato-conjunctivitis
DO67	Afghan × Setter	7 months	Experimental	STOCK	3 months	M. 0	None
DE48	Mongrel	7 months	Experimental	KEN	4 months	M. 1	None
DO24	Setter	1½ years	Natural	KEN	1 year	M. 1	None
DO28	Setter	1½ years	Natural	KEN	1 year	M. 1	None
DO78	Setter	6 months	Natural	KEN	3 months	M. 3	None
DO81	Setter	6 months	Natural	KEN	3 months	M. 3	None
DE54	Greyhound	4 months	None	.	.	.	Severe illness with recovery
DE54	Mongrel	3 months	None	.	.	.	Severe illness with recovery

Key (Tables 2–4). Severity of illness: M. 0 = inapparent; M. 1 = mild; M. 2 = moderately severe; M. 3 = severe; M. 4 = very severe and fatal.

virus infection and that hepatitis virus can occur in the peripheral blood of dogs 6 months after clinical recovery, for it is difficult to explain the presence of specific antigen without assuming the proliferation of virus.

D. *Presence of virus in lice from affected dogs*

Two 4-month-old red Irish setters, introduced into the stud kennel unit 2 months after the original epidemic, were found to be affected with a moderately severe form of virus hepatitis 6 weeks after their introduction. These dogs were found to be infested with dog lice, and a number of lice were collected from them during the first 3 days of clinical illness.

A methanol-precipitated antigen was made from a suspension of 200 lice, and this gave a positive complement-fixation reaction with canine virus hepatitis hyperimmune serum. We therefore inoculated a 'clarified' suspension, obtained by freezing and thawing and grinding together 150 lice, into a 3-month-old greyhound, DE 40, ♀, in which no antigen or antibodies had been detected in two samples of peripheral blood taken during the previous week; 0·05 ml. was injected into each eye and 10 ml. intraperitoneally. There were signs of moderate pyrexia on the 8th and 18th days, but otherwise there were no clinical signs of illness. However, serological examination of blood, the results of which are given in Table 1, demonstrated the presence of antigen during the 1st, 4th and 5th weeks, and antibodies from the 10th week.

These results indicate that the lice contained enough viable virus to cause infection and a mild disease.

E. *Response of clinically recovered dogs to challenge inoculation*

To determine whether the dogs inoculated in the previous experiments were immune to canine virus hepatitis, they were inoculated with a virulent BECK strain. At the same time survivors of the naturally occurring disease were also challenged.

A virulent BECK strain of virus was prepared. First passage virus material harvested from three acute fatal cases, and stored without preservative at −30° C. for 5 months, was passaged a second time to two 4-month-old greyhound pups, DE 52, ♂, and DE 53, ♀, which were inoculated intraperitoneally with 10 ml. of a 'crude' 10% liver suspension. Both dogs were normal for the first 48 hr., but they became severely ill between the 50th and 60th hr. (overnight). Dog DE 53 was found dead at 60 hr. and dog DE 52 had a temperature of 96·6° F., with diarrhoea and weakness; it died between the 64th and 66th hr. At autopsy very severe lesions of canine virus hepatitis were found, and microscopic preparations of their livers showed very numerous intranuclear inclusion bodies. No bacteria or Protozoa were found.

Virus material from the second passage was used for the challenge inoculation. The livers of dogs DE 52 and DE 53, were stored at −30° C. for 5 days; 10 ml. of a 'clarified' suspension was then injected intraperitoneally into eleven dogs (see Table 2). None of the dogs which recovered from the natural or experimental infections showed any evidence of ill-health, except dog DO 65, which developed

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a kerato-conjunctivitis in both eyes on the 7th day, which lasted for 14 days without pyrexia. The two control dogs, a 4-month-old greyhound, DE54, ♂, a litter-mate of dogs DE52 and DE53, and a 3-month-old mongrel, DE60, ♂, showed pyrexias of 104° F. after 48 hr., which continued until the 8th morning, with signs of severe hepatitis. Dog DE60, had short hysterical psychomotor seizures on the 6th and 8th days, after which it recovered completely. Dog DE54 showed a mild kerato-conjunctivitis of the left eye on the 14th day which had disappeared by the 21st day, since when the dog has been normal.

These results show that our inoculum was virulent. The dogs previously infected, apart from dog DO65, did not react and were presumably immune. The significance of the recrudescence of kerato-conjunctivitis in dog DO65 is at present uncertain, but it may possibly have been due to the 'lighting up' of a latent infection of the virus, as both eyes were affected after the primary infection.

F. *The occurrence of complement-fixing antigen and antibody in inoculated dogs*

In the experiments already described no specific antigen or antibodies against canine hepatitis virus were detected in the serum of any dog before inoculation, but both specific antigen and antibodies were demonstrated at various periods thereafter.

(1) *The occurrence of antigen*

The results of testing for antigen by the complement-fixation test using dog hepatitis virus hyperimmune serum are shown in Tables 1 and 3.

In the dogs inoculated with KEN, SYD and STOCK strains, in which the clinical illness was mild or inapparent, antigen was detected in the blood at periods varying from the 1st to the 8th week after inoculation. Sometimes this coincided with mild clinical signs, but clinical signs occurred when no antigen could be demonstrated.

In the sera of three dogs dying of an acute fatal illness (dogs DE35, DE37 and DE38) the test for antigen was strongly positive during the terminal phases of the disease within 24 hr. of death and in the livers after death. However, no antigen could be detected in dog DE35, 36 hr. before death, when the temperature was higher but the clinical illness much less severe.

To obtain more precise data on the occurrence of antigen in the peripheral blood during the acute phase of the disease, the sera from two dogs, DE54 and DE60, which developed severe non-fatal forms of the disease while being used to determine the virulence of the inocula used in the challenge experiments, were examined at approximately 4–6 hr. intervals (with the exception of early morning) for a week after the inoculation. The results with the two dogs were very similar; that for DE60 is shown in Fig. 1.

It will be seen that antigen could be detected in the blood within 3–7 hr. of the inoculation and thereafter at irregular intervals. The antigen persisted for only relatively short periods, because often a sample contained no antigen although it was taken 4–6 hr. after one known to contain antigen. The presence of antigen was usually followed within 4–8 hr. by a rise of temperature and exacerbation of the illness.

Table 3. *Occurrence of complement-fixing antigen in the blood of dogs after inoculation with different strains of canine hepatitis virus*

Strain	Dog no.		Period after inoculation: Days 4th	5th	6th	Weeks 2nd	3rd	4th	5th	6th	8th	11th
BECK	DE35	C.C.	M. 1	M. 3	Died	.	.	.	.	.	.	.
		C.F.T.	−	++++	.	.	.	.	.	.	.	.
	DE37	C.C.	M. 4	Died	.	.	.	.	.	.	.	.
		C.F.T.	++++	.	.	.	.	.	.	.	.	.
	DE38	C.C.	M. 4	Died	.	.	.	.	.	.	.	.
		C.F.T.	++++	.	.	.	.	.	.	.	.	.
KEN	DE34	C.C.	.	M. 0	M. 0	M. 2	M. 0	M. 0	.	M. 1	M. 0	M. 0
		C.F.T.	−	−	−	−	−	+	.	+	−	−
	DE48	C.C.	M. 1	.	.	M. 0	M. 0	.	.	.	M. 0	.
		C.F.T.	−	.	.	−	−	.	.	.	+	.
SYD	DO62	C.C.	.	.	M. 2	.	.	M. 0	.	.	.	.
		C.F.T.	.	.	−	.	.	−	.	.	.	.
	DO64	C.C.	.	.	M. 2	.	.	M. 0	.	M. 0	.	.
		C.F.T.	.	.	+	.	.	−	.	−	.	.
STOCK	DO65	C.C.	.	.	M. 1	.	M. 1	M. 0	M. 0	M. 2	.	.
		C.F.T.	.	.	−	.	−	.	.	−	.	.
	DO67	C.C.	.	.	.	.	.	M. 0	.	.	.	.
		C.F.T.	.	.	.	.	.	−	.	.	.	.

Key: C.C. = clinical condition; C.F.T. = complement fixation test for antigen; M. 0–4 = severity of illness, see key on Table 2.

Thus antigen occurred irregularly in the peripheral blood during the acute phase of the disease and for only a few hours at a time. In the convalescent dog, antigen may appear in the peripheral blood at irregular intervals up to 6–8 weeks after inoculation and 6 weeks after clinical recovery, and is often not associated with any clinical signs. These observations, taken in conjunction with our inoculation experiment with tissues from recovered dogs, suggest that recovered dogs may harbour the virus for considerable periods. Failure to demonstrate antigen during the recrudescences of clinical signs in dog DO 65 (see Table 3) was probably due to inappropriate timing of the bleeding.

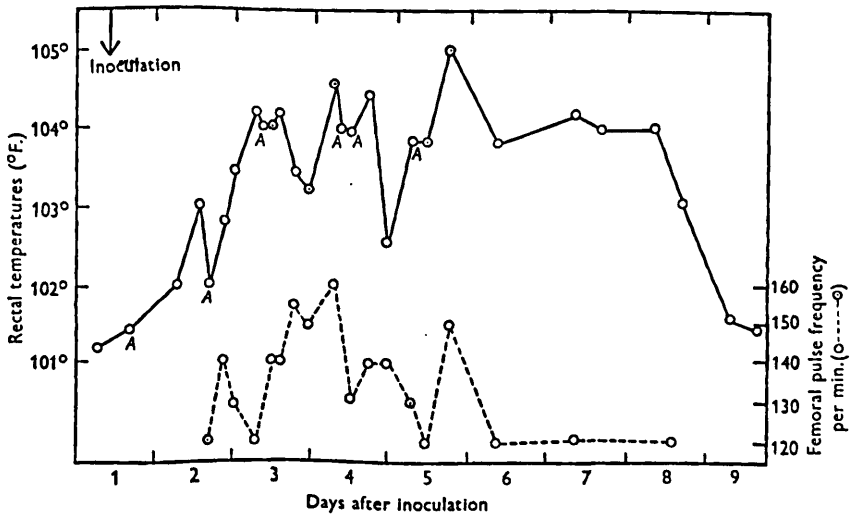

Fig. 1. The response of a dog to the intraperitoneal inoculation of canine hepatitis virus. Blood samples were taken at the time of the temperature recordings; *A* denotes the samples in which antigen was detected.

(2) *The development of antibodies*

The sera of inoculated dogs have been examined for specific antibodies using purified methanol precipitated antigen; the results are shown in Tables 1 and 4.

Antibodies were not detected in the sera of dogs dying of the acute disease, but in all dogs exhibiting a mild or even inapparent illness antibodies were detected first at periods of 21–70 days after the injection and 12–38 days after the onset of the illness. It will be noted that the antibodies usually appear more quickly the more severe the clinical disease.

IV. DISCUSSION

The disease produced experimentally in dogs had all the main clinical and pathological features seen in the naturally occurring epidemics of virus hepatitis. Table 5 shows the close similarity between the natural and the experimental disease in the frequency of the various clinical and pathological signs and in the occurrence of the principal pathological lesions in all the fatal cases irrespective of the route of infection. The minor signs were also alike. In many milder cases of the experimental and natural disease pyrexia was not accompanied by any

Table 4. *Occurrence of specific antibodies in the blood of dogs after inoculation with canine hepatitis virus*

Strain	Dog no.	Severity of illness	Days elapsing to first detection of antibodies: From inoculation	Days elapsing to first detection of antibodies: From onset of illness
KEN	DE 33	M. 1	51	12
	DE 34	M. 2	21	13
	DE 48	M. 1	23	19
SYD	DO 62	M. 1	21	18
	DO 64	M. 1	22	18
STOCK	DO 65	M. 2	42	36
	DO 67	M. 0	41	

M. 0–4: see Key, Table 2.

Table 5. *Occurrence of various clinical and pathological signs in experimental and natural infections of canine virus hepatitis in dogs*

	No. of cases showing various signs: In experimental infections: KEN strain	BECK strain	SYD strain	STOCK strain	Total: No.	Total: %	In a natural epidemic KEN strain*: No.	In a natural epidemic KEN strain*: %
Total no. of dogs ...	4	7	2	2	15	.	34	.
Severity of illness:								
Fatal	.	5	.	.	5	33	4	12
Severe	.	2	.	.	2	14	9	26
Moderately severe	1	.	2	.	3	20	9	26
Mild	3	.	.	1	4	26	12	36
Inapparent	.	.	.	1	1	7		
Clinical signs:								
Pyrexia (greater than 104° F.)	.	7	2	.	9	59	9	26
Icterus	.	1	.	.	1	7	2	6
Pallor of membranes	.	3	.	.	3	20	12	32
Rectal bleeding	1	2	.	.	3	20	5	15
Kerato-conjunctivitis (after I/P inoculation only)	.	2	.	.	2	14	1	3
Canine-hysteria	.	2	.	.	2	14	9	26
Pathological findings:								
Peritoneal effusion	.	5	.	.	5	100	4	100
Hepatitis	.	5	.	.	5	100	4	100
Gallbladder oedema	.	5	.	.	5	100	4	100
Blood in intestines		4	.	.	4	80	4	100
Lung haemorrhages	.	5	.	.	5	100	1	25
Cerebral haemorrhages	.	2	.	.	2	40	1	25
Intranuclear inclusion bodies	.	5	.	.	5	100	4	100
Absence of above in early convalescence	1	.	.	.	.	.	1	.

* Data taken from Parry (1950).

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apparent ill-health, or reduction of food intake, but slight cardiovascular signs were present. We have also occasionally observed thirst, kerato-conjunctivitis, transient ocular and nasal discharges, and retraction of the eyeball, signs observed occasionally in the natural disease. The kerato-conjunctivitis was unusual in that the corneal opacity developed very rapidly and disappeared without any vascular reaction. Kerato-conjunctivitis was expected 3–5 days after intraocular inoculation, but its appearance weeks after the primary ocular infection, and in dogs inoculated intraperitoneally, was not anticipated. The recrudescences of kerato-conjunctivitis in recovered dogs, either spontaneously or after challenge inoculation with virulent virus, are also of interest. We now regard this kind of eye reaction as strong evidence of active hepatitis virus infection.

That the four strains of virus produced the same disease, but of varying severity, has been confirmed by the resistance of recovered animals to challenge inoculation with virulent BECK virus, and by the similarity of the specific serological reactions.

The susceptibilities of individual dogs to the infection have appeared to vary. Rubarth (1947) noted similar variations in susceptibility. Although kittens can be infected with a mild disease, in which the virus has probably multiplied, they seem to be less susceptible than dogs. Natural cases of the disease in cats have not been reported as far as we know.

The serological tests developed in these laboratories (Larin, 1951*b*) have proved valuable in detecting antigen and antibodies specifically related to the virus in blood and tissues, and in establishing that inapparent and very mild infections have occurred. Such infections are probably common under natural conditions.

That antigen and probably virus can persist as an inapparent infection in clinically recovered animals for at least 6 months is of great importance, although serologically detectable amounts of the specific antigen only appear in the peripheral blood for a few hours at irregular intervals.

While we have not yet been able to demonstrate that the methanol-precipitated antigen is virus itself, it is capable of producing observable disease (Larin, 1951*a*). It is also difficult to explain the detection of antigen 3–7 hr. after intraperitoneal inoculation of virus and the periodical occurrence of antigen in the peripheral blood unless proliferation of the virus is assumed to occur within the animal.

At the same time, the recrudescence of kerato-conjunctivitis in recovered animals suggests that the infection has persisted. Such persistence would help to explain certain epidemiological features of the disease (Parry & Larin, 1951), while the demonstration of virus in lice indicates that they can be vectors of the disease.

The natural history of canine hepatitis virus shows many points of similarity to that of the human hepatitis viruses. Thus, the severity of the disease varies considerably in different individuals; the young and adolescents are more susceptible than adults; inapparent infections occur, probably frequently under natural conditions; virus and antigen specifically related to the virus persist in the blood and tissues of infected individuals for several months after clinical recovery.

V. SUMMARY

The main clinical and pathological features of the naturally occurring canine virus hepatitis were reproduced in dogs, using four strains of virus from different parts of the world and by various routes of inoculation. Young puppies seemed to be most susceptible. A kitten was also infected. An unusual type of kerato-conjunctivitis occurred after both intraocular and intraperitoneal injection.

Specific antigen was detected in the blood for short periods at irregular intervals during the acute stages of the illness and up to 6 months after complete clinical recovery.

Specific antibodies were detected in the blood of recovered animals 3 to 10 weeks after the inoculation and 2–6 weeks after the onset of clinical illness.

The four strains of virus were similar in their serological and immunological characters.

Evidence is presented that virus can persist for some months in recovered animals.

Virus was demonstrated in lice collected from affected dogs.

The importance of clinically inapparent infections is stressed.

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