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STUDIES ON THE VIRUS OF HEPATITIS CONTAGIOSA CANIS (HCC)

IV. THE PATHOGENESIS OF HEPATITIS CONTAGIOSA CANIS IN DOGS

By

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HCC virus infection in dogs has been studied from a number of viewpoints. Thus the clinical and the pathologic-anatomical sides have been very thoroughly described. The pathogenesis on the other hand, has scarcely been the subject for closer studies. Information concerning the pathogenesis can be obtained by qualitative and quantitative determinations of virus in organ material from different phases of the disease. The presence of HCC virus or antigen has also been shown by many authors, among them *Rubarth* (22), *Lehnert* (16), *Florent* and *Leunen* (9), *Baker et al.* (1), *Brunner* (3), *Larin* (15), *Parry et al.* (20), *Poppensiek* and *Baker* (21), *Schindler* (25), *Cabasso et al.* (4), *Fieldsteel* and *Emery* (8) and *Müller* and *Thordal-Christensen* (19). It can hardly be said that these investigations have explained pathogenesis. Generally, only a few organs have been examined. Only occasionally have the dogs been given virus per os, which probably is the natural route of infection. *Mansi* (17, 18) has tested the virus amount in various specimens from dogs inoculated in different ways. Thus a few dogs were infected by contact with sick animals. *Mansi* has chosen to take the test material in an advanced stage of the disease. The virus had by that time been distributed almost throughout the whole body and this has complicated the attempts to explain the pathogenesis. However, *Mansi*

made an important observation. The infected dogs, including those which contracted infection through contact, showed an acute tonsillitis. This was a confirmation of the earlier observations by *Rubarth* (22) and *Coffin* (5). The tonsils showed a high concentration of complement fixing antigen.

The experiments reported in this paper were undertaken with the purpose better to understand the pathogenesis of HCC virus infection in the dog.

MATERIAL AND METHODS

Virus. The virus strain used was SBL S1 isolated in this labora tory (23). A batch named pool 4559 was prepared as described earlier (24). The virus titre of the pool was $10^{7.6}$ $TCID_{50}$ per ml.

Tissue Culture. Roller tube cultures were prepared of trypsinized dog kidney cells as previously reported. For details the reader is referred to preceding papers (23, 24). The seeded cells were suspended in 1.0 ml. medium per tube and the addition of human embryo extract to the medium after the growth period was omitted or substituted by 0.5 % Bactotryptose or 5 % horse serum.

Inoculation of Dogs. The dogs, 2—4 months old, being serologically negative to HCC, were generally given 1.0 ml. of pool 4559 per os. Otherwise, the dose is stated in the particular experiment. The virus fluid was deposited in the oral cavity by means of a pipette.

Collection and Handling of Test Material. Blood from the dogs was obtained by venous puncture. Heparin was added in a final concentration of 1:2000. The animals were then anaesthetized and exsanguinated. From some of the dogs different kinds of organ materials were excised. Lymph was also taken. Heparin was added to this in the same concentration as to the blood. The organ materials were thoroughly cut and ground. During this procedure buffered saline was added to make a 10 % organ suspension.

The specimens from the small intestinal wall, from contents in the small intestine and from Peyer's patches (P. p.) were numbered in the order in which they were taken. Thus P. p. 1 was taken in the proximal part of the small intestine, P. p. 10 in the distal part close to valvula ileo-caecale.

Throat and rectal swabs were placed in tubes containing 1.0 ml. of tissue culture medium. After shaking for 5 minutes the

swabs were removed. Penicillin and streptomycin were added to give a final concentration of 1000 I.E. and 1000 μg., respectively. The tubes were kept at 37°C for 30 minutes and then centrifuged at 3000 r.p.m. for 10 minutes. The supernatant was pipetted off and used as inoculum.

Virus Isolations and Infectivity Titrations. Virus isolations and titrations on the specimens from guinea pigs and dogs were performed in dog kidney tissue cultures. Serial tenfold dilutions of the collected material were prepared and then inoculated into the tissue culture tubes, 0.1 ml. per tube. The tubes contained 1.0 ml. of medium. After an absorption time of 6 hours at 20°C, the medium in the tubes was changed in order to avoid toxic effects of the inoculum. The titre was computed according to *Kärber* (14) and expressed as log $TCID_{50}$ per ml.

Haemagglutination-Inhibition Test. The serological test was performed on serum from dogs. The titration was done as described earlier (7). Equal amounts (0.2 ml.) of serum dilution and antigen containing 4 HA units, were mixed. One hour later, 0.4 ml. of a 0.25 % suspension of human erythrocytes group 0 was added to the serum-antigen mixtures. After shaking, the test tubes were incubated in room temperature for 2 hours and then read. Non-specific inhibitors in the dog sera were removed by absorption of the sera with kaolin. Controls were included at the performance of the haemagglutination-inhibition test. Thus, a test antigen titration was run. Absence of non-specific agglutinins in the serum was checked for by mixing serum with saline instead of antigen. The HI titre was taken as the highest serum dilution which completely inhibited haemagglutination.

EXPERIMENTAL

A group consisting of 8 dogs was given HCC virus as described in Material and Methods. The temperature of the dogs was taken, and the values recorded as in Table I.

It seems as if the temperature in dogs 6, 7 and 8 had a diphasic course. This is usual in infections with HCC virus. Specimens were taken and titrated. The results are shown in Table II.

It is noticeable that virus could be detected at a very early stage in the tonsils, earlier than in any of the other materials tested. Virus was occasionally found in the contents and in the wall of the small intestine at a time when it had apparently not

Table I. Temperature scheme of dogs given HCC virus per os on day 0.

Dog number	Temperature on day 0	1	2	3	4	5	6	7	8
1	38.3	38.8	39.0[1])						
2	38.4	39.5	39.5	39.0[1])					
3	38.4	39.2	39.2	39.2[1])					
4	38.0	39.4	39.2	39.2	39.2[1])				
5	38.4	39.2	39.0	39.5	38.9	39.2[1])			
6	38.6	39.3	39.0	39.5	38.8	39.0	40.6[1])		
7	38.4	38.8	39.4	39.7	39.2	39.0	39.6	39.1	38.6[1])
8	38.4	38.8	39.0	40.2	38.8	39.0	40.2	39.0	38.6
Mean value	38.4	39.1	39.2	39.5	39.0	39.0	40.1	39.1	38.6

[1]) exsanguinated and specimens taken.

Table II. Virus titres of various organ materials from dogs given HCC virus per os on day 0. Each figure is the virus titre in one specimen.

Kind of specimen	Virus titre on day 2	3	4	5	6	8
Adrenals	—	—	—	1.7	1.7	—
Kidneys	—	—	—	1.7	2.3	—
Spleen	—	—	—	1.1	2.9	—
Liver	—	—	—	1.3	3.1	—
Lungs	—	—	—	0.7	1.9	—
Pancreas	—	—	—	—	—	—
Small intestinal wall 10	—	—	1.5	—	—	—
Peyer's patch 10	0	0	0	0	1.5	—
Contents from the last part of small intestine	—	1.5; 1.1	—	1.3	—	—
Tonsils	1.25	2.5; 3.0	2.9	2.7	2.1	—
Pelvic lymph glands	—	—	—	—	1.5	—
Popliteal lymph glands	—	—	—	—	1.3	—
Bone marrow	0	0	—	1.0	—	—
Blood	≤ 1.5	≤ 1.5	≤ 1.5	≤ 1.5	1.75	≤ 1.5
Lymph	≤ 1.5	≤ 1.5	≤ 1.5	3.3	3.7	≤ 1.5

0 = not tested
— = virus titre ≤ 0.5

yet generally disseminated. On the 5th day after infection virus was found in bone marrow, adrenals, kidneys, spleen, liver and lungs and in a high titre in the lymph, indicating a generalization of infection. On the 6th day the virus titer was usually higher than on the day before. However, the amount in the tonsils seems to

have decreased. For a more detailed analysis of the virus spread, a new group of dogs was infected and studied. This time examination of some of the organs studied in the previous experiment was omitted. Instead specimens from the lymph nodes draining the tonsillar region and those of the intestine, were included. The results of the virus titrations are given in Table III.

Table III. Virus titres of various organ materials from dogs given HCC virus per os on day 0.

Kind of specimen	Virus titre on day 1	2	3	4	5	6	7
Liver	—	—	—	—	0.9	1.7	1.7
Small int. wall 10	—	—	—	0.9	1.9	—	—
Rectal wall	—	—	—	—	—	—	—
Contents from the last part of the small int.	—	—	—	0.7	0.9	—	—
Contents from rectum	—	—	—	—	—	—	—
Peyer's patch 10	—	—	—	—	2.1	1.1	—
Mesenteric lymph glands	—	—	—	0.7	3.7	3.3	—
Tonsils	1.7	2.9	3.5	3.5	1.5	—	—
Superior deep cervical lymph glands	—	—	2.3	3.7	3.5	2.1	—
Paramandibular lymph glands	—	—	—	0.7	0.9	3.1	—
Blood	≤ 1.5	≤ 1.5	≤ 1.5	1.7	1.9	≤ 1.5	≤ 1.5
Lymph	≤ 1.5	≤ 1.5	≤ 1.5	≤ 1.5	3.1	2.5	≤ 1.5

— = virus titre ≤ 0.5

In this experiment it is obvious that virus was absorbed in the tonsils and that virus multiplication took place there at an early stage. Thus, already 24 hours after infection, virus was present in specimens from the tonsils. The titration showed that the virus amount increased to a maximum on the third and fourth day.

The draining lymph nodes of the tonsillar region seem to be the superior deep cervical lymph glands. The table also shows that there was virus in these glands rather early (3 days after infection), and that the virus amounts were the same as in the tonsils. Moreover, it is probable that virus multiplication in the superior deep cervical lymph glands was detected in a somewhat late phase judging by the relatively high virus titre on day three. In this experiment too, virus could be demonstrated in specimens

from the contents as well as from the wall of the small intestine. Thus virus was present in these materials 4 days after infection. The following day virus was found in the specimens from the wall and the contents of the small intestine, in the Peyer's patches, in the mesenteric lymph glands and in the lymph. On the same day virus was found in the liver. Two days later, virus was isolated from the liver specimen only. A viraemia of short duration (during days 4 and 5) was also revealed. HCC virus could on no occasion be recovered from the wall or contents of the rectum.

On the whole these experiments demonstrate the course of dissemination and multiplication of virus in the organism. Under natural conditions, however, the infecting dose of virus must be smaller than under the experiments referred to. An attempt to determine the oral MID was therefore undertaken. The appearance of HCC antibodies was considered as a criterium of infection. The results are reported in Table IV.

Table IV. HI-titres in serum from dogs given different amounts of HCC virus per os.

Dog number	Infectious dose in $TCID_{50}$	HI-titre Day of bleeding 7	13	16	22
4	40	< 1/10	< 1/10	< 1/10	< 1/10
5	400	1/10	1/40	1/40	1/160
6	4000	1/10	1/40	1/80	1/160
7	40000	1/10	1/80	1/160	1/320
8	400000	1/10	N.D.	1/160	1/320

N.D. = Not Done

It is obvious that infection was initiated by a virus dose of 400 $TCID_{50}$ but not of 1/10th of that dose. From the dogs material was taken from the tonsillar region by throat swabs and from the rectum by rectal swabs. Attempts to isolate virus from the specimens were made. The results are shown in Table V.

As might have been expected dogs given a large infectious dose began to excrete virus from the tonsils at an earlier stage than dogs given a low dose of virus. The virus amounts seem to be the same. The excretion continued for a long period — at least for 5 days. The first positive virus isolation from rectal specimens was made on the 4th day after infection. The material was taken from the dog given the highest concentration of virus. Dogs

Table V. Virus titres in specimens from dogs given different amounts of HCC virus per os.

Dog number	Test material	Virus titre on day 1	2	3	4	5	6	7	8	10
4	Throat specimen	—	—	—	—	—	—	—	—	—
5	„ „	—	—	—	—	N.D.	0.7	1.7	N.D.	—
6	„ „	—	—	1.25	1.5	1.5	1.7	2.3	—	—
7	„ „	—	—	≥1.5	1.9	2.1	2.1	2.5	—	—
8	„ „	—	0.9	1.3	1.5	1.9	1.5	—	—	—
4	Rectal „	—	—	—	—	—	—	—	—	—
5	„ „	—	—	—	—	—	—	—	N.D.	—
6	„ „	—	—	—	—	—	1.3	0.9	—	—
7	„ „	—	—	—	—	1.3	—	—	—	—
8	„ „	—	—	—	1.3	0.9	—	—	—	—

— = ≤ 0.5
N.D. = Not Done

number 6 and 7 showed the presence of virus in the rectal contents at a later stage. Somewhat remarkable is the fact that virus could be detected in rectal contents for only a few days. However, the virus titres in these specimens are rather low. It may happen that the specimens now accounted for as negative in reality contained a small, not titratable amount of virus. The last specimens were taken from the throat and rectum on the 10th day after infection. All of them were negative. Thus, excretion from the tonsils and from the intestine at this stage (10 days after infection) does not seem to play any rôle from an epizootiological point of view. As the relation between the obtained amount of test material and the volume of medium in which it was suspended, could not be the same from specimen to specimen, direct comparison between the different virus titres should be made with caution. During the experiment the temperature was recorded for 10 days after infection. Only dogs 7 and 8 showed a minor increase in body temperature between days 4 to 7 after infection.

The connection between the presence of virus in the tonsils and in the intestinal tissues was studied further in several experiments. Some of them are reported below. In the first each of six dogs were given 0.1 ml. HCC virus *per os*. The dogs were killed at an interval of 12 hours between each animal — the first taken 24 hours after infection. A series of specimens, especially from the small intestinal area, were excised and titrated. The results

Table VI. Virus titres in various specimens from dogs given HCC virus per os.

Test material	Virus titres Hours after infection 24	36	48	60	72	84
Tonsils	—	1.7	3.9	3.7	2.5	3.7
Small intestinal wall:						
Specimen 1—9	—	—	—	—	—	—
„ 10	—	—	—	—	1.0	1.0
Contents in small intestine:						
Specimen 1—6	—	—	—	—	—	—
„ 7	—	—	—	N.D.	0.75	—
„ 8	—	—	0.9	„	1.7	—
„ 9	—	—	1.1	„	1.5	—
„ 10	—	—	1.0	„	1.7	—
Peyer's patch:						
Specimen 1—8	—	—	—	—	—	—
„ 9	—	—	—	—	—	—
„ 10	—	—	—	—	1.0	—
Mesenteric lymph glands	—	—	—	—	3.5	2.5

N.D. = Not Done
— = ≤ 0.5

are shown in Table VI. As usual virus was found in the tonsils at an early stage (36 hours P.I.). Isolated virus findings in the intestinal wall and in the Peyer's patches were made 72 hours after infection. At the same time a high titre of virus was recorded in the mesenteric lymph gland. The activity detected in the intestinal contents on the second day after infection probably must be considered as originating from the virus administered. The next experiment was of the same type as the one just mentioned, with the exception that the specimens were taken at a later stage. The results of the virus titration are given in Table VII.

In all, 54 intestinal wall specimens were examined. Only two of them were positive. Of 57 specimens of Peyer's patches 9 were positive. Generally these were taken from the last half of the small intestine. In dog no. 2 virus was found in the mesenteric lymph glands in spite of the fact that no virus could be shown in the intestinal wall or in the Peyer's patches. The same has been the case in several other dogs, killed at the same time after infection (4 days). Apparently, the sites of virus growth in the intestine can only be scattered small areas.

Table VII. Virus titres of different specimens from dogs infected per os.

Test material	Virus titre on day 4		5		6	
	Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6
Tonsils	3.9	3.9	3.9	N.D.	3.1	1.9
Superior deep cervical lymph glands	3.7	3.3	3.9	1.9	2.7	1.5
Mesenteric lymph glands	—	1.5	4.7	3.7	3.7	4.5
Liver	—	—	—	—	—	2.2
Small intestinal wall:						
Specimen 1—8	—	—	—	—	—	—
„ 9	—	—	—	—	0.9	1.3
Peyer's patches:						
Specimen 1—2	—	—	—	—	—	—
„ 3	—	—	—	—	—	2.9
„ 4	—	—	—	—	—	—
„ 5	—	—	—	—	—	2.5
„ 6	—	—	—	—	—	N.D.
„ 7	—	—	—	—	—	1.7
„ 8	—	—	—	2.1	—	—
„ 9	—	—	—	0.9	1.7	—
„ 10	N.D.	—	2.7	N.D.	2.5	1.0

— = ≤ 0.5
N.D. = Not Done

In the last experiment reported here, dogs were given freeze-dried HCC virus in gelatine capsules per os. The purpose was to investigate whether dogs could be infected if the tonsillar area was excluded, *i.e.* if the virus could be absorbed by the intestinal epithelial cells or by the lymphoid cells in the intestine and multiply there. Twentytwo dogs were fed with HCC virus capsules. In fourteen of the dogs, virus was unfortunately brought into contact with the tonsils by mistake. Of the remaining 8 dogs 4 were completely negative (no virus could be found), while 4 showed the presence of virus in the mesenteric lymph glands but not in the tonsils or in the superior deep cervical lymph glands.

DISCUSSION

During the last years there has been reported findings (6, 10, 12, 26) which justify the inclusion of HCC virus in the adenovirus group. Of the pathogenesis of adenovirus infection in humans, certain features are known. Thus, virus has been isolated from

lymphoid tissue as the tonsils, the adenoids (11) and the mesenteric lymph nodes (13). Virus was also present in pharyngeal secretion and in human stools (2, 13, 27). Definite proof for association with enteric infection has also been furnished.

The pathogenesis of HCC seems in certain respects closely similar to that of adenovirus infection. Part of the orally given HCC virus was absorbed in the surface of the tonsils. There the multiplication started very soon and virus could be isolated 24—36 hours after infection. From the tonsils virus was obviously carried with the lymph to the draining superior deep cervical lymph nodes and was there detected on the third day. Virus was then transported to the supraclavicular lymph glands and then to the blood via the cervical lymph ducts and ductus thoracicus or right lymphatic duct. Viraemia was demonstrated on the 4th day after infection. On the following days virus was apparently spread over the whole body. Thus, virus was isolated for instance from the liver, spleen, lungs and kidneys on the 5th day.

Sporadic findings of virus in the Peyer's patches and in the small intestinal wall were made on the 3rd and 4th days. More regular findings were obtained only from the Peyer's patches on the 5th and 6th days. Material from mesenteric lymph glands was positive on the 4th day and exceptionally so on the 3rd day.

The fact that virus could be demonstrated at an early stage in the mesenteric lymph glands but not in the intestine, indicates that HCC virus multiplication might occur only in a few sites in the intestinal wall, and that these sites remain small and circumscribed. Consequently it seems as if the virus is regularly absorbed by the lymphatic tissue in the pharynx and occasionally also by cells in the small intestinal wall. After multiplication of the virus in the intestine, it is carried via lymph channels to the mesenteric lymph nodes. From them virus is transported to the blood via mesenteric lymph vessels and by ductus thoracicus, thus contributing to the viraemia.

The virus excretion in the faeces, sometimes also for a long time after recovery, at some adenovirus infections is very important from an epidemiological point of view. This can hardly be said to be the case in HCC. Table V shows that virus occurs only sporadically and only in minimal amounts in the faeces. This may perhaps be explained by the circumstance that the sites for virus growth in the intestinal tissue seemed to be small. The virus excretion was of short duration. Thus, ten days after infection feacal transmission of infection seems not to occur.

REFERENCES

1. *Baker, J. A., M. G. Richards, A. L. Brown and C. G. Rikard:* Infectious hepatitis in dogs. Proc. 87th Ann. Meet. Amer. Vet. Med. Ass. 1950, 242.
2. *Barr, J., L. Kjellén and A. Svedmyr:* Hospital outbreak of adenovirus type 3 infection. A clinical and virologic study on 38 patients partly involved in a nosocomial outbreak. Acta Paediat. 1958, *47*, 365.
3. *Brunner, K. T., M. Scheitlin and H. Stünzi:* Zum serologischen Nachweis der Hepatitis contagiosa canis. Schweiz. Arch. Tierheilk. 1951, *93*, 443.
4. *Cabasso, V. J., M. R. Stebbins, T. W. Norton and H. R. Cox:* Propagation of infectious canine hepatitis virus in tissue culture. Proc. Soc. Exp. Biol. Med. 1954, *85*, 239.
5. *Coffin, D. L.:* The pathology of so-called acute tonsillitis of dogs in relation to contagious canine hepatitis (Rubarth). A report of five cases. J. Amer. vet. med. Ass. 1948, *112*, 355.
6. *Davies, M. C., M. E. Englert, M. R. Stebbins and V. J. Cabasso:* Electron microscopic structure of infectious canine hepatitis (ICH) virus — a canine adenovirus. Virology 1961, *15*, 87.
7. *Espmark, J. Å. and C. R. Salenstedt:* Haemagglutination-inhibition test for titration of antibodies against Hepatitis contagiosa canis (infectious canine hepatitis). Arch. ges. Virusforsch. 1961, *11*, 64.
8. *Fieldsteel, A. H. and J. B. Emery:* Cultivation and modification of infectious canine hepatitis. Virus in roller tube cultures of dog kidney. Proc. Soc. Exp. Biol. Med. 1954, *86*, 819.
9. *Florent, A. and J. Leunen:* L'hepatite contagieuse du chien. Son existence en Belgique. Sa symptomatologie et son diagnostic. Essais de transmission expérimentale. Ann. Méd. Vét. 1949, *93*, 225.
10. *Heller, L. A. and C. R. Salenstedt:* One-sided immunologic relation between adenovirus and infectious canine hepatitis antigens. Virology 1960, *11*, 640.
11. *Huebner, R. J., W. P. Rowe, T. G. Ward, R. H. Parrot and J. A. Bell:* Adenoidal-pharyngeal-conjunctival agents. A newly recognized group of common viruses of the respiratory system. New Eng. J. Med. 1954, *251*, 1077.
12. *Kapsenberg, J. G.:* Relationship of infectious canine hepatitis virus to human adenovirus. Proc. Soc. Exp. Biol. Med. 1959, *101*, 611.
13. *Kjellén, L., G. Sterner and A. Svedmyr:* On the occurrence of adenovirus in Sweden. Acta Paediat. 1957, *46*, 164.
14. *Kärber, G.:* Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Arch. exp. Pharmak. 1931, *162*, 480.
15. *Larin, N. M.:* Studies on the agent of canine virus hepatitis (Rubarth's disease) I. Complement-fixation and precipitin tests. J. Hyg. 1951, *49*, 410.

16. *Lehnert, E.:* Der Wert der Komplementbindungsmethode bei der Hepatitis contagiosa canis. Skand. Vet.Tidskr. 1948, *38*, 94.
17. *Mansi, W.:* Rubarth's disease (canine virus hepatitis) I. Reaction of dogs to the conjunctival instillation of the virus. J. comp. Path. 1953, *63*, 236.
18. *Mansi, W.:* The value of the complement-fixation test in the study of canine distemper complex and Rubarth's disease. J. comp. Path. 1955, *65*, 291.
19. *Müller, J. and Aa. Thordal-Christensen:* Cultivation of the virus of infectious canine hepatitis (Rubarth) in tissue culture. Nord. Vet.-Med. 1954, *6*, 767.
20. *Parry, H. B., N. M. Larin and H. Platt:* 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. J. Hyg. 1951, *49*, 482.
21. *Poppensiek, G. C. and J. A. Baker:* Persistance of virus in urine as factor in spread of infectious hepatitis in dogs. Proc. Soc. Exp. Biol. Med. 1961, *77*, 279.
22. *Rubarth, S.:* An acute virus disease with liver lesion in dogs (Hepatitis contagiosa canis). A pathologico-anatomical and etiological investigation. Acta path. microbiol. scand. 1947, *24*, Suppl. 69.
23. *Salenstedt, C. R.:* Studies on the virus of Hepatitis Contagiosa Canis (HCC). I. The isolation of HCC-virus from the tonsils of a dog. Arch. ges. Virusforsch. 1958, *8*, 123.
24. *Salenstedt, C. R.:* Studies on the virus of Hepatitis Contagiosa Canis (HCC). II. Susceptibility of Guinea pigs to experimental infection with HCC virus. Arch. ges. Virusforsch. 1958, *8*, 600.
25. *Schindler, R.:* Serologische Untersuchungen über die Hepatitis contagiosa canis (HCC). Mh.prakt. Tierheilk. 1952, *4*, 481.
26. *Tajima, M., T. Motohashi and T. Samejima:* Electron microscopy of infectious canine hepatitis virus grown in cultures of canine kidney cells. Amer. J. vet. Res. 1961, *22*, 236.
27. *Van Der Veen, J. and G. Van Der Ploeg:* An outbreak of pharyngo-conjunctival fever caused by types 3 and 4 adenovirus at Waalwijk, The Netherlands. Amer. J. Hyg. 1958, *68*, 95.

SUMMARY

The pathogenesis of HCC virus infection in the dog has been investigated. Dogs were infected with virus given per os. After multiplication in the tonsils and in the draining lymph nodes virus was carried to the blood via the cervical lymph vessels. Virus was also absorbed to cells in the wall of the small intestine. In those cells as well as in the draining lymph nodes a multiplication took place and virus was then brought to the blood by the lymph in vessels from the mesenteric lymph nodes and via ductus thoracicus. By the blood virus was spread over the whole body and was detected in several organs among them the liver. This was shown 3-5 days after virus was detected

in the tonsils. It was also demonstrated that dogs could be infected when the virus was given in gelatine capsules. The tonsils thus were excluded from possibility to absorb virus and the infection probably was initiated by absorbtion of the virus to cells in the small intestine.

ZUSAMMENFASSUNG

Studien über das Virus des Hepatitis Contagiosa Canis (HCC).

IV. Die Pathogenese von Hepatitis Contagiosa Canis bei Hunden.

Eine Untersuchung in Bezug auf die Pathogenese der HCC beim Hunde wurde unternommen. Die Tiere wurden per os mit Virus infiziert. Nach einer Vermehrung in den Tonsillen und regionalen Lymphknoten gelangte das Virus über die Halslymphgefässe in den Blutkreislauf. Es wurde des weiteren an Zellen der Dünndarmwand absorbiert. In diesen Zellen wie auch in den regionalen Lymphdrüsen fand eine Virusvermehrung statt. Von dort aus gelangte der Infektionsstoff über Lymphgefässe der Mesenteriallymphknoten via ductus thoracicus in den Blutkreislauf. Mit dem Blute wurde das Virus im Gesamtorganismus verteilt und konnte in mehreren Organen, u. a. in der Leber nachgewiesen werden. Dies geschah 3—5 Tage nachdem das Virus in den Tonsillen festgestellt worden war. Es konnte weiterhin gezeigt werden, dass die Hunde auch mittels Virus in Gelatinekapseln per os infiziert werden konnten.

Mittels diese Methode konnte die Möglichkeit einer Absorption von Virus an die Tonsillen ausgeschlossen werden. Daraus ist zu schliessen, dass die Infektion durch das Virus nach dessen Absorption an Zellen der Dünndarmwand initiiert wurde.

SAMMANFATTNING

Studier över Hepatitis Contagiosa Canis virus (HCC).

IV. Patogenesen vid Hepatitis Contagiosa Canis hos hundar.

En undersökning över patogenesen vid HCC hos hund genomfördes. Hundarna infekterades med virus per os. Efter förökning i tonsillerna och i de regionära lymfkörtlarna fördes virus via halslymfkärl till blodet. Virus absorberades även till celler i tunntarmsväggen. I dessa celler ävensom i tarmens regionära lymfkörtlar ägde en virusförökning rum. Därifrån fördes infektionsämnet med lymfkärl från mesenteriallymfkörtlarna via ductus thoracicus till blodet. Med detta spreds virus över hela organismen och kunde påvisas i flera organ bl. a. i levern. Detta inträffade 3—5 dagar efter det att virus hade upptäckts i tonsillerna. Det kunde också visas att hundarna infekterades även då virus tillfördes per gelatinkapslar. Då så skedde uteslöts möjligheten för absorbtion av virus till tonsillerna. Infektionen bör då ha initierats genom att virus absorberats till celler i tunntarmsväggen.

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