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STUDIES ON THE VIRUS OF HEPATITIS CONTAGIOSA CANIS (HCC)

III. THE VIRAEMIC PHASE OF HCC VIRUS INFECTION IN GUINEA PIGS

By

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Appearance of virus or viral antigen in the blood in the course of HCC infection of dogs or guinea pigs was demonstrated by several authors, among them *Rubarth* (21), *Lehnert* (13), *Florent and Leunen* (9), *Baker et al.* (1), *Parry et al.* (18), *Parry and Larin* (17), *Poppensiek and Baker* (19), *Fieldsteel* (7) and *Salenstedt* (23). The relative virus content of plasma and blood seems not to have been determined.

However, viraemia has interested several authors in virus diseases other than HCC, especially in ephemeral fever, vaccinia, fowl plague, polio, and canine distemper. Papers on the subject have been published by *MacKerras et al.* (15), *Nicolle and Adil-Bey* (16), *Todd and White* (27), *Schein* (24), *Hornby* (10), *Todd* (26), *Smith* (25), *Bindrich* (2), *Horstman* (11), *Liu and Coffin* (14) and *Rockborn* (20). Most of them suggest or have shown that the virus was bound to the white cells during viraemia.

Papers concerning the virus amount of different types of white blood cells during the viraemic phase seem, however, to be very few. *Fenner and Woodroofe* (5) in their work on myxomatosis obtained results suggesting that most virus was associated with the lymphocytes. In addition, the investigations by *Yoffey and Sullivan* (28) should be mentioned. After instillation of vaccinia virus in the nose of rabbits virus was found in the cervical lymph. All evidence suggested the fixation of virus to the lymphocytes.

In a previous work (23) the susceptibility of guinea pigs to experimental infection with HCC virus was shown. This observation has been the basis for the experiments presented in this paper. Its purpose is to report results of investigations concerning viraemia in guinea pigs after inoculation of HCC virus.

MATERIAL AND METHODS

Virus. The virus strain was originally isolated in this laboratory (22). The same batch (pool 273) of virus material as reported in a previous paper (23) was used throughout this work. The titre of the virus pool was $10^{8.5}$ $TCID_{50}$ per ml.

Tissue Culture. Roller tube cultures were prepared of trypsinized dog kidney cells as previously described (23). During the outgrowth phase 0.5 % lactalbumin hydrolysate in Hank's solution with 5 % bovine serum was used as a medium. When the tubes were ready for inoculation the medium was changed to 0.5 % lactalbumin hydrolysate in Earl's solution with the addition of 2 % bovine serum. To both media penicillin and streptomycin were added in a concentration of 100 I.U. and 100 γ per ml. respectively. Each roller tube contained 1.0 ml. of nutrient fluid. For details see Tissue Culture in a preceding paper (23).

In several experiments tissue culture tubes prepared from cells obtained by versenation of tissue culture bottles were used.

Inoculation and Bleeding of Guinea Pigs. Guinea pigs weighing 275—325 grams supplied from three commercial breeders were inoculated subcutaneously in one hind leg with 0.2 ml. of pool 273. In each particular experiment guinea pigs from only one breeder were used. Blood specimens were drawn by heart puncture at different intervals after inoculation. Each guinea pig was bled only once.

Handling of the Blood

Whole Blood. To a portion of the drawn blood specimen heparin was added in a final concentration of 1:2000.

Serum. The clotted blood specimen was centrifuged and serum pipetted off.

Plasma. A portion of the heparinized whole blood was centrifuged and plasma was pipetted off.

Washed Red and White Blood Cells. Heparinized whole blood was washed three times with phosphate buffered saline (pH 7.4).

Each time the specimen was centrifuged for 10 minutes at 1500 r.p.m. A deposit mainly consisting of white cells had settled over the layer of erythrocytes after each washing. After the third washing saline was added in order to restore the specimen's original volume. The white and red cells were thoroughly and carefully mixed and a portion was pipetted off. This will be referred to as the *washed cell mixture.*

The remaining contents of the tube was centrifuged once more. The tip of a plastic tube was introduced into the layer of white blood cells and as much as possible of the white deposit was sucked up into the tube. An admixture of erythrocytes was obtained. The plastic tube was then placed in a spiral centrifuge according to *Danielsson and Lundmark* (4) and was centrifuged at a speed of 3000 r.p.m. for 4—5 min. A layer of white blood cells was formed centripetally over the red cell layer. The tube was cut immediately below the white layer and this was sucked off with a Pasteur pipette. The white blood cells were then resuspended in 1.0 ml. buffered saline. This specimen will be referred to as *washed white cells.* A specimen of *washed red cells* was taken from the glass tubes from which the white cells had been removed as described above. The red cells were sucked up with a Pasteur pipette, the tip of which had been carefully inserted to the bottom of the tube. The cells were resuspended in 1.0 ml. buffered saline.

A number of blood specimens were filtered according to a method given by *Fleming* (8) and later reviewed by *Fichtelius* (6). Heparin was initially added to the blood. The specimens were washed three times in the way described above. After the last washing saline was added to restore the specimen's original volume. The cells were resuspended and 2 ml. of the suspension was pipetted off. The rest, usually 4—5 ml., was passed through a filter of cotton. This was done in the following manner. A glass tube was fixed to an Erlenmeyer flask through a rubber stopper. The lower part of the tube was packed with cotton. The cell suspension was put onto the filter and soaked into the cotton. After incubation for 60 min. at 37°C and at a certain moisture (70 %) the filter was washed with 40—50 ml. buffered saline. The filtrate principally containing non-sticking white blood cells and erythrocytes was washed four times in the usual way. After the last washing buffered saline was added to the specimen in order to obtain the same volume as before filtration. The cell

suspension will be referred to as *non-filtered blood cells* and *filtered blood cells* respectively.

Cell counts of different cell suspensions were made in a haemocytometer.

Virus Isolations and Infectivity Titrations. Virus isolations and titrations were performed in dog kidney tissue cultures on material collected from the inoculated guinea pigs. From whole blood and some of its fractions serial tenfold dilutions were prepared in 0.5 % lactalbumin hydrolysate in Hank's solution. Each specimen, diluted or undiluted, was generally inoculated into 5 roller tubes, 0.1 ml. per tube. (In some cases only 4 roller tubes were used.) The titres were calculated according to *Kärber* (12) and expressed as log $TCID_{50}$ per 0.1 ml.

Neutralization Test. Neutralization tests were performed in dog kidney cultures. Before the test all sera were inactivated for 30 minutes at 56°C. Equal volumes of serum diluted in 4-fold steps and virus suspension containing about 100 $TCID_{50}$ per 0.1 ml. were mixed and kept at 37°C for 18 hours. Two roller tubes were inoculated with the serum-virus mixture, each tube receiving 0.2 ml. Final readings were taken when the virus titration showed a virus content of 100—320 $TCID_{50}$ per 0.1 ml., usually on the 6th to the 7th day. The antibody titre value was estimated as the highest final dilution of serum which inhibited cytopathogenic changes in at least one of the tubes at the final reading.

Statistical Assay. Statistical analysis was carried out according to the presentation of the theories of Fisher and others by *Bonnier and Tedin* (3).

EXPERIMENTAL

The virus distribution within different blood fractions. As previously described groups of guinea pigs were bled to death at predetermined intervals after inoculation with HCC virus. The bleeding times were principally chosen so that the interval between two groups of guinea pigs was 24 hours.

From some of them whole blood, serum, plasma and washed cell mixture were titrated while from other animals only three fractions were tested for virus content. In Table I details about the time of bleeding, number of guinea pigs and virus titres of different blood fractions are given.

In order to show the variation between the animals, and to give a typical example of the results obtained, all titrations on

Table I. Distribution of guinea pigs in relation to time of bleeding, virus titres and number of tested animals in respect to different blood fractions. The virus titres are expressed as mean values.

Time of bleeding (in hours)	Number of guinea pigs	Virus titres and number of tested guinea pigs respectively as to							
		Whole blood		Serum		Plasma		Washed cell mixture	
48	13	2.50	13	1.22	5	≤ 1.41	13	2.37	13
72	20	2.61	20	1.05	4	≤ 1.62	20	≥ 3.06	20
96	17	1.24	17	≤ 0.45	5	≤ 0.39	17	1.63	17
120	10	≤ 1.40	10	N.D.[1])	0	≤ 0.35	10	≥ 2.00	10
144	24	≤—0.19	24	≤—0.50	4	≤—0.33	24	≤ 0.56	24
168	4	≤—0.44	4	≤—0.50	4	≤—0.50	4	≤—0.31	4
192	10	≤—0.50	10	N.D.	0	≤—0.50	10	≤+0.04	10
240	4	≤—0.50	4	≤—0.50	4	≤—0.50	4	≤—0.44	4
Total examined	102		102		26		102		101

[1]) Not done.

material collected 72 and 96 hours after inoculation are recorded in Table II. The table shows the existence of large differences between individual animals. However, statistical analysis revealed that the titre differences between whole blood and plasma as well as between washed cell mixture and plasma were highly significant ($P < 0.001$). If the two groups, 72 and 96 hours respectively, were treated separately the difference between whole blood and plasma and between washed cell mixture and plasma were, with one exception, highly significant ($P < 0.001$). The difference be-

Table II. Distribution of virus titres of two groups of animals recorded in Table I. The first group bled 72 hours, the second 96 hours after HCC virus inoculation.

Virus titre	Whole blood	Plasma	Washed cell mixture
≤ 0.00	3	9	1
0.10—1.00	3	9	3
1.10—2.00	14	12	9
2.10—3.00	12	6	17
3.10—4.00	4	1	6
4.10—5.00	1	0	1

Differences: Whole blood — Plasma = 0.934 ± 0.237 ($P < 0.001$)
Washed cell mixture — Plasma = 1.347 ± 0.236 ($P < 0.001$)

tween whole blood and plasma as to the 72 hour test was of lower significance ($P < 0.01$).

Forty-three animals were tested for virus content in the washed cell mixture, washed red cells and washed white cells. Twenty of the animals were bled 72 hours after inoculation, 23 after 96 hours. The results of virus titrations are given in Table III. It

Table III. Distribution of virus titres of two groups of guinea pigs. One group bled 72 hours, the other 96 hours after inoculation.

Virus titre	Washed		
	Cell mixture	Red cells	White cells
≤ 0.00	0	0	0
0.10—1.00	5	36[1])	7
1.10—2.00	16	7	12
2.10—3.00	18	0	23
3.10—4.00	4	0	1

[1]) 24 of the values have the titre ≤ 0.50
Differences: Washed cell mixture — Washed red cells = 1.290 ± 0.122 ($P < 0.001$)
Washed white cells — Washed red cells = 1.252 ± 0.118 ($P < 0.001$)

shows that the variation in this experiment between the animals with respect to virus content is relatively small. Statistical analysis also revealed that the differences between washed cell mixture and washed red cells as well as between washed white cells and washed red cells were highly significant ($P < 0.001$).

Table IV. Distribution of white cell values for the guinea pigs recorded in Table III.

White cell number	Washed		
	Cell mixture	Red cells	White cells
0	0	39	0
1— 2000	3	4	6
2001— 4000	16	0	9
4001— 6000	12	0	11
6001— 8000	9	0	8
8001—10000	1	0	5
10001—12000	1	0	2
12001—14000	1	0	0
14001—16000	0	0	1
16001—18000	0	0	1

As shown in Table III 43 animals were examined as to the virus contents in three washed blood fractions. The number of white cells in these fractions were also determined. All results are recorded in Table IV.

Thirty-nine guinea pigs were bled 72 hours after inoculation with HCC virus. The virus titre and the number of mononuclear white corpuscles in non-filtered and filtered blood cell suspensions respectively was determined. The results are summarized in Table V. It shows that the virus titres and the cell numbers are

Table V. Distribution of the values of virus titres and the number of mononuclear cells in non-filtered and filtered blood cell suspensions. Guinea pigs bled 72 hours after inoculation with HCC virus.

Test material	Titre range					
	-0.40-0.00	0.10-0.50	0.60-1.00	1.10-1.50	1.60-2.00	2.10-2.50
Non-filtered blood	0	8	16	8	5	2
Filtered blood	11	16	7	5	0	0

Test material	Mononuclear cell value range			
	1-1000	1001-2000	2001-3000	3001-4000
Non-filtered blood	2	25	11	1
Filtered blood	35	4	0	0

lower in the filtered suspensions than in the non-filtered. Consequently, several mononuclear cells remained in the filter or were destroyed. In the latter case, presumably, virus was released. The filtrate, before the washing procedure, also contained a considerable amount of virus not associated with cells.

It will be mentioned in this connection that the introduction of the spiral centrifuge made it easy to prepare rather pure cell suspensions. It was said in Material and Methods that the white cell fraction contained some red cells. In the white cell specimens the relation between white and red cells varied between 1:1 and 1:40. The most common frequency was 1:5.

Simultaneous occurrence of viraemia and serum antibodies. From some of the guinea pigs in Table I not only whole blood, plasma and washed cell mixture were tested for virus content, but

also serum was examined for the presence of HCC antibodies. A total of 30 guinea pigs were tested. Of ten guinea pigs bled at 120 hours after inoculation, only one had serum antibodies. The number of animals with antibodies after 144 and 192 hours, were five of ten and six of ten respectively. Virus titres of the different blood fractions and neutralization titres of all guinea pigs with demonstrable HCC antibodies are recorded in Table VI.

Table VI. Distribution of virus titres of different blood fractions and serum antibody titres in a group of guinea pigs inoculated with HCC virus. Each animal was bled only once.

Time of bleeding in hours	120			144			192		
Virus titre	W. bl.[1])	Pl.[2])	W.c.[3]) mix.	W. bl.	Pl.	W.c. mix.	W. bl.	Pl.	W.c. mix.
≤ —0.50	1	1		3	5		6	6	
—0.40—0.00				2					3
0.10—0.50						1			1
0.60—1.00						1			1
1.10—1.50			1			2			1
1.60—2.00						1			
Antibody titre									
4					2			1	
≥ 16		1			3			5	

[1]) W. bl. = Whole blood
[2]) Pl. = Plasma
[3]) W. c. mix. = Washed cell mixture

DISCUSSION

From a pathogenetic point of view the viraemia phase of a virus infection must be considered very interesting. Thus, papers have been published on ephemeral fever, cattle plague, vaccinia, canine distemper, fowl plague, polio and infectious myxomatosis. The results of the investigations differ somewhat but as a whole it was found that the virus was bound to the cellular fraction of the blood during the viraemic phase. Some of the authors have obtained results, which suggest that the virus was bound to the mononuclear white cells. Thus, *Fenner and Woodroofe* (5) investigating the pathogenesis of infectious myxomatosis in rabbits obtained results suggesting the association with lymphocytes or

monocytes during the viraemia. However, their conclusions are based on materials from only one rabbit. *Liu and Coffin* (14) showed the occurrence of canine distemper viral antigen in the mononuclear leucocytes by the aid of fluorescein-labelled antibodies. It is, however, not certain that the viral antigen is the same as the virus itself.

The results obtained in the present work show with a high degree of probability that HCC virus during the viraemic phase is associated with the mononuclear white cells. The mathematical background for this assertion is the following.

The virus titres expressed logarithmically for washed cell mixture and washed white cells are symbolized with a and b respectively. The quotient is called α. In the same manner the number of white cells in the two blood fractions is called c and d respectively and the quotient β. On the assumption that virus is

Table VII. Statistical analysis of the difference between the quotients of virus titres and those of the number of white cells in washed cell mixtures and in washed white cell suspensions. Guinea pigs were bled 96 hours after HCC virus inoculation.

a = Virus titre in washed cell mixture.
b = Virus titre in washed white cells.
α = b — a
d = Number white cells in washed cell mixture. Expressed logarithmically.
e = Number white cells in washed white cells. Expressed logarithmically.
β = e — d

Guinea pig number	a	b	α	d	e	β
M 163	1.70	1.90	0.20	3.72	3.91	0.19
„ 164	1.10	1.70	0.60	3.36	3.77	0.41
„ 165	$\leq$ 0.50	0.70	$\geq$ 0.20	4.00	4.00	0.00
„ 167	2.30	2.10	—0.20	3.36	3.23	—0.13
„ 168	1.90	2.10	0.20	3.40	3.40	0.00
„ 169	2.30	2.70	0.40	3.66	3.78	0.12
„ 170	2.30	2.30	0.00	3.28	3.49	0.21
„ 171	1.50	1.50	0.00	3.67	3.52	—0.15
„ 172	2.30	2.70	0.40	3.54	3.91	0.37
$\bar{x}$			0.2			0.1275
m^2			0.008571			0.005517

Difference: $\alpha - \beta = 0.073 \pm 0.119$ $(0.6 > P > 0.5)$

associated with the white cells there will be no difference between the two series of α- and β values. In Table VII the values from a group of animals are given. Statistical analysis applied on the values showed that there was no difference between the α- and β-series, *i. e.* that the assumption concerning the virus association with the white cells was correct.

As seen in Table VII the virus in the washed cell mixture for the animal M 165 could not be estimated. In the whole test originally including 44 animals, this is the only uncertain value present. In order to simplify the calculation, the values of M 165 were excluded.

For the whole test, including 43 animals, all virus titres and number of white cells are recorded in Tables III and IV.

In this case the difference between the α- and β-series is 0.057 $\pm$ 0.081. T in the t-test will be 0.704 ($0.5 > P > 0.4$). Consequently, it thus seems from a statistical point of view that a high degree of probability exists for the correctness assuming that HCC virus during the viraemic phase was bound to the white cells.

Furthermore, if it is assumed that virus is bound to the mononuclear white cells, the ratio between the virus titres in non-filtered and filtered blood cell suspensions should be comparable to the ratio between the number of mononuclear white cells in those specimens. The statistical analysis was performed principally as said above. There was no significant difference between the two ratios. Obtained on the basis of the values given in Table VIII it was namely 0.018 ± 0.078 ($0.9 > P > 0.8$). The corresponding figures for the whole test, including thirty-nine animals, were 0.014 ± 0.055 ($0.8 > P > 0.7$). Obviously there seems to be a high probability that virus was associated with the mononuclear white cells. However, the method of filtration is not quite perfect for the present purpose. Thus, in twenty specimens out of thirty-nine there was an admixture of polymorph nuclear white cells. The extent of it was varying — sixteen specimens contained less than 15 % granulocytes. In all, nineteen filtrates did not contain any granulocytes judging by the cell count. It does not mean that those materials must be free from granulocytes. If a sufficient number of cells had been counted it seems likely that granulocytes should have been identified even in these filtrates. It was considered important to test the hypothesis that virus was exclusively associated with the granulocytes. For the sake of this it was considered arbitrarily that the 19 pure mononuclear specimens

Table VIII. Statistical analysis of the difference between the quotients of virus titres in non-filtered and filtered blood, and those of the mononuclear cell values in the materials respectively. The test applied to some of the values accounted for in Table V.

Guinea pig number	a*)	b*)	α*)	d*)	e*)	β*)
M 317	0.75	0.10	0.65	3.51	2.73	0.78
„ 318	1.25	0.90	0.35	3.18	2.60	0.58
„ 319	1.10	0.50	0.60	3.18	2.60	0.58
„ 320	1.90	1.10	0.80	3.18	2.37	0.81
„ 321	2.30	1.50	0.80	3.11	2.34	0.77
„ 322	1.50	0.75	0.75	3.26	2.48	0.78
„ 323	0.30	—0.10	0.40	3.30	2.78	0.52
„ 324	0.90	0.10	0.80	3.23	2.92	0.31
„ 325	1.10	0.50	0.60	3.09	2.43	0.66
„ 326	1.10	0.30	0.80	3.12	2.18	0.94
$\bar{x}$			0.655			0.673
m^2			0.002858			0.003278

$\beta - \alpha = 0.018 \pm 0.078$; $(0.9 > P > 0.8)$

*) a and b are the virus titres in non-filtered and filtered blood respectively.
d and e are values of mononuclear cells expressed logarithmically in non-filtered and filtered blood respectively.
$\alpha = a - b$
$\beta = d - e$

contained an admixture of 2 % polymorph nuclear white cells. The statistical analysis was performed as before. It could be shown with a high degree of probability ($P < 0.001$), that the hypothesis of virus association with granulocytes was wrong.

The virus titres in serum and plasma were determined in several cases. It was found that there did not exist great differences between the two fractions. The virus content was somewhat varying, in most cases between 1—10 % of that in the cell specimens. At repeated washings of cell mixtures containing virus it was found that each washing fluid showed the presence of virus. The explanation may be that some cells were destroyed during the procedure, thus releasing virus or that the association between virus and cells was weak. This opens the possibility that all virus originally was bound to the cells but owing to the experimental procedure part of the virus was later found in the fluid.

It must be kept in mind that the technique used for demonstrating antibodies is neither accurate nor sensitive. It is

rather probable that by a refined method it would have been possible to show the occurrence of HCC antibodies in many more instances than was the case. As shown in Table VI it has been possible to reveal HCC antibodies in serum 120 hours after virus inoculation at the earliest. However, the differences between virus titres in whole blood and in washed cell mixture (the values for these two fractions given in Table I) demonstrate the early appearance of a virus inhibitory factor. The differences seem to increase with time at least 6—7 days after inoculation. After this time the virus titres decreased and the values were unreliable. In reality, the differences must be somewhat higher than calculated. The cell mixture was namely washed three times. Thus, the difference in virus content should be as much higher as the loss by the three washings. A statistical analysis has been performed on the values at 120 and 144 hours. The test material is shown in Table IX.

Table IX. Statistical analysis of the difference in virus titre between whole blood and washed cell mixture in two groups of guinea pigs. The first group bled 120 hours, the second 144 hours after inoculation of HCC virus. The groups recorded in Table I.

Virus titre	Whole blood	Washed cell mixture
≤ 0.00	22	8
0.10—1.00	4	6
1.10—2.00	4	13
2.10—3.00	4	7
$\bar{x}$	0.2794	0.9853
m^2	0.029943	0.028318

Difference: Washed cell mixture — Whole blood = 0.706 ± 0.241 ($P < 0.01$)

The test revealed a significant ($P < 0.01$) difference between washed cell mixture and whole blood. The nature of the virus inhibitor is unknown. If antibodies are formed earlier than 120 hours after the antigen inoculation, they will be "masked" by virus simultaneously present in the serum. It was clearly shown that the difference in virus titre between whole blood and washed cell mixture increased with time. This seems to suggest that the inhibitor may be of antibody type.

From a pathogenetic point of view it seems likely that when

the virus enters the blood it is rapidly associated with the mononuclear white cells or that the virus enters the blood stream already within those cells. Probably the virus will then be distributed to different organs in the body and released from the mononuclear white blood cells. It was previously shown (23) that the immunity in guinea pigs after HCC virus administration is very strong and of long duration. This may perhaps be explained by the present experiments, which show that in the same animal serum antibodies and virus bound to the mononuclear white cells can exist simultaneously. Thus, the reason for the long-lasting and strong immunity could be that the virus lying in the white cells is not influenced by the antibodies, thus being distributed around the body during long periods stimulating antibody forming cells. It ought to be mentioned that several authors, among them *Parry et al.* (18), have found virus in the blood at a late stage of HCC virus infection in dogs and that virus was excreted for a long time after the dog had recovered (*Poppensiek and Baker* (19).

REFERENCES

1. *Baker, J. A., M. G. Richards, A. L. Brown & C. G. Rickard:* Infectious hepatitis in dogs. Proc. 87th Ann. Meet. Amer. vet. med. Ass. 1950, 242.
2. *Bindrich, H.:* Untersuchungen über den Virusgehalt des Blutes und der Organe bei Hundestaupe. Exp. Vet. Med. 1950, *2*, 73.
3. *Bonnier, G. & O. Tedin:* Biologisk variationsanalys, Svenska Bokförlaget, Bonniers 1957.
4. *Danielsson, H. & F. Lundmark:* En ny typ av centrifug (spiralcentrifug). Nord. Med. 1957, *57*, 175.
5. *Fenner, F. & G. M. Woodroofe:* The pathogenesis of infectious myxomatosis: The mechanism of infection and the immunological response in the European rabbit (Oryctolagus cuniculus). Brit. J. exp. Path. 1953, *34*, 400.
6. *Fichtelius, K.-E.:* A simple method to separate lymphocytes from the blood. Acta Soc. Med. Upsaliensis 1951, *56*, 27.
7. *Fieldsteel, A. H.:* Some aspects of infectious canine hepatitis virus in tissue culture. Amer. J. vet. Res. 1956, *17*, 380.
8. *Fleming, A.:* A simple method of removing leucocytes from blood. Brit. J. exp. Path. 1926, *7*, 281.
9. *Florent, A. & J. Leunen:* L'hépatite contagieuse du chien. Son existence en Belgique. Sa symptomatologie et son diagnostic. Essais de transmission experimentale. Ann. Méd. vét. 1949, *93*, 225.
10. *Hornby, H. E.:* The distribution of rinderpest virus in infected blood. J. comp. Path. 1928, *41*, 17.

11. *Horstmann, D. M., R. W. McCallum & A. D. Mascola:* Viremia in human poliomyelitis. J. exp. Med. 1954, *99*, 355.
12. *Kärber, G.:* Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Arch. exp. Path. Pharmakol. 1931, *162*, 480.
13. *Lehnert, E.:* Der Wert der Komplementbindungsmethode bei der Hepatitis contagiosa canis. Skand. Vet.Tidskr. 1948, *38*, 94.
14. *Liu Ch'ien & D. L. Coffin:* Studies on canine distemper infection by means of fluorescein-labelled antibody. I. The pathogenesis, pathology, and diagnosis of the disease in experimentally infected ferrets. Virology 1957, *3*, 115.
15. *MacKerras, J. M., M. J. MacKerras & F. M. Burnet:* Experimental studies of Ephemeral Fever in Australian cattle. VII. Distribution of the virus in the blood. Commonwealth Scientific and Industrial Research Organization. Bull. 136. 1940.
16. *Nicolle, M. et Adil-Bey:* Études sur la peste bovine. Ann. Inst. Pasteur 1902, *16*, 56.
17. *Parry, H. B. & N. M. Larin:* The natural history of virus hepatitis of dogs (Rubarth's disease). Vet. Rec. 1951, *63*, 833.
18. *Parry, H. B., N. M. Larin & 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.
19. *Poppensiek, G. C. & J. A. Baker:* Persistence of virus in urine as factor in spread of infectious hepatitis in dogs. Proc. Soc. Exp. Biol. Med. 1951, *77*, 279.
20. *Rockborn, G.:* Viraemia and neutralizing antibodies in experimental distemper in dogs. Arch. ges. Virusforsch. 1957, *7*, 168.
21. *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.
22. *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.
23. *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.
24. *Schein, H.:* Études sur la peste bovine. Ann. Inst. Pasteur 1917, *31*, 571.
25. *Smith, W.:* The distribution of virus and neutralizing antibodies in the blood and pathological exsudates of rabbits infected with vaccinia. Brit. J. exp. Path. 1929, *10*, 93.
26. *Todd, C.:* Experiments on the virus of Fowl Plague (I). Brit. J. exp. Path. 1928, *9*, 19.
27. *Todd, C. & R. G. White:* Experiments on Cattle Plague, Government Press, Cairo 1914.

28. *Yoffey, J. M. & E. R. Sullivan:* The lymphatic pathway from the nose and pharynx. The dissemination of nasally instilled vaccinia virus. J. exp. Med. 1939, *69*, 133.

SUMMARY

The distribution of virus during the viraemia phase of HCC infection in guinea pigs was studied. It was found that the virus was bound to the mononuclear white blood cells.

Antibody titration revealed that several of the animals with viraemia had HCC antibodies in serum.

By an indirect method it was shown that a virus inhibitor appeared in the blood between 2—3 days after inoculation. Its effect gradually increased as long as virus titrations could be performed with certainty as to mean titre value.

ZUSAMMENFASSUNG

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

III. Die Viraemische Phase der HCC-Virus Infektion bei Meerschweinchen.

Die Virusverteilung wurde in Hinsicht auf verschiedene Blutfraktionen und Blutzellen untersucht. Es wurde gefunden, dass das Virus an die weissen mononucleären Zellen gebunden war.

Eine Anzahl Tiere mit Viraemie besass gleichzeitig Antikörper gegen das Virus im Serum.

Mit Hilfe eine indirekten Methode konnte gezeigt werden, dass ein Virusinhibitor 2—3 Tage nach der Impfung im Blute auftrat. Diese nahm kontinuierlich zu und dies so lange, als Virustitrierungen mit einiger Sicherheit rücksichtlich Mittel-Titer-Wert durchgeführt werden konnten.

SAMMANFATTNING

Studier över Hepatitis Contagiosa Canis virus (HCC).

III. Virämiskedet vid HCC virus infektion hos marsvin.

Fördelningen av virus under virämiskedet med hänsyn till olika blodfraktioner och blodceller undersöktes. Det befanns att virus var bundet till de vita mononukleära cellerna.

Ett flertal djur med virämi hade samtidigt antikroppar mot virus i sina serum.

Genom indirekt metodik kunde det visas, att en virusinhibitor uppträdde i blodet 2—3 dagar efter ympningen. Dess effekt ökade kontinuerligt och så länge som virustitreringar kunde göras med någorlunda säkerhet med hänsyn till titermedelvärde.

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