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TRANSAMINASE AND TRANSFERASE  
ACTIVITIES IN BLOOD-PLASMA OF DOGS  
WITH EXPERIMENTALLY PRODUCED  
HEPATITIS CONTAGIOSA CANIS (H. c. c.)

By

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The determination of the plasma-levels of different tissue enzymes, which is now possible, has become extremely useful in the early diagnosis of certain tissue damage. In accordance with other authors (*Reichard 1957, Cornelius et al. 1959*) we have shown (*Lindblad & Persson 1962*) that glutamic-oxaloacetic transaminase (G.O.T.) and glutamic-pyruvic transaminase (G.P.T.), as well as ornithine-carbamyl transferase (O.C.T.) occur in high concentration in the dog's liver. In contradistinction to what has been shown for other animal species (*Wretling, Orstadius & Lindberg 1959*), G.P.T. in dogs has its highest activity in the liver. Determination of these enzymes should therefore be useful as a complement to earlier liver tests, which, in fact, concern primarily the function of the liver. Besides the clinical importance of these enzymes, the determination of their activity can be of value in experimental investigations, for instance in studying, by objective methods, the effect of different forms of therapy. For the purpose of making such studies in connection with the infectious hepatitis in dogs (H.c.c.), we have first investigated the plasma activity of G.O.T., G.P.T., and O.C.T. in this disease produced experimentally.

## MATERIAL AND METHODS

21 mongrel dogs of both sexes, of which 18 were less than 6 months old, were inoculated with 0.2 ml. of a virus suspension containing 6.1 TCID<sub>50</sub> per ml.<sup>1)</sup> Blood-sera from most of the dogs were examined for the presence of neutralizing antibodies to H.c.c. before and after the inoculation. Three of the inoculated dogs died 5, 6, and 8 days, respectively, after inoculation. The autopsy findings<sup>2)</sup> were typical of H.c.c. (*Rubarth* 1947).

Blood samples for study of plasma-enzyme activity were taken before inoculation and then, as a rule, every other day until the raised levels had returned to normal. The determinations of enzyme activity were carried out at the same laboratory and by the same methods as described in detail by *Wretling, Orstadius & Lindberg*. G.O.T. and G.P.T. were determined by the method of *Reitman & Frankel* (1957), and O.C.T. by *Reichard's* (1957) method.

On the same occasion as blood was taken for determination of enzyme activity in plasma, the bromsulphalein-retention test by *Møller-Nielsen's* (1952) method was carried out in order to obtain a measure of the liver function.

Routine examination of the blood was made to ascertain, in particular, the total number of leucocytes and the differential blood-count. The serum-proteins were studied electrophoretically.

For the technique used in the determination of neutralizing antibodies, the reader is referred to an earlier paper (*Persson, Persson & Sibalín* 1961). In testing sera for the presence of neutralizing antibodies, a "positive reaction" was neutralization of the antigen at dilutions higher than 1/10.

## RESULTS

Table 1 records the dogs used in the experiment and the results of inoculation with H.c.c. virus.

In estimating the transaminase activity normal values, published elsewhere (*Lindblad & Persson*), were used for comparison.

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<sup>1)</sup> The virus used was an SBL strain, grown in tissue culture from dog's kidney. It was obtained from Dr. *R. Salenstedt*, the State Bacteriological Laboratory, Stockholm.

<sup>2)</sup> The autopsies were performed at the Department of Pathology, the Royal Veterinary College, Stockholm.

Table I. 21 dogs inoculated with H.c.c. virus. An SBL strain, containing 6.1 TCID<sub>50</sub> per ml., obtained from Dr. *R. Sahlenstedt*, the State Bacteriological Laboratory, Stockholm, was used in all cases.

Dog	Age months	Dogs kept strictly isolated: +	Tested and without antibodies before inoculation: +. Not tested: -	Total number of leucocytes per c. mm. lowest value (number of days after inoculation)	Temperature °C highest value (number of days after inoculation)	Rise in transaminase activity: +. No rise: -	Bromsulphalein retention test; highest value mg. per 100 ml.
H:2	5	+	+	2700 (5)	40.8 (3)	+	
H:3	5	+	+	2700 (5)	40.5 (3)	+	
H:5	5	+	+	2100 (5)	40.5 (3)	+	
H:6	5	+	+	4600 (5)	40.8 (3)	+	
H:7	5	+	+	2400 (6)	41.2 (3)	+	
H:8	5	+	+	2800 (6)	41.0 (6)	+	
H:9	5	+	+	2800 (6)	40.7 (6)	+	
H:11	5	+	+	3200 (4)	40.8 (3)	+	
(H:21)	6	—	+	3100 (6)	40.2 (6)	—	
H:22	6	—	+	2500 (4)	40.8 (4)	+	
(H:23)	6	—	+	4700 (6)	40.3 (4)	—	
H:24	6	—	+	6300 (6)	40.5 (4)	+	
H:25	6	—	+	8800 (2)	40.2 (3)	+	
(Collie)	24	—	+	2900 (6)	39.9 (3)	—	
(Schäfer)	24	—	+	3000 (4)	39.1 (3)	—	
(Stövare)	24	—	+	8100 (6)	39.0 (3)	—	
Bellman	3	+	—	—	40.8 (3)	+	2.6
Ulla	3	+	—	—	40.8 (3)	+	2.5
(Schäfer I)	5	—	—	1400 (5)	40.8 (5)	—	0.4
(Schäfer II)	5	—	—	3000 (5)	39.2 (3)	—	0.2
(Schäfer III)	5	—	—	1300 (5)	41.3 (3)	+	0.7

Mean values for normal dogs are:

for G.O.T.:  $17.8 \pm 6.2$  s.d. enzyme units for 165 degrees of freedom;

„ O.C.T.:  $2.2 \pm 1.6$  s.d. enzyme units for 158 degrees of freedom;

„ G.P.T.: (under 6 months):  $10.3 \pm 4.3$  s.d. enzyme units for 21 degrees of freedom;

„ G.P.T.: (over 6 months):  $20.6 \pm 8.4$  s.d. enzyme units for 142 degrees of freedom.

Of the inoculated dogs, only 14 showed a rise of enzyme activity in blood-plasma. These 14 dogs reacted uniformly as regards general condition, body-temperature, decrease of leucocytes, and raised transaminase values. Two of the dogs, “H:25”

and "Ulla", developed opacity of the cornea of both eyes about 1 week after the onset of illness. The dogs placed in parentheses in the table exhibited fever but no other clinical symptom of disease. Nor did they show any rise of transaminase activity. The development of leucopenia in these dogs should be noted, however.

The results of the transaminase determinations are set out in Figs. 1, 2, and 3, which show altogether 232 values from the 14 dogs that fell ill with H.c.c. after inoculation.

For lack of space, detailed accounts of the clinical examinations cannot be given for all the dogs. "Ulla", "Bellman", and "Schäfer III" may, however, serve as models. They were subjected to full clinical examinations, the results of which are shown in detail in Figs. 4, 5, and 6.

To demonstrate the time-relationship between the various clinical data obtained, dog no. H:5 has been chosen as a typical

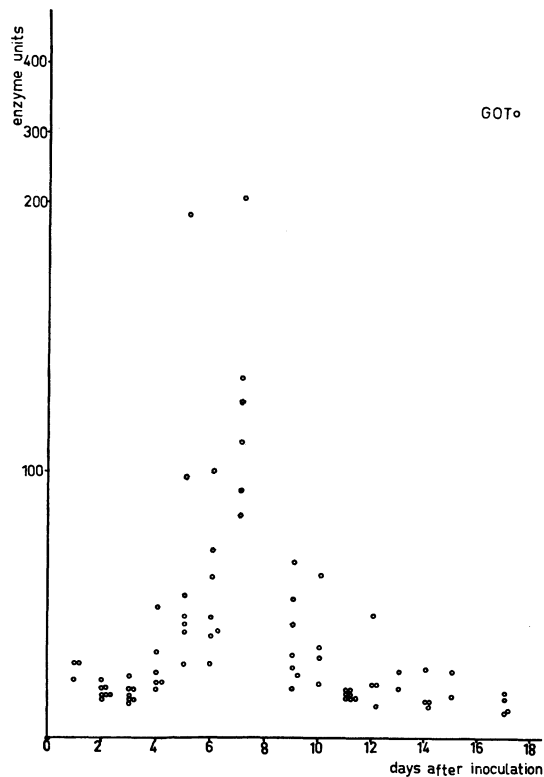


Fig. 1. Plasma-GOT levels at all determinations.

case. It will be seen from Fig. 7 that the leucopenia develops simultaneously with the temperature rise. Further, the lymphocyte-neutrophil ratio changes towards an increase in lymphocytes, in contrast to the findings reported by *Coffin & Cabasso* (1953).

The repeated electrophoretic studies of the serum-proteins show that in H.c.c. there is a general rise in globulins, which is striking for the  $\gamma$ -globulin. From normal values of about 0.2 g. per 100 ml. (4 per cent of the total protein) the  $\gamma$ -globulin rose continuously, and 14 days after inoculation it showed values of about 1 g. per 100 ml., equalling 18 per cent of the total protein. A corresponding decrease of the albumins was noted and, hence, the total-protein level remained unchanged.

#### DISCUSSION

Out of the 21 inoculated dogs 7 showed no increase of enzyme activity in the plasma. In 2 of these 7 dogs ("Schäfer I" and

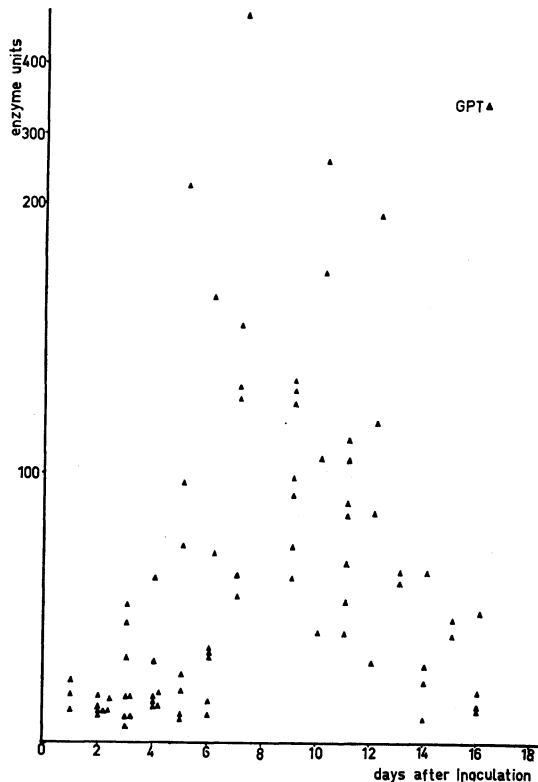


Fig. 2. Plasma-GPT levels at all determinations.

“Schäfer II”) the liver function was studied by means of the bromsulphalein-retention test. If the upper limit for normal values is set at 0.5 mg. per 100 ml., liver function, according to this test, was normal in 2 dogs. This observation may be taken as supporting evidence for the supposition that in these dogs, as well as in the other 5 without a rise in transaminase activity, liver damage had not been caused. In dogs “H:21”, “H:23”, “Collie”, and “Schäfer I”, however, the virus inoculation was accompanied by a significant temperature rise and leucopenia, while “Schäfer” and “Schäfer II”, showed no significant temperature rise above the normal but a marked leucopenia. As regards “Stövare”, the values for both the decrease in the leucocyte count and the temperature rise are not significant; the reaction of this dog is therefore doubtful. To obtain normal values of our own for the total number of leucocytes per c.mm., a statistical calculation was made of the original values for the dogs

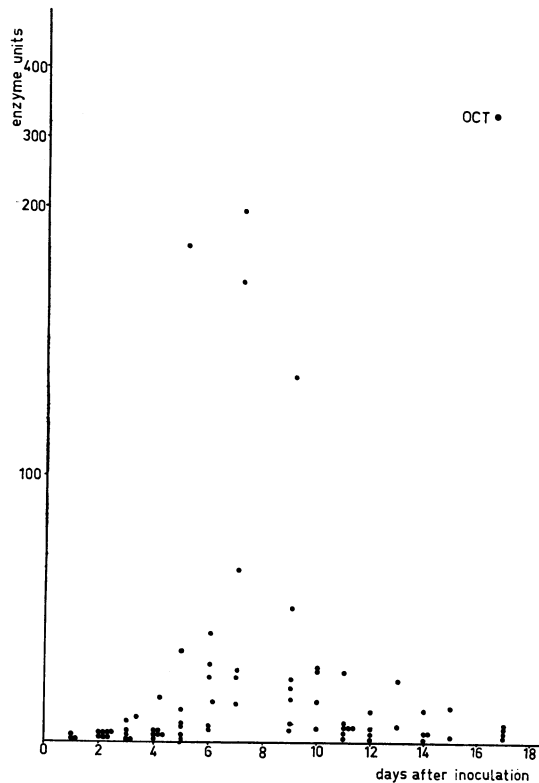


Fig. 3. Plasma-OCT levels at all determinations.

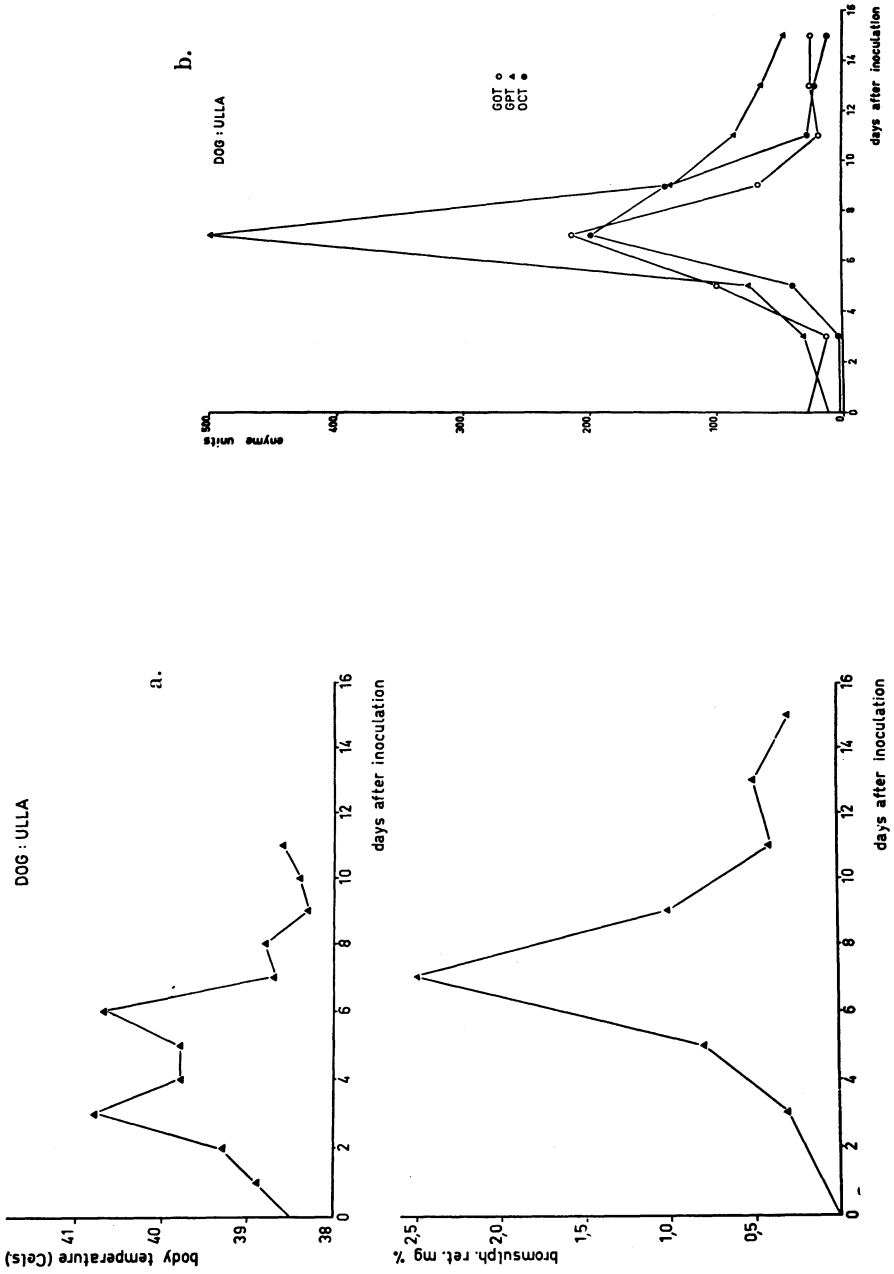


Fig. 4. Body temperature, bromsulphalein retention and plasma enzymes in an acute case.

concerned. For 20 degrees of freedom the mean value was  $10,132 \pm \text{s.d. } 2280$ . With a 95 % range the extreme values will thus be 5572—14,722 per c.mm.

The first question that arises regarding these 7 dogs is why they did not react with liver damage. Five of them were before the inoculation tested for the presence of neutralizing antibodies to H.c.c. and found to be negative. The other 2 can with good reason be presumed to have had no antibodies either, since they were litter-mates and had been reared together with "Schäfer III",

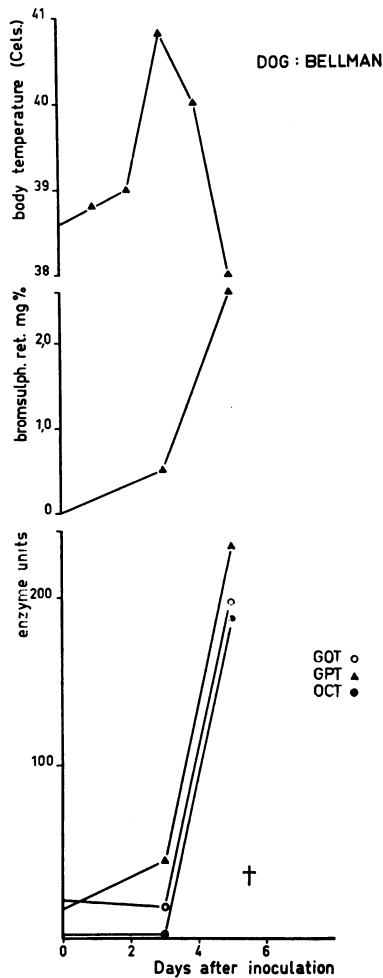


Fig. 5. Body temperature, bromsulphalein retention and plasma enzymes in a fatal case.



which became ill. As far as the older dogs are concerned, which had not been reared in strict isolation, a possible explanation why these 7 dogs did not fall ill with clinical symptoms characteristic of H.c.c. may be that they had earlier been infected with H.c.c. virus and remained resistant to the disease, and had again become free from demonstrable antibodies. *Larin* (1959) observed that 7 adult dogs, serologically negative as regards complement-fixing antibodies, did not fall ill after inoculation of antigen but showed a marked rise in titre. It is more difficult to explain why the younger ones did not become ill. As regards "Schäfer I" and "Schäfer II", however, the unfortunate fact remains that neutralization test was not made in these 2 cases. In dogs "H:21" and "H:23", on the other hand, an earlier infection should have led

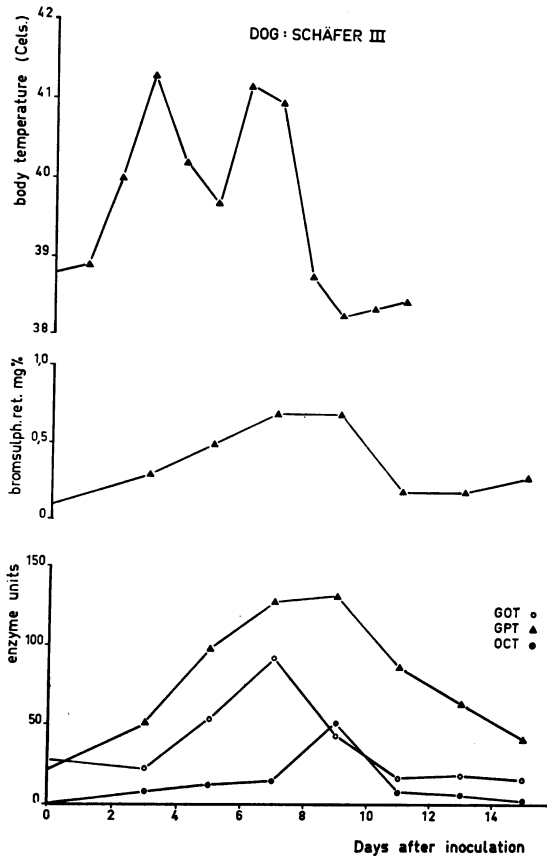


Fig. 6. Body temperature, bromsulphalein retention and plasma enzymes in an acute case.

to a rise in antibody titre, which would still have been noticeable. According to *Persson, Persson & Sibalín*, an antibody titre after a spontaneous H.c.c. infection is likely to persist for about 1 year. Nor can it be expected that passive immunity conferred from the mother could have given such long-lasting protection. It seems more probable that these dogs had some form of unspecific individual resistance which prevented the development of liver damage.

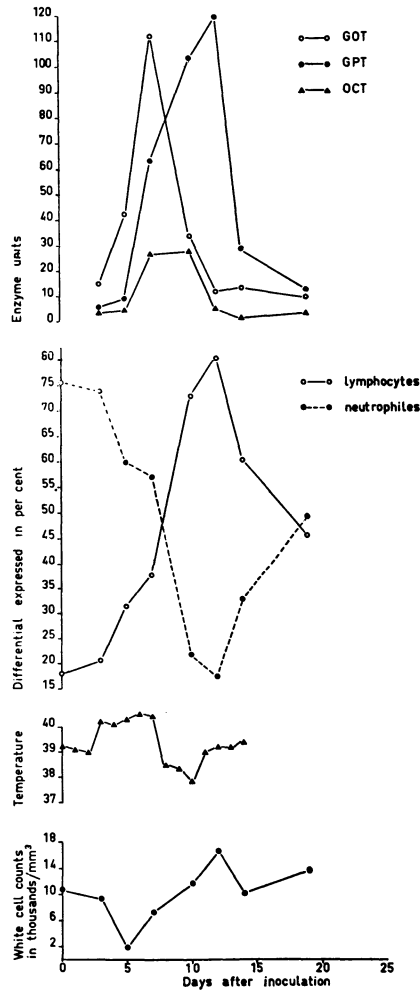


Fig. 7. Plasma enzymes, differential count between lymphocytes and neutrophils, white cell counts and body temperature in an acute case.

In 2 of the dogs an opacity of the cornea of both eyes was noted 11 and 13 days, respectively, after inoculation. This incidence of opacity of the cornea is in accordance with the findings of earlier workers (*Stünzi* 1954).

For determination of enzyme activity in blood-plasma in the infectious hepatitis, G.O.T. and G.P.T. have been studied by *Dal Santo* (1959) and *Hoe* (1961). The latter considers that estimation of G.P.T. activity gives a better measure of liver-cell damage than does G.O.T. estimation.

It will be seen from Figs. 1, 2, and 3 that the rise in plasma-enzyme activity in H.c.c. is marked. The rise in the different enzyme levels occurs about 4 to 5 days after the inoculation of virus, that is, about 48 hours after the first temperature rise. The enzyme activity continues to increase, reaching its maximum value 7 to 8 days after inoculation. At this time the temperature has begun to return to normal. The increased bromsulphalein retention correlates fairly well in time with the rises in plasma enzyme activity.

From Figs. 4, 5, and 6, it is seen more clearly than in Figs. 1, 2, and 3, that the increased G.P.T. activity, and to some extent the O.C.T. activity, persists longer than that of G.O.T. (It has been shown by, for instance, *Fleisher & Wakim*, 1956, that the elimination time is longer for G.P.T. than for G.O.T.) A general feature is also that the increase in G.P.T. activity is greater than that of the other enzymes. However, the observation that there seems to be some correlation between the G.P.T. and the O.C.T. rise is most interesting, especially as such a correlation could be demonstrated for the plasma-activity of these enzymes in healthy subjects (*Lindblad & Persson*).

A diphasic rise in temperature followed by a slight subnormal temperature is known to be characteristic of H.c.c. An interesting fact is, however, that in those cases in which the disease terminates in death, the second temperature spike fails to appear and, the reverse, that in those cases where the second phase does occur the prognosis seems to be good.

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#### SUMMARY

Continuous and comparative studies of the plasma-levels of glutamic-oxaloacetic transaminase (G.O.T.), glutamic-pyruvic transaminase (G.P.T.), and ornithine-carbamyl transferase (O.C.T.), as well as the concentration of bromsulphalein (B.S.P.) showed that in experimentally produced hepatitis contagiosa canis (H.c.c.) the plasma-activity of these enzymes and B.S.P. retention were raised and the increased B.S.P. retention correlated in time with the rises in plasma-enzyme activity.

A marked leucopenia developed simultaneously with temperature elevations. The lymphocyte/neutrophil ratio changed towards an increase in lymphocytes during the disease.

Electrophoretic studies of serum-proteins showed a rise of  $\gamma$ -globulins and a corresponding decrease of the albumins, that is, an unchanged level of total protein.

## ZUSAMMENFASSUNG

*Transaminase- und Transferaseaktivität der Plasma bei Hunden mit experimentell erzeugter H.c.c. (Hepatitis contagiosa canis).*

Kontinuierliche und vergleichende Untersuchungen über die Plasma-Aktivität von Glutaminsäure-Oxalessigsäure Transaminase (GOT), Glutaminsäure-Pyrotraubensäure Transaminase (GPT) und Ornithin Karbamyl Transferase (OCT) als auch die Konzentration von Bromsulfalein (BSP), zeigten bei experimentell erzeugter Hepatitis contagiosa canis (Hcc), dass die Plasma-Aktivität dieser Enzymen und die BSP Retention anstieg und dass die erhöhte BSP Retention mit den gleichzeitigen Erhöhungen der Plasma-Enzymen Aktivität übereinstimmte.

Eine ausgeprägte Leukopenie entwickelte sich gleichzeitig mit den Temperatursteigerungen. Das Verhältnis zwischen den Lymphozyten und Neutrophilen hat sich während der Krankheit zu der Lymphozyten verschoben.

Elektrophoretische Untersuchungen der Serumproteinen zeigten eine Erhöhung der  $\gamma$ -Globulinen und eine entsprechende Senkung der Albuminen, also einen unveränderten Totalproteingehalt.

## SAMMANFATTNING

*Transaminas- och transferasaktiviteten i blodplasma hos hundar med experimentell framkallad H.c.c. (Hepatitis contagiosa canis).*

Författarna har gjort kontinuerliga och jämförande undersökningar av plasmaaktiviteten av GOT (glutaminsyre-oxalättiksyretransaminas) GPT (glutaminsyre-pyrodruvsyretransaminas) OCT (ornithin carbamyltransferas), samt BSP (bromsulfaleinretention) och funnit dessa förhöjda och tidmässigt korrelerade vid experimentell hepatitis contagiosa canis (H.c.c.).

En uttalad leukopeni inträder samtidigt med temperaturstegring. Förhållandet lymfocyter-neutrofila leukocyter ökar under sjukdomen.

Elektroforetisk undersökning av serumproteinerna har visat en  $\gamma$ -globulinökning och motsvarande minskning av albuminerna, d. v. s. oförändrat totalprotein.

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