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THERAPEUTIC TRIALS IN DOGS WITH EXPERIMENTAL HEPATITIS CONTAGIOSA CANIS*)

By
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Because of the difficulties attached to the clinical diagnosis of Hepatitis contagiosa canis (H.c.c.) and the relatively large number of dogs that recover spontaneously from the disease, the assessment of the effect of therapeutic agents is a hazardous undertaking. The reported results vary between different published trials. *Craige* (1950) and *Chaddock & Carlsson* (1950) obtained good results by treatment with hyperimmune sera; an effect could be demonstrated within 24 to 48 hours. *Glover* (1959) reports that 75 % of the cases responded favourably to treatment with serum and that a 100 % result will be obtained by combined serum and prednisolone treatment. In trials carried out by *Parry & Larin* (1951), antiserum had no effect on the course of the disease.

According to *Craige* (1950), methionine and choline prevent the development of liver damage in H.c.c. *Stünzi* (1958) has shown that sulpha drugs and penicillin are ineffective. Several authors recommend treatment with circulatory stimulants. Transfusion of blood or blood plus plasma is suggested by, for instance, *Baker et al.* (1954). *Voss* (1961) reports good results with corticosteroids.

The diagnosis of H.c.c. has been discussed by *Lindblad &*

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Persson (1962). The trials reported here were undertaken to study the effect of hyperimmune serum, plasma volume expanders, and corticosteroids in experimentally produced H.c.c.

MATERIAL AND METHODS

47 dogs lacking antibodies^{*}) were used, they were of a mixed breed, males and females, and their ages ranged from 3 to 6 months. Undiluted virus suspensions of the virus strains SBL^{**}) (7.5 TCID₅₀/ml) and E 745^{***}) (6.3 TCID₅₀/ml) were inoculated intraperitoneally in doses of 2 ml. The development of the disease was followed as described earlier (*Lindblad & Persson* 1962). In the trials with hyperimmune serum we used the commercially available preparation Serasix (Pitman-Moore). The titre of antibodies against H.c.c. has been measured in the serum concerned and found to be 1:640^{*}) (*Espmark & Salenstedt* 1916). For the corticosteroid trials we chose prednisolone 1 % (ACO) and as plasma expanders dextran as Macrodex ® 6 % (Pharmacia) and Rheomacrodex ® 10 % (Pharmacia).

The treatments were in all the cases started on the 3rd post-inoculation day.

The hyperimmune serum was given in a single dose of 4 ml per kg body-weight to 4 dogs; 4 were left untreated. In a second trial, 5 dogs were given 2 ml of serum per kg body-weight twice at one-day intervals; the administration was on the first occasion by the intravenous and the second time by the subcutaneous route, and 4 dogs were left untreated.

In a group of 20 dogs, Macrodex was given by intravenous drip to 7 dogs, at an infusion rate of about 10 ml per minute, and the prednisolone was administered intramuscularly to 7. In most cases the infusions were given daily. Six dogs served as controls.

Rheomacrodex was administered by intravenous drip infusion (about 10 ml per minute) to 4 dogs and in this trials 2 dogs served as untreated controls.

The doses of plasma expanders given at each treatment averaged 10 ml, and those of prednisolone 0.4 mg per kg body-weight.

In a group comprising 8 normal dogs, Macrodex was given to 2, Rheomacrodex to 4, and Ringer's solution to 2. The infusions were made by intravenous drip at a rate of about 10 ml per minute. The dosage was about 10 ml per kg body-weight and day. Blood for transaminase determinations was drawn 24 hours after the treatment^s.

^{*}) The tests were carried out by *R. Salenstedt*, V.M.D., National Bacteriological Laboratory, Stockholm.

^{**}) Obtained from *R. Salenstedt*, V.M.D., National Bacteriological Laboratory, Stockholm.

^{***}) Isolated from a spontaneous case of H.c.c. by *M. Sibalin*, V.M.D., Department of Medicine for Non-Ruminants, Royal Veterinary College, Stockholm.

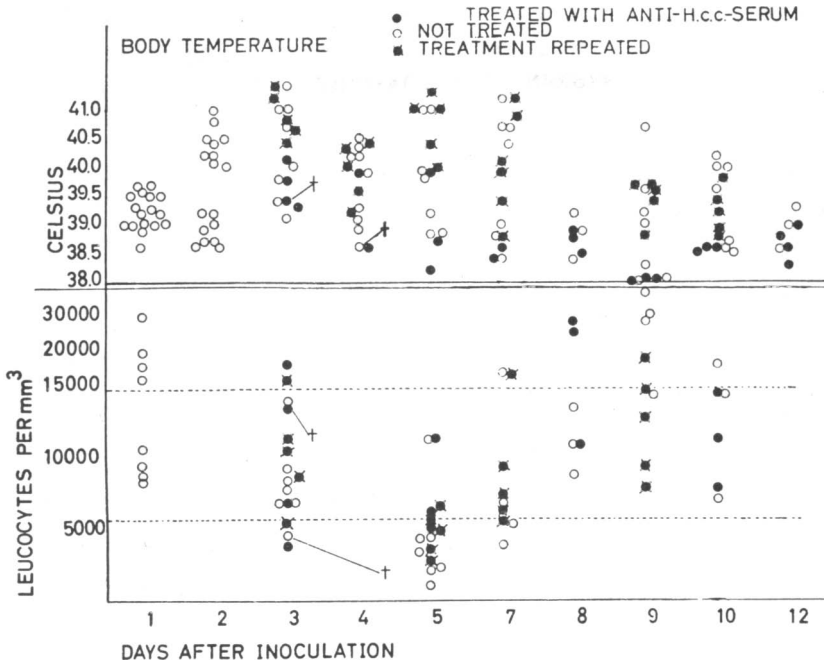


Fig. 1. Temperature and leucocyte counts in 17 dogs inoculated with H.c.c. virus, 9 of which were treated with antiserum. The dotted lines represent the mean value for total leucocyte count ± 2 s.d.

Table 1. Number of dogs having leucocyte counts and serum-enzyme activities either normal throughout or deviating from normal at least once during the experimental time. The values are extracted from figs. 1—3.

		Number of dogs treated with hyper- immune serum	Number of untreated dogs
Total leucocyte count	significantly low	6	7
	probably low	1	1
	normal	2	—
G.O.T.	significantly high	7	6
	—	—	—
	normal	2	2
G.P.T.*)	significantly high	2	3
	probably high	1	—
	normal	1	1
O.C.T.	significantly high	6	6
	probably high	1	1
	normal	2	1

*) Estimated in only 8 dogs.

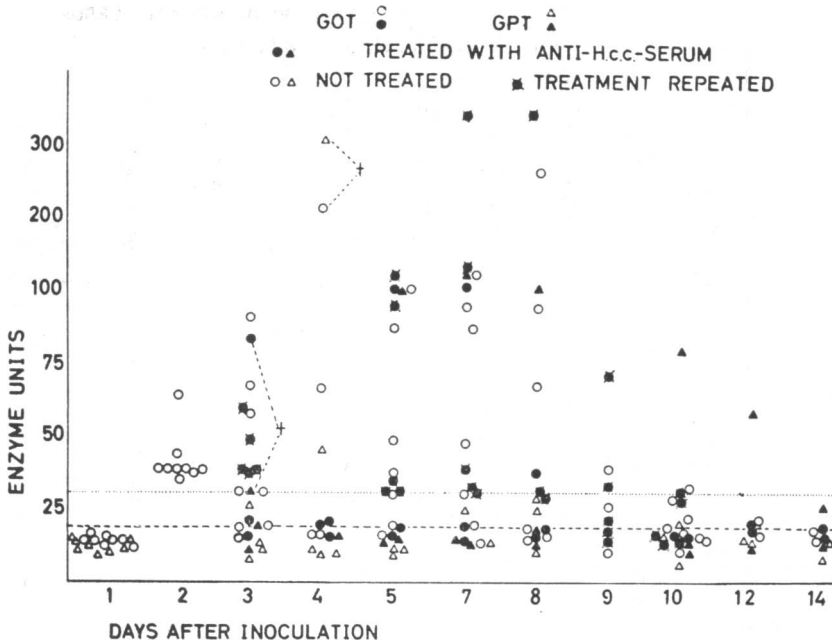


Fig. 2. G.O.T. and G.P.T. levels in 17 dogs inoculated with H.c.c. virus, 9 of which were treated with antiserum. The dotted line represents the mean value for G.O.T. + 2 s.d. The broken line represents the mean value for G.P.T. + 2 s.d.

RESULTS

Treatment with hyperimmune serum. Figs. 1—3 and table 1 show the results relating to the temperatures, total leucocyte counts, and glutamic-oxaloacetic transaminase (G.O.T.), glutamic-pyruvic transaminase (G.P.T.), and ornithine carbamyl transferase (O.C.T.) levels in 17 dogs treated with hyperimmune serum (because of the weights of the dogs — 1 to 3 kg — and the tendency to haematoma, sufficient amounts of blood for analyses could not always be obtained). Two dogs died within 3 and 5 days, respectively, of the inoculation. The autopsy findings were in both cases typical of H.c.c.*) One was an untreated and the other a treated case. The results for these two and another two dogs, one treated and one untreated, are recorded individually in figs. 4 and 5. As regards clinical symptoms, such as inappetence, apathy, thirst, and sluggishness, the onset was fairly

*) The autopsies were performed at the Department of Pathology, Royal Veterinary College, Stockholm.

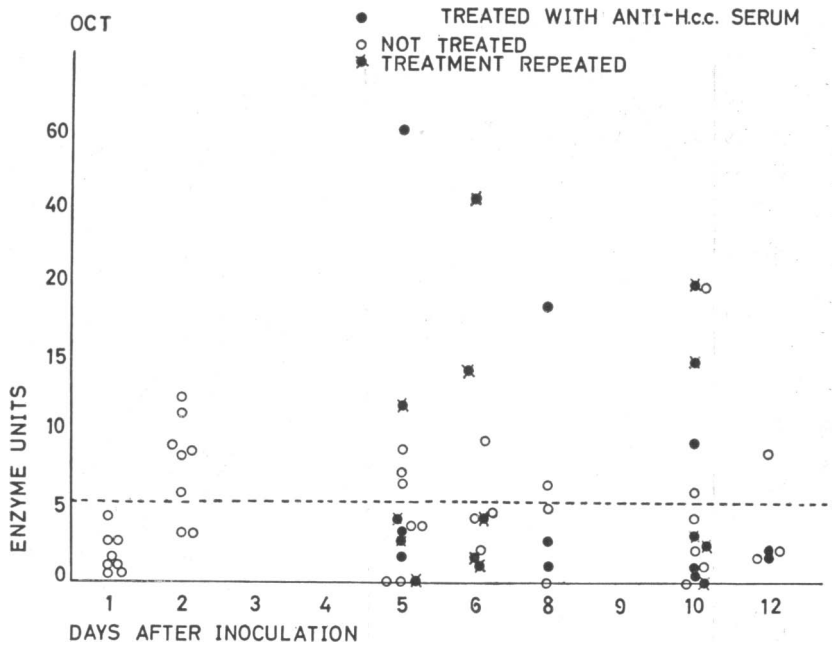


Fig. 3. O.C.T. levels in 17 dogs inoculated with H.c.c. virus, 9 of which were treated with antiserum. The broken line represents the mean value for O.C.T. + 2 s.d.

Table 2. Number of dogs having leucocyte counts and serum-enzyme activities either normal throughout or deviating from normal at least once during the experimental time. The values are extracted from figs. 6—9.

		Number of dogs treated with prednisolone	Number of dogs treated with plasma expander	Number of un- treated dogs
Total leucocyte count	significantly low	3	6	5
	probably low	3	1	—
	normal	1	—	1
G.O.T.	significantly high	5	7	3
	probably high	1	—	2
	normal	1	—	1
G.P.T.	significantly high	7	4	4
	probably high	—	3	2
O.C.T.	significantly high	6	5	5
	probably high	—	1	1
	normal	1	1	—

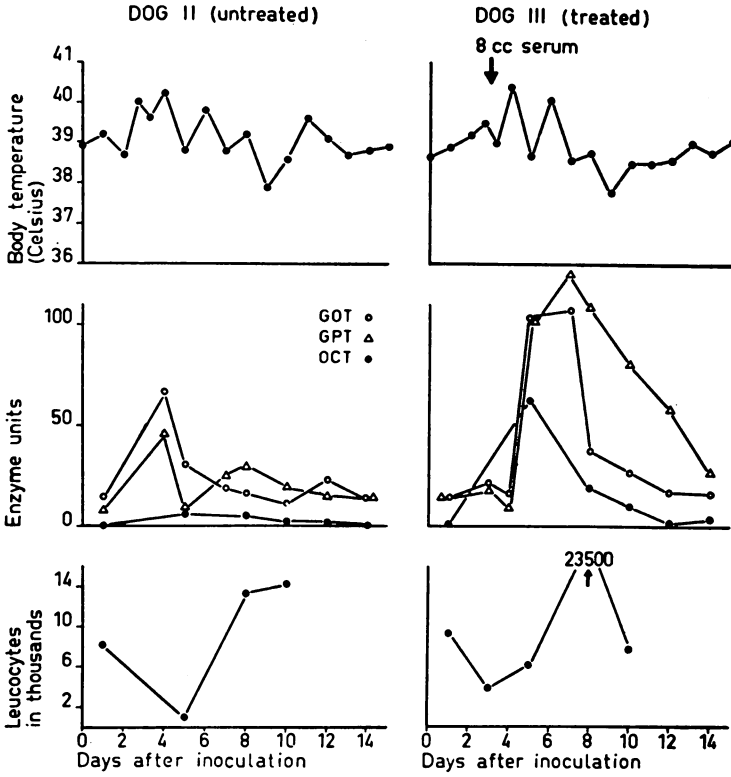


Fig. 4.

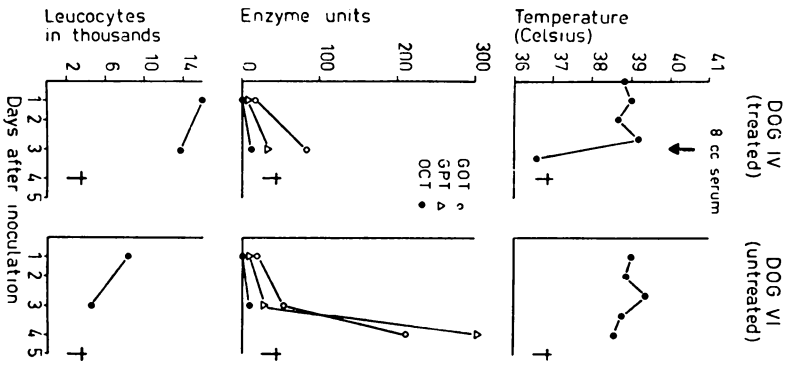


Fig. 5.

Figs. 4 and 5. Temperature, transaminase levels, and total leucocyte counts in 4 dogs inoculated with H.c.c. virus, 2 of which were treated with antiserum.

uniform in all the dogs, excepting 2 which did not respond in any respect. Opacity of the cornea occurred in 1 treated and 1 untreated dog on the 12th and the 10th postinoculation day, respectively.

Treatment with Macrodex® and corticosteroids. The results from the prednisolone and the Macrodex trials are shown in figs. 6—9 and table 2. The onset of clinical symptoms was similar in all the dogs in the three groups, as regards the poor general condition, inappetence, apathy, thirst, and temperature-rise. All the survivors showed diphasic temperature curves, with a fall occurring regularly on the 4th postinoculation day. Two dogs died, both belonged to the cortisone group. In one of these two, the temperature spiked a second time, which has earlier been stated to be a prognostically favourable sign (*Lindblad & Persson 1962*). The autopsy findings were in both cases typical of H.c.c.*)

Opacity of the cornea was noted in 9 dogs, including all the prednisolone-treated survivors (table 3).

Table 3. Occurrence of opacity of the cornea. 2 = both eyes, 1 = 1 eye. Figures in parentheses denote days after inoculation.

Controls	2(9, 14); 1(9)
Macrodex group	1(16); 1(14)
Prednisolone group	1(13); 1(18); 2(13, 16); 2(13, 9); 1(19)

Treatment with Rheomacrodex®. The dogs belonged to the same litter. Leucopenia and a temperature-rise occurred in all of them (table 4).

Table 4. Total leucocytes counts and temperatures in dogs with experimental H.c.c. treated with Rheomacrodex. Figures in parentheses denote days after inoculation.

Dog no.	Total leucocyte count (minimum value)	Temperature °C (maximum value)
1 (control)	4800 (4)	39.6 (6)
2	3100 (4)	40.9 (6)
3	4900 (4)	40.9 (6)
4 (control)	3700 (4)	39.9 (6)
5	2400 (4)	40.4 (6)
6	5600 (4)	40.9 (3)

*) The autopsies were performed at the Department of Pathology, Royal Veterinary College, Stockholm.

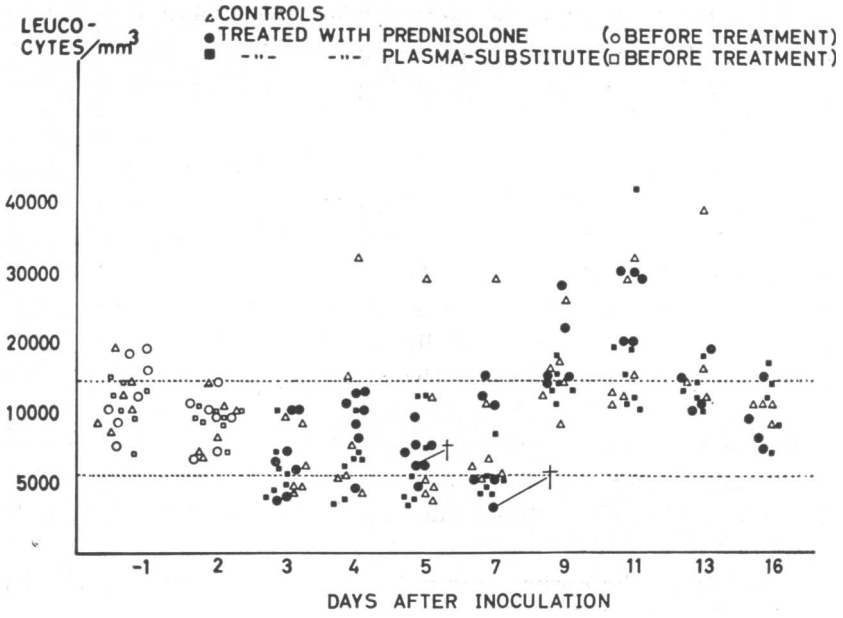


Fig. 6.

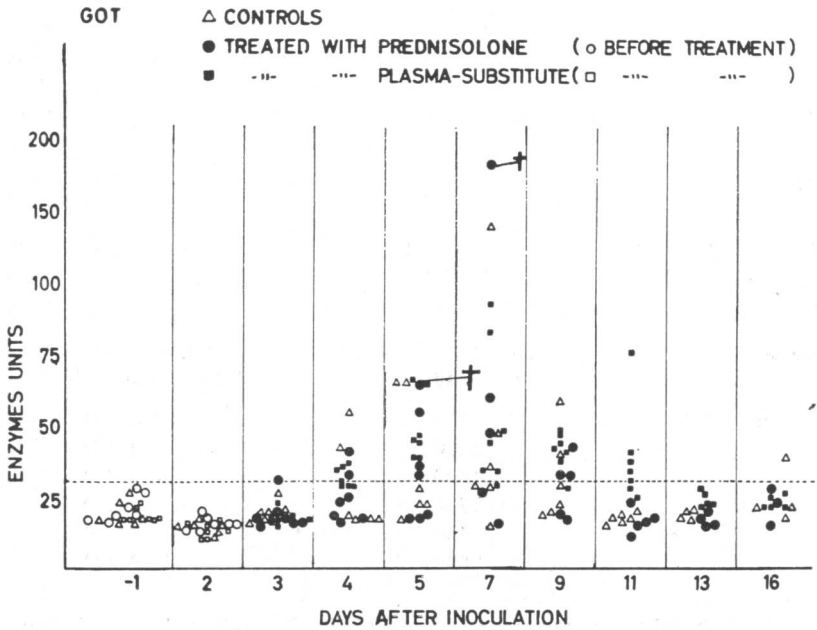


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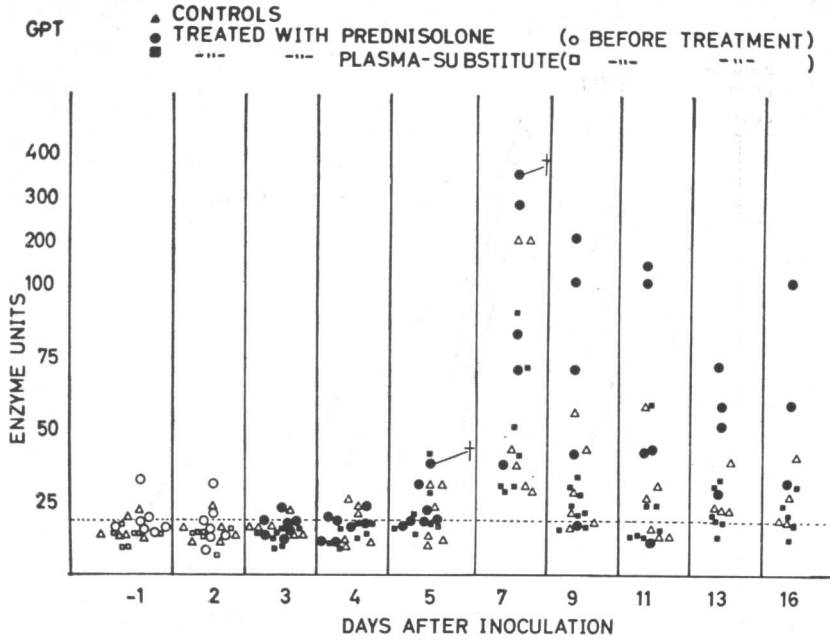


Fig. 8.

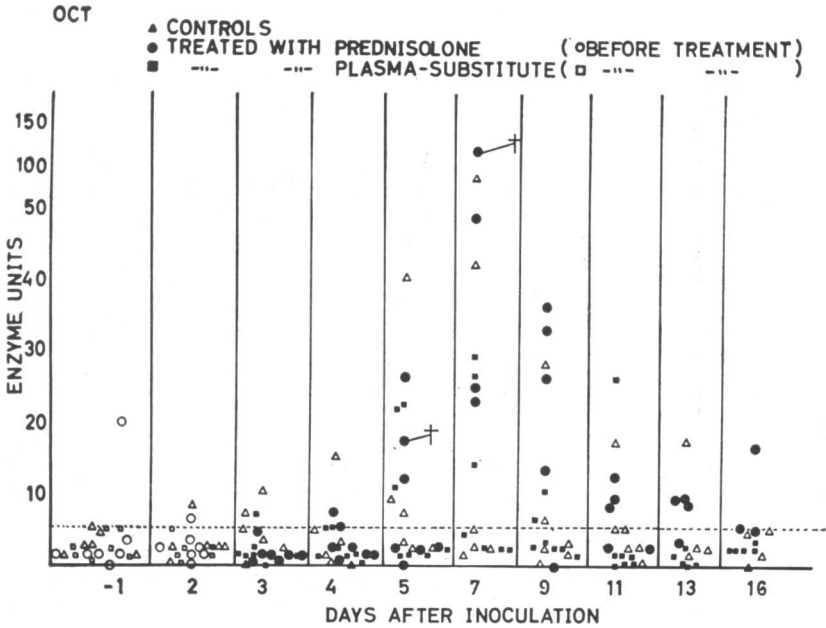


Fig. 9.

Figs. 6, 7, 8, and 9. Total leucocyte counts, G.O.T., G.P.T., and O.C.T. levels in 20 dogs inoculated with H.c.c. virus, of which 7 were treated with 6 % Macrodex, 7 with 1 % prednisolone, and 6 were left untreated. The broken lines represent the mean value for total number of leucocytes \pm 2 s.d. (Fig. 6), \pm 2 s.d. for G.O.T. (Fig. 7), G.P.T. (Fig. 8), and O.C.T. (Fig. 9).

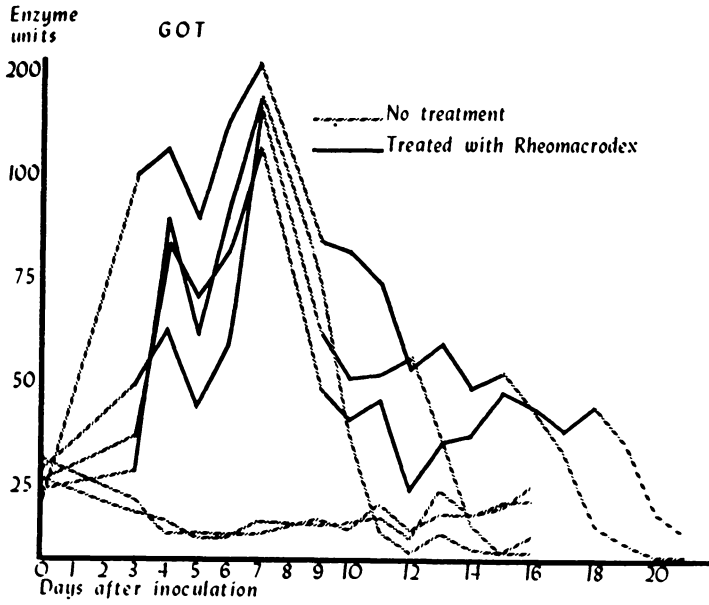


Fig.10.

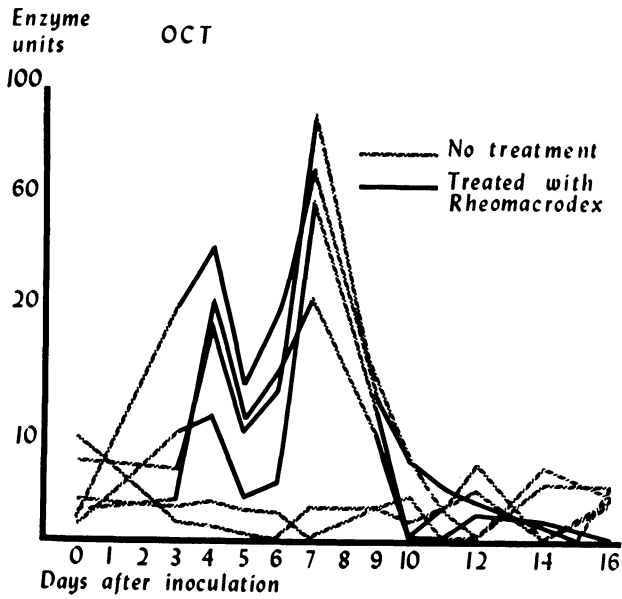


Fig.11.

Figs. 10 and 11. G.O.T. and O.C.T. in dogs treated with 10 % Rheomacrodex. The continuous lines represent daily treatment, the broken lines no treatment.

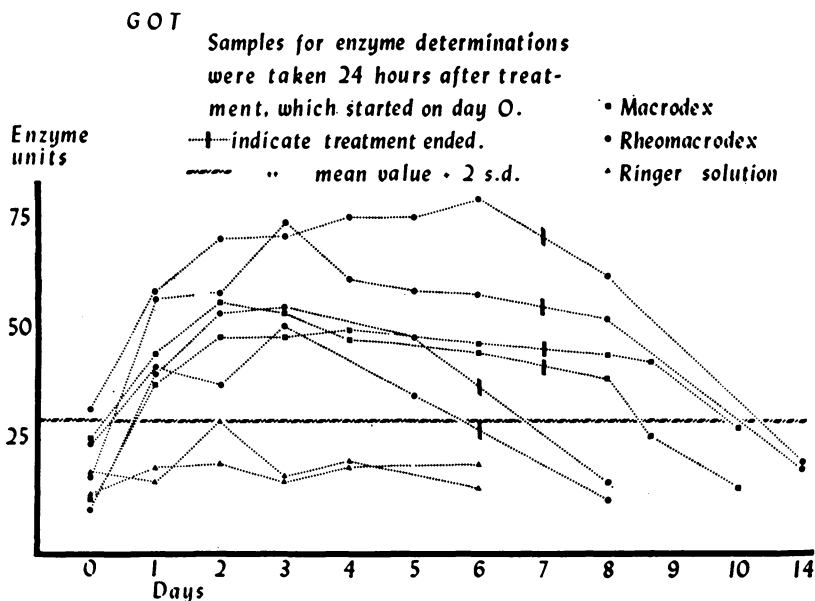


Fig. 12. G.O.T. levels in normal dogs treated with 6 % Macrodex, 10 % Rheomacrodex, and Ringer's solution.

The results relating to the G.O.T. and O.C.T. determinations are shown in figs. 10 and 11. It will be seen from the figures that high values for G.O.T. and O.C.T. were found in the treated dogs. It will also be noted that the return of the G.O.T. levels to normal was interrupted when the treatment was re-instituted and that the G.O.T. levels became normal when treatment was withdrawn. Opacity of the cornea did not occur in any of the cases.

In a control trial comprising 8 normal dogs, Rheomacrodex was given to 4, Macrodex to 2, and Ringer's solution to 2 (fig. 12). It will be seen from the figure that both Macrodex and Rheomacrodex caused a rise of the G.O.T. levels. Ringer's solution did not influence the G.O.T. activity. The O.C.T. levels, which were studied in parallel with the G.O.T. levels, were not influenced by the treatments.

DISCUSSION

In this trial we have studied the therapeutic effect of some substances on a few of the symptoms that are directly or indirectly caused by the H.c.c. virus. The treatments were started on the 3rd postinoculation day. This is the day on which the

Table 5. Normal values given by *Lindblad & Persson (1962)*, on which the comparisons in tables 1 and 2 are based.

<i>Total number of leucocytes</i>	10,132 ± s.d. 2280
Values below 7852 are designated as probably low	
Values below 5572 are designated as significantly low	
<i>G.O.T.</i>	17.8 ± s.d. 6.2
Values over 24.0 are designated as probably high	
Values over 30.2 are designated as significantly high	
<i>G.P.T.*)</i>	10.3 ± s.d. 4.3
Values over 14.6 are designated as probably high	
Values over 18.9 are designated as significantly high	
<i>O.C.T.</i>	2.2 ± s.d. 1.6
Values over 3.8 are designated as probably high	
Values over 5.4 are designated as significantly high	

*) This normal value was used because all the dogs were probably younger than 6 months.

first symptoms usually appear and, in practice, the day on which the question of treatment would be likely to arise. The temperature charts for treated dogs do not differ from those for untreated dogs. The diphasic curve described earlier (*Lindblad & Persson 1962*) was seen in all the survivors of the different groups, with the afore-mentioned exception.

On comparing the results shown in figs. 1—5 and table 1 with the normal values given by *Lindblad & Persson (1962)* and reproduced in table 5, it will be seen that there is no difference between dogs treated with hyperimmune serum and untreated dogs with respect to changes in the total number of leucocytes, and G.O.T., G.P.T., and O.C.T. levels. The transaminases are sensitive criteria of liver damage, and it is evident that this becomes manifest despite treatment with hyperimmune serum, whether it is given once or twice. Apparently, treatment with hyperimmune serum started on the 3rd postinoculation day has no effect on the course of H.c.c.

The similarities between the autopsy findings in cases of H.c.c. and of shock have been discussed by *Rubarth (1947)*. *Lindblad et al. (1964)* have shown that circulatory disturbances are present in experimentally produced H.c.c. Therefore, positive results could be expected from treatment with Macrodex®, and

perhaps particularly so with Rheomacrodex (*Gelin* 1962). Yet, none of these substances prevented a temperature-rise, the development of leucopenia, or transaminase elevations. Our control trial in normal dogs shows that both Macrodex and Rheomacrodex cause an increase of the G.O.T. activity in the plasma. Hence, the very high transaminase elevations recorded in these trials are presumably caused by the sum of the effects of plasma expanders and H.c.c., and when the disease ran a mild course the elevations would be attributable to the plasma expanders alone. (The transaminase values have, in fact, to some extent served as a measure of the severity of the disease.) It may be concluded that Macrodex and Rheomacrodex have no beneficial effects on the course of experimentally produced H.c.c.

Contrary to the reports of good results with corticosteroids, figs. 5—8 and table 2 show that prednisolone does not influence the course of H.c.c. in a positive direction. Two out of 7 treated dogs died. As regards leucopenia and transaminase elevations, there seems to be no difference between the survivors and the control group. The opacity of the cornea, as yet unexplained, which in our and other's experience occurs in about 20 % of survivors, was noted in all the 5 survivors in the prednisolone group. The opacity in itself has been regarded as a prognostically favourable sign (*Rubarth* 1947), but the results obtained in these trials would nevertheless justify a warning against the use of corticosteroids.

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SUMMARY

The effect of some substances on the temperature, total leucocyte count, and plasma levels of glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, and ornithine carbamyl transferase has been studied in dogs with experimentally produced Hepatitis contagiosa canis. Neither the hyperimmune serum Serasix (Pitman-Moore), nor 1 % prednisolone (ACO), nor 6 % Macrodex® (Pharmacia), nor 10 % Rheomacrodex® (Pharmacia) had any beneficial effect on the course of the disease. Opacity of the cornea arose in all the surviving prednisolone-treated dogs. In a group of normal dogs, treatment with Macrodex and Rheomacrodex led to a rise of the glutamic-oxaloacetic transaminase levels within 24 hours but caused no rise of the ornithine carbamyl transferase levels.

ZUSAMMENFASSUNG

Behandlungsversuche an Hunden mit experimenteller H.c.c.

Mittels der Registrierung der Temperatur, Gesamtanzahl der Leukocyten, der Plasmaaktivität von GOT, GPT und OCT, wurde die Wirkung gewisser Medikamente auf die experimentelle H.c.c. untersucht. Die angewendeten Mittel wie das hyperimmune Serum Serasix (Pitman-Moore), 1 % Prednisolon (ACO), 6 % Macrodex® (Pharmacia) oder 10 % Rheomacrodex® (Pharmacia), hatten keinen Einfluss auf den Krankheitsverlauf ausgeübt. Bei allen mit Prednisolon behandelten Hunden die überlebten, wurde eine Trübung der Hornhaut beobachtet. Die Behandlung der gesunden Hunde mit Macrodex und Rheomacrodex, ergab binnen 24 Stunden eine Erhöhung der Plasma-GOT-Werte, hatte jedoch keinen Einfluss auf die OCT-Werte.

SAMMANFATTNING

Terapiförsök på hundar med experimentell Hepatitis contagiosa canis.

Genom att följa temperatur, totalantalet leukocyter, plasmaaktiviteterna av GOT, GPT och OCT har effekten av några medel prövats vid experimentell H.c.c. Inget av de använda medlen hyperimmuneserum Serasix (Pitman-Moore), 1 % prednisolon (ACO), 6 % Macro-dex® (Pharmacia) eller 10 % Rheomacrodex® (Pharmacia) påverkade sjukdomsutvecklingen i positiv riktning. Samtliga överlevande prednisolonbehandlade hundar erhöilo corneagrümling. Macro-dex- och Rheomacrodexbehandlingar av friska hundar gav inom 24 timmar en höjning av plasma GOT-värdena, men påverkade inte OCT värdena.

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