RESEARCH ON THE TEST OF HEPATIC FUNCTION WITH CHROMAZUROL S.

REPORT I. MEASUREMENTS OF CHROMAZUROL S IN DOG SERUM.

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Chromazurol S (CS) which belongs to the triphenylmethane acid dye group, has been used for staining tissue sections as a polychromatic dye. Hosokawa and Saito (15) studied elimination of the dye from the liver with the vital microscopic technique, and found that CS was more rapidily transferred to bile canaliculi in high concentrations than other dyes, such as azorubin S and rose bengal, without influencing on the organism. They also found that decreased and prolonged excretion of CS corresponded to liver cell damage. Such a dye may be suited to test the excretory function of the liver. However, measurements of dye concentrations and excretion time in bile are very difficult and inpracticable clinically. Therefore experiments were carried out on the disappearance of CS from blood of healthy and diseased animals following an intravenous injection of this dye. The results obtained with dogs will be reported in this paper.

MATERIALS AND METHODS.

a) Measurement of Chromazurol S concentrations in the blood.

Healthy dogs, under 1 year of age, were used throughout the experiments. All the animals were intravenously injected with a 5% aqueous solution of CS. Samples of venous blood were drawn at 5, 10, 30, 60, and 120 minutes after the injection of the dye. Two-tenths milliliter of blood plasma was diluted with 1.6 ml. of distilled water in a small test tube, to which 0.2 ml. of ferric chloride was added to make the total quantity 2.0 ml. Blue color which developed immediately was compared with the standard CS solutions.

b) Preparation of the standard.

One ml. of 5% CS was diluted with 1 litter of distilled water (5 mg/dl) and tubes of various concentraions were prepared as follows:

No. of tube	Conc. (mg/dl)	5 mg/dl solu of CS (ml)	dist. water (ml)	Plasma* before injection (ml)	$FeCL_3$ (ml)
1.	0.0	0.0	1.6	0.2	0.2
2.	2.5	0.1	1.5	0.2	0.2
3.	5.0	0.1	1.4	0.2	0.2
4.	7.5	0.3	1.3	0.2	0.2
5.	10.0	0.4	1.2	0.2	0.2
6.	12.5	0.5	1.1	0.2	0.2
7.	15.0	0.6	1.0	0.2	0.2
8.	17.5	0.7	0.9	0.2	0.2

* Plasma was separated from the basal blood obtained before injection of CS.

c) Certain techniques of practical importance.

1. The blue color gradually fades and therefore the determination of color must be done within 5 minutes.
2. As soon as ferric chloride was added, tubes must be shaken rapidly to avoid coagulation of protein.
3. Since concentrations less than 5 mg/dl cause some error in measurement, it is desirable to have concentrations higher than 5 mg/dl in the aliquot.
4. Three tubes, No. 3, 5, and 7, are sufficient for standards.
5. Drugs which will react with ferric chloride (such as salicylates) should not be given to the patient prior to the test.

d) Hepatic functional tests.

Carbon tetrachloride administration and CS retention test: Three dogs were intramuscularly injected with $CC1_4$, 0.5 ml. per kg. Chromazurol S was given at the dose of 10mg. per kg, after varying intervals from the time of administration. The results were compared with the data of other hepatic function tests done on another group of 3 control dogs.

Fructose tolerance: A 30% fructose solution was instravenously injected at a dose of 0.1 g. per kg., and 1.0 ml. of blood was drawn at 5 and 15 minutes. Each blood specimen was poured into 4.0 ml. of 10% trichloro-acetic acid and was filtered with filter-paper. To 3 ml. of the filtrate was added 1.5 ml. of 0.2% resorcinol solution (dissolved in 25%HCL). The same amount of resorcinol solution was similary added to the standard fructose tubes. The tubes were heated in a boiling water bath for 25 minutes and cooled. The color was immediately measured colorimetrically against standards. Fructose concentrations in blood at 5 minutes after injection was under 30 mg/dl and under 15 mg/dl after 15 minutes. If the total of the both concentrations was less than 45 mg/dl, it was regarded as normal.

Bromsulphalein test: Bromsulphalein was injected, 5 mg. per kg, and blood was drawn after 30 minutes from the contralateral jugular vein. If the retention of BSP in blood was less than 5% after 30 minutes, it was considered as normal.

Takata-Ara reaction: Plasma was mixed with the reagent and incubated at 20°C for 3 hours. If complete turbidity was formed, even one such tube was taken as positive. The degree of precipitation was closely correlated with liver cell damage.

Urobilinogen: Ehrlich's aldehyd test was used.

Cholinesterase activity test: Takahashi and Sibata's method (10) was employed.

RESULTS

a) Suitable dose of chromazurol S.

Five % CS solution was injected intravenously to normal dogs at the levels of 5, 10, and 20 mg. per kg. Samples of venous blood were drawn at 5, 10, and 30 minutes after injection, and CS concentrations were measured according to the procedure mentioned above. As will be seen in **Table 1** which tabulates such results, the retention time of CS in blood was comparatively short. Hence, a large

Table. 1. Chromazurol S. concentrations in blood.

C. S. dose. per kg.	C. S. concentration. mg/dl.				Dogs.
	after 5 min.	af. 10 min.	af. 15 min.	af. 30 min.	
5 mg.	3~5	1~2	0~1	0	2
10 mg.	7~8.5	4~5	1.5~5.5	0.5~3	5
20 mg.	13.0	9~12	9~11	6~9	2

C S. concentrations following injection of 10 mg. per. kg.

Dog. No.	weight. kg.	C. S. concentration in blood. mg/dl.				
		after 5 min.	af. 15 min.	af. 30 min.	af. 45 min.	af. 60 min.
1	8.8	7.0	4.0	1.5	0.5	—
2	5.8	8.5	4.0	1.0	0.5	0
3	7.0	8.5	4.0	2.5	0.5	0
4	17.0	8.0	5.5	3.0	—	1.5
5	12.0	2.5	1.5	0.5	0.5	0
average	10.1	7.8	3.8	1.7	0.5	0.3

quantity of CS seems to be suitable for the test.

The CS concentration under 2.5 mg/dl were not accurately measured by this method. Therefore, 10 mg/dl may be considered as a suitable dose.

b) The change of the chromazurol S concentrations in blood of dogs with damaged liver as a result of carbon tetrachloride administration.

Results of individual dogs were presented in **Figures 2 through 4.**

In dog No. 1 which was treated with CCl_4, CS retention was 15 mg/dl at 24 hours after the administration. This figure fell to 12 mg/dl at 3 days and returned

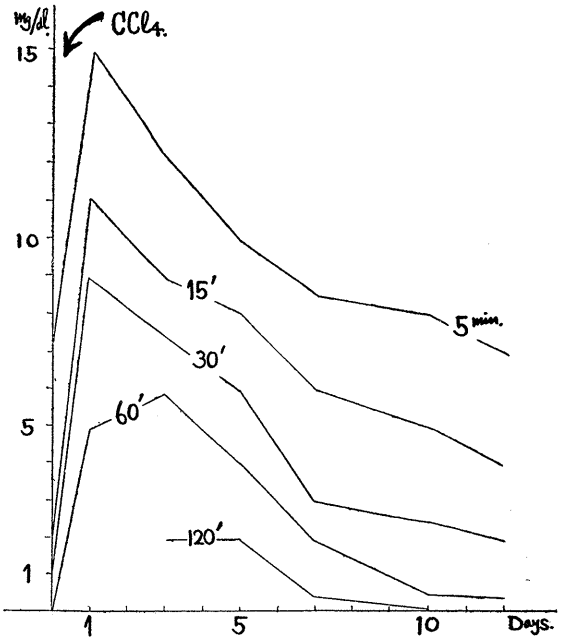

Fig. 2. C.S. concentrations in blood of dog treated with ccl_4. (Dog 1)

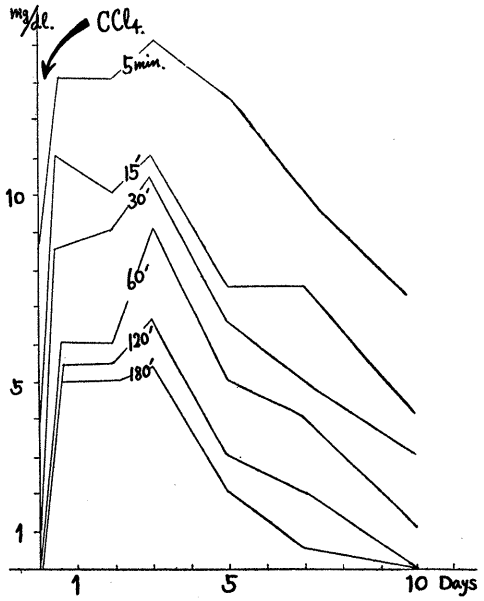

Fig. 3. C. S. concentrations in blood of dog treated with CCl_4. (Dog. 2)

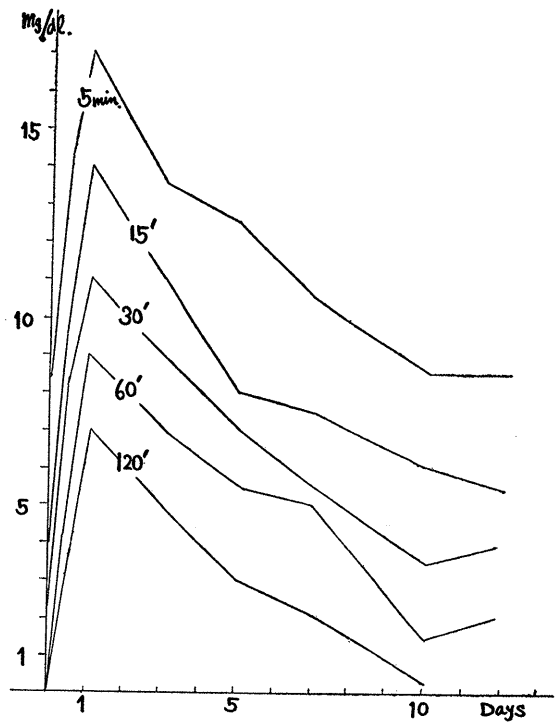

Fig. 4. CS. concentrations in blood of dog treated with CCl_4 (dog. 3)

to the normal range of 10 mg/dl 5 days after injection. Such figures were in close agreement with those assessed by other hepatic function test.

In dog No. 2, the retention was 13 mg/dl at 12 hours, reaching the greatest concentration at 3 days and recovering after the 7th days of injection. In dog No. 3, similar changes of concentration was obtained at 12 hours and the greatest retention was indicated at 24 hours. The concentration came back to the normal range at 7 days after CCl_4 injection. The average retention of CS was 7.8 mg/dl in the blood of dogs measured at 5 minutes after injection. Since the practical technical error is of the order of $\pm$ 2 mg/dl, the normal range of CS retention would be under 10 mg/dl at 5 minutes after CS injection.

c) Other liver function tests in CCl_4 poisoned dogs.

The results of various hepatic function tests are summarized in **Figs. 5, 6, & 7.** The fructose tolerance test showed very little impairment during the first 3 days. Takata-Ara reaction did not show marked turbidity during this period. An increase in turbidity was demonstrated after 3 days but a slight reduction occurred thereafter. Thus, a slight disturbance of albumin metabolism was indicated.

The BSP test indicated marked hepatic disturbances during 2 to 4 days. The lowest cholinesterase activity was found after 4 to 5 days, and recovery thereafter was

delayed. Urobilinogen in urine was positive from 2 to 4 days.

From these functional changes, it is presumed that the hepatic disturbance appears from the first day of CCl_4 administration and reaches the peak in 3 to 4 days, the disturbance disappearing gradually after then. The order of the appearance of functional changes is; fructose intolerance and BSP retention first, followed by impaired albumin metabolism and urobilinuria. Reduced cholinesterase activity follows somewhat later.

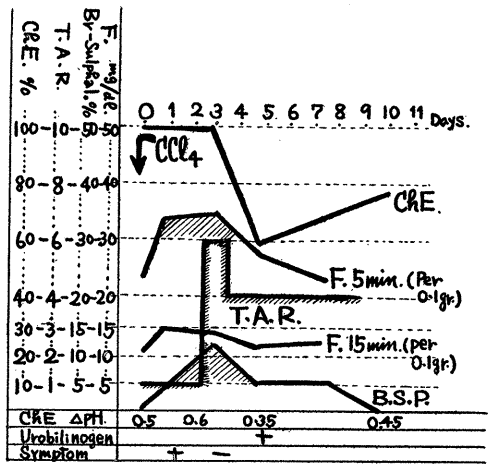

Fig. 5. Hepatic function tests in dog treated with CCl_4.

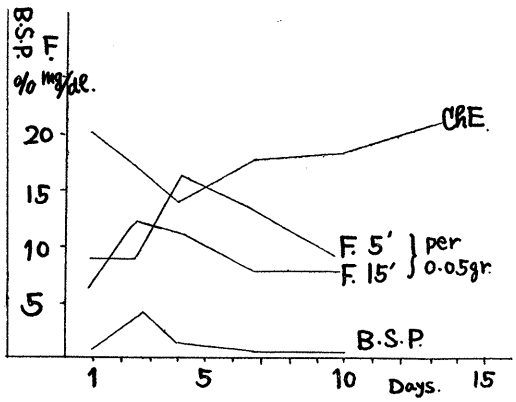

Fig. 6. Hepatic function tests in dog treated with CCl_4.

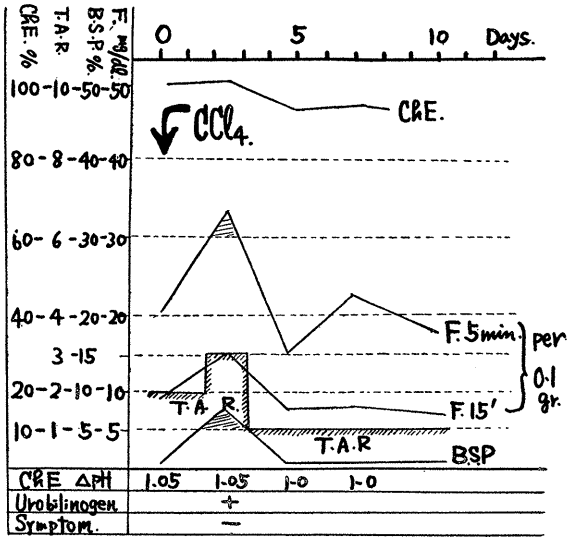

Fig 7. Hepatic function tests in dog treated with CCl_4.

DISCUSSION

The results of CS retention study agreed with other functional tests except that CS retention became demonstrable soon after CCl_4 injection. In other wards, this test is very sensitive and capable of detecting early hepatic dysfunctions. The normal average of its retention in blood was 3.8 mg/dl at 15 minutes of the load. Thereafter, values over 6 mg/dl may indicate hepatic disturbance. Every experimental dog showed marked changes at 12 to 24 hours and became normal after 7 days, with concomitant changes of CS retention measured at 5 minutes of load, The average of the retention in blood after 30 minutes was 1.7 mg/dl and might indicate a hepatic disturbance. The shift of the values with time elapsed after CCl_4 administration was very similar to that measured at 5 and 15 minutes. The mean concentration of CS in blood of normal dogs after 60 minutes wes 0.3 mg/dl therefore, the normal range was regarded as up to 2.5 mg/dl on the basis of the same experimental error of ± 2 mg/dl. Because, the CS concentration curve for 60 minutes was very similar to those for other times. The dye in the blood under normal conditions disappeared in 120 minutes of CS injection, whereas increased concentrations were noted in animals with damaged liver.

Chromazurol S retention in blood at 12 hours, one day, and 2 days, etc. after CCl_4 injection is shown in **Fig. 8.** The curves follow the pattern obtained in normal conditions. These findings seem to rationalize the conclusion that this CS retention test reflects hepatic disturbances precisely and sensitively. Measurement of the dye

in blood at 15 or 30 minutes after CS injection seems to be most suitable for that purpose.

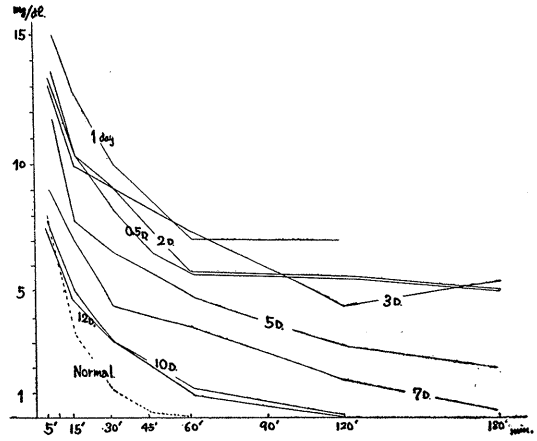

Fig. 8. Retention of the dye in blood of dog treaed with CCl_4.

SUMMARY

A new dye, chromazurol S (CS), was used in normal and CCl_4 injured dogs for evaluating the hepatic functions and the following conclusion was drawn:

The CS concentrations in blood measured at 15 to 30 minutes after an intravenous administration of CS at the level of 10 mg. per kg, reflected the hepatic functions more sensitively and accurately than other tests, such as fructose tolerance, Takata-Ara test, BSP test, cholinesterase activity, and urinary urobilinogen. This test has a great practical value in the evaluation of hepatic functions in the early stage of liver diseases.