

RESEARCH ON THE TEST OF HEPATIC FUNCTION WITH CHROMAZUROL S.

REPORT II. CHANGES OF LIVER FUNCTIONS IN CARBON TETRACHLORIDE DOSED GOATS.

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In the preceding report, chromazurol S (CS) was applied to dogs and its retention measured in the blood was found to reflect the liver functions. It was also pointed out that the measurement of CS retention in blood is a very sensitive and accurate test for determining the degree of liver damage. The value of this test has since been demonstrated in goats by comparing the CS retention and other liver function tests. Histological studies which will be reported in detail elsewhere were simultaneously conducted to confirm the correlation. Such results will constitute this second paper.

MATERIALS AND METHODS

a) Animals.

Five male goats, 2 to 3 months old and weighting about 8 kg, were used.

b) Administration of CCl_4 and the tests used.

Carbon tetrachloride was administered in the same way as report I. The following 12 tests were made to evaluate liver functions. Each test was done on 10 occasions, i. e., before injection, and 3, 6, 12, 24 hours, and 3, 5, 7, 10, 13 days after the injection of CCl_4 .

Fructose tolerance, BSP, Takata-Ara, and urinary urobilinogen tests were performed as described in the previous report. But BSP test, in some experiments, 5 minutes samples were obtained. If the retention of BSP in blood was less than 3% after 15 minutes, or 13% after 5 minutes, it was considered as normal.

A/G ratio: Saito and Yoshikawa's method (23) was used.

Sawada and So's mercuric chloride reaction: Mercuric chloride solution of 0.25% was added dropwise to 0.5 ml. of serum and stirred. The amount of mercuric chloride consumed to result continuous turbidity was measured.

Thymol turbidity test and Kunkel's zinc sulphate test: Since no remarkable changes were obtained throughout, the increase or decrease of the number of test-tubes with turbidity was regarded as indicating relative liver functions.

Cobalt reaction: This reaction was that developed by Inoue (24). For the same reason as above, the increase or decrease of the number of pseudo-positive tubes served as a reference.

Cadmium reaction: Two-tenths ml of 0.4% cadmium sulphate solution was added to 0.4 ml of serum and the tube was shaken. The cloudiness was grossly measured and divided into 4 grades of (++) , (+) , (\pm) , and (-) after 5 minutes.

Lugol's reaction: One drop of serum and one drop of Lugol's solution were mixed on a slide glass and appearance of sediment after 5 minutes was taken as positive.

Chromazurol S test: The normal values for CS retention in blood in dogs were less than 10 mg/dl at 5 minutes, less than 6 mg/dl at 15 minutes and less than 4 mg/dl at 30 minutes after injection of the dye. Greater retentions were considered as showing impaired liver functions.

RESULTS

a) Chromazurol S retention.

The retention of CS in the blood of normal goats was 11 mg/dl at 5 minutes, 7 mg/dl at 15 minutes, 3 mg/dl at 30 minutes and 0.5 mg/dl at 60 minutes after the injection of the dye as shown in **Fig. 1**. These figures are somewhat higher than those in normal dogs. The retention was in hepatic disturbance caused by CCl_4 in a similar rate as in dogs. In 6 hours after the toxin was administered, increased retention was already demonstrable and continued from 12 hours to 2 days, approaching normal levels after 3 days. A complete recovery was seen after 5 days. Thus, the curves of concentration were similar to those of dogs, the dye being more rapidly eliminated in goats. Comparison of the CS retention in the initial stage with various hepatic function tests revealed a good agreement with fructose tolerance, but CS retention being more remarkable and both showing recovery at the same time.

b) Other hepatic function tests.

The results of other tests obtained are shown in **Fig. 2-6**. Fructose tolerance test was not affected at 3 hours after the injection of CCl_4 , but demonstrable impairment was seen after 6 and 12 hours, reaching a maximum between 12 and 24 hours. A gradual improvement occurred after 3 and 5 days. Therefore, it was indicated that a slight metabolic disturbance in the handling of fructose appeared in the early stage of intoxication and it recovered soon.

The excretory function test as measured by BSP retention showed no change in

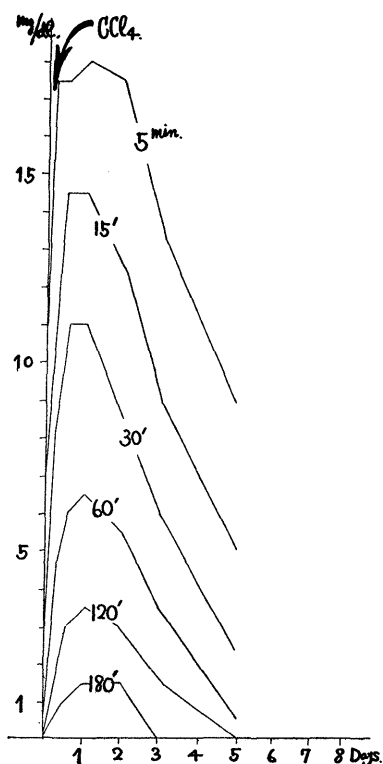


Fig. 1. C. S. concentration in the blood of goat treated with CCl_4 (♀. 43kg)

about one half of the animals. Even in the animals showing some abnormality, only a slight increase of the retention was recognized during a short period of time.

The tests on protein metabolism showed a dissociation of considerable degree among themselves from other tests. Namely, Takata-Ara reaction, mercuric chloride reaction and A/G ratio showed a remarkable change of hepatic function at 5 days, and their recovery was very slow. A marked decrease of A/G ratio was already noticed at 24 hours. The Takata-Ara test agreed well with the data of mercuric chloride reaction in terms of time and degree and therefore may be more significant for the evaluation of liver in the functions early stage.

Thymol turbidity test and Kunkel's zinc sulphate test did not show remarkable changes. The cadmium reaction indicated about the same reaction. The result of the cobalt reaction was equivocal. Lugol's reaction was negative, and the urinary urobilinogen was variable and unreliable.

Chromazurol S retention test showed an increase in blood from 6 hours to 5 days after CCl_4 injection, demonstrating its sensitiveness in an early stage.

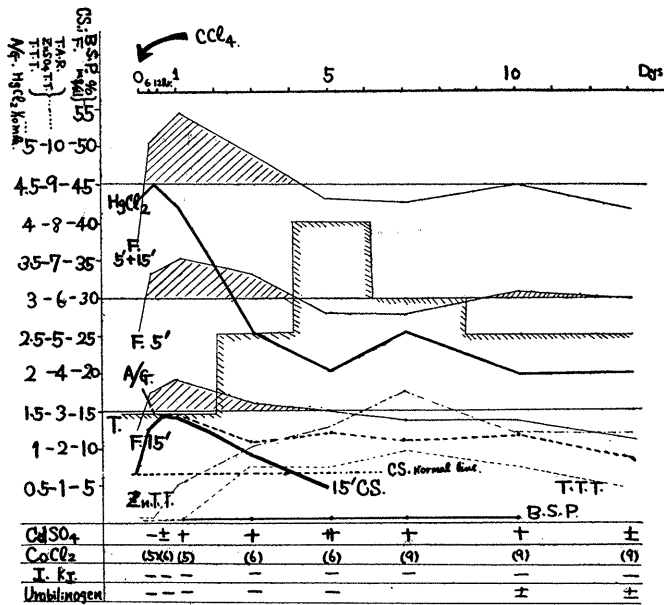


Fig. 2.

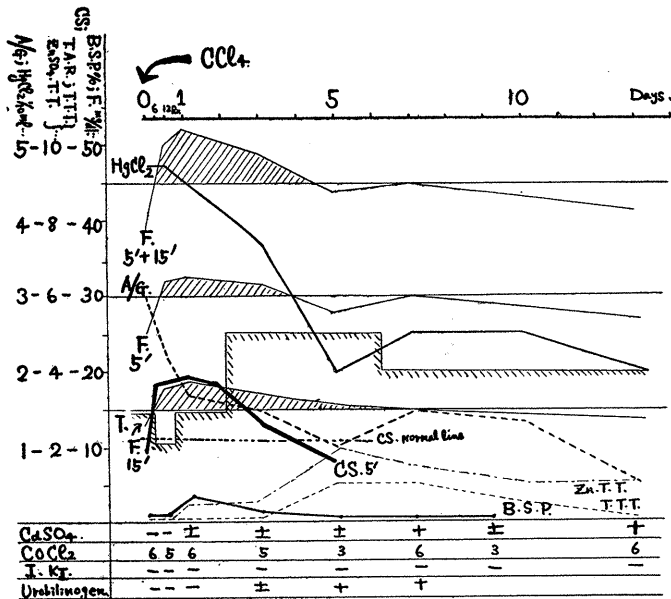


Fig. 3.

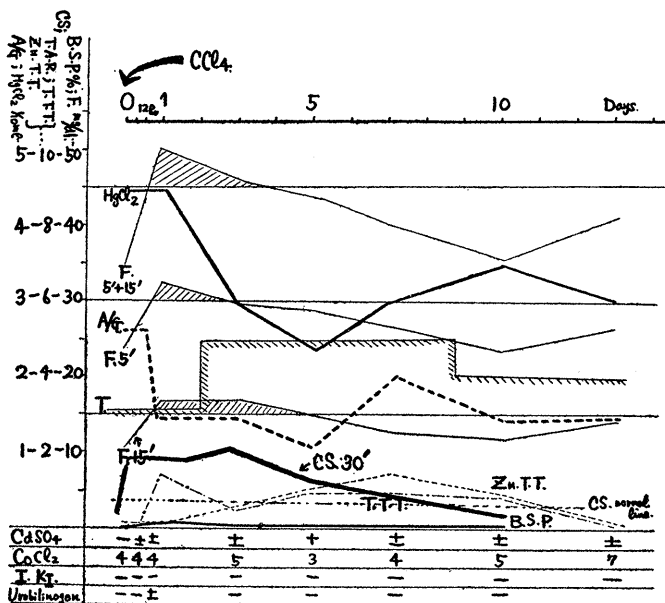


Fig. 4.

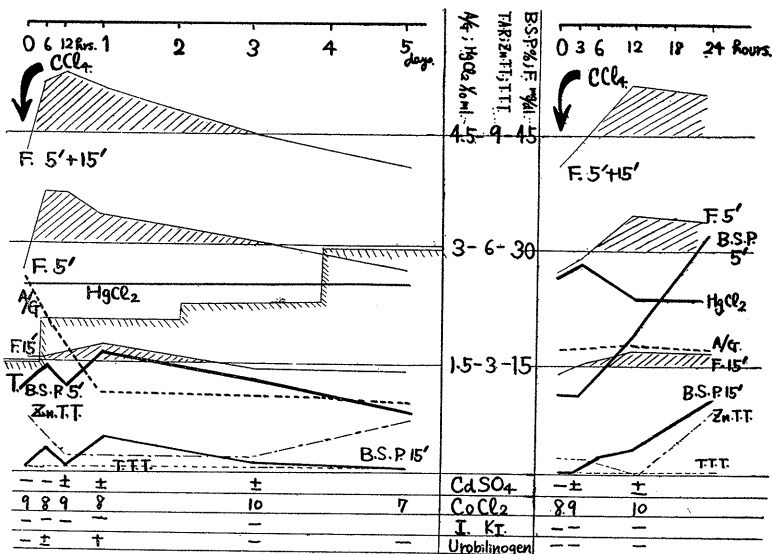


Fig. 5.

Fig. 6.

DISCUSSION

The change of CS retention appeared earlier following the administration of CCl_4 than various serum protein reactions generally used as liver function tests, and the times for recovery did not coincide. The excretion of BSP appeared to be faster in goats than in dogs, and showed a slight retention only between 12 to 24 hours of injection. In contrast, the CS test showed a markedly increased retention from 6 hours to 2 days, proving this method to be superior to BSP test.

In hepatic disturbances caused by CCl_4 , morphological degeneration was first seen in the peripheral zone of hepatic lobules as will be reported in the report to follow. Such degeneration reached the middle zone from 1 to 3 days and recovered after 5 days or so. These morphological findings corroborate the validity of this CS retention test as showing accurately the functional changes of the liver. However, the correlation between the morphological changes and the CS retention after 5 days was not significant.

These data may be interpreted as demonstrating that hepatic disturbance following an intermuscular injection of CCl_4 , 0.5 ml. per kg, to goats is not as marked as in dogs, and that its recovery is rapid. However, the protein metabolism seems to be so impaired as to require a considerable period of time for recovery.

In order to facilitate the evaluation of the hepatic functions in an early stage of liver damage, these tests would be graded in the following order in its reliability: CS retention test > fructose tolerance test > A/G ratio > Sawada's mercuric chloride reaction = cadmium reaction = Takata-Ara reaction > BSP test > Kunkel's reaction.

However, it has to be borne in mind that each test reflects certain aspects of metabolism and should not be interpreted on a same basis. Therefore, the data of these functional tests do not directly serve to conjecture the abnormal metabolism of glycogen, proteins and lipids in hepatic cells. Rather, the evaluation of the usefulness of these tests is based on the morphological changes of hepatic cells.

SUMMARY.

1. A slight hepatic disturbance occurred following an intramuscular injection of CCl_4 , 0.5 ml. per kg, in goats. The change of chromazurol S (CS) concentrations in the blood of goats loaded with 10 mg/dl CS per kg was the same as that in dogs, and reflected the degree of liver damage due to CCl_4 administration. However, disappearance of the dye from blood seemed to be rapid.
2. Using both CS and fructose tolerance tests, impairment of liver functions was demonstrated from 6 hours after the injection of CCl_4 , and recovery of the test results was seen after 5 days.
3. Protein metabolism as judged from various serum colloid reactions disclosed a dissociation among the tests results during 12 hours to 5 days after CCl_4 administra-

tion, and the recovery was very delayed. The sensitivity of these tests may be of the following order: CS retention test, >fructose tolerance test, >A/G ratio, >Sawada's mercuric chloride reaction, =cadmium reaction, =Takata-Ara reaction, >BSP, >Kunkel's zinc sulphate test, =thymol turbidity test, etc.

4. The BSP test showed a slight increase of retention during 12 to 24 hours in some animals and no changes in the other. Chromazurol S test is therefore superior to BSP test.

5. Urinary urobilinogen was variable and unreliable.

6. If these tests were analyzed on the basis of liver cell morphology, CS test was found to be correlated well with the degree of fatty metamorphosis. However, CS retention returned to normal levels earlier than the recovery of histological alterations.