CXRP IN RABBIT WITH ACUTE CCl₄ POISONING AND EXPERIMENTAL CHOLECYSTITIS

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It has been reported by the author¹,² that the C-reactive protein test becomes positive in patients with acute hepatitis and cholecystitis with or without gall stone. A positive CRP is closely related to the destruction and inflammatory changes in the liver as frequently seen in inflammation of other tissues³⁻⁵. Experimental work on the appearance or disappearance of this abnormal protein in the blood stream of animals is not possible using the regular CRP antiserum, since it is only positive in man and monkey.

In 1951, McCarty⁶ described an abnormal protein which appeared in the serum of the rabbit which reacts with a specific polysaccharide to make a precipitate, and he designated this as Cx polysaccharide.

The present paper deals with the alteration of this acute phase protein, CxRP*, in rabbits with acute CCl₄ poisoning and experimental cholecystitis. To rabbits with experimental cholecystitis, antibiotics were administered to observe the relation between the severity of the inflammation and the strength of the CxRP reaction. This work may afford experimental support to the clinical observations which the author previously reported.

Materials and Method

Male rabbits, weighing about 2 Kg., were used in this experiment. Acute CCl₄ poisoning was induced by a single intramuscular injection of 2.5 ml of 40% CCl₄ in olive oil per kilogram of body weight. Experimental cholecystitis was produced by ligating the cystic duct and inoculating a saline suspension of β-hemolytic streptococcus into the gall bladder. The sensitivity of the streptococci to chemotherapy was confirmed before the experiment. As a control, an operation was done in two rabbits simply opening and closing the peritoneal cavity.

Results

1. Acute CCl₄ poisoning:

As shown in Table 1, CxRP did not become positive for 24 hours. After 96 hours, in four out of five rabbits, CxRP became negative.

Table 1. CxRP in rabbits with acute CCl₄ poisoning

<table>
<thead>
<tr>
<th>Time</th>
<th>2 hrs.</th>
<th>6 hrs.</th>
<th>24 hrs.</th>
<th>48 hrs.</th>
<th>72 hrs.</th>
<th>96 hrs.</th>
<th>120 hrs.</th>
<th>168 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>4⁺</td>
<td>3⁺</td>
<td>1⁺</td>
<td>1⁺</td>
<td>−</td>
</tr>
<tr>
<td>No. 2</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>1⁺</td>
<td>1⁺</td>
<td>1⁺</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>No. 3</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>2⁺</td>
<td>1⁺</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>No. 4</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>1⁺</td>
<td>1⁺</td>
<td>1⁺</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>No. 5</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>1⁺</td>
<td>1⁺</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

* CxRP kindly supplied by Dr. M. McCarty Rockefeller Institute, New York City.
2. Experimental cholecystitis:

Laparotomy was done under anesthesia by sodium pentobarbiturate. After the ligation of the cystic duct, a small amount of saline suspension of hemolytic streptococci was injected into gall bladder. Prior to the inoculation, a small amount of bile was removed to avoid over distension of the gall bladder. In two rabbits out of five (No. 4 and 5), a mixture of 150,000 units of penicillin and 0.5 gm. of streptomycin was injected twice a day, beginning 18 hours after the inoculation and continuing through the 6th day. The data is shown in Table 2.

Table 2. CxRP in rabbits with experimental cholecystitis

<table>
<thead>
<tr>
<th>Time</th>
<th>before</th>
<th>24 hrs.</th>
<th>48 hrs.</th>
<th>72 hrs.</th>
<th>96 hrs.</th>
<th>144 hrs.</th>
<th>168 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1</td>
<td>—</td>
<td>5+</td>
<td>5+</td>
<td>5+</td>
<td>6+</td>
<td>over 6+</td>
<td>died</td>
</tr>
<tr>
<td>No. 2</td>
<td>—</td>
<td>died</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 3</td>
<td>—</td>
<td>over 6+</td>
<td>6+</td>
<td>4+</td>
<td>died</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 4*</td>
<td>—</td>
<td>4+</td>
<td>1+</td>
<td>1+</td>
<td>2+</td>
<td>3+</td>
<td></td>
</tr>
<tr>
<td>No. 5*</td>
<td>—</td>
<td>6+</td>
<td>6+</td>
<td>2+</td>
<td>2+</td>
<td>1+</td>
<td></td>
</tr>
</tbody>
</table>

* with administration of penicillin and streptomycin.

Discussion

Since the initial description of human C-reactive protein many of its properties have been defined, but virtually nothing has been determined concerning its function, site of origin, or method of disappearance from the body. One of the reasons for this has been the difficulty of experimental work using the usual CRP antiserum. In 1937, Abernethy7) was able to demonstrate the presence of a C-reactive protein in the sera of monkeys infected with pneumococci, but found that the acute phase sera of rabbits did not react with CRP. In subsequent work there were indications that rabbits formed a substance analogous to the C-reactive protein even though it could not be demonstrated by the precipitation reaction used with human sera. In 1951, McCarty6) confirmed the occurrence in the rabbits of an acute phase protein analogous to human C-reactive protein. He described the method of preparation and some of the properties of Cx polysaccharide. McCarty found the rabbit Cx-reactive protein was remarkably similar to human C-reactive protein in its general properties and in the conditions which govern its appearance in the blood. It is well known that carbon tetrachloride causes necrosis and fatty metamorphosis in the liver cells. Glynn and Himsworth5) demonstrated that a subcutaneous injection of 0.1 ml of CCl₄ causes cell damage in the liver of rat, 4 to 8 hours after the injection. Mylen6) found that subcutaneous injection of 0.002 ml of CCl₄ into mice resulted in diffuse cellular change in the liver as early as ten to sixty minutes.

In the present experiment, CxRP was negative two and six hours after the damage. It reached the maximal level 24 to 48 hours after the injection. This indicated that CxRP appeared only when severe necrosis occurred in the liver. After 96 hours, four out of five rabbits showed a negative reaction. One rabbit which showed still positive reaction after 96 hours, died on the next day. The finding of a positive reaction in
carbon tetrachloride poisoning continuing for a short duration agrees well with the clinical observation in patients with acute hepatitis in which a positive reaction is only seen in the very early stage of the disease.

McCarty demonstrated that in rabbits, as in man, the time of appearance of the acute phase protein in most instances is between 12 and 18 hours. Furthermore, he showed that the protein appears in response to three of the same stimuli that are known to produce CRP in human beings: namely, the hemolytic streptococcus, pneumococcus and typhoid vaccine. Following this observation, β-hemolytic streptococcus was used in this experiment of cholecystitis.

It was found that the laparotomy itself had no profound influence upon the CxRP reaction. In two rabbits with a sham operation, only once, 24 hours after the operation, the reaction became one plus. Thereafter the reaction was always negative. In rabbits without antibiotics administration CxRP continued to be strongly positive until death. The fact that all three rabbits without treatment died indicated that severe inflammation occurred. Administration of penicillin and streptomycin was found to be effective to reduce the positive reaction of CxRP, but the reaction had not become negative when our experiment was terminated at 6 days. This may indicate the cholecystitis persisted in these animals, especially true probably in No. 4. With the increasing reaction, one could expect that the infection was not being controlled by antibiotics. The data on the cholecystitis experiment indicates a close relation between the severity of the inflammation in the gall bladder and the intensity of the CxRP reaction.

Also, the data in this experiment shows the usefulness of CRP test in necrotic and inflammatory diseases of the liver or bile duct.

Summary

CxRP test, which is thought to be analogous to the CRP test in man, was determined in rabbits with acute carbon tetrachloride poisoning and cholecystitis caused by β-hemolytic streptococcus.

1. In acute carbon tetrachloride poisoning, CxRP became positive 24 hours after the onset of the damage. A positive reaction was seen only at the stage when the most severe necrosis probably occurred in the liver.

2. In cholecystitis, CxRP became strongly positive after 24 hours. A strongly positive reaction continued in rabbits without antibiotics administration until death, while in animals with treatment the reaction was found to be quickly reduced in No. 5. One could expect that the infection in No. 4 was not completely controlled by antibiotics. Thus CRP may be useful in following the effectiveness of antibiotic treatment in infection. The present data gives some experimental support to the usefulness of the CRP test in the differential diagnosis of patients with jaundice.

References


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(4)