

## STUDIES ON THE C<sub>x</sub>-REACTIVE PROTEIN

### I. THE EFFECT OF ADMINISTRATION OF C<sub>x</sub>-REACTIVE PROTEIN TO NORMAL RABBITS

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It has been demonstrated that rabbits produce an acute phase substance analogous to the human C-reactive protein (1). This rabbit protein, the properties of which are extremely similar to those of the human C-reactive protein, reacts with a special form of the somatic C-carbohydrate of the pneumococcus, the so called C<sub>x</sub>-carbohydrate. The human protein reacts equally well with either form of the carbohydrate, whereas the rabbit protein is precipitable in the presence of traces of calcium ion only by the C<sub>x</sub>-carbohydrate.

Many of the properties of the C<sub>x</sub>-reactive protein were defined at the time of its initial description by Anderson and McCarty (1). Like the human C-reactive protein, rabbit C<sub>x</sub>-reactive protein is not normally present in the blood but appears in response to stimuli of the same type which cause the appearance of the human protein; acute infections, the injection of triple typhoid vaccine, and the injection of many foreign substances, among them antigens such as the human C-reactive protein, and fractions of human gamma globulin (2). Rabbits respond to surgical injury and fracture by producing C<sub>x</sub>-reactive protein.

Earlier studies of the C-reactive protein and of the C<sub>x</sub>-reactive protein related to possible functions of these substances. In one of these studies it was shown that low concentrations of human C-reactive protein caused increased motility of normal human leukocytes (3). An extensive study of the relationship between the acute phase response and antibody production in the rabbit demonstrated a good correlation between early appearance of C<sub>x</sub>-reactive protein and the subsequent production of precipitating antibody to certain antigens (2). During the course of this study it was also found that the administration of adjuvant alone would cause the appearance of C<sub>x</sub>-reactive protein in large amount (4).

The present study stems from the observation that the C<sub>x</sub>-reactive protein when administered intravenously to rabbits causes them to produce the C<sub>x</sub>-reactive protein in amount larger than that injected, and from the observation that C<sub>x</sub>-reactive protein incorporated in adjuvant will, when administered intracutaneously, cause the development of extensive local inflammatory reac-

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tions in comparison with control animals given adjuvant containing normal rabbit serum albumin or adjuvant alone.

These inflammatory reactions were consistently obtained with three different preparations of Cx-reactive protein incorporated in sterile adjuvant. They were characterized by edema about the injection site and by the development of marked erythema over an area at least five times the diameter of the nodule in the skin caused by the injection of adjuvant. Suppuration was never seen. Further observations employing thorotrast suggested that the inflammatory reaction which occurred in response to the injection of Cx-reactive protein in adjuvant might be prevented by blockade of the reticulo-endothelial system.

#### *Materials and Methods*

*Preparation of the Cx-Reactive Protein.*—The Cx-reactive protein employed in these experiments was obtained from pooled sera obtained from many rabbits which were bled to death following dermal infection with pneumococcus Type I. Three separate preparations were made from three separate pools. Isolation and purification of the protein were carried out by the method described by Anderson and McCarty except that crystallization was not attempted. The preparations were tested for traces of normal rabbit serum protein with a guinea pig antiserum to normal rabbit serum, and the concentration of protein in the solutions was determined approximately by a spectrophotometric method.

*Quantitative Determination of the Cx-Reactive Protein.*—Sterile solutions of Cx-reactive protein in a solution 0.1 M in respect to both sodium chloride and sodium citrate which gave a capillary precipitin reaction against sheep antiserum to Cx-reactive protein of 4 mm. was used throughout the experiments. These solutions gave optical density readings of 0.400 to 0.410 at 280  $\mu$  in the Beckman D.U. spectrophotometer. This corresponded approximately to a protein concentration of 250 gamma/cc. when read on a calibration curve made with bovine Cohn fraction II.

*Preparation of Normal Rabbit Serum Albumin.*—Pooled normal rabbit serum was fractionated with ammonium sulfate. The protein fraction which precipitated at a concentration between 0.50 and 0.75 saturation with the salt was redissolved in a small volume of distilled water and dialyzed overnight against physiological saline. A solution containing 250 gamma/cc. was used for incorporation in adjuvant.

*Preparation of Sheep Antiserum to Cx-Reactive Protein.*—High titered precipitating antiserum to Cx-reactive protein was obtained from a sheep 4 weeks after the subcutaneous injection into six separate skin sites of purified Cx-reactive protein incorporated into an adjuvant consisting of an emulsion of two parts of antigen solution and two parts of heavy grade mineral oil to one part of the ointment base aquaphor. 0.5 mg. of dried, heat-killed Jamaica strain tubercle bacilli was incorporated in each 10 cc. of the adjuvant. 1 cc. of the adjuvant mixture was given in each subcutaneous injection.

*Precipitin Tests with the Antiserum.*—The precipitin tests with antiserum to Cx-reactive protein were carried out in capillary tubes according to the procedure described by Anderson and McCarty. This method is based on the capillary precipitin method for serologic typing of Group A streptococci described by Swift, Wilson, and Lancefield (5). One mm. of packed antigen-antibody precipitate was read as 1 plus. All readings were made with a millimeter rule. In each experiment all rabbits were bled every 4 hours for the first 36 hours, and every 12 hours thereafter.

*Preparation of Adjuvant Containing Cx-Reactive Protein and of Adjuvant Containing Normal Rabbit Serum Albumin.*—In all experiments employing the intracutaneous injection of

adjuvant containing either Cx-reactive protein or normal rabbit serum albumin the ointment base aquaphor was used in preparing the adjuvant. Two parts of a solution containing 250 gamma/cc. of Cx-reactive protein, or two parts of a solution containing the normal rabbit serum albumin, and two parts of heavy grade mineral oil were emulsified with one part of aquaphor. No heat-killed tubercle bacilli were added to these adjuvants. Control experiments other than the ones in which normal rabbit serum albumin was used, employed adjuvants which were made with physiological saline.

*Thorotrast.*—In one experiment thorotrast (24 to 26 per cent thorium dioxide) was given intravenously to three rabbits.

*Animals.*—Rabbits weighing between 2500 to 3500 gm. were employed in all experiments. A control bleeding was done on each animal to insure the absence of Cx-reactive protein at the beginning of each experiment.

TABLE I

*Capillary Precipitin Reaction Readings for Cx-Reactive Protein Obtained at Intervals Following the Intravenous Administration of Purified Cx-Reactive Protein*

Rabbits 1 through 4 received 1.2 mg. of one preparation of the protein. Rabbits 5 and 6 each received 0.64 mg. of another preparation.

Rabbit No...	1	2	3	4	5	6
Hrs.	Precipitin reactions: + = 1 mm. precipitin					
	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
0	—	—	—	—	—	—
2	++	+	++	++	+	++
5	—	—	—	—	—	—
24	++++	++++	+++++	+++++	++++	+++++
48	+++	+++	+++	+++	++	+++

## RESULTS

*Findings with Intravenously Administered Cx-Reactive Protein.*—Six rabbits were given Cx-reactive protein in single intravenous injections, four of them 1.2 mg. of one preparation, and the other two 0.64 mg. of a second preparation. In addition to taking hourly temperatures and determining hourly white blood cell counts for the first 8 hours following the injections, sera from the 1 hour, 5 hour, and from 24, and 48 hour bleedings were tested for Cx-reactive protein. No consistent effect of the injections on temperature or white blood cell count was noted. All six of the animals, however, responded to the injection of Cx-reactive protein by producing their own Cx-reactive protein. Table I shows the precipitin reactions obtained with the sera from these six rabbits at intervals. The calculated amount of circulating Cx-reactive protein at 24 hours in each rabbit was approximately 10 mg., many fold the amount injected intravenously 24 hours earlier. It is worthy of note that at 5 hours none of the injected Cx-reactive protein was any longer detectable in the serum.

*Findings with Cx-Reactive Protein in Adjuvant.*—Cx-reactive protein incorporated in adjuvant was injected intracutaneously into sixteen rabbits. Four control animals received normal rabbit serum albumin incorporated in adjuvant intracutaneously. The preparation of albumin had been subjected to the same fractionation procedure

used in obtaining Cx-reactive protein. Twelve rabbits were given adjuvant alone as a further control. All the animals given Cx-reactive protein in adjuvant developed extensive areas of erythema about the sites of injection within 16 hours following the injection. The three separate preparations of the protein were used in the experiments. At their height, these inflammatory areas ranged in diameter from 4.5 to 7.5 cm. The four rabbits given normal rabbit serum albumin in adjuvant and the twelve animals given plain adjuvant did not develop marked inflammatory reactions about the sites of the injections. In Table II the diameters of the cutaneous lesions obtained in eight rabbits given Cx-reactive protein in adjuvant are tabulated. These measurements were taken when the lesions were at their height. In all instances, the lesions began to develop at approximately 12 hours. They reached their maximum size and intensity of erythema and edema at approximately 16 hours. By 48 hours following injection the lesions had all decreased considerably in size and at 72 hours they were beginning to disappear.

TABLE II  
*Transverse Diameters of Inflammatory Lesions in Eight Rabbits 16 Hours Following the Intracutaneous Injection of Cx-Reactive Protein in Adjuvant*

Rabbit No.....	1	2	3	4	5	6	7	8
Diameters in cm.....	4.5 × 4.5	4.5 × 5.0	4.5 × 6.0	4.5 × 5.0	5.0 × 7.5	4.0 × 5.0	5.0 × 7.0	5.0 × 7.0

As in the instance of rabbits given Cx-reactive protein intravenously, rabbits given it in an adjuvant mixture responded with the appearance of Cx-reactive protein in the blood within 24 hours following injection. Since the intracutaneous injection of adjuvant without incorporated Cx-reactive protein can itself cause the appearance of Cx-reactive protein in the blood, the response of the rabbits cannot be attributed to the Cx-reactive protein alone but is certainly in part, at least, due to the effect of adjuvant.

*Prevention of the Inflammatory Response to Cx-Reactive Protein in Adjuvant by Thorotrast.*—Three rabbits which had responded to the injection of Cx-reactive protein in adjuvant by the development of typical inflammatory reactions, were injected over a period of days with 41 cc. of thorotrast intravenously. 72 hours after the final injection of thorotrast they were given 1 cc. of Cx-reactive protein in adjuvant intracutaneously. Marked differences in the response of these rabbits to the injection of the adjuvant mixture were noted following the thorotrast. No papular lesion occurred at the site of the injection and after 24 hours only a slight, faint erythematous halo was evident around the nodule which had been produced by the injection of adjuvant mixture.

#### DISCUSSION

The results of the experiments described in this study indicate that the Cx-reactive protein is a potent biological substance which, when administered

either intravenously, or intracutaneously in adjuvant mixture, causes animals of the same species from which the acute phase protein was derived, to respond by producing large amounts of the same acute phase substance. This appears to be a unique property of the Cx-reactive protein. When the purified substance is administered intracutaneously in adjuvant it causes the development of a characteristic surrounding area of inflammation which is not seen when concentrated rabbit serum albumin incorporated in adjuvant or when adjuvant alone is injected. Extensive studies of the cellular response in the inflammatory areas have not yet been carried out.

The findings in this study raise several questions of interest. There is as yet no evidence bearing on the fate of the intravenously injected Cx-reactive protein which elicited a Cx-reactive protein response in the recipient animals. Whether the injected protein was taken up by cells of the reticulo-endothelial system is not known. Further studies employing isotopically labelled Cx-reactive protein may elucidate this point. What direct role, if any, Cx-reactive protein may have in the development of inflammatory reactions caused by agents other than artificially administered Cx-reactive protein is still unknown. From work done with the human C-reactive protein it is evident that conditions in addition to acute inflammation may be characterized by the presence of C-reactive protein. This is seen in the instance of certain malignancies and some blood dyscrasias (6). It follows from this observation that the C-reactive protein and the Cx-reactive protein, although present in the blood in most acute inflammatory disorders, are not solely indicators of inflammation. It would appear that they represent the expression of activity of a widespread and generalized cellular system.

#### SUMMARY

It has been found that normal rabbits respond to the intravenous administration of Cx-reactive protein by producing this acute phase substance. When Cx-reactive protein incorporated in adjuvant is injected intracutaneously, in addition to producing Cx-reactive protein, the recipient animals develop a characteristic inflammatory reaction about the site of injection. This inflammatory reaction can be inhibited by the prior intravenous injection of thorotrast.

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