THE USE OF LECTINS TO MEASURE ACUTE PHASE PROTEINS IN THE SERUM OR PLASMA OF MAN AND ANIMALS DURING INFLAMMATION AND TISSUE BREAKDOWN

J. KOHN, J. WHICHER+, C. WARREN* and T. O'KELLY†

*Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH and Department of Clinical Pathology, Royal Marsden Hospital, Sutton, Surrey SM2 5PT, +Department of Chemical Pathology, Bristol Royal Infirmary, Bristol BS2 8HW, *Department of Chemical Pathology, Torbay Hospital, Torquay TQ2 7AA* and *†St. Luke's Hospital, Guildford, Surrey GU1 3NT, England*

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1. Introduction

Plant lectins have been extensively employed as probes to study the chemical nature of the glycoproteins of the cell surface of normal and malignant cells and for isolation and purification purposes [1,2]. The reactions with carbohydrate-containing polymers and serum glycoproteins have also been investigated and concanavalin A (con A), the Jack Bean lectin, which has a specific affinity for α-D-glucosyl residues and sterically related structures, has been found to bind and precipitate serum proteins including the acute phase reactants [3,4]. However, no attention has been paid to the value of lectin–serum glycoprotein interaction for the assessment of the biological response to physiological, pathological and 'pharmacological' agents.

Here we show that lectins and con A in particular can be employed as non-specific reagents in the assay of total serum glycoproteins. Almost all the acute phase proteins such as orosomucoid, haptoglobin, α_1-antitrypsin, α_1-antichymotrypsin and caeruloplasmin whose concentration in serum increases in response to inflammation, tissue damage and malignant tumours [5] are glycoproteins and bind in varying degree to con A and other lectins. Their measurement can be a useful aid in both the diagnosis and monitoring of certain inflammatory and neoplastic diseases, various glycoproteins being affected to a different degree in different conditions. We have studied the use of con A, *Ricinus communis* agglutinin (RCA) and *Abrus precatorius* lectin (ABL) as agents to measure acute phase proteins in man and animals during inflammation.

Address correspondence to: J. Kohn, Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH, England

2. Materials and methods

2.1. *Nephelometry*

The Hyland laser nephelometer PDQ system was used for the nephelometric assay of lectin binding. Con A reagent solution was made by dissolving 10 mg con A (Miles Labs., Slough) in 100 ml phosphate-buffered saline (pH 7.2) containing 30 g/l polyethylene glycol (mol. wt 6000). The parameters affecting this assay have been described [6]. ABL was used at 1.2 mg/100 ml and RCA at 2.0 mg/100 ml (both were a gift from Dr T. Forrester of the Chester Beatty Res. Inst., London).

2.2. *Laurell rocket electrophoresis*

The Laurell rocket technique [7] was used with a gel containing 0.1% (w/v) of lectin.

2.3. *Reference material*

A reference curve was prepared from a pooled human serum containing high levels of glycoproteins. Results were arbitrarily expressed as a % of this pool.

2.4. *The measurement of acute phase proteins*

All acute phase proteins were measured by the Laurell rocket technique [7] using antisera obtained from Seward Labs., Birmingham.

3. Results and discussion

We have measured the binding of con A, ABL and RCA to human serum glycoproteins by laser nephelometry and Laurell rockets, and have been able to show that this reaction can be used to quantitate changes in acute phase proteins (fig.1). As the method is based on the precipitation of protein by the reaction with the sugar residue and not with the species-specific protein part of the molecule the assay can be performed on any animal species. The dilution curves from human and animal sera both in normal and pathological conditions are parallel in spite of the presence of a mixture of glycoproteins varying in nature and amount (table 1).

We have found this assay to have similar value to individual protein measurements for monitoring disease in man. Cats, dogs and horses with inflammatory disease, and rats with experimentally-induced inflammation, show higher levels of con A binding than do normal animals (fig.2,3); this measurement has proved diagnostically useful in animals with non-specific signs of illness.

Table 1
Values obtained by assaying dilutions of sera from individuals with inflammation against a human serum pool for con A binding proteins

Serum	Con A binding as % of serum standard serum dilutions			
	Neat	1/2	1/4	1/8
Human A	87	81	82	86
Human B	61	59	58	60
Human C	61	62	64	72
Horse	–	109	100	96
Cat	78	79	80	80

Species-specific antisera to acute phase proteins are not widely available and protein analyses in animals have been rather neglected. Lectin-binding assays should therefore prove extremely valuable as a much

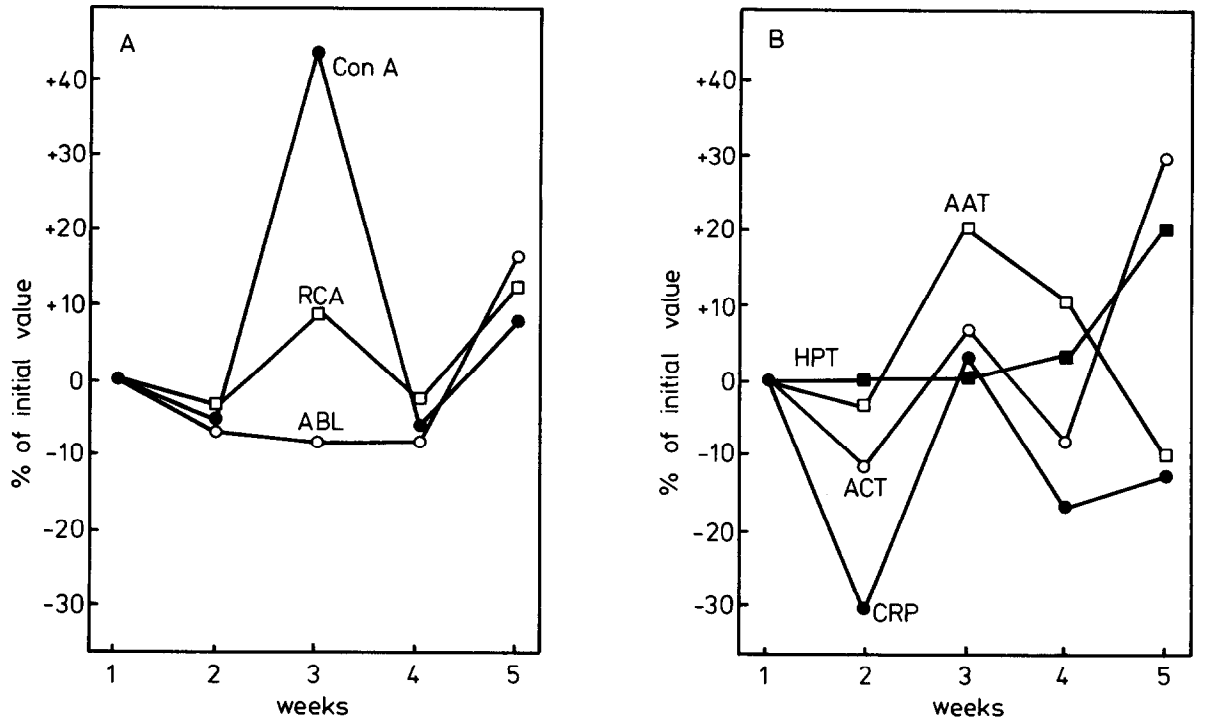

Fig.1. Sequential changes in lectin binding by and acute phase proteins in the serum of a patient with rheumatoid arthritis. (A) Lectin binding measured nephelometrically. (B) Acute phase proteins measured by rocket electrophoresis. AAT, α_1-antitrypsin; ACT, α_1-antichymotrypsin; CRP, c-reactive protein; HPT, haptoglobin. Con A binding integrates the changes in several acute phase proteins. Note that at 5 weeks con A binding has been suppressed by the falling level of AAT, a protein to which it binds strongly, whereas that of RCA has not. ABL reacts predominantly with HPT and hence shows a very similar pattern to that of this protein.

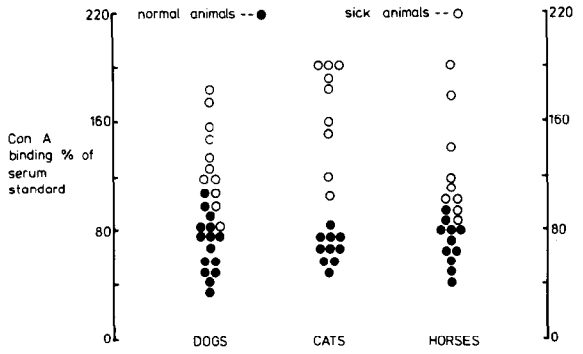

Fig.2. Con A binding in normal animals and animals with inflammatory disease. The inflammatory diseases were predominantly due to infection though some were immune complex or auto-immune in origin.

needed index of inflammation for evaluating the effect of both inflammatory and anti-inflammatory agents in the experimental animal. In veterinary practice we have found them to be of value in the diagnosis and management of inflammatory disease.

The lectin nephelometric and Laurell rocket assays are rapid, precise (CV 5%) and inexpensive. The nephelometric assays may also be automated. Unlike the erythrocyte sedimentation rate they are generally applicable; they may be performed on serum or plasma samples that have been frozen and require as little as 5 μl sample. In addition, con A has the advantage of being a single, relatively inexpensive, easily-characterised reagent of known specificity without the vagaries to which antiserum production is prone. The use of various lectins combining with different sugars may provide a means of identifying and measuring specific glycoproteins, and may also be applied following established separation methods, e.g., electrophoresis or chromatography. Using immunofixation techniques, certain characteristic patterns have already been observed in animals and it has been possible to demonstrate and identify the presence of carbohydrate moieties in a variety of biological materials.

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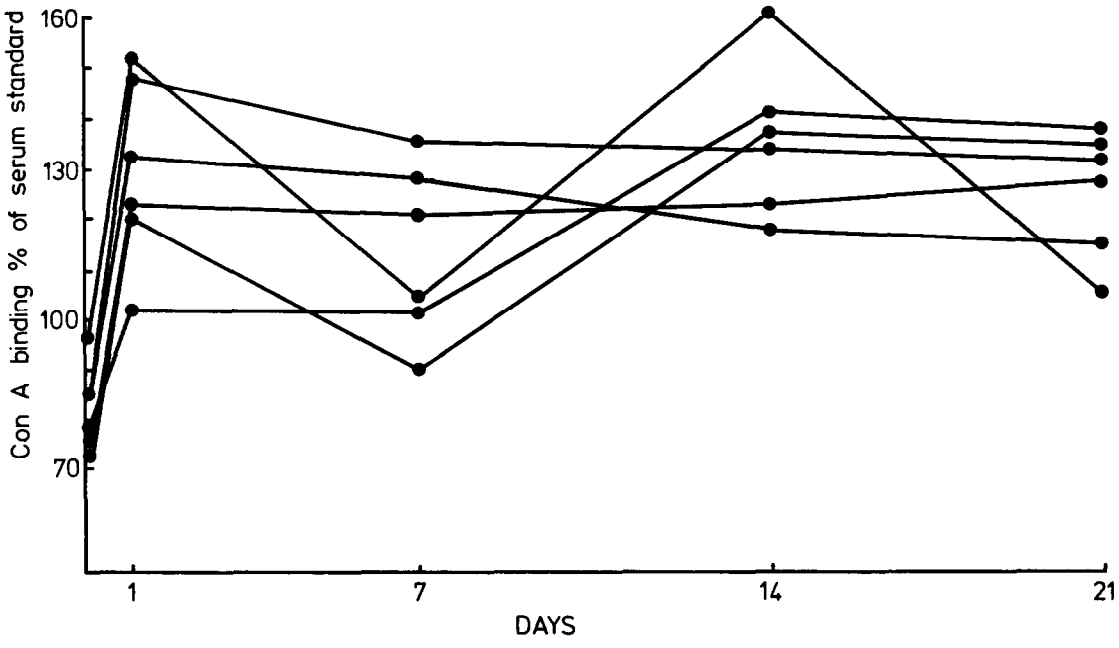

Fig.3. Sequential changes in con A binding in the plasma of 6 rats with adjuvant-induced arthritis. The initial sharp rise is associated with local inflammation at the site of injection of Freund's adjuvant. The increase at 14 days is associated with arthritis. Pre-treatment: $\bar{x}$, 80.5, SD 8.7; 1 day $\bar{x}$ 130, SD 18.2; 7 days $\bar{x}$ 113, SD 17.5; 14 days $\bar{x}$ 137, SD 15.1; 21 days $\bar{x}$ 126, SD 13.3.

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