Disappearance of congo red from the serum has long been considered by many investigators to be the best and most constant indicator of amyloidosis. The congo red test, as shown by Bennhold (1923), measures the proportion of this dye which is retained in the plasma one hour after its intravenous injection. The validity of this test has been the subject of intense discussion. Disappearance of 40-100 per cent. of the dye from the serum within one hour is generally regarded as a positive test, but according to Stemmerman and Auerbach (1944) and Taran and Eckstein (1942) the test should not be considered positive unless 90-100 per cent. disappears. In an experiment of Dixon, Ramcharan, and Ropes (1955), the congo red test tended towards positivity in 114 out of 227 patients with rheumatoid arthritis. In many cases of primary amyloidosis no affinity for congo red is found (Johansson and Pfeiffer, 1954), and Aegeert and Long (1949) demonstrated a positive congo red test in four out of five cases with systemic lupus erythematosus.

These observations lead to the suggestion that the disappearance of congo red from the serum is due not only to the presence of amyloid in the tissues, but also to other factors. One such possible factor that can be studied is the binding of congo red to the serum. Since congo red does not penetrate semipermeable membranes, equilibrium analysis could not be used in the present study. The method herein devised is based on a partition analysis system. Cellulose powder, which has a great affinity for congo red, was loaded with congo red. Upon shaking this congo red loaded cellulose powder with a buffered serum solution, congo red is taken up by the serum. The dye-binding capacity of serum from patients with rheumatoid arthritis and related diseases was compared to the serum content of glycoprotein and the results are reported below.

Material and Methods

A group of 83 sera with a positive streptococcal agglutination titration were kindly furnished by Statens Serum Institute, Copenhagen, where the streptococcal agglutination titrations (S.A.T.) were performed. The control group consisted of twenty sera with negative (S.A.T.), 37 sera from patients with manifest rheumatoid arthritis, and twenty normal sera collected by Dr. Jorgen Kryger. Other sera reported were from the University Hospital of Copenhagen.

Congo Red Suspension.—50g. cellulose powder (Schleicher and Schull, No. 123, from chromatography) are suspended in 200 ml. of a 0.5 per cent. congo red solution in water (Congo red: Grubler and Co.). After stirring for 2 hours, the suspension is filtered and washed with water until the filtrate is colourless. The washed red powder is then suspended in n/20 phosphate pH 7.4.

Procedure.—The congo red suspension is diluted 1:5 with water. From this diluted suspension 5 ml. are taken with a Krogh syringe during stirring, and to this 5 ml. are added 25 μl. serum with a micropipette. A dry serum solution is used as standard for every set of analyses. The test tubes with serum and congo red suspension are shaken together with a blank for one hour at room temperature. After centrifugation, the supernatant is read against the blank in a Beckmann B. spectrophotometer at 525 μm. The extinction of the blank read against water should not exceed 0.03. Having set the congo red affinity of normal sera at 100 per cent., the standard dry serum solution was found to be 85 per cent.

Serum Albumin.—This was determined by the micro method of Waddell (1956).

Determination of Serum Glycoproteins.—Periodic Acid-Schiff (P.A.S.) stainable serum proteins were determined by the method of Laurell and Skoog (1956). The intensity of the eluted fuchsinsulphite stain of 10 μl. serum spots on paper strips coloured by the P.A.S. staining procedure were read at 550 μm.
SERUM IN RHEUMATOID ARTHRITIS

TABLE I
MEAN CONGO RED AFFINITY FOR SERUM, SERUM CONTENT OF GLYCOPROTEIN, AND SERUM P.A.S. INTENSITY

<table>
<thead>
<tr>
<th>No. of Sera</th>
<th>Diagnosis</th>
<th>Congo Red Affinity (per cent.)</th>
<th>Protein-bound Hexose (mg. per cent.)</th>
<th>P.A.S. Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>83</td>
<td>S.A.T. Positive</td>
<td>83±1-1</td>
<td>184±4-2</td>
<td>195±6-7†</td>
</tr>
<tr>
<td>20</td>
<td>S.A.T. Negative</td>
<td>94±2-9</td>
<td>161±8-1</td>
<td>105±9-3†</td>
</tr>
<tr>
<td>35</td>
<td>Rheumatoid Arthritis</td>
<td>80±1-8</td>
<td>183±5-2</td>
<td>174±8-2</td>
</tr>
<tr>
<td>19</td>
<td>Controls</td>
<td>104±3-1</td>
<td>114±2-2</td>
<td>85±4-2</td>
</tr>
</tbody>
</table>

† 83 pairs of observations. † 20 pairs of observations.

CORRELATION COEFFICIENTS BETWEEN CONGO RED AFFINITY AND:

(a) Serum Glycoprotein (b) P.A.S. Intensity

<table>
<thead>
<tr>
<th>Group</th>
<th>r</th>
<th>Group</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.A.T. Positive</td>
<td>−0.376*</td>
<td>S.A.T. Positive</td>
<td>−0.407*</td>
</tr>
<tr>
<td>S.A.T. Negative</td>
<td>−0.019</td>
<td>S.A.T. Negative</td>
<td>−0.283</td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td>−0.213</td>
<td>Rheumatoid Arthritis</td>
<td>−0.109</td>
</tr>
<tr>
<td>Controls</td>
<td>−0.449</td>
<td>Controls</td>
<td>−0.244</td>
</tr>
</tbody>
</table>

* Statistically significant at the 0.05 level.

Total Protein-bound Hexose.—This was determined with anthrone. Using the method of Björnesjö (1955), an accuracy of 2 per cent. is obtained for each determination. The results are expressed in mg. protein-bound non-hexosamine hexose per 100 ml. serum.

Results

The mean values and corresponding standard errors of congo red affinity and the glycoproteins, determined both as P.A.S. stainable glycoprotein and as total protein-bound hexose, are summarized in Table I for the four groups of S.A.T. positive sera, S.A.T. negative sera, rheumatoid arthritis patients, and controls. It is demonstrated that the mean of the S.A.T. positive group is significantly lower than that of the S.A.T. negative group as regards congo red affinity, and significantly higher than that of the S.A.T. negative group as regards both serum glycoprotein and P.A.S. intensity. Comparison between the groups of sera from patients with rheumatoid arthritis and from the controls gives the same significance.

For the S.A.T. positive group there is a significant negative correlation between congo red affinity and the corresponding serum glycoprotein values (r = −0.376) and also between the congo red affinity values and the corresponding P.A.S. values (r = −0.407). For the other three groups the correlations were not significant; indeed, for the control group, between congo red affinity and serum glycoprotein there was a positive correlation but it did not attain significance level on such small numbers.

In Fig. 1 the correlation between congo red affinity and serum glycoprotein is shown graphically for eleven S.A.T. positive and eleven S.A.T. negative sera, selected at random from the two series for illustrative purposes. Where the values expressing the congo red affinity are progressing towards values of

Fig. 1.—Correlation between congo red affinity and serum glycoprotein content in eleven cases with S.A.T. negative sera and eleven cases with S.A.T. positive sera.
100 per cent., i.e. where they are tending towards the normal, the total of protein-bound hexose also tends towards the normal, 123 mg./100 ml. serum (Larsen, 1958), and the P.A.S. intensity shows the same fluctuations.

Determinations of congo red affinity were also carried out on sera from four patients with systemic lupus erythematosus, one with proven amyloidosis, and on nine sera from patients with diabetic nephropathy. Table II shows the decreased congo red affinities and the increased serum glycoprotein concentrations in these sera.

![Table II](image)

The result of dividing the sera into groups with increasing congo red affinity and determining the average glycoprotein content in these groups is demonstrated in Fig. 2 on the S.A.T. sera and in Fig. 3 (opposite) on sera from patients with proven rheumatoid arthritis. These Figures show an inverse ratio between congo red affinity and the serum content of glycoproteins.

The serum albumin concentration is plotted against the congo red affinity in Fig. 4 (opposite). Here some correlation is demonstrated between the albumin content and the degree of congo red uptake in the serum, but fluctuations in the congo red affinities are greater than the albumin variations.

### Discussion

The comparability of the congo red method in vitro with the original Bennhold method in vivo is favourable as regards the degree of dye retention in the serum. As shown by Sairanen, Koskiinen, and Holopainen (1955), normal subjects exhibit a disappearance of congo red of 33 per cent. and patients with rheumatoid arthritis of 48 per cent. The congo red affinity was lowered by 15 per cent. for patients with rheumatoid arthritis, and by the in vitro method this difference was of the same order of magnitude.

Among soluble proteins serum albumin is outstanding in forming reversible complexes with a variety of compounds of known structure, in particular with organic anions (Klotz and Urquhart, 1949). It was therefore thought that the low albumin content seen in the sera investigated could account for the lowered affinity of serum for congo red (Rawson, 1943). The variations in albumin concentrations showed a correlation with the congo red affinities.
Serum in Rheumatoid Arthritis

Serum in rheumatoid arthritis: the affinity for congo red. The control group comprises nineteen sera from healthy subjects.

![Graph showing congo red affinity](image)

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**Fig. 3.** Classification of 35 sera from patients with rheumatoid arthritis into four groups of increasing affinity to congo red as in Fig. 2.

**Fig. 4.** Comparison of congo red affinity for serum and serum albumin in groups of increasing congo red affinity, for 83 S.A.T. positive sera and twenty S.A.T. negative sera.

While the role of albumin in the binding of congo red cannot be excluded, other factors may also be operative. Organic ions are known to compete with different dye anions in their affinity for serum albumin; for instance, anionic dyes can compete with polysaccharide anions (Klotz, 1953). The same competitive effect may be demonstrated by the *in vitro* distribution method using congo red (Larsen, 1958).

It is therefore probable that the high content of glycoproteins in the serum may suppress the congo red affinity for the serum just as an increased glycoprotein concentration may reflect an increased concentration of polysaccharide anions.

**Summary**

A new method of determining the congo red affinity of serum has been devised, whereby the distribution of congo red between cellulose powder and serum is estimated.

The congo red affinity of sera from patients with rheumatoid arthritis, lupus erythematosus, diabetic nephropathy, and amyloidosis, was decreased. The serum glycoprotein content of the same sera was...
increased and the ratio between congo red affinity and serum glycoprotein content varied inversely.

It is suggested that the decreased congo red affinity for the sera investigated may be due not only to the lowered serum albumin content but also to the competition of congo red with polysaccharide anions.

The author wishes to thank Dr. K. Kalbak who gave him the impetus to start these studies.

REFERENCES

Relation entre l'affinité pour le rouge Congo et le contenu des glicoproteínas en el suero en la artritis reumatoide y las enfermedades afines

SUMARIO
Se inventó un nuevo método para determinar la afinidad para el rojo congo; este consiste en medir su distribución entre un polvo de celulosa y el suero.

La afinidad para el rojo congo de los sueros de enfermos con artritis reumatoide, lupus eritematoso, nefropatía diabética y amiloidosis se vio disminuida. En los mismos sueros las glicoproteínas se vieron aumentadas en razón inversa de la afinidad para el rojo congo.

Se sugiere que la afinidad disminuida para el rojo congo de los sueros estudiados se debe no sólo a una baja de la albumina sérica, sino también a la rivalidad entre el rojo congo y los aniones polisacáridos.
Relation between the Affinity for Congo Red and the Glycoprotein Content of Serum in Rheumatoid Arthritis and Related Diseases

Børge Larsen

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