SERUM DIPHENYLAMINE REACTION IN RHEUMATOID ARTHRITIS

BY

ELVIO CECCHI AND FABRIZIO FERRARIS

From the Rheumatic Centre, Rome. Director: Prof. T. Lucherini

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Normal human serum in contact with Dische’s diphenylamine reagent produces a reddish-purple colour; this is increased in such conditions as cancer, pulmonary tuberculosis, and rheumatic fever (Niazi and State, 1948). According to Coburn and others (1953) and Coburn and Haninger (1954), connective tissue is a rich source of the diphenylamine-reactive substance, the concentration of which in the blood stream as detected by the diphenylamine reaction increases principally during a sterile inflammatory reaction and appears to be conditioned by the intensity of the inflammatory process. In rheumatic fever, the diphenylamine reaction is much more intense in the acute phase, decreases with the lessening of the symptoms, and returns to normal with their subsidence; it parallels in most cases the erythrocyte sedimentation rate; it is inhibited by “antiphlogistic” drugs, such as salicylates, cortisone, and hydrocortisone (Ayala, Moore, and Hess, 1951). Therefore serial diphenylamine determinations are helpful both in estimating the degree of rheumatic activity and in evaluating the efficacy of therapeutic agents.

The substance in the serum which reacts with diphenylamine to produce the purple colour has not been certainly identified. It may be a mucoprotein, with a very low iso-electric point (less than 2), the reddish-purple colour being due to the carbohydrate of the mucoprotein found in the α-globulin fraction.

The present report includes a study of the diphenylamine values in thirty patients with rheumatoid arthritis, comparing the variations with changes in the clinical picture and erythrocyte sedimentation rate (Westergren measurements at one hour). The values in eight cases of ankylosing spondylitis, and one case of acute disseminated lupus erythematosus are also included.

Method

The technique used was the semimicro-procedure described by Ayala, Moore, and Hess (1951).

The readings were made with the Unicam spectrophotometer, model SP 350, with a wave length of 530 μm, using ½-in. test-tubes. The results are calculated in colorimetric units (log. I/I).

Our normal values differ from those of Ayala, Moore, and Hess (1951) who used a Coleman spectrophotometer, model 6A. We have therefore established our own “normal” values, using the serum of thirty individuals with no acute disease. The optical density obtained ranged from a maximum of 0·140 to a minimum of 0·120, with a mean of 0·134 and a maximum variation of 0·020 units. These figures were used as our basic values.

Results

The diphenylamine reaction was studied in thirty patients with rheumatoid arthritis, of whom eight were in the third and fourth stages,* and 22 in the first and second stages.*

In the first group (three men and five women between the ages of 25 and 65), colorimetric values within normal limits were constantly found, that is from 0·120 to 0·140 (mean 0·134); the erythrocyte sedimentation rate was also within normal limits (Table I).

![Table I](http://ard.bmj.com/annrheumdis/14/3/267)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>Erythrocyte Sedimentation Rate</th>
<th>Diphenylamine Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>m</td>
<td>56</td>
<td>8</td>
<td>0·125</td>
</tr>
<tr>
<td>m</td>
<td>40</td>
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</tr>
<tr>
<td>m</td>
<td>65</td>
<td>25</td>
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</tr>
<tr>
<td>f</td>
<td>32</td>
<td>36</td>
<td>0·137</td>
</tr>
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<td>f</td>
<td>30</td>
<td>10</td>
<td>0·140</td>
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<tr>
<td>f</td>
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<td>5</td>
<td>0·130</td>
</tr>
<tr>
<td>f</td>
<td>43</td>
<td>19</td>
<td>0·127</td>
</tr>
<tr>
<td>f</td>
<td>39</td>
<td>21</td>
<td>0·143</td>
</tr>
</tbody>
</table>

* Steinbrocker, Traeger, and Battermann (1949).
In the second group (fifteen males and seven females between the ages of 25 and 45), the course of the reaction was studied for a period of 30 days, determinations being done every 10 days (Fig. 1).

During this period the patients were under treatment with phenylbutazone by mouth, or hydrocortisone, or a combination of both. The dosage is set out in Table II, which also includes the values obtained at the beginning and end of the investigation.

At the beginning of our observations the colorimetric values of the diphenylamine reaction were consistently high in all patients (maximum 0·245, minimum 0·155, mean 0·190). On the 30th day the colorimetric values had fallen to normal in eighteen patients (above the continuous line in Table II; maximum 0·140, minimum 0·119, mean 0·135); the values remained above normal in four (below the continuous line in Table II; maximum 0·178, minimum 0·158, mean 0·170).

On the 30th day the erythrocyte sedimentation rate, which was high in all at the beginning of the testing period, had fallen to normal in twelve patients (above the dotted line in Table II, e.g. Figs 2a and b).

The course of the diphenylamine reaction was parallel to that of the clinical conditions. In the eighteen patients in whom the values returned to normal within 30 days, all presented a Grade I or II improvement, whereas the other four were Grade III and IV.

The erythrocyte sedimentation rate remained high in six of the eighteen patients, in whom the diphenylamine reaction had become normal (between the dotted and continuous lines in Table II, e.g. Fig. 2c).

The diphenylamine reaction returned to normal in all patients treated with hydrocortisone (e.g. Figs 2a, b, c), in all those treated with hydrocortisone and phenylbutazone, and in four of those treated with phenylbutazone only. The four patients in whom the diphenylamine reaction was...
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(a) Grade II, male aged 41.
(b) Grade I, female aged 29.
(c) Grade II, female aged 27.
(d) Grade II, male aged 27.

Fig. 2.—Diphenylamine reaction and erythrocyte sedimentation rate in four cases of rheumatoid arthritis (cf. Table II), showing sex, age, dosage, and grade of improvement.

higher than normal at the end of 30 days (below continuous line in Table II) had been treated with phenylbutazone alone (e.g. Fig. 2d).

In seven cases of ankylosing spondylitis (six males and one female, aged from 18 to 55), of whom two were in the relatively early stages (that is approximately 1 year from the onset of sacro-iliac and vertebral symptoms), moderately raised colorimetric values were found only in the two early cases. The values of 0.174 and 0.169 in these two patients (above the line in Table III) corresponded to a moderate rise in the sedimentation rate.

In one 18-year-old female patient with acute lupus erythematosus, continuous and very high levels were found (from 0.278 to 0.295) at monthly intervals; they did not correspond to the clinical picture, and were unaffected by the administration of therapeutic agents.

<table>
<thead>
<tr>
<th>Table III</th>
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<tr>
<td>SERIAL DIPHENYLAMINE REACTION AND E.S.R. DETERMINATIONS IN SEVEN PATIENTS WITH ANKYLOSING SPONDYLITIS</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>Before Treatment</th>
<th>After 30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Erythrocyte Sedimentation Rate</td>
<td>Diphenylamine Reaction</td>
</tr>
<tr>
<td>m</td>
<td>18</td>
<td>29</td>
<td>0.174</td>
</tr>
<tr>
<td>m</td>
<td>22</td>
<td>19</td>
<td>0.169</td>
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<td>38</td>
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<td>0.140</td>
</tr>
<tr>
<td>f</td>
<td>53</td>
<td>16</td>
<td>0.138</td>
</tr>
<tr>
<td>18-55</td>
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</tr>
</tbody>
</table>
Comment

This subject, which is still in its infancy but is full of scientific and practical interest in face of the specific immunological reactions caused by bacterial and virus infections, concerns the non-specific reactions not bound to anti-body production, which appear in the blood stream during the acute phase of infections. Reactions similar to the raising of the erythrocyte sedimentation rate occur even during non-infectious diseases and tend to persist in the blood as long as the disease remains active. They probably result from enzymatic adjustments which produce symptoms, anatomical changes, and finally recovery from the disease.

Among these acute-phase reactions, a particular significance seems to belong to the concentration in the blood of a diphenylamine-reactant substance; its exact chemical composition is not yet known, but it is probably a serum muco-protein. Recent studies of rheumatic fever have shown that the diphenylamine reaction is a valuable aid in determining the degree of activity of the disease and the efficacy of the drugs used in treating it.

Our research has shown that this reaction may also be used to test the course of rheumatoid arthritis; the diphenylamine reaction is constantly increased during the active phases of the disease and follows the clinical variations more faithfully and promptly than the erythrocyte sedimentation rate. Even though the absolute colorimetric value is not proportional to the gravity of the disease, it does show very clearly the degree of rheumatoid activity. It is less useful in diagnosis because it is also raised in other conditions, including rheumatic fever and disseminated lupus erythematosus, and is not sufficiently indicative in ankylosing spondylitis.

A most interesting feature was the rapidity with which the serum concentration of the diphenylamine-reactant substance fell with the use of hydrocortisone. This change was less marked with phenylbutazone.

Summary

The behaviour of the diphenylamine reaction is compared with that of the erythrocyte sedimentation rate in thirty cases of rheumatoid arthritis and seven of ankylosing spondylitis in various stages. The values found in the normal individual average 0·134 units with a maximum of 0·140 and a minimum of 0·120 (Unicam spectrophotometer, SP 350).

In active cases of rheumatoid arthritis (Stages I and II) these values were found to be constantly increased (mean 0·190), and tended to return to normal with improvement in the clinical picture; the reaction also seemed to be influenced by therapy, especially by hydrocortisone. As a measure of disease activity and of the efficacy of the therapy adopted, the reaction appears to be in general more sensitive than the erythrocyte sedimentation rate.

No significant alteration from normal was encountered in cases of rheumatoid arthritis in Stages III and IV or in ankylosing spondylitis.

The authors are greatly indebted to Dr. A. Mariani of the Superior Institute of Health in Rome for his aid with the biochemical section of this work.

REFERENCES


La réaction sérique de diphenylamine dans l'arthrite rhumatismale

RÉSUMÉ

On compare le comportement de la réaction de diphenylamine à celle de la sédimentation globulaire dans trente cas d'arthrite rhumatismale et sept cas de spondylarthrite ankylosante aux divers états de la maladie. Chez des sujets normaux ou trouvait le chiffre moyen de 0,134 unités, avec un maximum de 0,140 et un minimum de 0,120 (spectrophotomètre Unicam, Modèle SP 350). Dans les cas d'arthrite rhumatismale active (états I et II), ces chiffres augmentaient constamment moyennant 0,190 et tendaient à revenir à la normale avec l'amélioration du tableau clinique; cette réaction semblait également subir l'influence du traitement, surtout par l'hydrocortisone. En tant que mesure de l'activité morbide et de l'efficacité du traitement adopté, cette réaction paraît être, en général, plus sensible que la sédimentation globulaire.

On n'a pas trouvé de déviations significatives dans des cas d'arthrite rhumatismale aux états III et IV ou des cas de spondylarthrite ankylosante.

La reacción sérica de difenilamina en la artritis reumatoide

SUMARIO

Se compara el comportamiento de la reacción de difenilamina con la de la sedimentación eritrocitaria en treinta casos de artritis reumatoide y siete casos de espondilitis anquilosante en varios períodos de la enfermedad. En sujetos normales se encontraron valores medios de 0,134 unidades, con un máximo de 0,140 y un mínimo de 0,120 (espectrofotómetro Unicam, Modelo SP 350).

En los casos de artritis reumatoide activa (períodos I y II) estos valores aumentaban constantemente en promedio 0,190 y tendían a normalizarse con la mejoría del cuadro clínico; esta reacción parecía también estar bajo la influencia del tratamiento, particularmente de la hidrocortisona. Como medida de la actividad morbide y de la eficacia del tratamiento elegido, esta reacción parece, en general, más sensible que la sedimentación globular.

No se encontraron desviaciones significativas en los casos de artritis reumatoide en períodos III y IV ni en los de espondilitis anquilosante.