DIPHENYLAMINE REACTION IN RHEUMATOID ARTHRITIS

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From time to time new tests purporting to measure activity in rheumatic disease are described. Such a test is the colour intensity obtained when a protein-free precipitate of plasma or serum is treated with Dische's diphenylamine reagent under defined conditions (Ayala and others, 1951). The blood reactant is a mucoprotein or mucoprotein derivative, and the reaction has considerable theoretical interest. Coburn and others (1953), who found it to be a useful measure of activity in rheumatic fever, has reported interesting changes shown by this reaction in experimental arthritis (Coburn and Haninger, 1954).

For the practical purpose of measuring rheumatic activity in patients, however, any new test is acceptable only if it can be shown to be superior to the erythrocyte sedimentation rate which, for ease and simplicity, almost defies competition. We, therefore, decided to investigate the reaction in patients with rheumatoid arthritis and to compare it with the erythrocyte sedimentation rate (Westergren method). The objects of the investigation were as follows:

1. How does the diphenylamine reaction correlate with the E.S.R.?
2. What is the response in patients given steroid therapy?
3. Is the test of any value as a measure of activity in the small number of patients with clinically active rheumatoid arthritis and normal sedimentation rates?

Material

Controls.—32 subjects aged between 20 and 60 years, who had no evidence of disease.

Patients.—23 subjects with rheumatoid arthritis, aged between 20 and 70 years.

Method

The semimicro procedure of Ayala and others (1951) was used on heparinized plasma. Readings were made with a Unicam spectrophotometer at 530 μ. Results are given in optical density × 1,000. Duplicates gave an error of not greater than 2 per cent.

Results

Fig. 1 (opposite) shows readings obtained from 37 estimations on 32 healthy subjects, range 210-310 (mean 250; S.D. 26·6), and from 34 estimations on 23 patients with clinically active rheumatoid arthritis, range 230-420.

Fig. 2 (opposite) shows diphenylamine readings plotted against erythrocyte sedimentation rates of patients and controls (correlation coefficients were 0·66 and 0·60). It is thus apparent that diphenylamine levels are raised in patients with active rheumatoid arthritis, and that there is a reasonable correlation between the diphenylamine readings and the sedimentation rates.

Serial Observations.—Fig. 3 shows the results obtained in a male patient aged 26 who went into remission when given aspirin in a dosage of 100 gr. daily. It will be seen that a considerable fluctuation occurred in the diphenylamine level, but not in the E.S.R.

Fig. 4 shows the response of a female patient aged 31 with clinically very active rheumatoid arthritis, whose E.S.R. was barely raised and whose diphenylamine level was within normal limits when Acthar gel was started. It will be seen that both fell during therapy, which was attended by great clinical improvement.

Fig. 5 shows the response obtained in a male
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A patient aged 21 with Reiter's syndrome who was treated with Acthar gel. In this patient the E.S.R. and the plasma diphenylamine level fell in parallel. Study of the chart suggests that this patient's arthritis was probably going into remission before therapy was started.

Fig. 6 (female aged 65) and Fig. 7 (male aged 38) show the changes encountered in two further patients with rheumatoid arthritis treated with Acthar gel. In both, and especially in the latter, there were unexplained fluctuations in the diphenylamine level which were not paralleled by the E.S.R.

Fig. 6 shows that the E.S.R. and the diphenylamine level both rebounded sharply when the dosage of Acthar gel was reduced. Replacement by "Butazolidin" then appeared to cause a reduction in both levels, but this drug was not continued long enough to determine whether the downward trend would have been maintained.

Relation between Diphenylamine Level, E.S.R., and Clinical Activity

In 34 determinations on patients with clinically active rheumatoid arthritis, 27 were found to have a raised erythrocyte sedimentation rate (over 15 mm./hr, Westergren) and 25 of these also had a raised diphenylamine level (over 304—optical density $\times 1,000$); of the seven arthritics with a normal E.S.R., only two had a raised diphenylamine level.

Fig. 1.—Diphenylamine levels in (A) 32 normal subjects (37 readings) and (B) 23 arthritic subjects (34 readings).

Fig. 2.—Correlation between erythrocyte sedimentation rate and P.D.T. in normal subjects and arthritics.
Comment.—In something like 10 per cent. of patients with rheumatoid arthritis who show clinical evidence of activity, the erythrocyte sedimentation rate lies within the generally accepted range of normality. This is not taken to mean normality for a given individual, as it is obvious that a Westergren E.S.R. reading of 14 mm./hr would represent an elevation for a patient whose E.S.R. was normally 2 mm./hr. However, in the majority of instances, the "normal" reading for a given individual who has rheumatoid arthritis cannot be known. It seemed possible that the diphenylamine test might be a more sensitive indication of rheumatic activity than the E.S.R., and, hence, of value in the study of patients who fall into the above group. This has not been so in the small number of cases studied by us.

The fluctuations of the diphenylamine level in some patients under steroid therapy were not reflected by similar fluctuations of the E.S.R. This seems to be a further disadvantage of the test considered as a practical measure, but is of some theoretical interest. We have no explanation for these fluctuations, but further investigations might be rewarding.

Fig. 3.—Results in a male patient aged 26.

Fig. 4.—Results in a female patient aged 31.
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In conclusion, we would emphasize that any test of rheumatic activity must be shown to have some advantage over the E.S.R. before it can be recommended as a practical measure.

Summary

(1) The diphenylamine reaction has been investigated as a measure of activity in rheumatoid arthritis and has been compared with the erythrocyte sedimentation rate with which it correlates, but over which it seems to have no particular advantage.

(2) The diphenylamine level fell in patients undergoing steroid therapy, but in some patients there were unexplained fluctuations, not reflected by the erythrocyte sedimentation rate.

(3) In patients with clinically active rheumatoid arthritis but with normal erythrocyte sedimentation rates, the diphenylamine reaction was not of much value as an indication of activity.

REFERENCES
La réaction de diphenylamine dans l'arthrite rhumatismale

RESUMÉ

(1) On étudia la réaction de diphenylamine comme mesure d'activité de l'arthrite rhumatismale et on la compara à la vitesse de sédimentation globulaire à laquelle elle est liée, sans lui être supérieure d'une manière quelconque.

(2) Le taux de diphenylamine chez des malades soumis à la thérapie stéroïde baissait, mais chez certains d'entre eux il y avait des fluctuations inexplicables qui ne correspondaient pas à la vitesse de sédimentation globulaire.

(3) Chez des malades atteints d'arthrite rhumatismale cliniquement active avec une sédimentation normale, la réaction de diphenylamine comme indicatrice d'activité était peu utile.

La reacción de difenilamina en la artritis reumatoide

SUMARIO

(1) Se estudió la reacción de difenilamina como medida de actividad de la artritis reumatoide y se la comparó a la velocidad de sedimentación eritrocitaria a la cual esta reacción está ligada, sin superarla de manera alguna.

(2) La cifra de difenilamina bajó en enfermos sometidos a la terapia esteroide pero en algunos hubo fluctuaciones inexplicables que no correspondieron a la velocidad de sedimentación eritrocitaria.

(3) En enfermos con artritis reumatoide activa y con velocidad de sedimentación eritrocitaria normal, la reacción de difenilamina fue de poca utilidad para indicar la actividad.
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Fig. 7.—Results in a male patient aged 38.
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