INTRA-CUTANEOUS CONGO RED IN RHEUMATOID ARTHRITIS

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It has been claimed that, in patients with amyloidosis, the presence of amyloid material in the skin may be demonstrated by the intracutaneous injection of Congo red (Philpott and Freshman, 1936; Nomland, 1936). In the presence of amyloidosis, the area of staining is said to be more intense and to persist for a longer time than in normal skin.

Recently this technique has been employed by Laine, Holopainen, and Mäkinen (1956) in a series of patients with rheumatoid arthritis. The fixation of the dye in the skin was measured by the time taken for the stained area to fade to a standard intensity and this was found to be significantly longer in 55 patients with rheumatoid arthritis than in thirty normal control subjects. From these results, Laine and his co-workers concluded that this phenomenon might be due to the presence of amyloid in the skin of patients with rheumatoid arthritis. This seemed quite possible since, with the decline of tuberculosis, rheumatoid arthritis is one of the chief causes of secondary amyloid. At the Postgraduate School of Medicine, London, amyloid was found in eight out of 48 autopsied cases (Missen and Taylor, 1956).

Using a modified method of assessment, we have confirmed Laine's results, but our experience leads us to differ somewhat from Laine and his colleagues in their tentative conclusions cited above.

Methods

An autoclaved solution of 1 per cent. Congo red in normal saline was used, since the aqueous solution used by Laine and others was found to produce some pain on injection. This solution may be autoclaved repeatedly without change in its staining properties and no discomfort or untoward reactions occurred after its injection. A tuberculin syringe was used and exactly 0.1 ml. was injected into the skin of the flexor surface of the forearm of each subject.

During the first 24 hours, the pink stain spread to an area of between 1 and 2 cm. diameter, slightly larger in children than in adults, and usually, contrary to expectation, less intense in adults. After 24 hours there was no further spread and the stain gradually faded. It was always more marked at the periphery and the fading was complete by about 9-12 days in the "control" cases, but lasted for a longer period—between 17 and 20 days—in the cases with rheumatoid arthritis. The initial intensity of the staining and the rate at which it subsequently faded varied from individual to individual. The intensity of the staining at any stage was estimated by matching with a series of arbitrary standards consisting of strips of filter paper (Whatman No. 1) which had been soaked for about 2 minutes in an aqueous solution of Congo red, and then dried and kept in the dark. In these test specimens no fading occurred within 7 weeks. Eight solutions were used of the following dilutions: 1.0, 0.5, 0.25, 0.125, 0.062, 0.031, 0.016, and 0.008 per cent. For convenience, these standards were numbered 1 to 8, the last numeral corresponding to the weakest solution of Congo red (0.008 per cent.). All readings were made in daylight and recorded in terms of these standard colour units.

Interpretation

It was found that, when readings were made in this manner, different observers might match the same stained area with different standards, especially as the staining became less intense. In order to assess the size of this observer variation, some preliminary trials were carried out. Two groups of ten patients each (five with Still's disease and five convalescent from rheumatic fever) were injected: two observers made "blind" independent daily readings (*i.e.* without reference to preceding readings) for 5 to 7 days. In the first group (read by one medical and one lay observer), 30 per cent. of the ninety readings differed by one colour unit and about 3 per cent. differed by two units. In the second group (read by the same medical observer and another), the readings also differed by one unit in

30 per cent. of cases, but differences of more than one unit did not occur. One observer tended to read consistently higher than the other.

To minimize this slight observer error, to compensate for the differing initial intensity of staining in different subjects, and because readings made during the first week were subject to less variation than those made later, it was decided to utilize the difference between the reading at 24 hours after injection and another reading of the same spot by the same observer 5 to 7 days later. The difference between these two readings, divided by the time in days which elapsed between them, gives an estimate of the amount of fading which has taken place each day. This "fading rate" varied from 0 to 1.6 units daily in the 120 subjects tested. A rate of 0 indicates no fading over the week, and one of 1.6 indicates rapid fading in 5 to 6 days. When this rate was calculated for the two preliminary trial groups, the same rate was given in five of the ten duplicate readings in the first group, the mean rates for each observer being 0.32 colour units/day and 0.42 colour units/day respectively. In the second group, the same rates were given in eight of the ten duplicate readings, the means being 0.60 and 0.53 units respectively. No rates differed by more than 0.17 units, and over the whole group of twenty subjects the means of the two duplicate sets of readings were 0.46 and 0.47 colour units/day respectively.

Obviously the method does not lend itself to precise estimation in single cases, but it was considered accurate enough when means of groups are considered.

Results

The fading rate was estimated on 120 subjects. These comprised 32 cases of rheumatoid arthritis (adult and juvenile) and 88 cases regarded as controls, which included forty cases of rheumatic fever (mostly convalescent) or chorea, and 21 cases of pulmonary tuberculosis. When the mean intensity of the staining at each day after the injection is plotted for the rheumatoid and the control group (Fig. 1), it is seen that the rheumatoid group

fade more slowly and that the rate of fading in both groups is almost constant over the period observed.

Fig. 1.—Mean intensity of staining of rheumatoid group (upper line) and control group (lower line). Numbers in brackets indicate number of patients observed each day.

When this difference is expressed in terms of the mean fading rate, the 88 controls faded at 0.67 units daily and the 32 rheumatoid patients at 0.30 daily.

There was considerable scatter of fading rates in both groups (Table I), but the difference between them is significant (approximately seven times the standard error of 0.05). Because of this scatter, an attempt was made to correlate the fading rate with certain other clinical and pathological data.

TABLE I
DISTRIBUTION OF FADING RATES IN EACH GROUP

Group	Fading Rate			
	0.0-0.17	0.33-0.50	0.66-0.83	1.0+
CONTROL (88 subjects) ..	1	35	39	13
RHEUMATOID (32 subjects) ..	15	13	3	1

(If these figures are converted to a fourfold table by combining the two outer with the corresponding inner columns, then $\chi^2 = 18.4$ and $P = < 0.001$.)

Table II shows a progressive increase in the fading

TABLE II
EFFECT OF AGE ON MEAN FADING RATE

Group	Age (yrs)			Total
	0-9.9	10-15.9	16+	
RHEUMATOID (32 subjects) ..	0.17 9 subjects average age 7.05	0.32 14 subjects average age 12.1	0.42 9 subjects average age 38	0.30 ± S D 0.24
CONTROLS (88 subjects) ..	0.47 6 subjects average age 7.5	0.65 30 subjects average age 13	0.69 52 subjects average age 38	0.67 ± S D 0.24

rate with increasing age in both groups, the distinction between the rheumatoid and control groups being preserved in each age bracket. This is significant on a χ^2 test ($P=0.05-0.02$) for the control group, but the numbers are insufficient in the rheumatoid group to allow examination in this way.

A less definite correlation but one of some interest was found in cases of rheumatic fever excluding chorea; seven of 26 such cases were considered to have active disease, both clinically and on the basis of the Westergren sedimentation rate. The nineteen inactive cases had a mean fading rate of 0.72, while the seven active cases had a mean rate of 0.47 (the difference is just over twice the standard error of 0.12). The ages of the two groups were comparable.

No correlation with duration, activity, or severity of the disease process could be found either in the patients with rheumatoid arthritis or in those with pulmonary tuberculosis. The presence or absence of specific skin rashes in the rheumatoid and rheumatic fever group showed no correlation, nor did the presence of an abnormal electrophoretic serum protein pattern or a positive Rose-Waaler test. Two patients with clinical and proven amyloidosis as a complication of rheumatoid arthritis showed a fading rate no slower than many other rheumatoid arthritis patients in whom this complication was not present.

In ten patients (five rheumatoid and five control), an injection of 1 per cent. Evans blue was made into one forearm, at the same time as the Congo red was injected in the other (Fig. 2, overleaf). The rate of fading of the Evans blue (as measured by similar staining standards) was almost identical in the two groups, *i.e.* rheumatoids 0.22 and controls 0.25 colour units daily. The rate of fading of the Congo red showed a difference: rheumatoids 0.20 and controls 0.55 colour units daily.

Discussion

These results confirm the report of Laine and others (1956) that the skin of patients with rheumatoid arthritis shows a more prolonged fixation of injected Congo red than the skin of normal controls. However, it was felt that several facts made it unlikely that this phenomenon was due to deposits of amyloid as Laine and his co-workers suggested.

Those patients who had had extensive involvement for a long time and who could reasonably be expected to be more likely to have developed amyloid, showed no greater fixation than those in whom the disease was relatively mild and early. Furthermore, the two cases of proven amyloidosis

tested did not show any more prolonged fixation than other cases of rheumatoid arthritis of the same age. The increased fixation shown by cases of active rheumatic fever and the demonstrable difference in the three age groups of the control series also suggest that the explanation lies not in amyloid deposition in the skin but rather in a change in the nature or metabolism of one of the normal skin constituents.

Equally, the similarity of fading rates shown by rheumatoid and control patients when Evans blue was injected, and the difference in the same patients with Congo red, make it unlikely that the explanation lies in a difference in peripheral circulation.

Dixon, Ramcharan, and Ropes (1955) have reported an increased rate of loss of Congo red from the plasma in adult rheumatoid arthritis patients compared with normal subjects. Thus normal subjects retained in the plasma 70 per cent. of the amount injected after one hour, but the patients with amyloid retained 50 per cent. or less. 114 out of 227 rheumatoid arthritis patients gave values between 50 and 70 per cent. It was possible, they thought, that this represented a subclinical level of amyloidosis or an analogous alteration of the polysaccharide and protein constituents of connective tissue, since amyloid was found in 24 per cent. of eighty autopsied rheumatoid patients and some decreased Congo red plasma retention has also been found in other patients without clinical evidence of amyloidosis but with diseases which could be associated with that complication. Against this interpretation, however, was the failure to find any evidence at all of amyloidosis at autopsy in two patients with Congo red retention of 50 per cent. or less after one hour within 2 weeks of death.

It appears possible that this low plasma retention might be explained by the appearance of a Congo red-avid material in the tissues of rheumatoid subjects. Another abnormality affecting the skin has been described in rheumatoid arthritis and in the acute stages of rheumatic fever (Bywaters, Holborow, and Keech, 1951), and this may be a related phenomenon. A prolonged reconstitution of the substrate for hyaluronidase was found. In young normal subjects, this substrate reformed more quickly than in the more elderly. If these two phenomena relate to similar substances, one might postulate that in rheumatoid arthritis there is an increased amount in the skin and that degradation is very slow. In young normal subjects there is more present than in the old, but the turnover rate is quicker.

These data confirm the widespread involvement of connective tissue in rheumatoid arthritis and rheumatic fever, and suggest that further attention

Fig. 2.—Congo red test at the first and eighth day in a case with convalescent rheumatic fever (left) and in a case with Still's disease (right).

should be paid to the apparently unaffected tissues in these diseases.

Summary

(1) The vital staining of the skin after intracutaneous injection with Congo red has been studied in 120 subjects.

(2) There is an increased and prolonged fixation of this dye in the skin of patients with rheumatoid arthritis and active rheumatic fever compared with convalescent rheumatic fever patients and others.

(3) There is a well-marked change with age, younger subjects showing a slower fading rate.

(4) This phenomenon is thought to be due to a change in the nature or metabolism of the connective tissue constituents rather than to altered vascularity or to the deposition of amyloid.

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Rouge Congo intradermique dans l'arthrite rhumatismale

RÉSUMÉ

(1) La coloration vitale de la peau après injection intradermique de Rouge Congo fut étudiée chez 120 sujets.

(2) Il se produit une fixation prolongée et augmentée de ce colorant dans la peau des malades atteints d'arthrite rhumatismale et de rhumatisme articulaire aigu, en comparaison avec les convalescents de rhumatisme articulaire aigu et autres.

(3) Il existe une différence très nette selon l'âge, les sujets plus jeunes se décolorant plus lentement.

(4) On pense que ce phénomène est dû à un changement dans la nature ou le métabolisme des constituants du tissu conjonctif plutôt qu'à une altération de la vascularité ou à un dépôt amyloïde.

El rojo Congo intradérmico en la artritis reumatoide

SUMARIO

(1) La coloración vital de la piel después de inyecciones intradérmicas de rojo congo fué estudiada en 120 sujetos.

(2) Se produce una fijación prolongada y aumentada de este tinte en la piel de enfermos con artritis reumatoide y con reumatismo poliarticular agudo, en comparación con los convalecientes de reumatismo poliarticular agudo y otros.

(3) Existe una diferencia muy marcada según la edad, produciéndose la descoloración más lentamente en sujetos más jóvenes.

(4) Se cree que este fenómeno se debe más bien a un cambio en la naturaleza o en el metabolismo de los constituyentes del tejido conjuntivo que a una alteración de la vascularidad o a un depósito amiloide.