GREEN COLOUR IN RHEUMATOID SERUM

BY

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The existence of green coloration in the human body is well recognized in chloroma, a rare type of leukaemia with greenish tumour material. Green colour has been studied in the sputum (Robertson, 1962) and its presence noted in the serum of patients with rheumatoid arthritis and those suffering from myeloid leukaemia (Bodman, 1960). The green colour of chloroma and sputum was found to be due to verdoperoxidase (Schultz and Schwartz, 1956; Robertson, 1962), but the nature of green colour in patients suffering from rheumatoid arthritis remains unknown. The subject has been studied in this department by Swinburne, Losowsky, and Hall (1963), who found that a large percentage of patients with rheumatoid arthritis demonstrated the colour in the serum, but were unable to characterize it further. We have endeavoured to assess the incidence of green serum in patients with and without rheumatoid arthritis and to discover any correlation between it and the clinical, radiological, and serological features of rheumatoid patients. We have also examined the effects of therapy and have investigated the cause of the green colour.

Material and Methods

We examined 104 patients with definite rheumatoid arthritis by the criteria of the American Rheumatism Association (Ropes, Bennett, Cobb, Jacox, and Jessar, 1959) and 150 patients without rheumatoid arthritis. Blood was taken and the serum separated and graded for colour:

- (1) Definite green;
- (2) Green-green-yellow;
- (3) Yellow-yellow-green;
- (4) Yellow.

The serum was then stored in the deep freeze.

A complete clinical examination of the patients with rheumatoid arthritis was performed and the functional status assessed in five grades—from normal (1) to completely crippled (5).

The extent of the disease was graded by the number of joints involved in the upper and lower limbs, taking no account of small joint involvement:

Mild = one or two large joints,
Moderate = three or four large joints,
Severe = more than four large joints.

Joint involvement was taken to mean either deformity or the presence of two of the following: swelling, tenderness, and limitation of movement.

A special note was made of the therapy which the patient was currently receiving and had received in the past.

Radiographs of the hands and feet were taken of all patients and classified as follows:

- (1) Severe
Four or more major erosions,
or deformity and at least two major erosions,
or bony ankylosis.
- (2) Moderate
Two or three major erosions,
or one major erosion and deformity.
- (3) Mild
One major erosion,
or unequivocal minor erosions.
- (4) Doubtful
Juxta-articular rarefaction only.

The haemoglobin level, erythrocyte sedimentation rate (ESR) and sheep cell agglutination titre (SCAT) were estimated in all cases.

Spectrographic curves were obtained for the sera on a Unicam S.P. 800 machine with light passing through a cell of 1 cm. thickness.

The simple physical properties of the colour were determined by exposing the serum to sunlight and ultra-violet light, and by dilution with normal saline. To observe which protein fraction contained the green colour the serum was analysed by electrophoresis and separated on Sephadex and D.A.E.A. cellulose columns. Protein digestion was performed by incubation of the serum with pronase in a water bath at 37° C. for 3 hours. Verdoperoxidase was estimated by the method of Schultz and Schwartz (1956).

Results

The sera were ranked by the authors according to their colour on two separate occasions without knowledge of the patient's name. On a third occasion they were ranked independently by a third observer (Dr. Brian Payne). It was found that the ranking for definite green and definite yellow was fully reproducible, but it was impossible to separate yellow-yellow-green from green-green-yellow with any certainty and these are henceforward referred to collectively as "the intermediate group". The intermediate group was not always clearly distinguished from the definite yellow group, and for clinical correlations the definite green group has therefore been contrasted with the rest of the rheumatoid population.

Table I shows the incidence of green serum. No example of definite green was found in the 150 patients without rheumatoid arthritis, who included patients with osteo-arthritis and patients whose sera had been sent to the Department of Chemical Pathology for tests connected with other diseases. Green serum occurred more frequently in women, being present in 14 per cent. of women with rheumatoid arthritis compared with 5 per cent. of men.

TABLE I
INCIDENCE OF GREEN SERUM

Diagnosis	No. of Cases	Green Pigmentation			
		Definite		Intermediate	
		No.	Per cent.	No.	Per cent.
Rheumatoid Arthritis	104	13	12.5	11	10.5
No Arthritis	150	0	—	3	2.0

Clinical Correlations

Green serum did not occur more frequently in patients with more extensive arthritis clinically or radiographically (Tables II and III), but it was found more frequently in those with arthritis of longer duration. 18 per cent. of those with arthritis of more than 10 years' duration had green serum compared with 7 per cent. of the remainder. The lower the functional status, the more often was green serum observed; it was twice as common in severely disabled patients (Grades 4 and 5) as in those with mild disability (Grades 1 and 2). It was found less frequently in patients with nodules.

To check that the inclusion of "the intermediate group" with the "definite yellow" group was not producing bias, the results were re-calculated with the intermediate group excluded. The comparisons were essentially the same: green serum was present in 22 per cent. of those with arthritis of more than 10 years duration compared with 9 per cent. of the remainder. Similarly 18 per cent. of those with severe disability had green serum, compared with 8 per cent. of those with mild disability.

Green serum was found with equal frequency (13 per cent.) in patients who had and had not received phenylbutazone. It was found in a similar number of those treated with anti-malarial preparations (10 per cent.) and those not so treated (13 per cent.), and in those who had received corticosteroids (17 per cent.) and those who had not (15 per cent.). The incidence was also similar (13 per cent.) in those who had had chrysotherapy.

Green serum was commoner in patients with anaemia than in those with a haemoglobin of over 85 per cent. and in patients with an SCAT reciprocal titre of 128 or more (Table IV, opposite).

TABLE II
INCIDENCE OF GREEN SERUM RELATED TO CLINICAL FEATURES OF RHEUMATOID ARTHRITIS

Green	Age at Onset (yrs)*					Duration (yrs)†					Function (grade)‡					Extent of Disease†			Nodules‡	
	-29	-39	-49	-59	60+	<1	1-5	5-10	10-20	20+	1	2	3	4	5	Mild	Moderate	Severe	Present	Absent
Absent	15	11	32	22	10	8	22	19	23	17	7	15	39	25	3	8	10	71	24	64
Present	2	2	6	1	2	1	3	—	6	3	—	2	6	5	—	1	1	11	1	11

*In one patient not recorded.

†In two patients not recorded.

‡In four patients not recorded (one had green serum).

TABLE III
INCIDENCE OF GREEN SERUM RELATED TO RADIOGRAPHICAL SEVERITY OF RHEUMATOID ARTHRITIS

Green	Hands*					Feet†				
	Normal	Doubtful	Mild	Moderate	Severe	Normal	Doubtful	Mild	Moderate	Severe
Absent	11	10	8	11	48	13	10	7	17	37
Present	2	1	1	5	3	2	—	1	5	3

*Four films not available for grading.

†Nine films not available for grading.

TABLE IV
INCIDENCE OF GREEN SERUM IN RHEUMATOID ARTHRITIS RELATED TO HAEMOGLOBIN LEVELS AND SCAT

Green	Haemoglobin (per cent.)*			SCAT†			
	<70	71-84	85	0-16	32-64	128-256	512+
Absent	12	28	43	20	27	17	15
Present	3	6	4	1	1	3	1

*In eight patients not recorded.

†In nineteen patients not recorded.

Physical and Chemical Properties

The colour was photosensitive. Exposure to sunlight resulted in decolorization of the serum in 2 to 3 hours, and it was decolorized by ultraviolet light in 30 minutes.

Dilution of potassium dichromate was found to give a green appearance to the previous yellow solution. Dilution experiments were therefore performed on yellow serum and on pure bilirubin, but in none was a green colour produced.

Separation of the serum protein by electrophoresis and on Sephadex and D.A.E.A. cellulose columns demonstrated that the green colour travelled with the albumin fraction of the serum proteins. Digestion with pronase did not destroy the colour. After digestion, heating of the serum produced no coagulation in contrast with the control experiments, demonstrating that digestion was complete. No free veridoperoxidase could be demonstrated in any green or yellow sera.

Spectrographical Studies

The absorption curve of green serum on spectrographical examination was similar to that of yellow serum (Fig. 1). This was confirmed by testing on a more sensitive machine (Bush and Lumb, Spectronic 505), by courtesy of Dr. I. D. Rattee, professor of colour chemistry. Decolorization of the serum

by exposure to light did not alter the absorption spectrum obtained. Fig. 1 shows the absorption curves of two specimens of yellow sera in patients with rheumatoid arthritis and osteo-arthritis respectively compared with green serum. The form of the curves were similar, but the area under the curve in the green serum was less than that under the yellow curve.

To analyse this further, absorption at 430 m μ was measured in green and yellow sera, since it is in the region of 410-460 m μ that most substances producing a yellow colour occur (*e.g.* bilirubin and carotene). Absorption at 520 m μ was also measured, since this is the region in which green colour absorption occurs. The results are shown in Fig. 2, and in Fig. 3, overleaf.

Fig. 2.—Absorption at 430 m μ in green and yellow sera.

Fig. 1.—Absorption curves of green and yellow sera.

Fig. 3.—Absorption at 520 mμ in green and yellow sera.

There was little difference. Results were then plotted as a ratio of the absorption at 430 and 520 mμ (Fig. 4). It was found that the green sera showed a lower ratio than yellow sera and that the intermediate group came in between. It is concluded that the appearance of green is not due to a specifically-occurring green pigment, but to a relative absence of yellow compared with green in the serum.

Fig. 4.—Absorption ratio 430/520 mμ in green and yellow sera.

Discussion

The finding of green serum in patients with rheumatoid arthritis previously reported from this department (Swinburne and others, 1963) has been confirmed. A definite green colour was present in 12.5 per cent. of the rheumatoid population, being more common in women than men. It was difficult to grade the sera intermediate between green and yellow with certainty and these were included with the yellow group in the clinical correlations. Separate analysis after exclusion of this group revealed no significant difference in the results.

The colour was photosensitive in common with many bile pigments, and travelled with the albumin fraction of the serum proteins. This suggests that

it was not an iron compound which would be more likely to have gone with the globulin. The possibility was raised by the more frequent occurrence of green pigmentation in the sera of patients with anaemia. Green coloration is also found in the body in chloroma, myeloid leukaemia, and infected sputum. In chloroma and in sputum the green colour appears to be due to free verdoperoxidase, but this did seem to be so in the sera of patients with rheumatoid arthritis. It did not appear to be a dilution effect, such as that which produces the green appearance of potassium dichromate when it is diluted. The green colour appeared to be due to a relative absence of yellow compared with green, rather than a specifically-occurring green pigment. In preliminary experiments we have found a decreased amount of carotene in these patients (Shah and Wright, unpublished data) and this required further investigation. The green serum was not related to therapy. It was of interest that it was found to be more common in long-standing cases of arthritis, in patients whose functional status was worse, and in patients with a higher titre of rheumatoid factor in the blood.

Summary

The sera of 104 patients with definite rheumatoid arthritis and of 150 patients without rheumatoid disease have been examined for green pigmentation. Definite green serum was present in 12.5 per cent. of the rheumatoid group only, and was present more often in women (14 per cent.) than men (5 per cent.). It was not related to the clinical or radiographical extent of the arthritis, the presence of subcutaneous nodules, or to previous treatment with phenylbutazone, antimalarial drugs, corticosteroids, or gold. It was commoner in patients who had had arthritis for more than 10 years, whose function was worse, who were anaemic, and who had a higher rheumatoid factor titre in the serum.

The green colour was photosensitive. It was not produced by dilution of yellow sera or of pure bilirubin, and did not appear to be due to verdoperoxidase, the cause of green coloration in chloroma and sputum. It travelled with the albumin, but was not destroyed by digestion with pronase. The absorption curve was the same in form as that of yellow serum, although the area under the green curve was less. Measurement of the absorption ratio at 430 and 520 mμ suggested that the green coloration was due to a relative deficiency of yellow pigment.

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La couleur verte dans le sérum rhumatoïde

RÉSUMÉ

On rechercha la pigmentation verte dans les sérums de 104 patients atteints de polyarthrite rhumatoïde et de 150 patients sans atteinte rhumatoïde. Le sérum vert n'était présent que dans le groupe des polyarthritiques en proportion de 12, 5 pour cent et on le trouvait plus souvent chez des femmes (14 pour cent d'entre elles) que chez des hommes (5 pour cent d'entre eux). On ne trouva aucun rapport entre cette pigmentation et l'intensité de l'atteinte clinique et radiologique de l'arthrite, l'existence des nodules sous-cutanés, ou le traitement par la phénylbutazone, les médicaments antipaludiques, les corticostéroïdes ou les sels d'or. Le sérum vert était plus fréquent chez des malades atteints de polyarthrite depuis plus de 10 ans, chez ceux dont la fonction était plus affectée, chez les anémiques et chez ceux qui accusaient un titre plus élevé du facteur rhumatoïde dans le sérum.

Le couleur verte était photosensible. Elle ne se produisait pas lorsqu'on diluait les sérums jaune ou la bilirubine pure et elle ne semblait pas être due à la verdoperoxidase qui confère la coloration verte à l'expectoration et dans le chlorome. Elle se déplace avec l'albumine mais n'est pas détruite par digestion en présence de la pronase. La courbe d'absorption a la même forme que celle du sérum jaune, bien que l'aire sous la courbe verte soit moindre. La détermination de la raison d'absorption à 430 et 520 m μ suggère que la couleur verte est due à l'insuffisance relative du pigment jaune.

El color verde en el suero reumatoide

SUMARIO

Se investigó la pigmentación verde en los sueros de 104 pacientes con poliartritis reumatoide y de 150 pacientes sin enfermedad reumatoide. El suero verde fué presente sólo en un 12, 5 por ciento del grupo poliartítico y se manifestó con más frecuencia en las mujeres (un 14 por ciento de estas) que en los hombres (un 5 por ciento de ellos). No se encontró relación alguna entre esta coloración y la intensidad de la afección clínica y radiológica de la artritis, la existencia de nódulos subcutáneos o el tratamiento con la fenilbutazona, los antipalúdicos, los corticosteroides o sales de oro. El suero verde fué más frecuente en enfermos afectados de poliartritis desde más de diez años, en aquellos con función empeorada, en los anémicos y en aquellos cuyo título sérico del factor reumatoide fué más elevado.

El color verde era fotosensible. No se producía con dilución de los sueros amarillos o de la bilirubina pura y no parecía deberse a la verdoperoxidasa que da la coloración verde al esputo y en casos de cloroma. Se mueve con la albumina pero no se ve destruida por la digestión con pronasa. La curva de absorción tiene la misma forma que la del suero amarillo, aunque la superficie debajo de la curva verde sea menor. La determinación de la razón de absorción a 430 y 520 m μ sugiere que el color verde se debe a una deficiencia relativa del pigmento amarillo.