Sero-negative.—The sero-negative patients with probable or definite rheumatoid arthritis in 1961 remained sero-negative and the two sero-positive remained sero-positive. During this interval, one man aged 43, who had complained only of mild shoulder pain, then described as "fibrositis", with negative serological tests and a normal erythrocyte sedimentation rate, developed definite sero-positive rheumatoid arthritis.

It is our intention to re-examine those with definite sero-positive rheumatoid arthritis, the false-positives, and those with plasma protein abnormalities each year, and the whole population every 5 years.

Conclusion

Our results are somewhat contradictory and do not confirm the findings of many previous workers, including Ziff, Schmid, Lewis, and Tanner (1958), McKusick (1959), Lawrence and Ball (1958), Lawrence (1961), and Scotch and Geiger (1962). They are also at variance with some of our own earlier findings (de Blécourt, Polman, and de Blécourt-Meindersma, 1961; de Blécourt, Westendorp Boerma, and Vorenkamp, 1962). The numbers involved are too small to draw any definite conclusions, but regular examination will show the further progress of those with abnormal plasma proteins, and also which members of the population developed into new cases of rheumatoid arthritis.

The detection of "rheumatoid factor" or other abnormal plasma proteins does not lead to the detection of disease, which suggests that screening of populations or of patients' relatives by examining the plasma proteins is of little value unless combined with a physical and, if possible, radiological examination. Our results also failed to show any correlation between the erythrocyte sedimentation rate, blood groups, and rheus factor with the presence of rheumatic disease.

PART II. ANTINUCLEAR FACTORS AND ANTITHYROID ANTIBODIES

BY

R. L. F. NIENHUIS, E. MANDEMA, AND A. JANsz

Tests for antinuclear factor and antithyroid antibodies were also carried out in the population described above.

Methods

The presence of the antinuclear factor (ANF) was demonstrated by Coons' indirect fluorescent antibody technique (Coons and Kaplan, 1950) by the method of Mandema, Pollak, Kark, and Rezaian (1961). Smears of human buccal mucosal epithelial cells were used as a substrate. The globulin fraction of rabbit anti-human gamma globulin conjugated with fluorescein isothiocyanate (commercially produced by Sylvania Chemical Co.) was used as an antiserum.

Before use the conjugated antiserum was absorbed twice with acetone-dried rat-liver powder. The smears were incubated with the test serum for 90 min. and with the fluorescein-conjugated globulin fraction of rabbit anti-human gamma globulin serum for 30 min.

All sera were tested undiluted in duplicate, and if they were positive titrations were made as described above.
Results

Of the 431 sera tested, 76 (17·6 per cent.) were positive and 355 (82·4 per cent.) were negative (Fig. 1). Two sera first considered positive when tested undiluted, turned out to be negative on re-testing for titration. On titration thirteen sera were found to be positive only when undiluted, 46 had titres ranging from 1 : 4 to 1 : 64, and seventeen (nearly 4 per cent. of the total number investigated) had a titre of 1 : 128 or higher; three sera were still positive in very high dilutions.

Of the 76 sera found positive for ANF by this method, only three showed a positive Waaler-Rose test and/or latex-fixation test.

This probably means that, in a certain population, the distribution of ANF as estimated by our technique does not show a positive correlation with the occurrence of the gamma globulins which are responsible for positive Waaler-Rose and latex-fixation tests.

48 sera positive for ANF with human buccal mucosal epithelial cells were all negative when either dep smears of rat liver or guinea-pig kidney were used as a substrate. This result agrees with that found previously, when frozen sections of rat kidney were compared with human buccal mucosal cells. In the previous study, however, in which the sera of patients with systemic lupus erythematosus (S.L.E.) were tested, some of the sera were positive with both substrates but some were positive with human buccal mucosal cells only.

49 sera positive for ANF with human buccal mucosal cells were also tested by the Coombs' consumption test (CCT), with chicken red-cell nuclei as a substrate, using the technique described by de Rooy (1960), which is a slight modification of Steffen's original method: fifteen were positive (Fig. 2, opposite), four were doubtful, and thirty were negative.

Of these fifteen positive sera, seven had a titre with the immunofluorescence technique of lower than 1 : 64, and eight had a titre of 1 : 64 or higher. Of the thirty negative sera, thirteen had a titre of lower than 1 : 64 and seventeen of 1 : 64 or higher. The thirteen sera which gave the highest ANF titre with the immunofluorescence test (Fig. 2) were all negative in the Coombs' consumption test.

The absence of correlation between the immunofluorescence test and the Coombs' consumption test is not easy to understand; it may be that these two methods detect gamma globulins which are at least partially different.

All sera were tested for the presence of antibodies against human thyroglobulin, using the passive haemagglutination technique of Boyden (1951), and 39 out of 431 (9 per cent.) were positive. The positive sera were titrated; in fifteen of them (3·5 per cent.) the titre was 1 : 1,600 or higher, in six it was 1 : 12,800 or higher. Again there was no correlation between the presence of ANF, antithyroglobulin antibodies, and rheumatoid factor indicated by a positive Waaler-Rose and/or latex-fixation test (Fig. 3, opposite).

Discussion

To explain these results is not easy. Although it is difficult to exclude either subclinical (latent) S.L.E. or Hashimoto's disease, no patient with either of these diseases has so far been found in the population under study.

Two suggestions may, however, be advanced:

1) That ANF and antithyroglobulin antibodies may not uncommonly be found in an apparently healthy population, even sometimes with antibodies in high titres;

2) That there is no correlation between these various antibodies.

One may speculate, first, that an auto-immune diathesis may exist in apparently healthy persons, which cannot be detected by studying the occurrence of multiple circulating auto-antibodies.

Secondly, perhaps no auto-immune diathesis exists at all, and the so-called auto-immune antibodies are due to exogenous antigens.

Thirdly, an auto-immune antibody diathesis may exist in apparently healthy persons in whom the
RHEUMATIC DISEASE ON A COASTAL ISLAND

1. In 1951 and 1961 the population (about 500 persons over 15 years of age) of a small island (Schermonnikoog) was investigated for the presence of rheumatic disease. About 25 per cent. of them had been born on the island.

The results of the two surveys were about the same. About 25 per cent. complained of rheumatism, and of these about 6 per cent. had rheumatoid arthritis, about 50 per cent. had osteo-arthritis, and about 44 per cent. had non-articular rheumatism. In 1961 2·27 per cent. of the population had a positive Waaler-Rose and/or latex-fixation test, and 1·8 per cent. were “false-positives”.

Positive serological reactions were not more common in relatives up to the third generation of patients with rheumatoid arthritis or of “false-positive” cases. Rheumatoid arthritis and “false-positive” reactions were not more frequent in that part of the population that could be regarded as autochthonous.

II. Tests for anti-nuclear factor and antithyroid antibodies gave a number of positive reactions, but there was no correlation between the immuno-fluorescent technique, the Coombs’ consumption test, and Boyden’s passive haemagglutination technique, or between the presence of antibodies and the diagnosis of clinical rheumatic disease.

No patients with systemic lupus erythematosus or Hashimoto’s disease were found in the island.

REFERENCES

ANNALS OF THE RHEUMATIC DISEASES


Discussion

PROF. E. G. L. BYWATERS, DR. W. HUMANS, and DR. G. LOEWI stressed the importance of choice of substrate as well as kind of conjugate for antinuclear factor, in view of this high incidence of positives in a normal population.

DR. J. S. LAWRENCE congratulated the speaker on the persuasive powers which had succeeded in obtaining repeated follow-up examinations on the same population.

In reply to a question, DR. DE BLÉCOURT said that there had been no cases of rheumatic fever in the last survey and that no active steps had been taken on previous occasions to treat or suppress rheumatic fever.

Maladies rhumatismales dans la population d'une île littorale

RÉSUMÉ

I. En 1951 et un 1961 on procédé à des enquêtes parmi les habitants (environ 500 personnes âgées de plus de 15 ans) d'une petite île (Schiermonnikoog) sur la maladie rhumatismale. Près de 25 pour cent d'entre eux n'ont jamais vecu en dehors de l'île.

Les résultats de ces deux enquêtes furent à peu près les mêmes. Près de 25 pour cent des habitants souffraient de rhumatisme, parmi ceux-ci 6 pour cent était atteints d'arthrite rhumatismale, environ 50 pour cent d'ostéo-arthrite et près de 44 pour cent de rhumatisme non-articulaire. En 1961, 2,27 pour cent de la population avait la réaction de Waaler-Rose et/ou la réaction de fixation au latex positive et 1,8 pour cent d'entre eux étaient de "faux positifs".

Des réactions sérologiques positives n'étaient pas plus communes chez des consanguins, jusqu'à la troisième génération, malades atteints d'arthrite rhumatismale ou des "faux positifs". L'arthrite rhumatismale et les réactions "falsas positivas" n'étaient pas plus fréquentes parmi ceux qui n'avaient jamais quitté l'île.

II. Des réactions recherchant le facteur antinucléaire et les anticorps antithyroides ont donné quelques résultats positifs, mais il n'y avait pas de corrélation entre le procédé d'immuno-fluorescence, la réaction de consommation de Coombs et le procédé d'hémagglutination passive de Boyden, ou entre la présence des anticorps et le diagnostic des maladies cliniques.

On ne trouva pas sur l'île de malades atteints de lupus érythémateux disséminé ou de maladie de Hashimoto.

Enfermedades reumáticas en la población de una isla costera

SUMARIO

I. En 1951 y en 1961 se investigó la población (cercia de 500 personas de más de 15 años de edad) de un isote (Schiermonnikoog) respecto a la enfermedad reumática. Cerca del 25 por ciento de ellos nunca vivieron fuera de la isla.

Los resultados de ambas investigaciones fueron aproximadamente iguales. Cerca de un 25 por ciento de los habitantes se quejaron de reumatismo; un 6 por ciento de estos fueron afectos de artritis reumatoide, cerca de un 50 por ciento de osteoartritis y en los demás un 44 por ciento el reumatismo fue no articular. En 1961 la frecuencia en un 2,27 por ciento de la población acusó la reacción de Waaler-Rose y/o la reacción de fijación sobre latex positiva y en un 1,8 por ciento se obtuvieron "falsos positivas".

Reacciones serológicas positivas no fueron más comunes en parientes, hasta la tercera generación de los, enfermos con artritis reumatoide o de los casos "falsos positivos". La artritis reumatoide y las reacciones "falsas positivas" no fueron más frecuentes entre aquellos que nunca habian dejado la isla.

II. Reacciones para evidenciar el factor anti-nuclear y los anticuerpos antitiréoides dieron algunos resultados positivos, pero no hubo correlación entre el procedimiento de inmunofluorescencia, la reacción de consunción de Coombs y el procedimiento de hemaglutinación pasiva de Boyden, o entre la presencia de anticuerpos y el diagnóstico de enfermedades clínicas.

No se encontraron en la isla enfermos con lupus eritrematoso diseminado o con la enfermedad de Hashimoto.