that observation I notice that you found C₁q precipitins in one patient where the DNA binding was going down. Was this patient on corticosteroid therapy?

DR. HUGHES Yes.

DR. K. WHALEY (*Glasgow*) Could this relate to the presence of free DNA in the serum, as this binds with C₁q under the same conditions as it binds to aggregated gamma globulin?

DR. HUGHES Yes. We have looked at it; as you know we described this effect that steroids have of releasing DNA into serum (Hughes, Cohen, Lightfoot, Meltzer, and Christian, 1971b) and we wondered if the fall might be related to release of DNA and subsequent complexing. We haven't been able to show that in any of the 6 patients in whom C₁q studies were performed serially. Our results conflict with those of Agnello who did claim a close association between disease activity and C₁q precipitins. I cannot explain the difference except perhaps C₁q precipitins do not relate to the main disease entity in SLE.

DR. K. WHALEY (*Glasgow*) Yes; could I ask one more question? Have you followed the course of titrations for the total DNA binding capacity by the standard Farr technique and the antigen binding capacity as a measure of disease remission? We find in the acute sera it is a far better index of response to therapy, it falls far quicker than the DNA binding capacity and in our experience, which is somewhat limited, patients who are going to respond to corticosteroid therapy appear to reduce their total DNA binding capacity much faster than those who don't reduce it.

DR. HUGHES I agree.

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Antibodies to RNA in the Connective Tissue Diseases.
By P. DAVIS and G. R. V. HUGHES (*Department of Rheumatology, Royal Postgraduate Medical School, London*)

The finding of antibodies to double stranded RNA in SLE (Koffler, Carr, Agnello, Thoburn, and Kunkel, 1971) has led to speculation concerning the possible role of RNA viruses in the aetiology of this disease (Talal, 1970). This report describes the application of an immunoassay for the measurement of these antibodies and its use in SLE and other connective tissue diseases.

Methods

Antibodies to polynucleotides were measured by the modified Farr technique using the synthetic polynucleo-

tides poly I:poly C:poly A:poly U, and viral RNA, previous studies having shown cross-reaction between viral-RNA and poly I:C antibodies. Optimum experimental conditions were determined using polynucleotide conc. 0.01 µg/50 µl and serum diluted 1:10. Precipitation was maximal with 50% saturated ammonium sulphate. Reproducibility within 5% was obtained. Binding was inhibited by an excess of cold RNA. Inhibition studies with sera of patients with virus infections are in progress.

Cases

104 serum specimens from 75 patients with SLE have been measured. Serial studies have been performed on 4 of them. RNA antibodies have also been measured in 20 patients with RA, 20 with DLE, 20 with chronic active hepatitis, and 40 normals. Parallel DNA antibody measurements were performed in all patients.

Results

Viral RNA antibodies were found in 21 of the 75 patients with SLE (36%). In the serial studies RNA-Ab were not found to be as sensitive a clinical guide as DNA-Ab measurements and 60% of the active SLE group had no RNA antibodies. High titres of viral RNA-Ab were not found in patients with RA, though there was a high incidence of antibodies to poly I:C.

Discussion

DR. G. S. PANAYI (*Guy's Hospital*) The interest of these antibodies is that they may reflect persistent infection with an RNA virus and yet people who have recovered from diseases with RNA virus do not show any increase in these antibodies. Can you explain this?

DR. DAVIS Yes. The majority of antibodies in the recovery stages of virus infections are not directed against the nuclear core of RNA or DNA which they may contain, but the protein which surrounds them. The suggestion has been made that with persistent infection some breakdown in the protein coat may occur exposing the antigenic sites on the RNA.

DR. K. WHALEY (*Glasgow*) I would like to point out that human RNA has a very well-defined secondary structure with extensive double-stranded regions, and until you perform inhibition studies it is rather dangerous to assume that this is an antibody to any sort of viral antigen.

DR. DAVIS Yes, I agree. We are not supporting the concept that SLE is a virus infection or that these antibodies are directed necessarily against an RNA virus. Our interest in RNA antibodies is firstly in their clinical usefulness and secondly in their possible role in immune complex formation.

DR. R. N. MAINI (*London*) Can we also correct for the record the impression you created about the possible aetiological significance of raised antibodies to viruses in SLE? The work of Phillips and Christian (1973) has emphasized that the antibody rise in SLE to certain viruses is correlated to the rise in gamma globulin also observed, and does not indicate that a virus is implicated as a specific aetiological factor.

DR. DAVIS I think you have missed the point. Our main concern has not been the measurement of viral antibodies as such, but antibodies against RNA; the two are not synonymous as I have explained.

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Abnormalities in Rheumatoid Synovial Collagen: Studies on Synovial Polymeric Collagens (PC) from Normal Individuals and Patients with Rheumatoid and other Arthropathies. By JACQUELINE WEISS, S. J. LEIBOVICH, J. A. A. HUNTER, and M. I. D. CAWLEY (*Rheumatism Research Centre, University of Manchester*)

Enzymic degradation and electron microscope studies have been used to detect abnormalities in polymeric collagen. The results indicate that rheumatoid synovia contain two distinct abnormal forms of polymeric collagen, one of which (F_2) represents only a small proportion of the total and which is also found in all inflamed synovial membranes. The other, which constitutes between 15–70% of total PC is, characteristic of rheumatoid arthritis synovia.

Methods

Synovial membrane samples were obtained at biopsy or necropsy from 18 patients with classical or definite rheumatoid arthritis, 18 normal controls, and 13 patients with other arthropathies. Polymeric collagens were prepared by the EDTA method (Steven, 1967). Supernatant and insoluble fractions resulting from pepsin digestion (pH 3.1, 15°C) were quantitated as hydroxyproline. Soluble collagen present in the supernatant was precipitated as fibrils and examined in the electron microscope (Figure).

Abnormality characteristic of RA

An abnormal form of polymeric collagen which was susceptible to pepsin digestion was found in all 18 patients with RA. Normal adult human PC is not susceptible to pepsin and the abnormality could not be reproduced by action of synovial collagenase (Leibovich and Weiss, 1971). The products of pepsin digestion when reconstituted and examined in the electron microscope showed that both N- and C-terminal regions of the molecule had been removed (Leibovich and Weiss, 1970). Immature normal polymeric collagen is also susceptible to pepsin digestion, but in this case only the C-terminal region is removed.

General abnormality associated with inflammation (F_2)

A polymeric collagen, different from normal in its polarity and slowness of aggregation from a neutralized acid suspension, constitutes up to 10% of total synovial collagen in acute inflammatory arthritides and less in rheumatoid arthritis depending on the degree of inflammation. This second abnormal collagen is resistant to pepsin degradation and may represent reassociated products of lysosomal degradation of collagen and matrix (Figure).

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Biosynthesis and Maturation of Skin Collagen in Scleroderma. By C. M. HERBERT, K. A. LINDBERG, M. I. V. JAYSON, and A. J. BAILEY (*Department of Medicine, University of Bristol, Royal National Hospital for Rheumatic Diseases, Agricultural Research Council, Meat Research Institute, Bristol*)

The aetiology and pathogenesis of scleroderma are still unknown. The main characteristic of the disease is severe rigidity and thickening of the skin. Since the mechanical stability of the skin devolves almost entirely on the fibrous protein collagen, qualitative and quantitative changes in the collagen, and particularly of the crosslinks stabilizing the fibre, could lead to the observed symptoms.

The present study was carried out on skin biopsy material obtained from 15 cases of scleroderma, 6 of whom had systemic sclerosis and 9 cutaneous scleroderma as defined by changing physical signs. Controls matched for age, sex, and site were obtained from autopsies on patients who had no disease or medication known to interfere with the metabolism of connective tissue.

The biopsy material was analysed for the presence of reducible aldimine crosslinks (Herbert, Jayson, and Bailey, 1973). Normal skin shows a pattern of maturation of these crosslinks. When collagen is newly formed during the early growth period it contains a high proportion of the aldimine bonds, which are readily cleaved by mild chemical agents. At maturity when growth ceases, the crosslinks become stabilized to an as yet unknown form and are no longer detectable by reduction.

Analysis of the sclerodermatous skin collagen from patients with active scleroderma revealed the presence of the aldimine crosslinks normally absent in adult subjects. Biopsy material from the active edge of the plaque showed