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.

References

Abnormalities in Rheumatoid Synovial Collagen: Studies on Synovial Polymeric Collagens (PC) from Normal Individuals and Patients with Rheumatoid and other Arthopathies. 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 (F2) 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 arthopathies. 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 (F2)
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).

References
Steven, F. S. (1967) Ibid., 140, 522

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 aldime 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 aldime 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 aldime crosslinks normally absent in adult subjects. Biopsy material from the active edge of the plaque showed
a higher proportion of the aldimine crosslinks than the centre of the plaque, and, furthermore, the proportion was considerably reduced in inactive scleroderma patients. The presence of these aldimine crosslinks establishes that new collagen is being laid down in the skin of sclerodermatous patients.

Cleavage of the crosslinks stabilizing the collagen fibre may relieve the symptoms by softening the skin. D-penicillamine is capable of cleaving the aldimine crosslinks of newly formed collagen but not the stable crosslinks of mature collagen. Clearly, D-penicillamine will only be effective on active scleroderma, and previous conflicting evidence on the value of this drug may be partly due to a failure to differentiate its effects on mature and immature collagen. In addition, in vitro tissue culture studies on sclerodermatous skin have shown a partial inhibition of collagen synthesis in the presence of D-penicillamine (Herbert, Jayson, Bailey, and Lindberg, 1974).

Two patients with active scleroderma were treated with D-penicillamine (1.5 g/day) and followed with sequential biopsies over 9 months. In both cases a significant decrease in the proportion of aldimine cross links was observed, together with a definite improvement in the clinical manifestations of the disease.

References

Discussion on both papers

DR. W. C. DICK (Glasgow) May I congratulate this team on a valuable piece of work in a field which badly needs this kind of study. I am interested in the mechanism which turns a person from being normal to producing a more soluble collagen which has more crosslinks. Do you think this could be a depressor mechanism?

DR. HERBERT This is, of course, the most important question to answer. At present we are only showing the changes that have taken place, and not the initiating mechanism.

DR. ALNOFF (Brussels) You have chosen scleroderma as a model. Would you think that in situations, like in keloids, you would have something of the same sort?

DR. HERBERT I think it would be very well worth looking at.

DR. R. N. MAINI (London) One of the problems in dealing with patients with scleroderma is the clinical assessment of disease activity. Do you think your investigations might provide a basis of such an assessment?

DR. HERBERT Two patients were referred to us with exactly this problem and we were able to show the presence of new collagen, thus establishing that both patients had active disease. Whether this is always a justifiable conclusion, I don't know as yet.

DR. D. J. WARD ( Oswestry) Would measurements of hydroxyproline help to assess disease activity and the effect of penicillamine?

DR. HERBERT Urinary hydroxyproline determinations would be valueless as they would give no indication of the precise biochemical processes taking place in the sclerodermatous skin.

DR. S. D. ROBERTS (Belfast) You said that there were in the 'active' cases areas of skin where the activity was clinically less obvious. Is it true then to say that in the patient with active scleroderma there is also inactive scleroderma; and that there are areas in patients with inactive scleroderma that may be active?

DR. JAYSON This differentiation of areas was only possible in certain patients who had well-defined plaques and it was in these that we took separate biopsies from the centre and the edge of the lesion, and it showed that in the centre of the lesion the collagen had apparently matured, whereas at the edge of the lesion there was still proliferation of new collagen being laid down. This was possible in only a small number of patients. The sequential biopsies were taken from adjacent sites. These were not close enough so that new scar tissue might be taken, but otherwise were closely comparable. Incidentally, we have had the problem in a patient who presented with an acute connective tissue disease with some debatable changes in the skin and there was some difference of opinion as to whether this was an acute scleroderma, but in fact the analyses did show a massive increase in the production of new collagen which confirmed the diagnosis.


The acid proteinase cathepsin D can degrade proteoglycan, a major constituent of cartilage. The erosion and loss of articular and patellar cartilage observed in rheumatoid arthritis may be related to the excessive extracellular activity of this and other proteinases released from cells in rheumatoid synovia. Up to the present time there has been no available means of detecting the local extracellular release and presence of this and other enzymes. Techniques recently developed in the Strangeways Research Laboratory have made it possible to capture enzymes as they are released from cells.

This paper reports the first application of this method to an investigation of cathepsin D release from normal and rheumatoid synovia. So far tissues have been removed from knee joints of 3 patients undergoing meniscectomy (normal) and 11 patients undergoing synovectomy (rheumatoid), and immediately cultured for up to 24 hours with a specific antiserum to human cathepsin D. While cell viability was maintained, extracellular cathepsin D was trapped by antibody with the formation of insoluble immunoprecipitates: these were subsequently localized at or near cell surfaces with immunofluorescence microscopy.

There was relatively little evidence for cathepsin D release from cells in synovia of nonrheumatoid joints: only occasionally were intimal lining cells and histiocytes in the capsular tissue suspected of secreting cathepsin D and then only in small amounts relative to synovia from many rheumatoid patients.