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 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 (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 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 (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 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
a higher proportion of the aldimine crosslinks than the
centre of the plaque, and, furthermore, the proportion
was considerably reduced in inactive scleroderma pa-
tients. The presence of these aldimine crosslinks establishes
that new collagen is being laid down in the skin of sclero-
dermatous 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 cross-
links of newly formed collagen but not the stable cross-
links 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, to-
gether with a definite improvement in the clinical manifesta-
tions of the disease.

References
Dis., 32, 510

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 ex-
actly 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 con-
clusion, 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 sclero-
dermatous 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.

Extracellular Release of Cathepsin D from Cells in Human
Normal and Rheumatoid Synovial Membranes. By A. R.
Poole, R. M. Hembrv, J. T. Dingle, I. Pinder, E. F. J.
Ring, and J. Cosh (Strangeways Research Laboratory,
Cambridge, and Royal Hospital for Rheumatic Diseases)
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 Labora-
tory 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 (nor-
mal) and 11 patients undergoing synovectomy (rheuma-
toid), 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 micro-
scopy.

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.
Proceedings: Biosynthesis and maturation of skin collagen in scleroderma.
C M Herbert, K A Lindberg, M I Jayson and A J Bailey

*Ann Rheum Dis* 1974 33: 404-405
doi: 10.1136/ard.33.4.404-b

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