

what extent is this independent of the serum fibrinolytic activity. When you improve the fibrinolytic activity of the serum, does the fibrinolytic activity of the vessel improve and is it dependent or secondary to the serum?

DR. CUNLIFFE We are finding a relationship between tissue activity and plasma fibrinolytic activity. Furthermore, patients with vasculitis have no tissue fibrinolytic activity whatsoever in their lesions, and the skin nearby where there is no rash also shows a reduced fibrinolytic activity. The fibrinolytic activity of the tissues improves with treatment.

DR. H. L. F. CURREY (*London*) In patients with vasculitis and raised euglobulin lysis times, what is the effect on the euglobulin lysis time of giving steroids?

DR. CUNLIFFE I have not done any work on steroids, but we found that in four patients who were on steroids there was some improvement in the fibrinolytic activity and in two there was not; but the patients who had rheumatoid arthritis were also on other drugs.

DR. A. ST. J. DIXON (*Bath*) Would it not be equally logical to conclude from the data that patients who start with a prolonged euglobulin lysis time have a good prognosis irrespective of treatment?

DR. CUNLIFFE This we have not found. We have been studying patients over a period of several years and at the moment one cannot draw this conclusion.

DR. M. I. V. JAYSON (*Bath*) Do you think that the prolonged euglobulin lysis time could be due to excessive fibrin deposition and be the result of vasculitis rather than being concerned in pathogenesis?

DR. CUNLIFFE This is our conclusion.

DR. D. A. PITKEATHLY (*Manchester*) Are you using this treatment as the treatment of choice in patients with severe vasculitis?

DR. CUNLIFFE I should treat the acute vasculitis with steroids, but if the patient has had it for many years and the fibrinolytic activity is impaired then I would go straight on to phenformin and an anabolic steroid.

Reference

Parish, W. E. (1972) *Proc. roy. Soc. Med.*, 65, 276

Immunohistological Studies of the Kidney in Systemic Lupus Erythematosus and Systemic Sclerosis. By D. G. SCOTT and N. R. ROWELL (*Leeds*)

The kidney specimens examined were obtained from seven patients with systemic lupus erythematosus and eight with systemic sclerosis. Fourteen of the specimens were obtained at autopsy, and one at biopsy from a patient with systemic sclerosis.

Sections from all specimens were reacted in immunohistological staining experiments with antisera to IgG, C'3, and human renal glomeruli. Sections of kidney from three patients with systemic lupus erythematosus and three with systemic sclerosis were reacted also with antisera to IgM, IgA, and fibrinogen.

In systemic lupus erythematosus, granular staining for IgG and C'3 was found in the walls of arterioles in five kidneys and the glomerular mesangium or along glomerular capillary basement membranes in six. In two instances

granular basement membranes staining was superimposed on smooth linear staining of the basement membrane. The presence of granular basement membrane staining appeared to correlate with clinical evidence of renal failure, but staining confined to the mesangium did not. This relationship has been previously described by Koffler, Agnello, Carr, and Kunkel (1969).

In systemic sclerosis, IgG was detected in arterioles and in glomerular capillary basement membranes in one kidney and IgG and C'3 in a second. Staining for fibrin was found at these sites in three other kidneys.

In studies with antiglomerulus antisera, systemic sclerosis kidneys showed alterations in the pattern of intimal and medial staining of arterioles in seven instances and broadening of glomerular capillary basement membranes in four. In systemic lupus erythematosus abnormal antiglomerulus staining was not found in the absence of immunoglobulin deposition.

It is suggested that these observations provide further evidence that immunological processes are not involved in the pathogenesis of the renal lesions of systemic sclerosis. They appear to indicate also that the vascular deposition of fibrin in systemic sclerosis occurs in previously damaged vessels.

Discussion

DR. P. J. L. HOLT (*Manchester*) I am not quite sure what your antiserum is raised against, because it does not seem to be basement membrane antigen and it seems to be staining the arterioles rather than the rest of the glomerulus.

DR. SCOTT The antiserum was raised against the whole of the glomerulus. Glomeruli were stained in the sections.

DR. P. J. L. HOLT (*Manchester*) So it is fairly crude.

DR. SCOTT Yes, it is a crude antiserum.

DR. P. J. L. HOLT (*Manchester*) And it stains the arterioles of the kidney in systemic sclerosis. Can you find staining in other organs in systemic sclerosis?

DR. SCOTT The antiserum will produce staining of reticulin in the media of normal arteries. In systemic sclerosis there are abnormalities in the distribution of this staining. These abnormalities are not associated with the deposition of globulin, but may be associated with the deposition of fibrin.

DR. P. J. L. HOLT (*Manchester*) So it is the pattern of the staining rather than the presence or the absence of the staining that is important?

DR. SCOTT Yes.

DR. M. I. V. JAYSON (*Bath*) Dr. Dubois has presented a series of patients with systemic sclerosis which showed the features of S.L.E., and these features responded to steroid therapy, whereas the systemic sclerosis features did not. Were you able to find any S.L.E.-like signs and symptoms in this group of systemic sclerosis patients?

DR. R. N. MAINI (*London*) Was there any correlation with immunoglobulin or complement levels in the pattern of deposition which you saw?

DR. ROWELL This work has been done over about 10 years. Serum complement levels were not available in this hospital in the early days.

DR. R. N. MAINI (*London*) How did you diagnose systemic sclerosis and was there any information on antinuclear factors available on any of these patients?

DR. ROWELL All the patients had Raynaud's phenomenon followed by characteristic cutaneous changes and multisystem involvement of systemic sclerosis. Antinuclear factor was done on all cases. There was no consistent pattern to suggest any relation between antinuclear antibodies and the pattern of staining seen in the sections. All this series had one or more antinuclear factors. It has been shown that nearly 70 per cent. of patients with systemic sclerosis have antinuclear factor of one pattern or another (Rowell and Beck, 1967).

DR. G. HUGHES (*London*) I should like to ask you again about your controls, because this sort of straining is not specific. The second point is that mesangial deposition is found in patients with systemic lupus without clinical evidence of renal disease and is of uncertain significance.

DR. SCOTT I agree. In our study, too, mesangial staining had little clinical significance. The question is why is this so? Are there two different types of complexes involved in systemic lupus erythematosus, one which lodges in or on capillary basement membranes and one which lodges in the mesangium? As to the controls used; the antglomerulus conjugates were absorbed with normal human serum and Group A and B red cells before use. The usual blocking procedures were run in parallel with staining experiments. The staining seen in the systemic sclerosis and systemic lupus erythematosus material was compared with that seen in sections of normal kidney.

References

- Koffler, D., Agnello, V., Carr, R., and Kunkel, H. C. (1969) *Amer. J. Path.*, 56, 305
Rowell, N. R., and Beck, J. S. (1967) *Arch. Derm. (Chicago)*, 96, 290

Occurrence of DNA-antibodies in Antinuclear Factor containing Sera and a Comparison with Immunofluorescence. By D. N. GLASS, J. CAFFIN, H. J. ANDREWS, R. N. MAINI, and J. T. SCOTT (*Kennedy Institute of Rheumatology and Charing Cross Hospital*)

HeLa cell C₁₄ DNA at a final concentration of 0.5 µg./ml. was used in a 'Farr' type ammonium sulphate assay for the detection of DNA binding in ANF positive sera. The sera had been obtained from a range of connective tissue diseases including systemic lupus erythematosus (SLE). ANF was detected by the usual immunofluorescent technique using rat liver sections. Of the 150 ANF positive sera tested, 25 showed raised DNA binding correlated with clinically active SLE.

The relationship of DNA binding to ANF tested at serum dilutions of 1:10 and 1:1,000 is shown below:

DNA binding	No. ANF positive at a dilution of:	
	1:10	1:1,000
Positive	25	9
Negative	125	17
Total sera	150	26

Eleven out of 26 sera with ANF at 1:1,000 dilution were derived from patients with SLE and of these seven were positive and four negative for DNA binding. Two other sera with ANF from non-SLE patients gave low

DNA binding values and were negative on repeating the test. It will be noted that there was a better correlation between DNA binding and sera positive for ANF at a 1:1,000 dilution than at 1:10. 25 DNA antibody positive sera gave homogenous pattern fluorescence in most instances and comet or membranous in the rest. No particular pattern of fluorescence correlated specifically with DNA antibodies, each pattern also being found in DNA antibody negative sera.

DNA of high purity (containing RNA protein contamination of less than 0.1 per cent.) gave very similar results to those obtained with the crude DNA preparation. DNA from other sources (kindly supplied by Dr. H. Harley, University College Hospital Medical School) such as CVI (monkey) cells was also undertaken and the results were comparable. However, the ammonium sulphate precipitation technique has several unsatisfactory aspects for routine use. The technical problems include a necessity to work at 4°C. for separation and difficulty in centrifuging down the whole of the rather fragile ammonium sulphate precipitation, giving an unsatisfactory extrapolation of results by having to count the upper half of the supernatant as well as the remainder of the precipitate and supernatant. The latter occasionally gave rise to DNA binding with a negative value. A lack of precision due to large replicate variation between duplicate samples, especially at the low level of binding, was noted and accounted for values above the normal range (false positives) in normal sera. For some investigative and research purposes a more precise assay is necessary, and a double antibody technique developed in this laboratory might be suitable.

Reference

- D. N. Glass, J. Caffin, R. N. Maini, and J. T. Scott (1973) *Ann. rheum. Dis.*, 32, 342

A Study of Depression in Rheumatoid Disease. By G. ZAPHIROPOULOS and H. C. BERRY (*Department of Rheumatology, Guy's Hospital, London*)

It has long been suggested that patients suffering from rheumatoid disease may suffer a mild depressive reaction which can materially impede their progress and delay rehabilitation.

We have endeavoured to determine the incidence of depressive reaction in patients suffering from rheumatoid disease admitted for in-patient therapy to the Hume-Kendall Unit at New Cross Hospital. For the purpose of this study the Beck Depression Self-Assessment Inventory was used. This is designed to incorporate all symptoms relevant to the depressive constellation of symptoms, at the same time providing for grading of the intensity of symptoms. With this method the highest score (26-45) corresponds to the clinical rating of 'moderate to severe' degree of depression, the middle range (15-25) to 'mild to moderate', and the lowest range (0-14) to 'no-depression'. Symptom complexes which could have had a physical rather than psychological basis were not scored, e.g. work inhibition, weight loss, or loss of libido.

Fifty unselected patients with rheumatoid disease fulfilling the A.R.A. criteria for definite or classical RA were included in the study. They completed the inventory