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A Pyroninophilic Component in Pathological Serum Proteins and Urinary Proteins

PYRONINE-METHYL green is frequently used in histological investigations for staining ribonucleic acid and deoxyribonucleic acid¹.

Yeoman^{2,3} applied this staining method to serum proteins and urinary proteins from patients with multiple myeloma. In each of the 11 cases examined, the abnormal protein fraction stained red-violet with pyronine-methyl green after paper electrophoresis of both serum and urine. This staining reaction was negative in 100 cases of hyperglobulinæmia and proteinuria. Yeoman presumes that the staining is specific for true myeloma proteins.

For some considerable time, investigations in our laboratory have been aimed at the chemical and immunological characterization of paraproteins. Consequently we have at our disposal a collection of sera from patients whose blood plasma contains one or several abnormal protein fractions. In addition, a few Bence Jones proteins are available. The specificity of the above-mentioned staining was tested in this material. A sample of each serum and each Bence Jones protein was submitted to electrophoresis at pH 8.6 in agar gel⁴, and then stained with pyronine-methyl green in an acetate buffer at pH 4.8.

Table 1. STAINING OF PARAPROTEINS WITH PYRONINE-METHYL GREEN

	Positive	Negative
Multiple myeloma	9	—
Bence Jones proteins	8	—
Waldenström's macroglobulinæmia	13	19
Atypical macroglobulinæmia	4	3
Unexplained paraproteinæmia	1	7

The results obtained (see Table 1) show that the abnormal serum protein fraction stained with pyronine-methyl green in all cases of myeloma. Eight different isolated and purified Bence Jones proteins likewise produced a positive reaction.

A positive staining reaction was found in about half the cases of Waldenström's macroglobulinæmia and of so-called atypical macroglobulinæmia⁵.

Sera from patients with an unexplained paraproteinæmia failed to stain, with one exception.

Therefore, this staining is unfortunately not specific for myeloma proteins from serum and urine, although these proteins showed a positive staining reaction both in the series examined by Yeoman and in our own material.

It is remarkable, however, that only abnormal protein fractions from serum and urine can be stained with pyronine-methyl green. This stainability might be taken to denote the presence of ribonucleic acid or deoxyribonucleic acid in these pathological protein fractions.

In order to verify this, the ultra-violet absorption spectrum of the electrophoresis-spots of these fractions

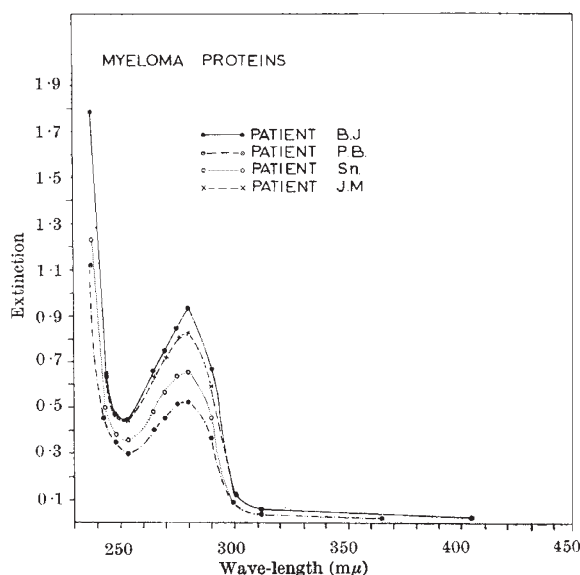


Fig. 1. Ultra-violet absorption spectrum of the pathological proteins in the serum of four myeloma patients

was determined. This proved to be identical with that of proteins; ribonucleic acid or deoxyribonucleic acid was not demonstrable in these fractions (see Fig. 1).

Both exposure to the influence of the enzyme ribonuclease (0.1 per cent in acetate buffer pH 6.0 at 37° C. for 30 min. and 7 hr., respectively) and treatment with 1 N perchloric acid at 4° C. failed to influence the stainability of the pathological protein fractions. The Feulgen reaction was also negative.

This warrants the conclusion that these proteins contain no ribonucleic acid or deoxyribonucleic acid.

Further investigations will be aimed at a definition of the component responsible for the positive staining reaction with pyronine-methyl green.

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¹ Trevan, D. J., and Sharrock, A., *J. Path. Bact.*, **63**, 326 (1951).

² Yeoman, W. B., *Lancet*, **i**, 263 (1960).

³ Yeoman, W. B., Eighth Colloquium, Bruges (1960).

⁴ Wieme, R. J., thesis, Ghent (1959).

⁵ Jahnke, K., and Scholtan, W., *Verh. deutsch. Ges. inn. Med.*, **61**, 312 (1955).

Transferrin in Normal Cerebrospinal Fluid

PREVIOUS work has shown differences between some of the proteins in cerebrospinal fluid and those found in serum¹⁻³. Thus paper-electrophoresis of concentrated normal cerebrospinal fluid may show a peak in the intermediate area between the beta- and gamma-fraction ('tau-fraction'), which is not seen in paper-electrophoresis of normal human serum⁴⁻⁶. The similarities and differences between transferrin in cerebrospinal fluid and in serum have been further investigated.

The cerebrospinal fluid was obtained by lumbar puncture from individuals without any neurological