

the collagen is dissolved, and in 60 min. (complete relaxation) 75 per cent of the nitrogen of collagen goes into solution. By heat relaxation the same dissolution of protein takes place. At 67° C. in 2 min. 14 per cent, in 5 min. 47 per cent and in 10 min. 73 per cent of the nitrogen of the collagen is dissolved. The gross structure of the collagen fibre remains, as if the protein has dissolved only from the inside.

With elastase at pH 10, only 5–10 per cent of the native fibres were dissolved. From the contracted fibre the elastase dissolves the outer phase, which accounts for 15–20 per cent of the entire collagen nitrogen, while the internal phase remains intact. Fig. 4 shows a contracted fibre after treatment with elastase. The relaxed fibres are dissolved with elastase without residue.

The contraction process remains reversible if the fibres, just after contraction, were removed from the solution, washed, stretched and dried in the stretched state. But after relaxation, contraction could no longer be observed.

In a previous experiment, one of us (Banga<sup>2</sup>) showed that during the heat contraction of Achilles tendon a mucoid is dissolved which contains 6–8 per cent of carbohydrate. In those experiments the relaxation is already beginning and the analytical results may have related to the combination of two substances. It is to be noted that in Achilles tendon of cattle the heat contraction–relaxation processes follow much more slowly than in the collagen fibre of rat tail tendon.

Our experiments seem to prove that the collagen fibre is not built up from a single protein but at least of two different protein components. In our experiments the chondroitin sulphuric acid content of the ground-substance which stabilizes the collagen fibre does not play a part in the process of contraction–relaxation. This is shown by the following experiment. We treated the native collagen fibres with a concentrated aqueous solution of hyaluronidase, which dissolves chondroitin sulphuric acid. After treatment for 24 hr., contraction and relaxation took place in the usual way. This indicates that neither of the two proteins described corresponds to chondromucoid.

A paper containing the detailed results of the experiments will be published in the *Acta Morph. Acad. Sci. Hung.*

I. BANGA  
J. BALÓ  
D. SZABÓ

First Department of Pathological Anatomy  
and Experimental Cancer Research,  
University of Budapest.  
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<sup>1</sup> Partridge, S. M., *Biochem. J.*, **43**, 387 (1948).

<sup>2</sup> Banga, I., *Nature*, **172**, 1099 (1953).

### A Lipid Component of Reticulin

IN a previous communication in *Nature*<sup>1</sup>, renal cortical reticulin was described as consisting of randomly orientated collagen fibrils embedded in a structureless matrix. X-ray diffraction photographs of these reticulin specimens showed a diffraction pattern quite typical of that of collagen, together with some additional rings; this suggested that the reticulin consisted of collagen together with an unidentified material.

Further studies on the chemical composition of this reticulin, isolated from kidneys as before, indicated that a fat component was present.

The X-ray diffraction pattern of this isolated fat has been compared with that of a series of saturated fatty acids, and the spacings and intensities show close agreement with the series. The precise member of the series present in reticulin was not identified from the diffraction pattern alone, but a melting point over the range 49°–52° C. suggested a mixture of fatty acids, with myristic acid predominating. Analysis by Imperial Chemical Industries, Ltd., using vapour phase chromatography, has given more than 90 per cent myristic acid.

In the X-ray diffraction pattern of reticulin, the broad collagen band at 4.5 Å. somewhat masks the bands seen at 4.5 and 3.8 Å., which are very intense when the isolated lipid component is examined alone. Outside the classical 2.9 Å. collagen band seen in reticulin are other rings at 2.5, 2.35 and 2.2 Å.; and these outer rings correspond very closely with those seen with saturated fatty acids.

From a reassessment of the electron microscope results, it is probable that the lipid was present in those regions which were too thick to give clear photographs. The reticulin as isolated was therefore a mixture of three components—collagen, fatty acid and polysaccharide-containing matrix. From the extraction methods used for isolating the reticulin samples, it was clear that the fatty acid was an essential component of the reticulin, and not a contaminant from extra-reticular sources.

K. LITTLE

Atomic Energy Research Establishment,  
Harwell, Berks.

G. M. WINDRUM

Radcliffe Infirmary,  
Oxford.

<sup>1</sup> Little, K., and Kramer, H., *Nature*, **170**, 499 (1952).

### Staining Reactions of Elastic Fibres with Special Reference to 'Elastotic Degenerations' in the Human Skin

PART of our research programme is devoted to clarifying various aspects of skin carcinoma in man and in experimental animals. Careful study of the dermal accumulations of morphologically unusual fibrillar material, consistently found in our specimens, and generally referred to by other authors<sup>1</sup> as "an increase in elastic fibres", led us to suspect that these accumulations of unusual fibrils were not, in fact, unmodified elastic fibres. We conducted a systematic survey of the staining reactions of the fibrillar masses in the dermis and in diseased blood vessels, always carefully comparing our findings in the abnormal with those of the elastic and other fibres in apparently healthy human skin, aorta and other vessels.

The unusual clumps of dermal fibres, referred to above, as well as certain fibres which may appear in vessel walls during degenerative vascular diseases, stain with some of the dyes (for example, Weigert's, Verhoeff's, orcein and Gomori's aldehyde-fuchsin, in particular), but not with all the stains which sharply delineate apparently 'normal' elastic fibres. On the other hand, these same fibre clumps may stain with several dyes, or dye-mixtures, which do not stain 'normal' elastic fibres (see Table I).

The conclusion, therefore, seems to be justified that the accumulations or clumps of morphologically