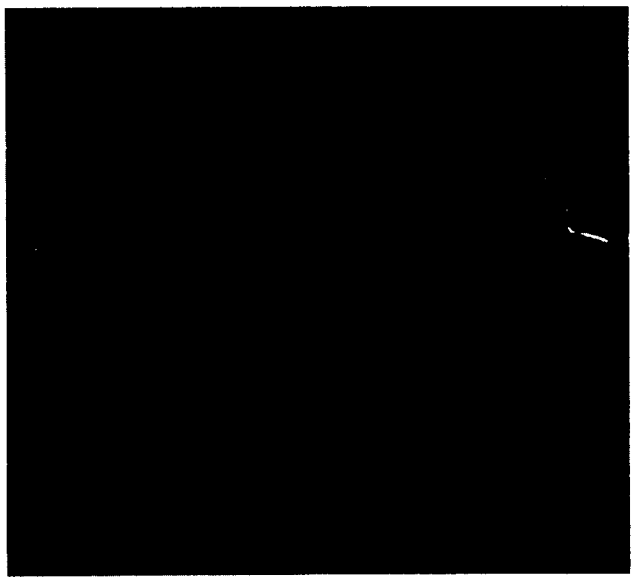
Formation of fibrils from extracts of skin

As part of a physico-chemical investigation of extracts of skin, the method of HIGHBERGER *et al.*[1] for the reconstitution of collagen was applied to the precipitates obtained on saturating certain extracts of whole cow hide with ammonium sulphate. The finely ground hide from a freshly killed cow was extracted successively at 0–5° C for three days with 10% sodium chloride and then *M*/15 sodium phosphate[2]. The precipitates were first suspended in water, dialysed thoroughly against several changes of water and the insoluble portions extracted with citrate buffer (I = 0.2, pH = 3.8). The citrate extract was then dialysed against water. Specimens for examination in the electron microscope were obtained by drying a drop of a suspension of the final precipitate in water on a collodion-covered grid. After drying in a desiccator the specimens were chromium shadowed. The formation of "heat-precipitated" collagen[3,4] was prevented by working at 0–5° C.

Electron microscope examination of several preparations from two sodium chloride extracts showed, in addition to some illdefined fibrils and amorphous material, helical twisted fibrils of the type shown in Fig. 1 (a, b). These fibrils, which appear to be composed of ribbon-like material twisted in the manner of a rope, ranged in diameter from 2,000 to 4,000 A, but no obvious repeat distance, as found with collagen was apparent.

The insoluble fraction (0/40) obtained from the sodium chloride extract at 40% saturation with ammonium sulphate also gave fibrils. These were in general of the helical twisted type, but in addition a few untwisted fibrils (Fig. 2), which were similar in diameter to collagen (*ca.* 1100 A) but without the characteristic fine structure, occurred. Amino-acid analysis[5] by two-dimensional paper chromatography, of both the 0/40 and 40/100 fractions showed the presence of proline but the absence of hydroxyproline.

Superficially the helical twisted fibrils appear similar to the non-striated rope-like fibrils, described by NODA AND WYCKOFF[6], which are precipated from acetic acid solutions of rat tail collagen, and to degraded forms of collagen (*e.g.*[7]).



a

b

Fig. 1 (a and b). Fibrils prepared from whole sodium chloride extract. Magnification × 21,500. 1 cm = 310 mμ.

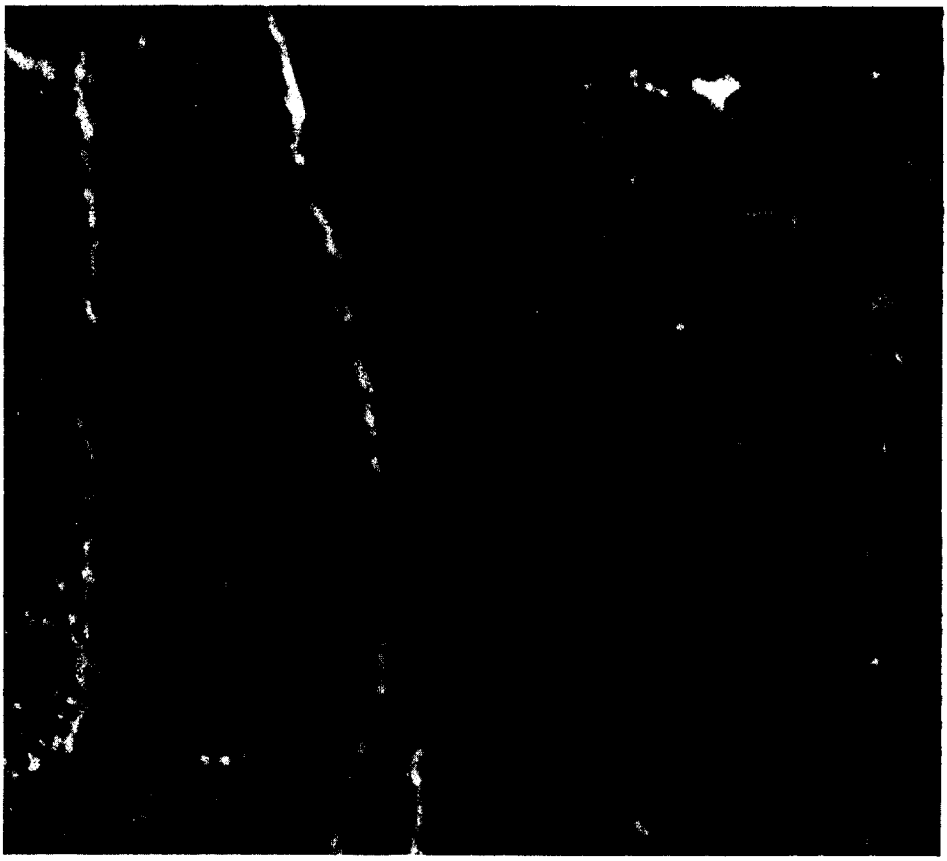


Fig. 2. Fibrils prepared from 0/40 fraction of sodium chloride extract. Magnification × 80,000. 1 cm = 125 mμ.

Reconstituted fibrils were also obtained from the sodium phosphate extract and these resembled the untwisted fibrils from the saline extract. No amino-acid analysis of the phosphate extract was made but, as phosphate buffer is generally regarded as a solvent for collagen[1,2], the extract would be expected to contain hydroxyproline. This, and the somewhat higher content of carbohydrate reactive to hyaluronidase in the phosphate extract, may account for the different fibrils obtained from the two extracts.

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