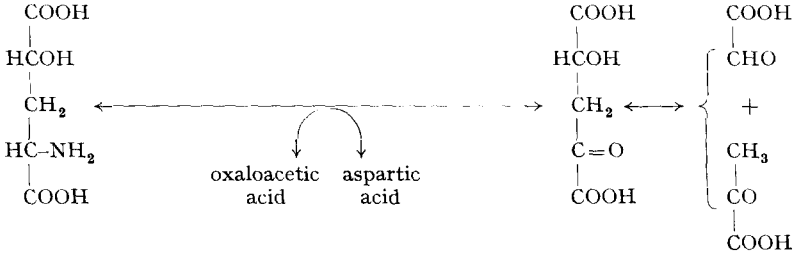
enzyme. In the transaminase reaction, the acceptors of the amino group of γ-hydroxyglutamic acid were oxaloacetic acid or α-ketoglutaric acid, from which aspartic acid or glutamic acid was formed respectively. Formation of amino acids was confirmed by paper chromatography with four different solvent systems. γ-Hydroxy-α-ketoglutaric acid formed by the transaminase had the same properties as the keto acid formed by condensation of pyruvic acid and glyoxylic acid enzymically, and was split into pyruvic and glyoxylic acid in equimolar amounts by adding the condensing enzyme (free of the transaminase). Accordingly, one of the metabolic pathways of γ-hydroxyglutamic acid in rat liver may be shown as follows:



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Calcification *in vivo* of implanted collagen

On the basis of nucleation experiments[1-3] *in vitro*, it has been suggested that formation of the mineral phase of bone is initiated by an interaction between calcium, phosphate and some as yet unidentified template on the collagen polymer. It has further been postulated that inhibitors[4] or promoters[5] of calcification, or differences in the reactivity of hard- and soft-tissue collagens[6], may be factors determining which of the collagenous tissues do or do not undergo mineralization. None of these ideas have been substantiated by evidence gained from experimentation *in vivo*.

In recent studies carried on as part of an investigation of collagen catabolism, we observed that rat-tail tendon and reconstituted collagen implanted in the peritoneal cavities of rats became calcified. The possibility of using a peritoneal implant

technique for studies *in vivo* of the mechanism of calcification was recognized, and the exploratory experiments described here were undertaken. Collagen preparations of several types were implanted into rats and rabbits, and the material was recovered at various intervals for chemical analysis, and for electron microscopy and diffraction. The specimens destined for electron microscopy and diffraction were fixed in an OsO_4–$K_2Cr_2O_7$ mixture, embedded in methacrylate, and sectioned on a Porter-Blum microtome with glass and diamond knives.

Strips of tail tendon from mature rats were implanted into the peritoneal cavities of female Sprague-Dawley rats weighing 100–150 g. Samples of tendon removed up to 30 days appeared unchanged under the electron microscope, did not give electron-diffraction patterns and had essentially the same calcium:hydroxyproline ratio as the material before implantation. Samples removed at 100, 120 and 160 days were

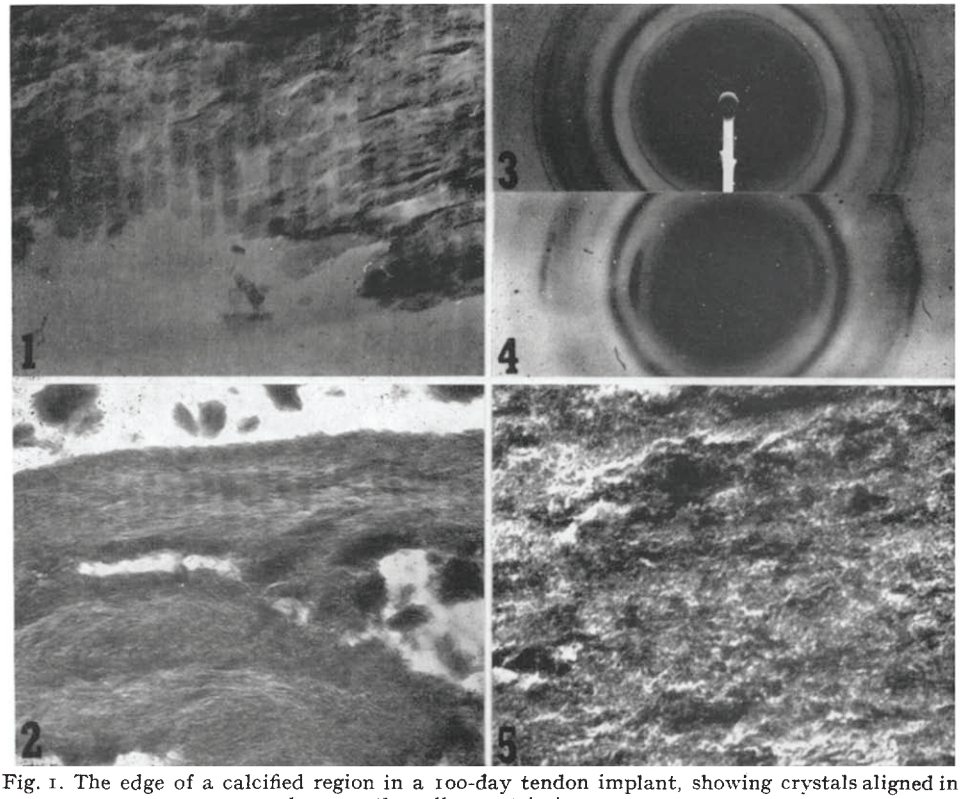

Fig. 1. The edge of a calcified region in a 100-day tendon implant, showing crystals aligned in correspondence to the collagen striation pattern. 50,000 ×.

Fig. 2. An 18-day direct implant of reconstituted collagen, in which the striation pattern is almost entirely masked by mineral. 40,000 ×.

Fig. 3. A standard transmission diffraction pattern obtained from a relatively large area of cross-sectioned fibrils from the same direct implant of reconstituted collagen. Unoriented patterns made in this fashion demonstrate clearly that the mineral present is apatite.

Fig. 4. A selected area diffraction pattern obtained from longitudinally sectioned fibrils in a different region of the preceding preparation. Preferred orientation of the crystals is evident.

Fig. 5. A very heavily mineralized area of reconstituted collagen implanted 160 days in a dialysis bag. Individual fibrils and crystals can no longer be distinguished. 35,000 ×.

found to be hard on gross examination, and marked calcification was indicated by the typically altered calcium: hydroxyproline ratio, and by the presence in the collagen fibrils of microscopically visible crystals, which were identified by diffraction as hydroxyapatite. The crystals were obviously laid down in an array conforming to the pattern established by the periodic spacings of the fibrils (Fig. 1), and the diffraction patterns further indicated that they were preferentially oriented, with their c-axes paralleling the fibril axes. Tail tendon dissected from these same animals at the times when calcified tendon implants were removed showed no evidence of mineralization.

Reconstituted rabbit-skin collagen, in the form of heat-precipitated gels, was implanted either directly or in dialysis bags into the peritoneal cavities of 2-kg female New Zealand white rabbits and 100–150-g female Sprague-Dawley rats. The collagen gels were precipitated at pH 7.4 in either 0.16 *M* NaCl or 0.4 I phosphate buffer. The fibrils reconstituted in these two solutions appeared identical under the electron microscope, and showed the 640-Å periodicity characteristic of native collagen. Mineralization of the directly implanted gels prepared with the phosphate buffer was observed after as short a period as 16 days in both the homologous (rabbit) and heterologous (rat) animals (Fig. 2). Crystals were again seen on the surfaces of and within the fibrils of all the reconstituted collagen implants which calcified. The diffraction patterns indicated that these crystals were also hydroxyapatite (Fig. 3), and that they were likewise oriented with their c-axes paralleling the fibril axes (Fig. 4). Throughout the 160-day course of the experiment, calcification was not detected in the directly implanted gels of collagen reconstituted from the NaCl solutions. Although the results were erratic, some of the gels prepared from NaCl solutions and implanted in dialysis bags were found to be calcified at the end of this period (Fig. 5).

These preliminary results indicate that normally non-mineralizing collagen fibrils can be induced to calcify in an ordered pattern through transplantation to the peritoneal cavity either in their natural state or after purification by means of reconstitution techniques. Challenging possibilities now arise for experimentation *in vivo* with collagen preparations which may be subjected to various treatments prior to implantation, or implanted in combination with agents thought to influence mineralization.

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