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Department of Pathology, Keio University, School of Medicine, Tokyo[1]);
Department of Pathology, Tokai University, School of Medicine, Tokyo[2]);
Department of Obstetrics and Gynecology, Keio University, School of Medicine, Tokyo[3])

An experimental model of calcification in the vessel wall

By H. YAMAGUCHI[1]), T. TAJIMA[2]), M. MORISADA[3]) and H. SAKAGUCHI[1])

With 5 figures

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Address for correspondence: H. YAMAGUCHI, Associate Professor, Department of Pathology, Keio University, School of Medicine, 35 Shinano-machi, Shinjuku-ku, Tokyo, Japan

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Summary

Multiple angiolytic changes of the mesenteric arteries due to administration of vast amounts of Na_2EDTA into the peritoneal cavity of guinea pigs have already been demonstrated by YAMAGUCHI et al. (1981 a, b).

In this experiment, one week after Na_2EDTA administration calcium lactate was administered in the same manner. As a result calcium deposition was observed in the intimal elastic fibers, in and at the collagenous fibers and intermuscular spaces of lytic and ensuing dilatated areas of the vascular wall. This suggests that dystrophic calcification might be induced by conjugation of calcium ions to the free negative charges of acid mucopolysaccharides (aMPS) which are the main component of the above structural elements.

Introduction

Administration of vast amounts of Na_2EDTA leads to various morphological changes in the artery and lung which are thought to be due to the calcium removal from the molecular structure of acid mucopolysaccharides (aMPS) which results in loosening of the matrix and other elements.

In the present investigations the precise mechanism of calcification in relation to the molecular structure of aMPS is studied.

Materials and Methods

Forty guinea pigs of both sexes (body weight 250—300 g) were used. 4—5 ml of 6% Na_2EDTA (approx. 0.178 Mol) saline solution were administered intraperitoneally. The average serum calcium level in normal guinea pigs used in this experiment was 6.8 mEq/l and it dropped down to 4.7 mEq/l within 20 min after injection. All the animals treated in this way suffered from tetanic shock. About 80% of the animals died immediately after administration and the remaining ones recovered within several hours.

One week after Na_2EDTA administration, 5 ml of 6% calcium lactate saline solution were injected into the peritoneal cavity. These animals were sacrificed on the 3rd and 7th d. The samples for light microscopy were prepared using routine techniques and stained with HE and Kossa staining.

The samples for electron microscopy were fixed in phosphate buffered OsO_4 for 2 h, dehydrated in gradual concentrations of alcohol and embedded in epoxy resin. After sectioning, these materials were stained with uranyl acetate and lead citrate and were observed under a JEM 100 B electron microscope.

For control, the same amount of calcium lactate was administered to normal guinea pigs.

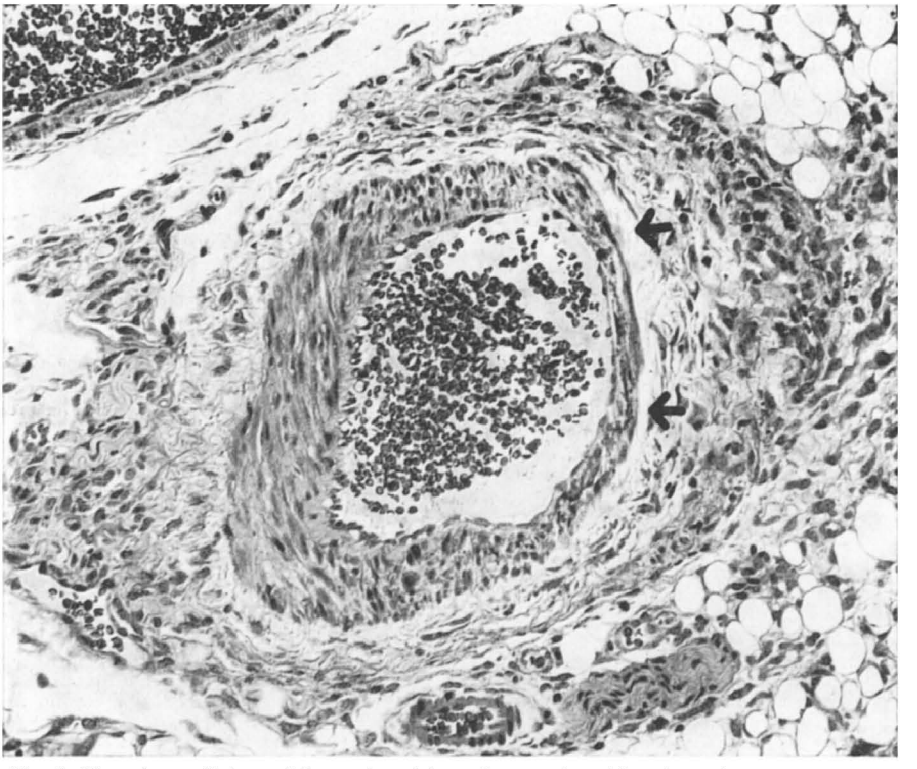

Fig. 1. Vascular wall (arrow) becoming thin and somewhat dilated. ×200.

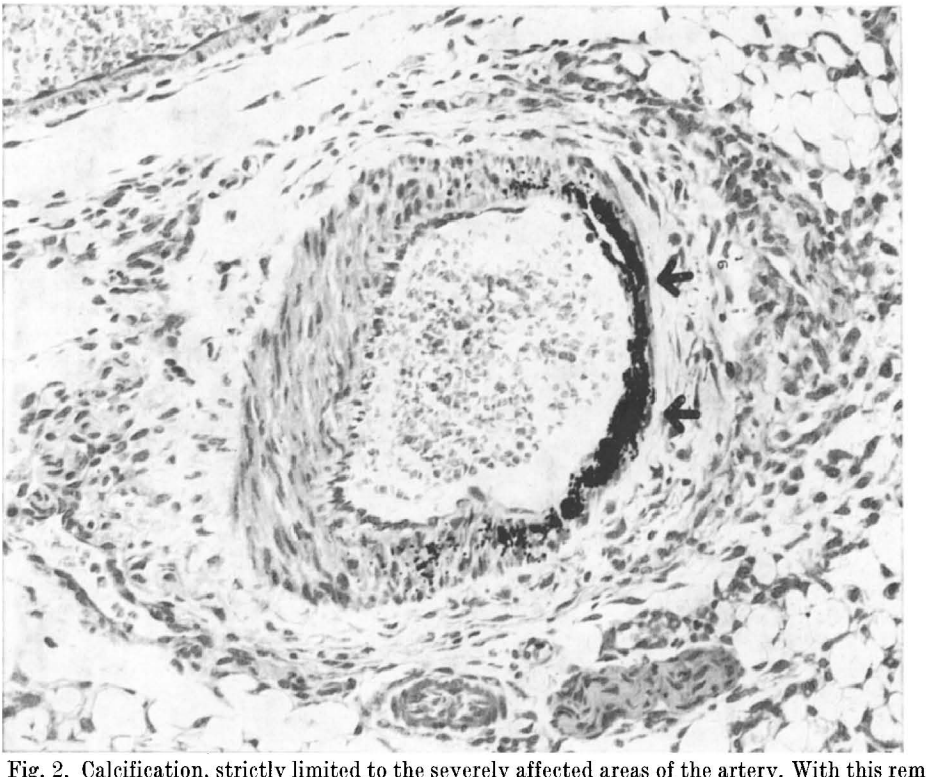

Fig. 2. Calcification, strictly limited to the severely affected areas of the artery. With this remarkable calcification, the cellular elements in the vascular wall become covered. ×200.

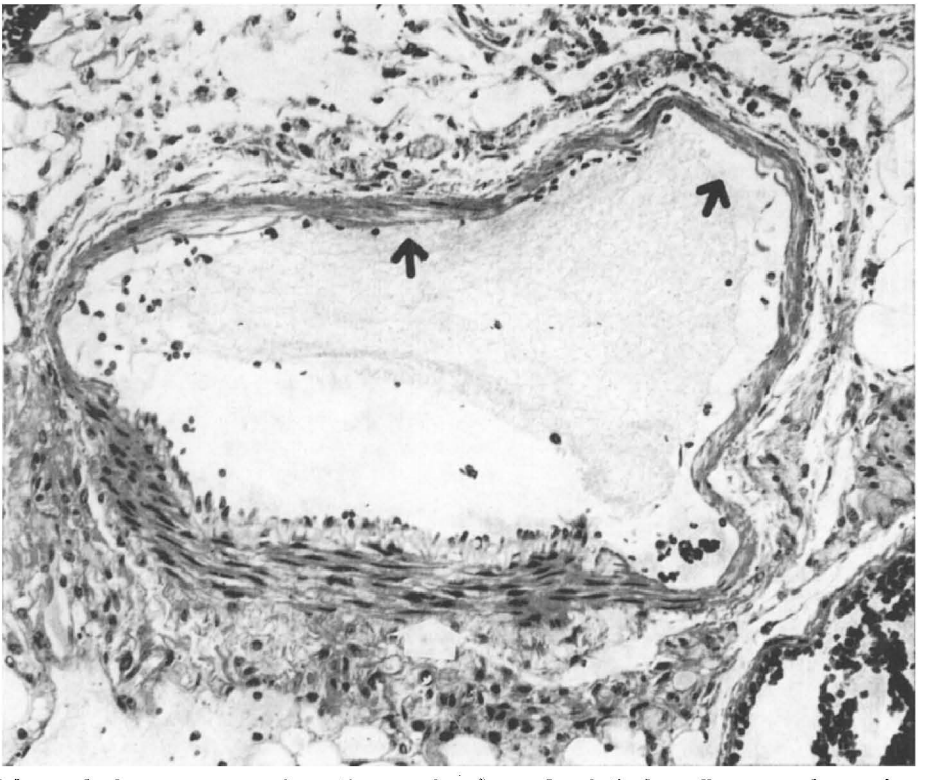

Fig. 3. Advanced phase, aneurysm formation can be observed, relatively well preserved vascular wall (arrow). In the dilated area the vascular wall is remarkably thin. ×200.

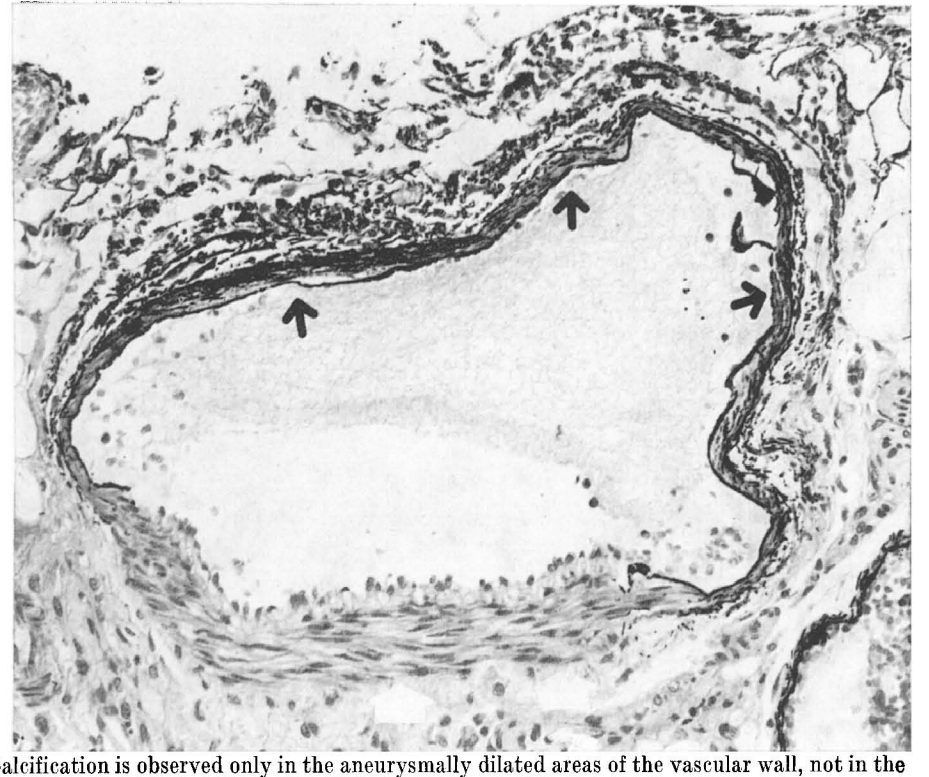

Fig. 4. Calcification is observed only in the aneurysmally dilated areas of the vascular wall, not in the relatively well preserved vascular wall. ×200.

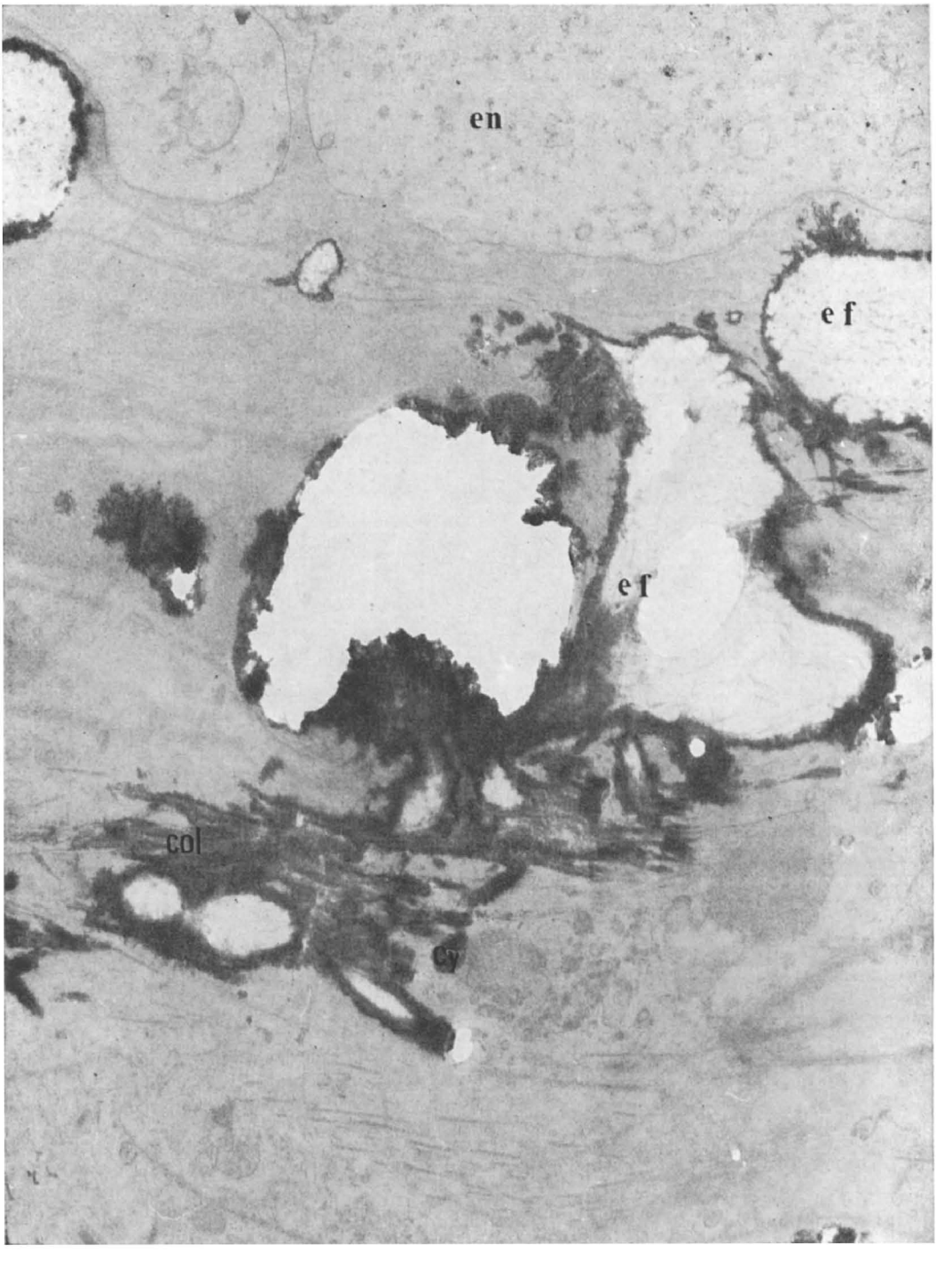

Fig. 5. EM study, calcium deposits are demonstrated in the elastic fibers (ef), the electron lucent core is uncalcified. Calcium deposits are also noted in collagen fibers (col) and form crystalloids (cy) in the intercellular spaces. ×14,000.

Results

As reported in the previous papers, administration of vast amounts of Na_2EDTA intraperitoneally led to multiple angiolysis and/or aneurysm formation of mesenterial arteries.

In our present experiments with administration of 5 ml of 6% calcium lactate saline i.p. injections, we made the following observations:

In the initial phase, calcium deposition could light microscopically be observed along the intimal elastic lamellae and some calcium was scattered in the intermuscular spaces. In the advanced phase, calcium accumulation became remarkable along the elastic fiber, collagen and intercellular spaces, and the cellular components in the vascular wall became indistinct following Kossa staining (figs. 1—4).

Electron microscopic studies of the specimens taken from relatively less calcified areas revealed that in these areas of the vascular wall, the intermuscular spaces were expanded and the smooth muscle cells became spindle-shaped. Elastic lamellae fragmented into pieces and appeared worm-eaten as reported in the previous papers. The calcium deposition accumulated remarkably around the elastic fibers and the electron lucent core remained uncalcified. But in some areas calcium was deposited in the electron lucent core of the elastic fiber, being tooth-comb like in appearance. Calcium deposits were also noticed in collagen fibers. Calcium crystalloids were also observed in the expanded intercellular spaces (fig. 5).

Discussion

As shown in the previous papers, administration of vast amounts of Na_2EDTA led to angiolytic and/or aneurysmal changes without degenerative changes in the constituent cells. These changes were suggested to be due to the rapid removal of calcium ions from the molecular structure of aMPS of the matrix of the vessels. These changes provoke the loosening of the vascular wall which results in loosening and weakening of their structure, i.e. angiolysis and/or aneurysm formation of the affected vascular walls.

Adding of the same amount of calcium lactate to these animals led to calcium deposition strictly limited to the affected areas of the vascular walls.

The calcification is known to take place in the ground substance (Utermann et al. 1966), in and at the collagenous (Keeley et al. 1974) and elastic fibers (Banga 1969; Meyer et al. 1968). However, as a matter of fact, the precise mechanism of the initial calcification has not been clarified yet.

Dead or degenerating tissue in the body often becomes impregnated with deposits of calcium salts. There are two main types of pathological calcification, dystrophic and metastatic calcification.

Phosphatase (Gomori 1943), phosphorylase (Berthet et al. 1951), glycolytic enzymes, structural characters of organic matrix and local changes in *p*H have been thought to be concerned in calcification, but the precise roles of these factors have not been determined yet.

There is little doubt that calcification can be favoured by other local changes which have not yet been demonstrated. A local increase in the concentration of free calcium ions, as well as in the concentration of phosphate, can help in the formation of calcium salt deposits. Dystrophic calcification might be favoured by the breakdown of some substances able to bind calcium in non-ionic form. It might be favoured by the uncovering of tissues that provide a suitable matrix for the initial deposition of calcium phosphate. Calcification may be initiated by a reaction between the organic matrix and the ions to be deposited, which provides a nucleus for the formation of a crystal hydroxyapatite ($3\ Ca_3(PO_4)_2Ca(OH)_2$); once formed, the crystal can be expected to grow at a rate which depends on the concentrations of calcium and phosphate.

Gutman and Gutman (1941) found that each of the sulphuric acid groups and glucuronic acid groups of chondroitin sulphate, which are present in the ground substance, can bind calcium ions and subsequently phosphate can be taken up until the Ca/P ratio is that of

hydroxyapatite. The importance of these particular reactions *in vivo* is uncertain. Considering our experiment in which the animals were administered vast amounts of Na_2EDTA, calcium removal from the matrix is expected. Accordingly, it is also expected that the matrix among the vascular wall components becomes loosened and the negative charges of aMPS become apparent.

Morphologically, angiolysis and/or aneurysmal changes of the vascular wall were observed. Calcium ions added to the affected vessels might bind to the negative charges of aMPS of the matrix, because this negatively charged aMPS could provide the suitable site for conjugation of calcium ions and lead to calcification.

Our results might explain the mechanism of dystrophic calcification in relation to the altered connective tissue.

Literature

Banga, J., Biochemistry of elastin and its quantitative proportion in the vessel wall. In: Biochemie der Gefäßwand. Hrsg. M. Comél and L. Laszt. Karger, Basel-New York 1969, pp. 18—92.

Berthet, J., and C. de Duve, Tissue fractionation studies. I. The existence of a mitochondria-linked, enzymicly inactive form of acid phosphatase in rat liver tissue. Biochem. J. **50**, 174—181 (1951).

Gomori, G., Calcification and phosphatase. Am. J. Path. **19**, 197—209 (1943).

Gutman, A. B., and E. D. Gutman, A phosphorylase in calcifying cartilage. Proc. Soc. exp. Biol. **48**, 687—691 (1941).

Keeley, F. W., and S. M. Partridge, Amino acid composition and calcification of human aortic elastin. Atherosclerosis **19**, 287—296 (1974).

Meyer, W. W., Calcinose der "foetalen Strecke" der Beckenarterien im Kindesalter. Beitr. Path. Anat. **138**, 149—162 (1968).

Utermann, D., C. Erich, K. Martens and H. Hilz, Über Veränderungen des Gefäßstoffwechsels bei experimenteller Rattenarteriosklerose vom Mönckeberg-Typ. Klin. Wschr. **44**, 1028—1032 (1966).

Yamaguchi, H., H. Usui and T. Tajima, Multiple angiitis of mesenterial arteries of guinea pigs induced by intraperitoneal administration of vast amounts of Na_2EDTA — light microscopic study. Exp. Path. **20**, 26—30 (1981).

— — — and H. Sakaguchi, Multiple angiitis of mesenterial arteries of guinea pigs induced by intraperitoneal administration of vast amounts of Na_2EDTA — electron microscopic study. Exp. Path. **20**, 31—40 (1981).