$^{3}$H-reserpine (corresponding to those taken up by the membranes of intact organelles), only about 30% of the drug go to the membrane, whereas 70% remain in the supernatant. The different distribution of $^{3}$H-reserpine and $^{14}$C-tyramine within the 5-hydroxytryptamine organelles of platelets subjected to the drugs in vitro and in vivo might therefore indicate that reserpine acts mainly at the level of the membrane and tyramine preferentially in the interior of the 5-hydroxytryptamine organelles.

According to recent findings[12], 5-hydroxytryptamine and adenosine-triphosphate of the organelles of platelets form mixed micelles of high molecular weight. Tyramine might replace the 5-hydroxytryptamine of these micelles. Reserpine, in contrast, possibly acts by altering the membrane of the organelles. The nature of this postulated change as well as its possible consequences with regard to the stability of the 5-hydroxytryptamine-adenosine-triphosphate micelles remains to be investigated. Up to now, no evidence exists that the storage of 5-hydroxytryptamine is a process depending on an active (i.e. energy-dependent) transport through the membrane of the organelles which would be impaired by reserpine[13,14].

In conclusion, the experiments with subcellular fractionation of blood platelets show that reserpine probably acts at the level of the membrane, whereas tyramine seems to have its site of action in the interior of the 5-hydroxytryptamine organelles.

*Zusammenfassung.* Die subzelluläre Lokalisation von Reserpin und Tyramin in Blutplättchen von Kaninchen erfolgt in vitro und in vivo grösstenteils in den 5-Hydroxytryptamin (5HT)-Organellen. Dabei reichert sich Reserpin vorwiegend in der Membran, Tyramin hauptsächlich im Inneren der Organellen an. Es wird vermutet, dass Reserpin in bezug auf 5HT-Freisetzung an der Membran und Tyramin im Inneren der 5HT-Organellen angreift.

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## Calcification in Implants of Tendon

In vitro studies[1-5], conducted over a decade ago, demonstrate that calf tail tendon and reconstituted collagen fibers could nucleate and crystallize mineral, chemically and radiographically similar to bone salt, from metastable solutions of calcium and phosphate ions. When nuclei are present, secondary nucleation and crystal growth follows, even at calcium and phosphate ion concentrations nearly as low as in normal blood serum[6,7]. The theory is that the formation of crystals of hydroxyapatite occurs by heterogeneous nucleation[1-9], but the question whether a collagen-calcium, or a collagen-phosphate, or a collagen-calcium-phosphate complex, or collagen itself is the nucleation site cannot be answered by experiments with solutions containing both calcium and phosphate ions in systems free of inhibitors in vitro.

Some of the objections to in vitro methods can be overcome by experiments on calcification of implants of normal tendon in rats with normal body fluid organic constituents and ion concentrations of calcium and phosphate of only 1 mmole/l. Implants of KCl-extracted tendon and various forms of reconstituted tendon collagen do not calcify with any degree of consistency, or to any appreciable extent, under these conditions in vivo. Implants of tendon calcify consistently when implanted in the anterior eye chamber of the rat after they are exposed to solutions containing 25 mmoles $Ca^{++}$/l, but not after exposure to phosphate ion concentrations even as high as 500 mmoles/l[10-12]. These observations on calcification in implants of calcium-treated tendon in vivo are corroborated by WADKINS[13] in a recent report of experiments on calcification in vitro.

To quantitate the process of calcification, experiments were performed to determine the relative amount of mineral per total mass of tendon deposited in uncalcified, partially calcified, and completely calcified implants after periods as long as 35 days. In addition, experiments were also performed to determine the effect of nuclei of apatite produced in vitro upon the quantity of mineral deposited in vivo in tendon over a similarly long period of time.

Samples of Achilles tendon of young New Zealand rabbits were cut into segments, about 1 cm in length, and equilibrated at 2 °C: (a) in 1.5, 5.0, 10.0 and 15.0 mmolar solutions of calcium for 10 h, or (b) in nucleating and precipitating mixtures of calcium and phosphate ions, pH 7.4 and ionic strength 0.16, for 10 days. Nucleation and precipitation were assessed by methods presented in previous publications[2,4]. Experimental and control (0.9% NaCl-treated) preparations of tendon were aseptically implanted into pouches in the anterior abdominal wall muscles of allogeneic rabbits as previously described[14]. Implants were recovered at 5–7 weeks after

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the operation, examined roentgenographically and histologically (von Kossa stain), ashed, and analyzed for calcium by atomic absorption spectrophotometry[15].

Figure 1 demonstrates the relationship between the calcium content of the implants, or the percentage of the dry weight of the implant that is mineralized, on the one hand, and the concentration of calcium solutions to which tissues were exposed before implantation, on the other. Chemically and roentgenographically positive evidence of calcification was obtained when tendons were treated with 15.0 mmolar solutions of calcium, but generally not with 10.0 mmoles $Ca^{++}$/l or less, prior to implantation. Even with 15.0 mmolar calcium solutions, only 8–23% of the dry weight of the tendon was calcified.

In Figure 2 are presented data relating nucleation and precipitation of apatite mineral by or in the presence of tendon, before implantation, to the ash content of the implants. In the absence of tendon (open circles in Figure 2), mixtures of solutions of calcium and phosphate do not precipitate until the concentration product Ca X P, expressed in mmoles/l[16], exceeds the precipitation point ($\cong$11.8 at 2 °C). When precipitation takes place, a solid phase of low solubility is obtained. A similar precipitate (hydroxyapatite) has been also obtained at room temperature and at 37 °C[1,2]. In the presence of tendon (closed circles in Figure 2), nucleation of mineral occurred at concentration products of Ca X P at least as low as 5.9; precipitation was initially inhibited (unpublished observations), but the total amount of precipitate formed after 10 days was of the same order of magnitude with or without tendon. Calcification, estimated from the percentage of ash per dry weight of the implants, was in no case less than 50%. Thus, despite the looser weave pattern and the relatively long periods of exposure to body fluids (7 weeks), tendon never calcified as fully as compact bone (73% ash)[17].

The data presented in this study suggest that calcification requires initially relatively large quantities of calcium and phosphate to transform tissue into calcifiable matrix. Exposure of tendon to calcium ions produces calcification of 8–23% of an implant of tendon. Simultaneous exposure to both calcium and phosphate ions in nucleating and/or precipitating solutions of physiological pH and ionic strength produces calcification of 60–80% of the dry weight of the tendon[18].

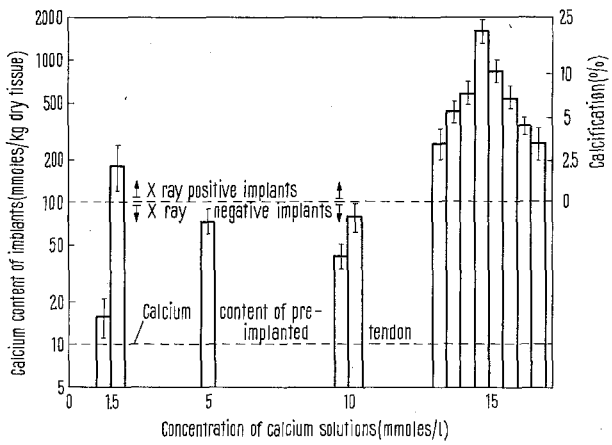


Fig. 1. Priming of tendon with calcium ions and calcification in vivo. Each bar represents average values for 3 implants.

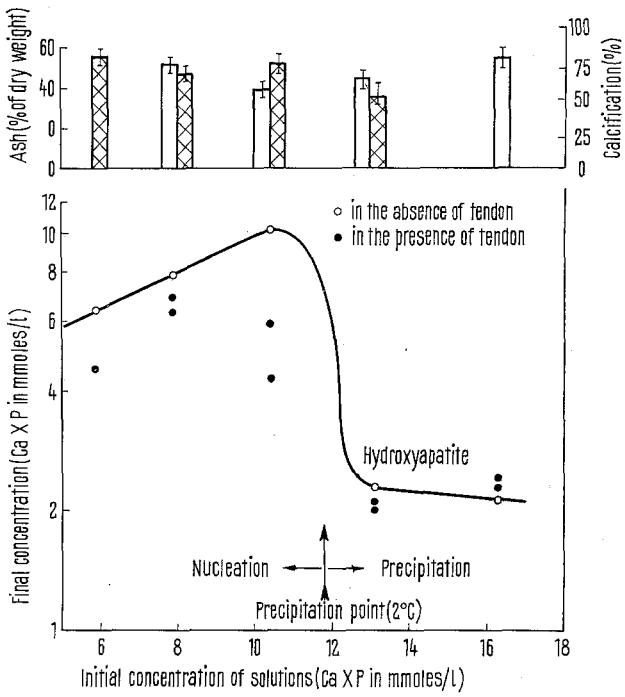


Fig. 2. Nucleation and/or crystal formation of apatite in tendon before and after implantation. All solutions had an ionic strength of 0.16 and were buffered at pH 7.4 with 0.02 moles/l barbital acid. Initial concentration refers to the concentration product CaXP before equilibration, final concentration to the same product after an equilibration period of 10 days. Horizontal arrows point to areas of concentration products CaXP at which nucleation or precipitation of hydroxyapatite occurs. Each point is average of triplicate determinations and each bar represents averaged values for 3 implants. Open bars are for 5-week-old implants, bars with cross-hatched lines for 7-week-old implants.

*Zusammenfassung.* Behandlung von Hasenmuskeln vor der Implantation mit Kalzium- und Phosphatlösungen bei 2 °C ergibt Mineralisierung der Sehnen bis zu 60–80%, mit 15 molaren Kalziumlösungen bis zu 8–23%. Zur Kalzifikation sind grössere $Ca^{++}$-Mengen nötig: werden gleichzeitig Kalzium- und Phosphationen verwendet, so sind kleinere Mengen zur vermehrten Apatitablagerung notwendig.

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