Spontaneous Mineral Deposition in Sponge Biopsy Connective Tissue.* (26323)

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(Introduced by R. J. Boucek)
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Adequate amounts of homogeneous connective tissue are unobtainable for most electrolyte metabolism studies. Tendon, skin and cartilage are heterogeneous structures with variation in electrolyte composition from one region to another, and pooling of samples often is required to supply sufficient material for quantitative analysis. Introduction of polyvinyl alcohol sponges beneath the dorsal skin of rats, guinea pigs and rabbits is followed by ingrowth of an essentially reproducible and relatively non-inflammatory connective tissue(1). The suitability of this experimental system for study of connective tissue electrolytes is herein evaluated.

Methods. Employing the technic of Boucek and Noble(1), Ivalon† polyvinyl alcohol sponges and other plastics were implanted in 50 Sprague-Dawley rats weighing 180-240 g each, including both sexes. Granulomas, harvested at 40 and 90 days, were weighed, dried overnight in an oven at 110°C, extracted with ether and acetone, and ashed in a muffle furnace at 700°C for 36 hours. The ash was dissolved in hydrochloric acid and subjected to the following analyses: 1) sodium and potassium, using the Baird flame photometer with internal standards(2); 2) calcium, by titration with potassium permanganate after precipitation with ammonium oxalate(3); and 3) phosphorus by the method of Fiske and Subbarow(4). Determinations were run in duplicate, usually agreeing within 3%. Subsequent to fixation of tissues for 24 hours in 10% neutral formalin and conventional processing, sections were cut at 6 μ thickness, mounted, stained by a Harris hematoxylin eosin sequence and toluidine blue. Fresh frozen sponge tissue was treated in an absolute alcohol-formalin xylol sequence, cut at 6 μ, mounted on quartz slides and microincinerated. Histochemical identification of inorganic phosphate was made by the Von Kossa staining procedure applied to absolute alcohol-fixed tissue. Polyvinyl alcohol sponge, soaked in silicone-resin‡ and dried overnight, as well as 3 additional plastics were implanted in separate groups of animals (Table II); the resulting tissue ingrowths were analyzed histologically as above.

Results. The high mean calcium and phosphorus content of the sponge tissue is apparent (Table I). Calcium to phosphorus ratios in 40 and 90-day sponges were 2.15 and 2.37 respectively—slightly above the limit expected if the salt were hydroxyapatite(5). "Bone salt" content approximately doubled in the period from 40 to 90 days. Two sponges indicated a 3- to 4-fold further rise of calcium and phosphorus content after 6 months of implantation. Another sponge, removed after one year, had become a solid block of mineral deposits. Sodium and potassium levels in the 40-day sponge were similar to those of other dense connective tissues (Table I)(6), and did not alter in the 90-day sponge, except for an insignificant decrease of sodium.

Histologic sections from 40 and 90-day sponges revealed a dense ingrowth of homogeneous connective tissue sparsely populated with fibroblasts, but lacking signs of inflammation. There was no evidence of systemic or local infection. Bluish granules in the hematoxylin-stained tissue were observed along the plastic margin in some sections. However, Von Kossa stains indicated a strongly positive dark granular band along the entire plastic-tissue interface of all 60 slides studied. An artifact related to fixation technic could not have caused this phenome-

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TABLE I. Electrolyte Content of Sponge Biopsy Connective Tissue.*

Tissue		Calcium	Phosphorus	Sodium	Potassium
		mM		meq	
40-day sponge	Mean	594	276.0	73.2	5.28
n = 9	S.E.	67.8	40.8	4.26	.78
	95% C.L.	438–750	182–369	63.3–83.1	3.42–7.14
90-day sponge	Mean	1224	516	60.6	4.68
n = 12	S.E.	153	42.3	3.00	.51
	95% C.L.	888–1560	423–609	54.0–67.2	3.54–5.82

* Per kg wet tissue. Data calculated on basis of fat-free dry tissue showed no greater homogeneity.

non inasmuch as microincineration studies of 40-day sponges confirmed the presence of a dense collection of inorganic material distributed along the plastic margin. Also, microincinerated and Von Kossa-stained sections from 4 unimplanted sponges were negative before and after incubation at 5° C for 48 hours in normal rat serum. In contrast to findings of Rubin and Howard(7), who studied a variety of pathologic calcifications, no metachromasia by toluidine blue staining was found in either the 40 or 90-day granulomas after removal of mineral deposits with dilute acid.

Other plastics evaluated in regard to deposit of mineral salts were studied at an early phase of tissue ingrowth. After 14 days implantation, polyvinyl alcohol sponges reveal a peripheral invasion by connective tissue for a distance of approximately 3-4 mm (Table II). Von Kossa stains were positive along the plastic margin only where contiguous with new cellular tissue (Fig. 1). Central region of the sponge containing lymph had no trace of Von Kossa staining. The silicone-coated sponges showed a similar ingrowth of tissue, but the Von Kossa staining was dense along the outside margin of the sponge, whereas little staining appeared along the invading tissue margins. The polyurethane, cellulose acetate sponges and polyethylene sheets induced a slight marginal reaction by the Von Kossa method. Of these plastics, only cellulose acetate induced granulomas comparable to those in polyvinyl alcohol sponge implants, but in contrast to the latter, showed a considerable polymorphonuclear leucocyte infiltration and local edema.

Discussion. As a model system for studying electrolytes in dense connective tissue, the sponge biopsy deviates from the ideal because of the deposition of "bone salt" accumulating at the plastic surface. Hydroxyapatite is believed to be the principal component of the lining material, inasmuch as the calcium and phosphorus content is high and hydroxyapa-

TABLE II. Histologic Studies of Plastic Implants under Dorsal Skin of Rats.*

Plastic	Duration of implant (days)	Tissue ingrowth (mm)	Von Kossa stain
Polyvinyl alcohol (sponge)	14	3–4	Strongly positive reaction distributed along entire plastic-tissue interface.
Polyvinyl alcohol (silicone coated sponge)	14	3–4	Moderately positive reaction only in scattered peripheral surface areas of sponge.
Cellulose acetate (sponge)	14	3	Moderate positive reaction in scattered peripheral surface areas of the plastic. No positive reaction seen in new granulation tissue.
Polyurethane (sponge)	14	.5–1	Negative reaction.
Polyethylene (sheets)	14 and 50	Layer of tissue (.25 mm thick)	*Idem.*

* No. of implants = 4 for each plastic in 10 rats.

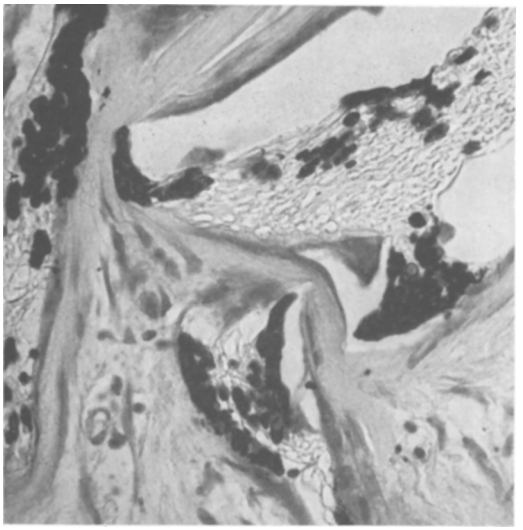

FIG. 1. 132×. Photomicrograph of 14-day sponge implant. Note Von Kossa positive staining granules confined to tissue-plastic interface.

tite is the prevailing salt found in mammalian tissues. Sodium and potassium content of the connective tissue as well as its histologic appearance was similar to that of other dense connective tissues, such as corium and tendon (8).

"Bone salt" accumulation could not be accounted for in the present experiments. Polyvinyl alcohol§ is a linear polymer with hydroxyl groups as the only regions of the molecule which would seem possible sites for nucleation of inorganic salts from ions in the adjacent lymph. In regard to these phases (9), interactions such as enzymatic esterification of hydroxyl groups with phosphate, or linkage of the plastic with calcium, phosphate or preformed hydroxyapatite are possibilities which have not been investigated. The data in Table II suggest that salt formation of the type demonstrated in this study requires the presence of living tissue adjacent to the plastic. Possibly an enzymatic reaction or other local conditions set by the presence of cells, fibers or their associated matrix is needed to induce the "bone salt" deposits. Decrease of the mineral deposition in silicone-coated sponges and failure of mineral deposition to occur in other plastics favor the view that polyvinyl alcohol engenders this phenomenon with some degree of specificity. The current data suggest that surgical use of polyvinyl alcohol sponges as pliable prosthetic devices in repair of heart valves, septal perforations and cosmetic defects, might show evidence eventually of significant mineral deposits(10).

§ Polymerized with formaldehyde.

Summary. Calcium and phosphorus content of connective tissue grown in rats implanted with polyvinyl alcohol sponges for 40 and 90 days was at high levels, which when correlated with histologic findings were indicative of salt formation. It appears that polyvinyl alcohol sponge has a greater tendency, from short term *in vivo* observations, to induce mineral deposits along its surfaces than other plastics tested. The data indicate a potential disadvantage to the usage of this plastic as a pliable prosthesis in human surgery.

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1. Boucek, R. J., Noble, N. L., *Arch. Path.,* 1955, v59, 53.

2. Baird Associates Flame Photometer Manual. Cambridge, Mass., Baird Assoc. Inc., 1956, 32.

3. Willard, H. H., Furman, N. H., *Elementary Quantitative Analysis,* Van Nostrand, 1940, 235.

4. Consolazio, C. F., Johnson, R. E., Marek, E., *Metabolic Methods,* C. V. Mosby, St. Louis, 1951, 109.

5. Neuman, W. F., Neuman, M. W., *The Chemical Dynamics of Bone Mineral,* Univ. of Chicago Press, Chicago, 1958.

6. Manery, J. F., *Physiol. Rev.,* 1954, v34, 334.

7. Rubin, P. S., Howard, J. E., Josiah Macy Jr. Foundation, Tr. 2nd Conf. on Metab. Interrelations, 1950, v155.

8. Audia, M., *Am. J. Physiol.,* 1958, v195, 702.

9. Elden, H. R., Howell, D. S., *Fed. Proc.,* 1960, v19, 142.

10. Fitch, E. A., Denman, F. R., Waldron, G. W., *Arch. Surg.,* 1960, v81, 164.

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