Influence of Age on Calcification in Homologous Transplants in Rats

It is well known that with advancing age, the calcium content of soft tissues increases[1-4]; gross calcification often occurs in the cardiovascular system, periarticular tissues, tendons and urinary passages whereas bones become demineralized and brittle[5]. Nevertheless, old animals are resistant to many types of experimental ectopic calcification[6-10].

Preliminary experiments have shown that, in the rat, cutaneous homografts from young donors to young recipients exhibit constant and widespread calcification of the surface epithelium and hair follicles[11]. The influence of age on the calcification of skin and aorta transplants is further examined in the present experiment.

Materials and methods. Young (28 days old and with a mean body weight of 90 g) and old (retired breeders, approximately 1 year old and with an average weight of 360 g) female Sprague-Dawley rats (Holtzman Farms, Madison, Wisc., USA) were used. The animals were maintained on Purina Laboratory Chow and tap water.

Four groups, each consisting of 10 young and 10 old rats received transplants from 10 young and 10 old donors. Cutaneous homografts were taken from the shaved dorsal skin and introduced into the recipients according to the technique for subcutaneous transplants previously described[11]. Specimens of the thoracic aorta were also implanted using the same technique.

The recipients were killed 7 days after receiving the homografts which were removed at autopsy for demonstration of calcification by means of the von Kóssa technique. Total calcium and inorganic phosphorus in the skin and aorta were determined by atomic absorption spectroscopy[12] and the FISKE and SUBBAROW technique[13] respectively. The means and standard errors of the groups were calculated and the data were then compared by means of the Student's *t*-test.

Results. Table I shows that cutaneous homografts from young (28 days) or old (360 days) donors implanted subcutaneously into young recipients exhibit constant and widespread calcification which is limited to the surface epithelium and the hair follicles (groups 1 and 3). Comparable skin transplants virtually failed to calcify in old recipients (groups 2 and 4). On the 7th day after transplantation, when these observations were made, leucocytic infiltration was present but no necrosis of the connective tissue elements was evident. The calcium and phosphorus content of the skin implanted into young recipients significantly increased ($P < 0.001$) after transplantation (groups 1 and 3). Only the calcium content of the grafts into old recipients (groups 2 and 4) increased ($P < 0.001$) but to a lesser degree ($P < 0.001$) than in those implanted into young rats.

Similar results were obtained in aorta transplants (Table II): sections of aorta from young or old donors calcified only if implanted into young recipients (groups 1 and 3). Histologically, the calcium deposits were located in the elastic lamellae and extended into the interlamellar smooth muscle layers. The calcium and phosphorus content increased significantly ($P < 0.001$) when compared with the values obtained before transplantation. When aortas were placed into old recipients (groups 2 and 4), only mild edema and fragmentation of elastic

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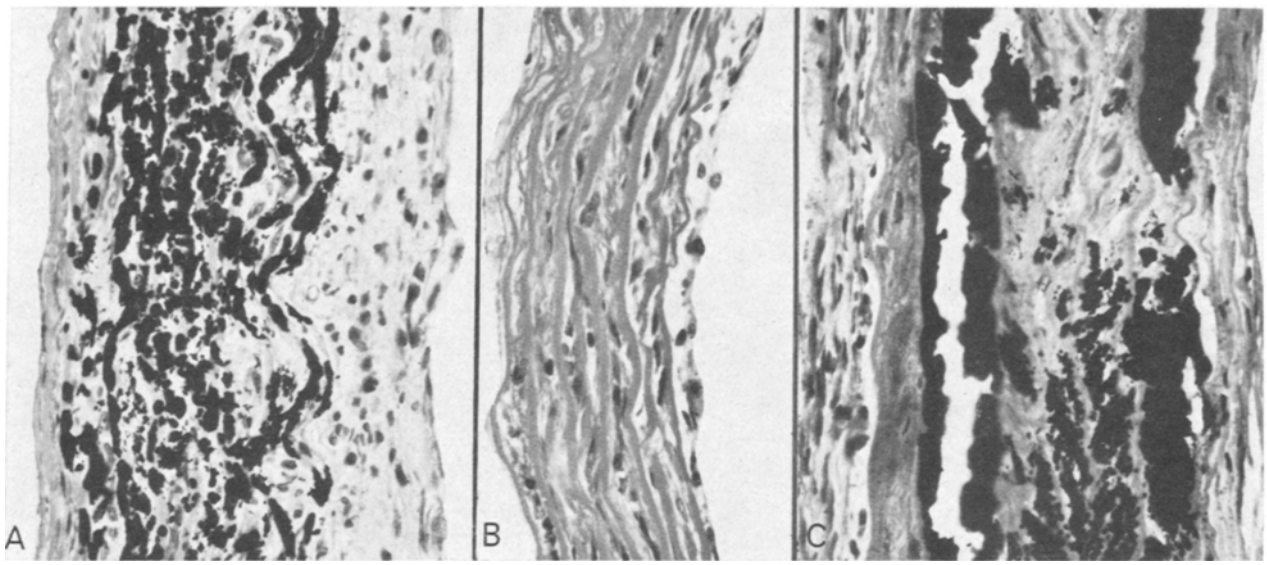
Table I. Influence of age on calcification in cutaneous homografts

Group	Age (days) Donors	Recipients	Histological analysis (scale 0–3)	Chemical analysis Calcium (mg/g dry tissue) Before transplant	Calcium (mg/g dry tissue) After transplant	Phosphorus (mg/g dry tissue) Before transplant	Phosphorus (mg/g dry tissue) After transplant
1	28	28	2.3	0.4 ± 0.02	11.4 ± 1.0	3.7 ± 0.2	10.4 ± 0.7
2	28	365	0	0.4 ± 0.22	1.0 ± 0.06	3.7 ± 0.2	4.4 ± 0.2
3	365	28	2.0	0.3 ± 0.02	10.0 ± 0.9	2.3 ± 0.1	8.4 ± 0.3
4	365	365	0	0.3 ± 0.02	0.8 ± 0.04	2.3 ± 0.1	2.2 ± 0.3

Table II. Influence of age on calcification in aorta transplants

Group	Age (days) Donors	Recipients	Histological analysis (scale 0–3)	Chemical analysis Calcium (mg/g dry tissue) Before transplant	Calcium (mg/g dry tissue) After transplant	Phosphorus (mg/g dry tissue) Before transplant	Phosphorus (mg/g dry tissue) After transplant
1	28	28	3.0	1.0 ± 0.1	97.0 ± 8.7	2.9 ± 0.1	56.8 ± 5.2
2	28	365	0	1.0 ± 0.1	5.9 ± 0.8	2.9 ± 0.1	5.9 ± 0.6
3	365	28	2.8	1.4 ± 0.1	77.0 ± 4.4	3.1 ± 0.2	53.3 ± 5.3
4	365	365	0.2	1.4 ± 0.1	6.8 ± 1.8	3.1 ± 0.2	7.4 ± 1.1



Influence of age on calcification in aorta transplants. (A) Aorta from a young rat showing calcium deposits in elastic lamellae after transplantation into a young recipient (von Kóssa, × 320). (B) Aorta from a young donor failed to calcify after implantation into an old recipient (von Kóssa, × 320). (C) Pronounced foci of mineralization in an aorta from an old donor after transplantation into a young recipient (von Kóssa, × 320).

fibers were observed (Figure). The mineral content increased significantly ($P < 0.005$) but less ($P < 0.001$) than in the aortas implanted into young rats.

Discussion. It appears from our results that the age factor is important in the calcification of skin and aorta transplants. Previously, it was reported that collagen calcifies more markedly in the peritoneal cavity of young than of old rats[14].

Probably the changes in calcium and phosphorus metabolism occurring with advancing age play a more important role than local factors in the resistance of old animals to the experimental skin calcinosis induced by metallic salts[10]. A similar mechanism may account for the results obtained in the present experiments. URIST et al.[15] found that aorta transplants calcify slightly if obtained from rats up to 3 weeks old, while those from mature and older rats calcify in all instances. Here we show that the calcification of aorta or skin transplants depends also on the age of the recipient. It is noteworthy that the aorta or skin from an old donor does not calcify in an old recipient, even though local factors such as chemical changes of elastin[16] or collagen[17,18] should favor its calcification. The mechanism of this resistance is now being investigated[19].

Résumé. Chez le rat, des homogreffes de peau ou d'aorte implantées dans le tissu souscutané ne se calcifient que si les receveurs sont jeunes (28 jours), quel que soit l'âge du donneur. Les dépôts calcaires sont localisés dans l'épithélium et les follicules pileux de la peau ainsi que dans les lamelles élastiques de l'aorte.

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The Effects of Prostaglandin E_1 on the Systemic and Pulmonary Circulations of Intact Dogs. The Influence of Urethane and Pentobarbital Anesthesia[1]

In our initial studies of the effects of prostaglandin E_1 (PGE_1) on the pulmonary and systemic circulations of intact dogs anesthetized with urethane, results were obtained which differed considerably from those reported by others who used barbiturate anesthesia[2-6]. However, barbiturates have been reported to influence the response of the dog to infusion of PGE_1[7]. The present study was performed to learn if the hemodynamic effects of PGE_1 are influenced by pentobarbital and urethane anesthesia in dogs and to study the physiologic mechanisms responsible for the differences. The effects of PGE_1 on the pulmonary circulation, particularly the small pulmonary veins was also studied.

Methods. 14 mongrel dogs (average weight, 16.99 kg) were used for these experiments. 8 dogs were anesthetized with urethane (1.5 g/kg) i.v. and 6 dogs were anesthetized with sodium pentobarbital (22 mg/kg) i.v. Catheters were placed in the aorta, right atrium, right ventricle, pulmonary artery, a peripheral leg vein and transeptally into a small vein draining the left lower lobe