Bone Formation in Heterotopic Implants of Kidney Tissue[1] (35638)

MEHDI TAVASSOLI AND WILLIAM H. CROSBY

Blood Research Laboratory, New England Medical Center Hospitals and the Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts 02111

Osteogenesis has been observed in ischemic renal tissues in the rabbit, cat, guinea pig, and rat. Bridges (1) has reviewed this subject and added some of his own observations. In the usual investigative model, ischemia is induced by ligation of the renal artery; osteogenesis then appears, first in the medulla and then in other parts of the kidney. It has been suggested that the transitional epithelium of the pelvis is the stimulus to bone formation.

We have observed bone formation in subcutaneous implants of kidney fragments in rats. This model has allowed observations of the osteogenic potential of different parts of the kidney separately.

Materials and Methods. Male Wistar rats (300–400 g) were used. Nembutal was given for anesthesia and all operations were carried out under aseptic conditions. Four groups of animals were studied as follows:

Group I. Right nephrectomy was performed in 78 rats; and the kidney, in whole or part, was implanted in a pocket in the subcutaneous tissue of the abdomen. In 23 of these animals, the kidney was sliced longitudinally into two halves and each half was implanted separately. In 37 animals, the kidney was sliced into wedge-shaped pieces and each piece was implanted separately. In 18 rats, the whole kidney was implanted. The implants were removed every 2 days up to 20 days for short-term studies and every 2 months up to 7 months for long-term studies.

Group II. In 20 rats, fragments of the cortex, at least 5–6 mm in diameter, were removed from the kidney which had been sliced longitudinally into two halves, care being taken not to include any medulla. They were then implanted in a subcutaneous site.

Group III. Subcutaneous implants of fragments of medulla were made in another 20 rats, with care being taken not to include any cortical tissue. Separation of the medulla from the rest of renal tissue was not as easy as separation of the cortex.

Implants in Groups II and III were all removed after 5 months for examination.

Group IV. In seven animals, ligation of the right renal pedicle was performed and the kidney was removed at 4 and 8 days, 3, 5, and 7 months after operation.

Tissues were fixed in 10% buffered formalin, decalcified, and sections were stained with hematoxylin and eosin for microscopic examination.

Results. The early response of the kidney after subcutaneous implantation or ligation of renal pedicle is coagulation necrosis. The general architecture of the kidney remains well defined but the cells lose their nuclei and by day 8, glomerular and tubular structures were dead (Fig. 1). The transitional epithelium of the pelvis does not appear to take part in the necrotic process; its fragments remain scattered at the border of the implant wtih occasional underlying capillaries. Although the supportive tissue surrounding the implant has many capillaries, typical of granulation tissue, no invasion of the implant by these vessels is noted. The cortex appears to become necrotic somewhat earlier than medulla. During the second week, when the necrosis is complete, fibroblasts richly interspersed with capillaries invade the implant. During the third week many macrophages are seen. Occasionally, small, localized areas of abscess formation are observed, especially in the cortex.

[1] These studies were aided with funds from U.S. Public Health Service Grant number AM 12444 from the National Institute of Arthritis and Metabolic Diseases and Atomic Energy Commission Contract number AT (30-1) 3808.

TABLE I. Bone Formation in the Kidney Implants.[a]

Group	No. of implants	Positive for bone formation	Positive (%)
I. cortex and medulla	123	46	37
II. cortex only	40	0	0
III. medulla only	40	17	42.5
IV. ligation of renal pedicle	3	3	100

[a] Only implants removed 1 month or more after implantation are included.

Bone formation never occurred in implants of isolated cortical tissue (Table I). Osteogenesis, however, did occur in more than one-third of the cases where medulla was implanted alone or in combination with the rest of the renal tissue. In both of these types of implants transitional epithelium was present. The results of bone formation observed in the microscopic sections is summarized in Table I.

Microscopically, osteogenesis appeared to begin in the medulla and was often seen in the vicinity of the transitional epithelium (Fig. 2). The bone was trabecular and sometimes a loose connective tissue resembling marrow was present in its interstices. Vascular structures similar to marrow sinusoids were often seen in this connective tissue (Fig. 3).

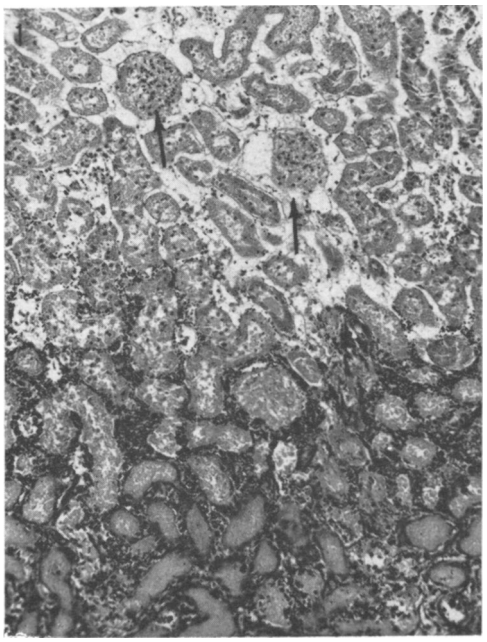

FIG. 1. Kidney implant after 6 days: Necrosis is evident throughout the tissue but the general architecture of the kidney remains distinguishable (coagulation necrosis). Note the two glomeruli (arrows). Invading granulation tissue is seen in the lower part; ×100.

FIG. 2. Kidney implant after 5 months: The kidney is replaced by fibrous tissue. Note the fibroblasts richly interspersed by collagen fibers and a few large vascular channels (arrows). On the left, transitional epithelium is seen in close association with a fragment of bone; ×225.

The earliest bone formation was observed in microscopic sections 14 days after implantation. On gross examination, calcified areas, recognized by their consistency, appear in multiple foci. These foci grow in size; and, although they may coalesce, they always display a multifocal architecture. When the implants were examined early (*e.g.*, 1 month), small fragments of bone were missed unless

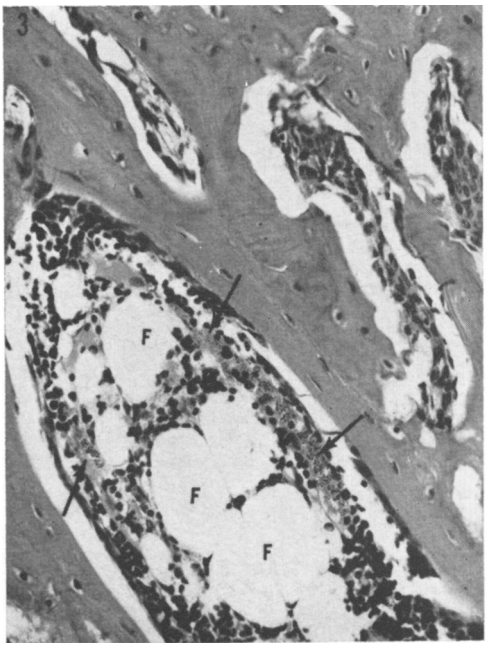

FIG. 3. Bone formation in the kidney 5 months after implantation: The bone is trabecular. In the lower part, a loose connective tissue resembling bone marrow is seen within the interstices of trabecular bone. Note a few erythrocyte-filled channels similar to marrow sinusoids (arrows). Large empty-looking areas are likely fat cells (F). No definite megakaryocytes are seen and other blood cell precursors cannot be distinguished with any degree of certainty in this type of preparation; ×225.

multiple sections through the implant were obtained. As a result the likelihood of detection of bone in microscopic sections increased with the time elapsed between implantation and examination.

Discussion. Heterotopic transplants of renal tissue used in the present study has allowed us to determine the osteogenic potential of separated parts of the kidney. These results eliminate the cortex as a source of contribution to renal osteogenesis. Osteogenesis, however, was observed in implants of medulla alone or in combination with the cortex. Microscopic sections suggest that the osteogenic potential of renal tissue depends upon the presence of viable transitional epithelium of the pelvis. Urinary bladder epithelium (2–5) and other epithelium (6) have been shown to possess osteogenic inductive capacity. Osteogenesis in the heterotopic kidney implants, therefore, is attributed to the induction by the transitional epithelium of the pelvis present in the implants.

The early events in the implants of kidney fragments consist of coagulation necrosis followed by fibroblastic invasion. The necrotic process in the implant is similar to that occurring after the ligation of renal vessels indicating that the necrosis in the implant is caused by ischemia. Furthermore, these early events are similar to those observed in implants of liver fragments where necrosis supervenes because the implant fails to establish vascular connections with the supporting tissue (7). Kidney tissue, as in the case of the liver, seems not to elicit a vascular response with prompt penetration of the implant by capillaries. The necrotic process in the kidney implant is complete after 8 days, whereas in the liver implant, this process takes 6–8 weeks (7). This may reflect higher susceptibility of renal tissue to ischemia compared to hepatic tissue. Differential susceptibility to ischemia may also account for our observation that the cortex becomes necrotic before the medulla. The sequence of events leading to bone formation, therefore, occurs as follows: ischemia of the kidney after implantation or ligation of the renal artery gives rise to necrosis of glomerular and tubular tissue. Granulation tissue ultimately replaces the necrotic kidney. Transitional epithelium of the renal pelvis which has remained viable induces bone formation in the invading fibroblasts.

Summary and Conclusion. From these observations, we conclude:

1. Fragments of kidney, implanted in subcutaneous tissue undergo coagulation necrosis similar to necrosis which supervenes after ligation of the renal artery. The necrosis may result from failure of the kidney to elicit a prompt vascular response in the surrounding tissue within the implant. Scattered fragments of pelvic transitional epithelium remain viable.

2. Osteogenesis is observed in more than one-third of the implants as early as 14 days after implantation; yet the size of bone tissue is small at this time and a single section through the tissue may not disclose it.

3. Implantation of cortex alone results in no bone formation. The likely stimulus to bone formation is the transitional pelvic epithelium which is implanted along with the medulla.

4. Implantation of kidney fragments in subcutaneous tissue seems to unify two previous models frequently used to study heterotopic osteogenesis; *i.e.*, ligation of renal artery and transplanation of bladder epithelium.

1. Bridges, J. B., Int. Rev. Cytol. **8**, 253 (1959).

2. Huggins, C. B., Arch. Surg. **22**, 377 (1931).

3. Huggins, C. B., McCarroll, M. R., and Blacksom, B. H., Arch. Surg. **32**, 915 (1936).

4. Johnson, F. R., and McMinn, R. M. H., J. Anat. **90**, 106 (1959).

5. McLean, F. C., and Urist, M. E., *in* "Bone," p. 196. Univ. of Chicago Press, Chicago (1968).

6. Huggins, C. B., and Sammet, J. F., J. Exp. Med. **58**, 393 (1933).

7. Tavassoli, M., and Crosby, W. H., Anat. Rec. **166**, 143 (1970).

Received Jan. 25, 1971. P.S.E.B.M., 1971, Vol. 137.