and they are probably a more mature population of stem cells than the bone marrow PHSC, of which they are the progenies [9]. The present investigation showed that the colony-forming ability of splenic PHSC, unlike that of bone marrow PHSC, does not increase during interaction with thymus cells. Since the increase in colony formation is not in this case the result of increased migration of CFU into the spleen, for in that case an increase in the number of exogenous colonies would be expected in R_1 spleens but not in R_2, it can be tentatively suggested that the effect of thymocytes is linked with their action on proliferative activity of PSHC. Probably in the course of their ontogeny hematopoietic stem cells lose their ability to respond to the proliferative stimulus arising from T cells. It may be that the higher proliferative activity of splenic than of bone marrow PHSC is an obstacle to their interaction with T cells.

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PROPERTIES OF BONE TISSUE INDUCED BY TRANSITIONAL EPITHELIUM

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The transitional epithelium of the urinary tract possesses marked osteogenic activity: in adult mammals bone tissue is formed around grafts of this tissue and in the pelvis of the kidneys after ligation of its blood vessels, and ectopic bone marrow organs appear [2]. If the epithelium is transplanted in diffusion chambers bone is formed on the outer surface of the millipore filters, direct proof that it is formed by inducible osteogenic precursor cells (IOPC) of the recipient [1, 4].

Bone is formed in guinea pigs after both autografting and homografting of epithelium. In the first case, however, it persists for many months, but in the second case only for a few weeks. It has accordingly been postulated that transitional epithelium evokes the formation of a nonself-supporting, inductor-dependent bone which exists only while the inductor continues to act [3, 4]. The aim of this investigation was to test whether the cause of resorption of ectopic bone tissue is in fact immunologic rejection of the allogeneic epithelium.

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TABLE 1. Results of Simultaneous Autologous and Homologous Transplantation of Urinary Bladder Wall in Guinea Pigs

Grafts	Time of immunization (time after transplantation, in days)	Time of fixation (time after transplantation, in days)	Beginning of softening of graft (time after transplantation, in days)	State of graft on fixation: bone	epithelium	bone marrow
1 AG	30	37	26	+	+	
1 HG				−	−	
2 AG	30	90		+	+	+
2 HG				+	−	+
3 AG	21	42		+	+	+
3 HG				+	+	+
4 AG	21	56	33	+	+	+
4 HG				−	−	−
5 AG	21	74	33	+	+	
5 HG				−	−	−
6 AG	21	47	39	+	+	
6 HG				−	−	−
7 AG	—	37	26	+	+	
7 HG				−	−	−
8 AG	30	72	39	+	+	
8 HG				−	−	−
9 AG	—	42	33	+	+	
9 HG				−	−	−

Legend to Tables 1 and 2: +) bone, epithelium, and bone marrow present in grafts on fixation; −) bone, epithelium, and bone marrow absent in grafts on fixation: bone and epithelium only present in HG on fixation.

EXPERIMENTAL METHOD

Homologous and autologous transplantation of the urinary bladder wall into muscles of the anterior abdominal wall was performed on noninbred adult guinea pigs weighing 200-300 g. Under pentobarbital anesthesia (40 mg/kg body weight) half of the urinary bladder was resected and used for transplantation, and a purse-string suture was applied to the rest. In experiment 1 each recipient received one autograft (AG) or one homograft (HG), in experiment 2 each recipient received two grafts (AG + HG); the resected part of the bladder was cut into two halves which were transplanted in the form of an AG and an HG; in experiment 3 each recipient received three grafts: the resected parts of the bladders were cut into fragments measuring 0.5-1 mm and divided into three parts, one of which was transplanted as an AG, another as an HG, and the third was mixed with fragments of the same homologous bladder and transplanted in the AG + HG form. On the 21st-57th day most recipients were given an intraperitoneal injection of 10^8 spleen cells from the donor of the bladder used for HG in order to give additional immunization. On the 14th-96th day the grafts were fixed with alcohol-formol, decalcified, embedded in paraffin wax, and cut into series of sections which were stained with hematoxylin–eosin and by the PAS method. The state of the grafts before fixation was regularly assessed by palpation.

EXPERIMENTAL RESULTS

In the AG ectopic bone developed starting from the 9th day around epithelial cysts and growing sheaths of epithelium, as has been fully described previously [1-4]. By the second week bone formed dense sheaths around the epithelium; cartilage development was rarely observed. After 3 weeks foci of myeloid hematopoiesis appeared in some of the grafts. In experiment 1 all 10 AG and nine of the 10 HG contained epithelium and bone tissue on the 14th-15th day. The epithelium in some HG was in a state of immunologic resorption, accompanied by lymphoid infiltration; the bone in such grafts was not surrounded by a lymphoid barrier, but showed evidence of adsorption, reflected in the absence of an osteoblastic layer and unmasking of collagen fibers. The size of the induced bone corresponded to the size of the masses detected by palpation of the grafts in living recipients. These masses appeared after

TABLE 2. Results of Simultaneous Autologous, Homologous, and Mixed Auto-homologous Transplantation of Urinary Bladder Wall in Guinea Pigs

Grafts	Time of immunization (time after transplantation, in days)	Time of fixation (time after transplantation, in days)	Beginning of softening of graft (time after transplantation, in days)	State of graft on fixation: bone	State of graft on fixation: epithelium	State of graft on fixation: bone marrow
1 AG			60	−	−	−
1 HG	53	96	44	−	−	−
1 AG+HG				+	+	
2 AG				+	+	
2 HG	52	74		±	±	
2 AG+HG				+	+	
3 AG				+	+	
3 HG	48	69	44	−	−	−
3 AG+HG				+	+	+
4 AG				+	+	
4 HG	46	67	32	−	−	−
4 AG+HG				+	+	
5 AG				+	+	
5 HG	57	67	44	−	−	−
5 AG+HG				+	+	
6 AG				+	+	
6 HG	34	55	32	−	−	−
6 AG+HG				+	+	
7 AG				+	+	
7 HG	34	55	After 43	−	−	−
7 AG+HG				+	+	+
8 AG				+	+	
8 HG	33	55	After 43	−	−	
8 AG+HG				+	+	
9 AG				+	+	
9 HG	33	50	17	−	−	−
9 AG+HG				+	+	

the 7th day around AG and HG. In the first 2 weeks, bone thus succeeded in developing around most AG and HG. This is in agreement with previous results [2, 4] showing that osteogenesis in AG and HG of transitional epithelium in guinea pigs follows a similar course and leads after 10-14 days to the formation of typical bone tissue with osteocytes, osteoblasts, and proliferating preosteoblasts.

The results of experiment 2, in which nine recipients were grafted simultaneously with AG and HG, are given in Table 1. On the 37th-90th day all AG and 1 HG (32nd day) contained epithelium and bone tissue. Besides, 1 HG (90th day) contained a bone organ with medullary cavity and bone marrow, but no epithelium. The remaining 7 HG were completely resorbed, although after 10 days distinct masses of condensation were observed around each of them. These masses softened between the 26th and 39th days and then disappeared. Table 2 gives the results of experiment 3. Eight of the 9 AG and all 9 AG + HG, and also 1 of the 9 HG consisted of epithelium and bone tissue on the 43rd-96th day. In 8 HG the masses of condensation softened between the 17th and 44th days, and disappeared by the 50th day; at the time of fixation these grafts contained neither epithelium nor bone. One AG + HG on the 69th day contained cartilage and bone marrow besides bone tissue.

Ectopic bone is thus formed during the first 2 weeks both in AG and in HG. In HG, however, it undergoes resorption by the end of the 2nd month; meanwhile bone induced in AG in the same recipients persists for at least 3 months (according to other observations, at least 9 months).

HG differ from AG in the fact that the former are subjected to an immunologic reaction directed against antigens of allogeneic epithelium.

During induction of osteogenesis the bone on the outer surface of diffusion chambers containing allogeneic epithelium is not adsorbed, and the induced bone is therefore not exposed to immunologic rejection.

Persistence of induced bone tissue in AG + HG showed that the process of immunologic resorption of the epithelium does not itself lead to destruction of bone adjacent to the epithelium. Disappearance of bone tissue in HG after resorption of the epithelium thus proves that induced osteogenic tissue is unable to support itself after the action of the inductor ceases. This distinguishes it from bone formed by determined osteogenic precursor cells [3, 4].

In transplantations between noninbred guinea pigs cases can naturally arise when the time of rejection of allogeneic epithelium is delayed, even in spite of reimmunization. It is evidently in such cases that both epithelium and bone tissue persist for a long time in HG (42 days in one HG in experiment 1 and 74 days in one HG in experiment 2). In one of the 18 HG studied in this investigation preservation of bone with bone marrow was observed in the absence of epithelium. Such cases are interesting for the elucidation of the mechanisms of adsorption of bone formed from IOPC after the action of the inductor has ceased.

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