FUNCTION OF THE GALL BLADDER EPITHELIUM AS AN OSTEOGENIC STIMULUS AND THE PHYSIOLOGICAL DIFFERENTIATION OF CONNECTIVE TISSUE

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PLATES 17 TO 19

Recent investigations have shown (1–3) that the epithelium lining the kidney pelvis, ureter and urinary bladder is capable of directly inciting osteogenesis in certain connective tissue areas in the dog, guinea pig and rat. This can be demonstrated most easily by removing it from its normal environment by transplantation. Provided the transplanted epithelium survives, it proliferates to form a cyst containing brown cloudy fluid, lined with epithelium and encapsulated by fibroblasts. In the parietal connective tissues, for example those of the abdominal wall, extremities, neck and others, the fibroblasts surrounding a portion of the cyst readily and always undergo osteoblastic transformation and true bone is formed, containing fibrous and later blood-forming marrow. Other connective tissues such as those surrounding the epithelium in the urinary tract, the fibroblasts *within* the kidney cortex, liver and spleen, do not react to this epithelial stimulus, although the other conditions remain the same. Thus the evidence suggests that the mechanism preventing the formation of bone normally in the urinary tract lies in the presence of connective tissue functionally variant from the fibroblasts of the more parietal regions.

In the present communication, evidence will be presented to demonstrate that a similar osteogenic function is inherent in the epithelium of the gall bladder. During the course of the experiments, calculi composed chiefly of calcium carbonate were frequently observed and this phenomenon will also be briefly discussed, as will be further evidence that physiological variations exist in connective tissues, which appear morphologically similar.

The Gall Bladder and Bone Formation.—The gall bladder has been transplanted in three previous experiments. Nakamoto (4), investigating carcinoma of the gall bladder, transplanted this organ to the abdominal wall in 18 guinea pigs and observed the formation of bone and cartilage within the transplanted organ; this author presented his results in brief form (one sentence) and made no mention of a causal histogenetic relationship. In the transplantation experiments of Stater (5) and Bauer and Hakki (6) bone was not observed.

A spontaneous occurrence in man of a process closely analogous to the present experimental findings was observed by Micseh (7) who reported a necropsy where carcinoma of the gall bladder was found with metastases to the liver, lung and lymph glands. Both the original tumor and the metastases contained true bone and hematopoietic bone marrow. On microscopic examination the tumor presented a papillary pattern in great part and the heterotopic bone was situated in connective tissue closely adjacent to the epithelial cells; in the tumor wherever bone was present, neoplastic epithelial cells were in intimate relationship to it.

Bone in the human gall bladder in close association with calculi as described by Phemister, Rewbridge and Rudisill (8) is probably not related to the phenomenon under present consideration.

The Gall Bladder and Calcium Carbonate Calculi.—The occurrence of these calculi in man has been considered by Phemister (8, 9, Lit.) who has constructed a clinical syndrome associated with calcium carbonate deposition in the gall bladder. Askanazy (10) has discussed the finding of $CaCO_3$ microliths at necropsy.

These stones have been observed experimentally by several investigators. Carmichael (11) introduced sterile and infected foreign bodies into the gall bladder of dogs and rabbits and found that calcium carbonate was deposited on the foreign surface. Rous, McMaster and Drury (12) found calcium carbonate in the proximal portion of the tubing used by Rous and McMaster in their device for collecting bile under sterile conditions. Walsh and Ivy (13) found calculi in the gall bladder of 3 dogs with occluded cystic duct. Phemister, Day and Hastings (9) described calcium carbonate calculi in rabbits and a dog following cystic duct ligation and injection of streptococci into the gall bladder.

Methods

Dogs and guinea pigs were used. The technique was that of transplantation. The animals were operated upon under ether, with asepsis, in 3 groups. In Group 1 the dome of the gall bladder was excised and replaced by a circular, free patch of connective tissue covering the rectus abdominis muscle, about 2.5 cm. in diameter; the repair was made by continuous suture with one layer of fine silk. Group 2 was identical to Group 1 except that the patch consisted of connective tissue, muscle and peritoneum obtained from the dome of the urinary bladder by denuding its mucosa. It was sutured in place so that the serosa faced the peritoneal cavity. In Group 3, the gall bladder was excised, opened and sutured to the fascia of the

anterior abdominal wall and to the wall of the stomach. At the termination of the experiment x-ray photographs of the specimens were taken, after which serial sections of the material were made for histological examination.

RESULTS

Group 1. Transplantation of Abdominal Connective Tissue to Gall Bladder.—Four experiments were done and the dogs were killed at 46, 50 and 56 days. At necropsy in every case the transplant was found surrounded by omental adhesions, markedly shrunken, very hard to the touch and showing an area of calcium density in the x-ray photograph. Microscopic examination showed that the cylindrical epithelium had grown over the patch in a single layer of cells much less papillary than the original lining of the gall bladder but otherwise identical with it. This difference together with the presence of the sutures and the absence of smooth muscle clearly demarcated the transplant. In each instance there was found a thin plaque of bone with marrow spaces filled with connective tissue, confined strictly to the transplant. A narrow zone of fibroblasts, two to twenty cells in depth, separated the epithelium from the bone. The surface of the bone was covered with a palisade of osteoblasts and a few osteoclasts.

The possible influence of bile on this process was excluded by emptying the gall bladder and ligating the cystic duct at the time of transplant in 3 other dogs. In 2 dogs epithelium grew across the patch and bone was found in the subepithelial layers of the transplant, identical with that found in bile-containing gall bladders. In the third dog, due to infection, a pyogenic membrane formed on the surface of the transplant and bone was not found in this experiment. In each instance the gall bladder was found filled with mucus, in one instance thin and limpid, and in the others thick, tenacious and inspissated. In this mucus in all of the dogs, and embedded in the pyogenic membrane of the infected transplant in one, microliths were found and chemical examination of the larger of these showed them to be composed chiefly (91.4 per cent) of $CaCO_3$ with no trace of inorganic phosphorus. The largest stone was 3 x 2 x 1 mm. but the great majority were microscopic in size, 10–50μ in diameter. Some of these stones took a purple stain with hematoxylin, while others were colorless. Most of the stones were spherical and laminated in shape, but there

also occurred a wide variety of configurations, ellipsoid, budding forms, etc.

Group 2. Transplantation of Urinary Bladder Connective Tissue and Muscle to Gall Bladder.—This procedure was carried out in 3 dogs and necropsy was made 36 days later. The gall bladders were not unduly distended, contained 8–15 cc. of bile. In each case there were adhesions about the gall bladder, and omentum was attached to the fundus. X-ray photographs in contrast to the previous cases failed to show bone. On section the surface of the patch was glistening; in

TABLE I

Results in Group 3. Transplantation of Gall Bladder to Abdominal Wall in Guinea Pigs and Dogs

Guinea pigs	Dogs
13 days: Osteoid tissue	7 days: No bone
14 days: True bone present	16 days: No bone
15 days: No bone—infection	18 days: No bone
15 days: Osteoid tissue	29 days: No bone
17 days: True bone present	41 days: Bone present
17 days: True bone present	46 days: Bone present
19 days: True bone present	48 days: No bone
19 days: No bone	
21 days: Bone present	
23 days: Bone present	
23 days: Bone present	
27 days: Bone present	
34 days: Bone present	

one instance the suture material had partially extruded into the lumen at one point and the epithelium here was edematous.

Microscopic examination showed that cylindrical epithelial cells of the gall bladder had grown over the patch. There was a marked tendency of the epithelium to grow down along the silk between the patch and the gall bladder wall and in several places it reached the serosa. A partial atrophy of the smooth muscle of the transplant occurred, but numerous compact bundles of these cells could be identified easily. The epithelium covering the transplant rested on connective tissue. There was no formation of bone at any place.

Group 3. Transplantation of Gall Bladder Epithelium to Connective

Tissue of the Abdominal Wall.—Table I gives a resumé of the results in this experiment. Necropsy in each instance showed that the transplanted epithelium had decreased greatly in amount and was represented by a small mass of grouped cysts, with thin wall containing a colorless thin mucoid fluid. Microscopic examination showed spicules of bone in close apposition to the epithelium in many cases in the guinea pig, less often in the dog. The cysts in part multiloculated, in part single, varied in size. The diameter of most of the cysts was approximately 50μ–2 mm. and of the largest 1.2 cm. The cysts were lined with a single layer of cylindrical epithelium morphologically identical with the original transplant. The epithelium was decidedly less papillary than the normal relaxed gall bladder epithelium. The cyst fluid had a high protein content, contained polymorphonuclear leucocytes in the experiments of shorter duration, later only pyknotic cells, obviously degenerated epithelial and phagocytic cells.

In the 13 and 15 day experiments the bone was not calcified; in the 17 and 23 day experiments the central portion was calcified while a considerable number of cells at the periphery were not; in the older experiments the uncalcified portion of the bone became less noticeable and disappeared. The bone was true fibrous bone with anastomosing osteocytes; cartilage was never observed. It is important to note that the bone spicules occurred only in proximity to the epithelium and that many of the vesicles were unsurrounded by bone.

DISCUSSION

It is apparent from these experiments that the epithelium of the gall bladder is capable of inducing bone formation and in this way it is similar to the urinary epithelium as cited above. The evidence is strongly in favor of the view that there are two components in the tissue reaction that produces bone; namely, (a) epithelium and (b) certain kinds of connective tissue. The connective tissue adjacent to the epithelium of the gall bladder, and similar cells of the urinary bladder do not normally form bone. These cells are identical in morphological and tinctorial reactions with the fibroblasts of the abdominal wall which readily become osteogenetic under the stimulus of these epithelia. There is thus a physiological difference between morphologically similar connective tissue cells of certain great areas of

the organism, manifesting itself in potency or impotence to form bone. Further data on physiological differences of fibroblasts have been obtained in other ways by Parker (14–16) and by the one of the present authors (2, 17). It is of interest that carcinomatous gall bladder epithelium has once been observed (7) to cause bone to form from the connective tissue of the gall bladder.

It is our concept derived from the evidence obtained in these experiments that this bone is not an unusual, bizarre response to the adjacent, geographically abnormal epithelium, but that it is a normal and usual reaction of certain connective tissues. Thus the evidence shows that it is the subepithelial connective tissue which fails to respond to the osteogenic stimulus of the overlying epithelium and thus prevents the formation of an osseous layer in the gall bladder under normal circumstances.

The total absence or the small size of the bone fragments in Group 3 must be considered. In each case the fibromuscular mechanism of the gall bladder was not dissociated from the epithelium. It is probable that growth of these transplanted fibroblasts, which are incapable of forming bone, occurred, sequestering the growing epithelium in large part or completely from the fibroblasts capable of forming bone.

The osseous transformation of the connective tissue develops after its cells have swollen and become basophilic to hematoxylin so that they correspond to osteoblasts. Osteoblasts are not present in these cell areas of the abdominal wall, and this observation is evidence that the osteoblast is derived by metaplasia of certain connective tissue cells.

The formation of the calcium carbonate microliths in the gall bladder mucus is related, as Phemister has shown, to the cystic duct occlusion. It is apparent that an abnormal surface such as the cannula system of Rous, the glass beads of Carmichael and the connective tissue patches as here, facilitates deposition and growth of the calculi.

SUMMARY

Evidence is presented that the proliferating gall bladder epithelium in the dog and guinea pig is capable of stimulating bone formation in certain connective tissues such as the abdominal wall. Other connective tissue areas such as the subepithelial connective tissue of the gall bladder and urinary bladder do not share in this tissue reaction and

resist the bone stimulus of the epithelium. The formation of bone in these circumstances is thus biphasic.

A difference between connective tissues morphologically identical can be proven physiologically, by their response to the osteogenic stimulus of appropriate epithelia.

Calcium carbonate microliths occurred in the mucus of the occluded gall bladder in which there was transplanted connective tissue forming part of the wall.

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EXPLANATION OF PLATES

The stain used in preparing the photomicrographs was hematoxylin and eosin.

PLATE 17

FIG. 1. Transplantation of connective tissue of the abdominal wall to the fundus of the gall bladder at 46 days. The epithelial surface is above, and the serosa (S) below. A thin layer of bone confined to the transplant is seen beneath epithelium. The bone has been decalcified. × 15.

FIG. 2. A higher magnification of a part of the epithelium and bone (B) of Fig. 1. × 185.

FIG. 3. Somewhat tangential section of abdominal wall transplant to fundus of gall bladder with occluded cystic duct, showing bone (B) in close association with the gall bladder epithelium. The bone has not been decalcified. × 70.

PLATE 18

FIG. 4. Transplantation of connective tissue and muscle of the urinary bladder to the dome of the gall bladder at 36 days. One-half of the transplant is shown with the serosal surface (S) below and the epithelium above. The normal gall bladder wall is at the left and between this and the transplant there has been an invagination of epithelium along the sutures. Smooth muscle bundles of the bladder wall may be seen. The epithelium has grown across the fibroblasts of the transplant but bone has not formed.   × 15.

FIG. 5. Transplantation of gall bladder epithelium to abdominal wall of guinea pig at 17 days. Vesicles lined with gall bladder epithelium are seen with closely adjacent islands of partly calcified bone (B).   × 125.
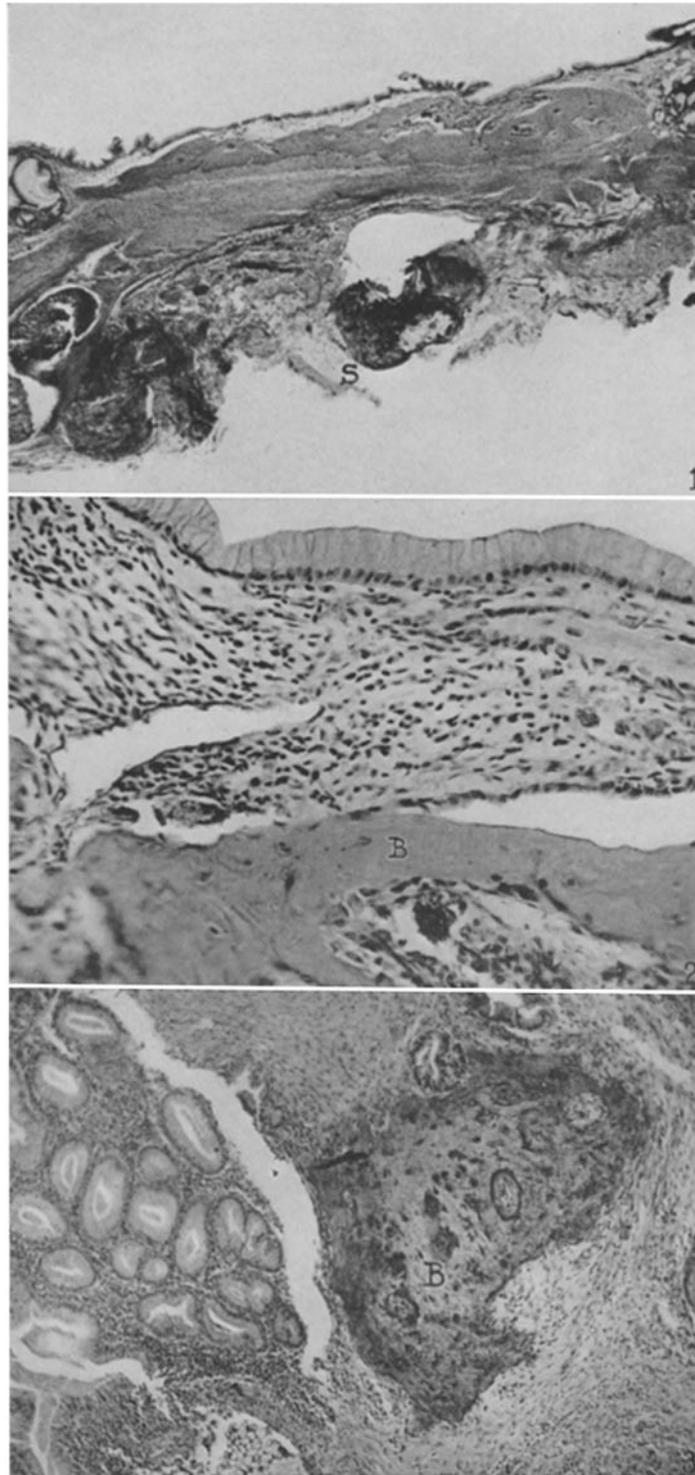
FIG. 6. Transplantation of gall bladder epithelium to abdominal wall in the dog at 42 days. Calcified bone is seen separated from the epithelium by a thin layer of fibroblasts.   × 250.

PLATE 19

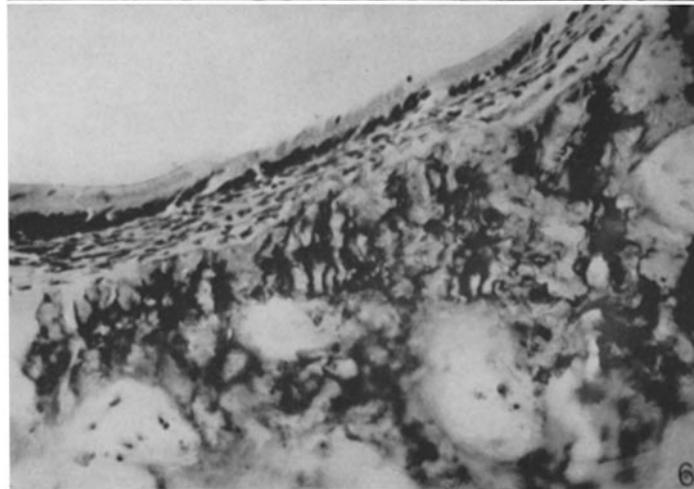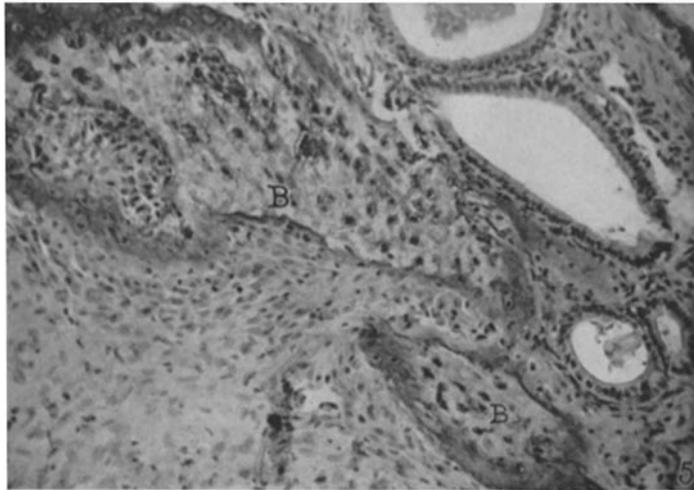FIG. 7. Microliths composed chiefly of calcium carbonate embedded in granulation tissue of a fascial transplant to the gall bladder for 40 days.   × 300.

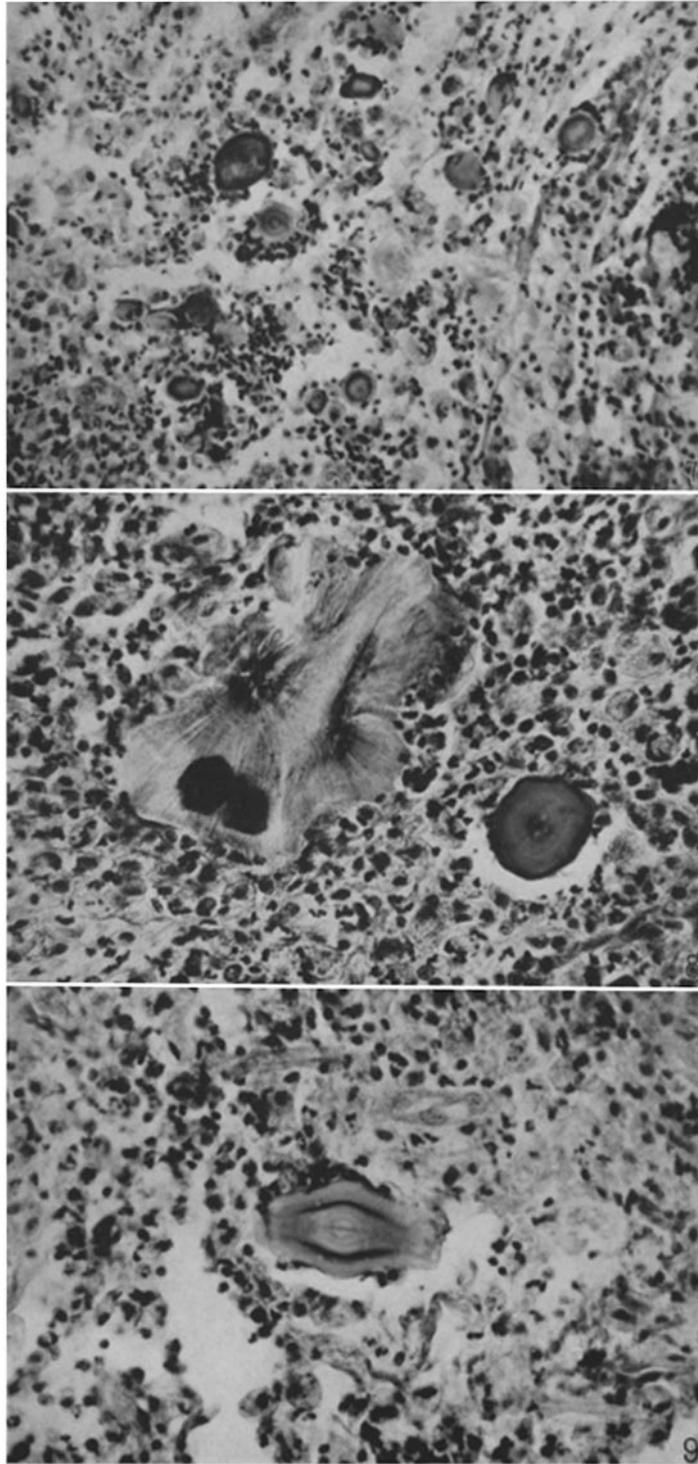FIG. 8. Same as Fig. 7, showing various shapes of the microliths.   × 185.

FIG. 9. Same as Fig. 7.   × 360.

(Huggins and Sammet: Gall bladder epithelium)

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