LXXX. THE PHOSPHATASE ACTIVITY OF TRANSPLANTS OF THE EPITHELIUM OF THE URINARY BLADDER TO THE ABDOMINAL WALL PRODUCING HETEROTOPIC OSSIFICATION.

By CHARLES BRENTON HUGGINS.

From the Biochemical Department, The Lister Institute, London¹.

(Received March 24th, 1931.)

THE presence of an enzyme capable of hydrolysing certain phosphoric esters has been demonstrated in bone and ossifying cartilage by Robison [1923], who with his collaborators has presented evidence that it is an active agent in ossification.

In general, this evidence has been adduced from three lines of observations: (a) survey of the normal distribution of the enzyme; (b) study of calcification in bone and cartilage in experimental rickets; (c) tissue culture *in vitro* of cartilage and bone. Some of the more important observations may be briefly cited. Normally phosphatase was found to occur in the ossified portions of young human embryos and infants, while non-ossifying cartilage was in all cases devoid of it [Martland and Robison, 1924]. When severely rachitic bones were immersed in solutions of calcium hexosemonophosphate or glycerophosphate, deposition of calcium phosphate took place in the hypertrophic, but not in the small-celled, or articular cartilage [Robison and Soames, 1924, 1930]. The addition of very small amounts of a phosphoric ester to solutions of calcium salts and inorganic phosphate in concentrations otherwise inadequate for deposition of calcium phosphate in rachitic bone led readily to calcification [Robison and Soames, 1930; Robison, Macleod and Rosenheim, 1930]: desiccation for 24 hours or the addition of such protoplasmic poisons as chloroform, acetone or potassium cyanide inhibited calcification in inorganic solutions but did not destroy the enzyme, and calcification resulted in the presence of phosphoric ester [Robison, Macleod and Rosenheim, 1930]. In tissue culture of the embryonic femur and mandible of the chick, phosphatase could be demonstrated in large amounts when hypertrophic cartilage and bone were present; in the absence of these and in certain areas which do not normally ossify in these structures phosphatase was not present in detectable amounts [Fell and Robison, 1929, 1930].

¹ The surgical work herein reported was carried out in the Department of Physiology, University College, London.

An osteogenic effect has been demonstrated in the epithelium of the urinary bladder, ureter and kidney pelvis of the dog [Huggins, 1930, 1, 2; 1931]. When, for example, a portion of the bladder epithelium is transplanted to the connective tissue of the abdominal wall, ossification occurs regularly after 18 to 20 days in the connective tissue, provided the epithelium survives. The bone so formed has a constant relationship to the epithelium; the epithelium never ossifies. The bone gives the typical crystallographic appearance of true bone when examined spectroscopically by means of the X-rays [Roseberry, Hastings, and Morse, 1931].

The object of the present experiment was (a) to ascertain if phosphatase occurs normally in the urinary bladder epithelium or fibrous tissue of the abdominal wall and whether it is synthesised in the bone formed by transplantation of the former to the latter, and (b) to study the progress of its synthesis in relation to the ossification.

METHODS.

Surgical. Under aseptic precautions and ether anaesthesia the abdominal cavity was opened through a small lower mid-rectus incision, and the urinary bladder delivered through the wound. A preliminary incision was made in the dome of the bladder down to the mucosa, and a portion of the epithelium, approximately 2.5×2.5 cm., stripped from the muscular wall by blunt dissection and cut away. The wound in the dome of the bladder was closed with a fine silk suture, in two layers. The excised epithelium was transplanted to the top of the sheath of the rectus abdominis muscle, sewed in at four corners with a silk interrupted suture. The abdominal wall was closed, without drainage, with three silk sutures.

At operation small portions of the whole thickness of the bladder wall, the epithelium alone, the muscular wall of the bladder alone, and the connective tissue of the rectus sheath were excised for chemical examination as control specimens. The dogs were killed at intervals of 5, 9, 13, 16, 18 and 26 days after operation. At necropsy, many samples of tissue in the region of the transplant were removed and divided into nearly equal portions, the one half for histological examination and the other for phosphatase determinations.

Chemical. The method for determination of phosphatase activity was similar to that employed by Fell and Robison [1930]. The tissue was transferred to a cover-slip and dried for 18 hours over sulphuric acid in an evacuated desiccator. It was then weighed on a micro-balance after attaining constant weight in air and transferred to a tube containing 2 cc. of water and a small drop of chloroform. The cover-slip was then reweighed and the weight (W) of tissue thus obtained. The tissues were kept in the chloroform water at room temperature for 3 days; 1 cc. of 0.2 M solution of sodium glycerophosphate (p_H 8.7) was added together with a small drop of chloroform. The further procedure and calculation of the index (A/W) of phosphatase activity

has been described by Fell and Robison [1929]. The ratio A/W expresses the amount of hydrolysis as mg. P effected by 1 mg. of tissue (dry weight) at a temperature of 37° and p_H 8.6.

RESULTS.

In all cases the transplants survived as proved by histological examination and presented the characteristic histological appearance already described [Huggins, 1931]. In the 16-day specimen "osteoid" tissue or fibroblasts with large, swollen, deeply staining cytoplasm surrounded by eosinophilic oedematous tissue was found near the transplanted epithelium: no bone was found at this time or before. In the 18- and 26-day specimens, a rather large amount of bone was found adjacent to the transplanted epithelium. This had the characteristic features of membrane bone.

In the preliminary control tissues (Table I) phosphatase activity could not be demonstrated in the fibrous tissue of the rectus sheath or the muscular portion of the bladder wall. A definite but small value (about 0.03) for A/W was obtained for the bladder epithelium. Variations between A/W values of 0.01 and 0.03 in the whole bladder wall can probably be explained by variations in the amount of epithelium included, since the muscle does not contain the enzyme.

Table I. *Phosphatase activity of various tissues removed at operation as controls.*

Tissue	W mg.	A mg. P	A/W
Whole thickness of bladder wall	3.39	0.11	0.03
"	19.1	0.05	<0.01
"	14.2	0.10	<0.01
Bladder epithelium alone	5.04	0.14	0.03
"	4.5	0.10	0.02
"	3.7	0.09	0.03
"	3.0	0.05	0.02
"	3.6	0.12	0.03
"	3.7	0.55	0.01
"	13.0	0.06	<0.01
Bladder musculature alone	6.6	0.02	<0.01
"	9.3	0	0
Connective tissue rectus sheath	10.6	0.01	<0.01
"	3.5	0	0
"	5.9	0	0
"	8.9	0	0
"	5.7	0	0

Of the tissues of the transplant containing bladder epithelium histologically (Table II *b*) no activity could be demonstrated at 5 or 9 days. In similar specimens at 12, 13 and 16 days small amounts of phosphatase were demonstrated and the A/W value was of the order of 0.01 except in one instance where it was 0.05.

Specimens of connective tissue adjacent to but not including bladder epithelium (Table II *a*) from 12 to 26 days contained even smaller amounts of the enzyme, ranging from 0 (14 times) to a maximum value of 0.05 (once).

Table II.

<i>Phosphatase activity of connective tissue adjacent to bladder epithelium transplant.</i>				<i>Phosphatase activity of bladder epithelium transplanted to connective tissue.</i>			
Time after operation	W mg.	A mg. P	A/W	Time after operation	W mg.	A mg. P	A/W
(a) <i>No bladder mucosa or bone present.</i>				(b) <i>Bladder mucosa but no bone present.</i>			
12	5.0	0.25	0.05	5	1.8	0	0
12	7.0	0.07	0.01	5	3.0	0	0
13	19.7	0.25	0.01	9	3.8	0	0
13	22.2	0.51	0.02	9	3.3	0	0
13	13.2	0.06	<0.01	9	5.9	0	0
16	36.0	0.02	<0.01	12	10.0	0.113	0.01
16	12.0	0.20	0.02	12	11.0	0.09	<0.01
16	42.0	0.005	<0.01	12	23.0	0.22	0.01
16	14.0	0.05	<0.01	12	4.5	0.055	0.01
16	9.0	0.13	0.01	13	14.5	0.78	0.05
18	5.07	6.192	0.04	16	10.0	0.13	0.01
18	6.0	0.187	0.03	16	22.0	0.18	<0.01
18	2.7	0	0	(c) <i>Bladder mucosa and bone present.</i>			
18	12.0	0	0	18	1.0	0.41	0.41
18	2.0	0	0	18	0.8	0.099	0.12
18	4.2	0	0	18	3.1	6.80	0.22
26	3.6	0	0	18	0.8	0.198	0.25
26	4.4	0	0	26	1.3	0.20	0.16
26	2.2	0	0	26	1.0	0.13	0.13
26	1.4	0.042	0.03	26	1.5	0.18	0.13
26	7.0	0.033	<0.01	26	2.7	0.57	0.21
26	5.2	0.033	<0.01	26	1.6	0.25	0.16
26	4.0	0.055	0.01				

Contrasting sharply with these index values in unossified tissues and controls, the 18- and 26-day tissues containing bone (Table II c) without exception contained large amounts of phosphatase; the *A/W* value varied from 0.12 to a maximum of 0.41. These values are of the same order of magnitude as those obtained by Fell and Robison [1929, 1930] for normal and embryonic ossifying cartilage and bone. The variations can be accounted for in part by the different amounts of soft tissues in relation to bone in the material examined. In the presence of bone, therefore, phosphatase was present in amounts 2.4 to 8 times the maximum present in the highest of non-ossified tissues and 10 to 20 times the mean values for these tissues (Table III).

Table III. *Summary of ratios of phosphatase to dry weight A/W.*

	Controls		Transplantation experiment		
	Bladder mucosa	Rectus sheath	Rectus sheath adjacent to transplant	Rectus sheath bladder epithelium.	Rectus sheath bladder epithelium.
				No bone	Bone
Maximum	0.03	0	0.05	0.05	0.41
Minimum	<0.01	0	0	0	0.12
Mean	0.02	0	0.01	0.02	0.20

SUMMARY.

The histological and chemical relationships of the enzyme (phosphatase) effecting hydrolysis of certain phosphoric esters were studied in the various stages of heterotopic osteogenesis in transplants of the urinary bladder epithelium to the abdominal fibrous tissue in the dog. In the presence of bone so formed the ratio of phosphatase activity to dry weight was very high, at least 2.5 to 8 times higher than the maximum, and 10 to 20 times the mean values obtained for the control tissues without bone.

The writer is greatly indebted to Dr R. Robison for many kindnesses, suggestions and criticism. He also wishes to thank the Lister Institute by whom the expenses of this investigation were defrayed and Prof. C. L. Evans for the use of the facilities of the Department of Physiology, University College, London, where the surgical work was done.

REFERENCES.

- Fell and Robison (1929). *Biochem. J.* **23**, 767.
— — (1930). *Biochem. J.* **24**, 1905.
Huggins (1930, 1). *Proc. Soc. Exp. Biol. Med.* **27**, 349.
— (1930, 2). *Proc. Soc. Exp. Biol. Med.* **28**, 125.
— (1931). *Arch. Surg.* **22**, 577.
Martland and Robison (1924). *Biochem. J.* **18**, 765.
Robison (1923). *Biochem. J.* **17**, 286.
— Macleod and Rosenheim (1930). *Biochem. J.* **24**, 1927.
— and Soames (1924). *Biochem. J.* **18**, 740.
— — (1930). *Biochem. J.* **24**, 1922.
Roseberry, Hastings, and Morse (1931). *J. Biol. Chem.* **90**, 395.