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Explanation of Figures

- Fig. 1: Succinic dehydrogenase. $\times 200$
 Fig. 2: Latic dehydrogenase. $\times 200$
 Fig. 3: Malic dehydrogenase. $\times 200$
 Fig. 4: α -Glycerophosphate dehydrogenase. $\times 100$
 Fig. 5: β -Hydroxybutyric dehydrogenase. $\times 100$
 Fig. 6: Aconitase. $\times 100$

Histochemistry of Heterotopic Hard Tissue Formation

Transplantation of Urinary Bladder Epithelium

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The present work deals with a histochemical analysis of the bone formation which is induced by implantation of the epithelium of urinary bladder.^{8-14,20)}

The observations were primarily focused on the hyalinized tissue and what is called "abnormal bone tissue"¹⁴⁾ which appear during the bone induction, and some intrinsic difference in nature between the two tissues was indicated.

Methods

The animals used were guinea pigs which were from 3 to 5 months of age. They were divided into two groups, A and B. In group A the animals were anesthetized with a 1 : 2 : 3 mixture of alcohol, chloroform and ether, their abdomen opened, small piece of the urinary bladder excised and autotransplanted into the abdominal subcutaneous tissue.¹⁴⁾ In group B, 10-20mg. of aminoacetoneitrile (AAN) was given to each animal by intramuscular injection every day from 5 days after the transplantation.¹⁴⁾

Three weeks after the operation, the implant was quickly excised with the surrounding tissue, and prepared into a serial fresh frozen sections of 15 μ thick in a -20°C cryostat. The sections were used for the demonstrations of alkaline phosphatase,¹⁹⁾ acid phosphatase,³⁾ esterase,¹⁵⁾ aminopeptidase¹⁶⁾ and succinic dehydrogenase.¹⁷⁾ For the preliminary observations, additional sections were also examined under the hematoxylin eosin, van Gieson, von Kossa¹⁸⁾ and PAS stainings.¹⁸⁾

Results

1. Hematoxylin-eosin, von Kossa, van Gieson and PAS staining.

In group A all cases showed a typical cyst formation due to an active proliferation of the implanted bladder epithelium. Beneath the epithelial lining of the cyst, a newly formed bone tissue was seen in the connective tissue. Fairly large amount of hyalinized tissue was also seen as a thin layer along the epithelial lining. It showed a rather homogeneous eosinophilic structure, containing a few cellular elements, stained red by van Gieson's method and was positive to PAS reaction.

In group B, to which AAN had been administrated, the bone formation was seen to have been more active, and the bone was frequently noticed to be accompanied by a large amount of "abnormal bone tissue". No mineralization was determined in the "abnormal bone tissue" by von Kossa staining and the staining reactions to hematoxylin-eosin, van Gieson and PAS of this tissue were essentially the same with those of the hyalinized tissue seen in group A.

2. Enzyme activities

The present results concerning the activities of various enzymes in the bone induced by the urinary bladder epithelium were in agreement with many earlier works carried out with normally developing bone.^{1,2,4-7,21)} The mineralized bone matrix showed no activities of alkaline phosphatase, aminopeptidase and succinic dehydrogenase, while rather strong activities of acid phosphatase and esterase were confirmed in it.

One of the interesting features of the present study was the difference in the enzyme activities between the hyalinized tissue and the "abnormal bone tissue". As was explained before, there was no difference in the staining reactions to hematoxylin eosin, van Gieson and PAS, between these two tissues. Enzyme histochemically, however, a remarkable difference between the two was confirmed. While the hyalinized tissue did not show any enzyme activity, a strong activity of alkaline phosphatase was always determined in the "abnormal bone tissue". Moderate or weak activities of acid phosphatase and esterase were also detected in it.

Discussion

In his recent report Kobayashi¹⁴⁾ has assumed that the hyalinized tissue is possibly brought about as a result of some modification of the connective tissue and that what is called the "abnormal bone tissue" may be attributed to a

transitional or intermediate type between the bone and the hyalinized tissue. No intrinsic difference, however, has so far been observed between the hyalinized and the abnormal bone tissues.

Through the present study, a remarkable difference in the enzyme reactions was determined between the two tissues. The enzyme reactions in the "abnormal bone tissue" is basically same with those observed by the author in the pre-mineralized matrix which appears in a close relationship with the experimentally formed heterotopic hard tissue.²²⁾

The "abnormal bone tissue" would be attributed, therefore, to the osteoid tissue which is ready to be mineralized. The hyalinized tissue is thought to be a mere collagenous matrix and to have no direct relation with the bone formation.

Acknowledgement

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Explanation of Figures

Bone (Figs. A), hyalinized tissue (Figs. B) and "abnormal bone tissue" (Figs. C) induced by transplention of the urinary bladder epithelium. $\times 65$.

Figs. 1. Hematoxylin-eosin, Figs. 2. Alkaline phosphatase,
Figs. 3. Acid phosphatase, Figs. 4. Esterase.

- A:- The bone (b) is shown beneath the epithelial lining (e) of the cyst (c). Serial fresh frozen sections, 3 weeks after transplantation.
- B:- The hyalinized tissue (h) runs along the epithelial lining (e) of the cyst (c). Serial fresh frozen sections, 3 weeks after transplantation.
- C:- The "abnormal bone tissue" (a) is seen to be located between the epithelial lining (e) of the cyst (c) and the bone (b) in the AAN-administrated animal. Serial fresh frozen sections, 3 weeks after transplantation.

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Discussion

Dr. Chin

When intra-ocular inflammation continues for long period of time, it is not rare to recognize intra-ocular ossification.

How do you think about the mechanisms of inducing osseous tissues with the transplantation of urinary bladder epithelium ?

Dr. Yoshiki

Though various factors are considered to be responsible for inducing osseous tissues with the transplantation of epithelial tissues, I have not enough data to give satisfactory answers to your question. In this study I have intended to compare the histochemical findings of normal osseous tissues with those of induced osseous tissues.

Histochemical Studies on Transition of Phosphatase Activities in the Processes of Bone Repair

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Director. Prof. Dr. Kensaku Kawakatsu

On the bone repair many investigations have been done with histological, physical and chemical techniques, however, there is a few report about histochemical demonstration, especially, as to the acid phosphatase activity in repairing process of bone.