Work is continuing in an attempt to elucidate more precisely the nature of the protective action which tiger snake venom exhibits against staphylococcal alpha toxin.

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Organic Composition of Bone: Localization of Isocitric Dehydrogenase in Femurs

THE original observation of Dickens[1] that calcified tissues contain the majority of the body's citric acid has raised the question of the mechanism by which this occurs. Currently, hypotheses to explain bone citrate levels are based on the findings of Dixon and Perkins[2], who observed relatively little isocitric dehydrogenase in rabbit femurs. We felt that these values[2] might be minimal, since manganese ions and triphosphopyridine nucleotide were not used as supplements. Furthermore, citric, rather than isocitric, acid was used as the substrate in the assay system. In the present work, tissues were assayed[3] for isocitric dehydrogenase activity using a spectrophotometric method. Extracts of soft tissues (weight × 5 of 0·1 *M tris*, *p*H 7·4; 15 min. at 1,100*g*) from exsanguinated animals were assayed. The femurs were frozen in 'dry ice', cleaned of adhering muscle, connective tissue and periosteum, then sawn into five transverse sections. The marrow was removed from the shaft marrow space. Each section was crushed in 'dry ice' to an eight mesh. 1-gm. samples were extracted in the refrigerator with 2·0 ml. of 0·1 *M tris*, *p*H 7·4 for 4 hr., then centrifuged. The extracts were assayed and the rate of reaction was determined from the linear portion of the reaction curve using the molecular extinction coefficient[4] for reduced triphosphopyridine nucleotide of 6·22 × 10^6 cm.^{2}/mole.

The values presented in Table 1 indicate that isocitric dehydrogenase activity many times greater than that previously reported was found in all sections of the femurs, with highest activity in those areas containing spongy bone. Contrary to Dixon and Perkins[2], who failed to demonstrate activity in bone marrow, our data show considerable isocitric dehydrogenase activity in this tissue. Since trabeculated bone of the femur is surrounded by marrow elements, which were not separable from the calcified areas, the observed enzyme activities are partly a reflexion of the marrow enzyme.

Table 1. ISOCITRIC DEHYDROGENASE ACTIVITY*

Tissue	Growing dogs (approx. 9 months old)		Growing rabbits (8–9 weeks old)	
	Act./gm.	Act./mgm. protein	Act./gm.	Act./mgm. protein
Kidney	418	5·90	742	8·96
Liver	578	7·72	835	10·14
Submaxillary	158	3·25	325	3·07
Femur†, Sect. 1	26	1·36	76	4·18
,, ,, 2	23	1·38	43	4·65
,, ,, 3	5	1·43	9	3·34
,, ,, 4	19	1·44	16	3·77
,, ,, 5	24	1·41	58	4·17
Marrow	48	1·57	115	4·08

* μMoles triphosphopyridine nucleotide reduced/hr. expressed either as per gm. fresh tissue or per mgm. protein. Values are means of at least four separate preparations.
† Section 1, proximal head but not including the lesser trochanter; section 2, extended distally to the marrow space; section 3, midshaft (compact bone); section 4, midshaft to approximately the midpoint of the popliteal surface; section 5, remainder of the femur.

In view of the above findings of considerable isocitric dehydrogenase in the head of the femur, it would be of interest to investigate factors which have been shown to influence the apoenzyme or triphosphopyridine nucleotide-levels in other tissues (that is, vitamin D[5], œstradiol[6], etc.).

The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the U.S. naval service at large.

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Importance of the Synthesis of Acidic Polysaccharide for Wound Healing

Weiss and Matoltsy[1] have reported that wounds in chick embryos fail to exhibit healing prior to about the twelfth day of incubation, but that by the sixteenth day of incubation the mechanisms of healing appeared to be fully developed. This observation appears to have additional significance in relation to some of our work on the synthesis of sulphated polysaccharides in the tissues of embryos and young animals.

We have presented experimental evidence in support of our suggestion[2–4] that the healing of open wounds is dependent upon the ability of the tissues to synthesize, at the site of injury, certain sulphated mucopolysaccharides. Administration of cortisone elicits a reduction in the synthesis of acid mucopolysaccharides, possibly by interfering with hexosamine synthesis in the tissues[5], and concomitant with this inhibition there is a failure of wounds to heal[6]. It was shown also that there is a reduction in the synthesis of sulphated polysaccharides in the tissues of the scorbutic guinea pig[3]. Studies on wound healing in normal animals, in animals treated with cortisone, and in scorbutic guinea pigs indicated a relationship between fibroblastic activity and the synthesis of sulphated mucopolysaccharide. Vascularization is partially responsible for the increased synthesis in wound tissue, but the acidic sulphated polysaccharide observed in the metachromatically stained ground-substance of granulation tissue appears to have been secreted by, or to be associated with, the fibroblasts and mast cells.

In order to determine the relationship between developmental stages and the capacity of chick tissues to synthesize sulphated mucopolysaccharides, skeletal