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Effect of Parathyroid Extract on Succinic Dehydrogenase Activity in Young Rat Bone and Skin.* (29817)

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While studying the mechanism of parathormone action on bone matrix, it was observed that at very early time intervals enzymatic changes could be detected in bone. Goldhaber, using mouse calvaria in tissue culture found that bone resorption was oxygen dependent (1) and that malonate inhibited bone resorption presumably by inhibition of succinic dehydrogenase(2). He also observed large amounts of succinic dehydrogenase in osteoclasts associated with active bone resorption after addition of parathyroid extract (PTE) to culture medium(3). This increase in succinic dehydrogenase has also been reported by other investigators(4-6) employing histochemical techniques. However, Laskin and Engel(7) observed a decrease in succinic dehydrogenase in bone in weanling rabbits following injection of parathyroid extract. This effect of PTE was measured only at 28 hours after the last injection. It would appear that this difference in effect of PTE could be accounted for by the length of time after the hormone injection. The investigations of Jeffay and Bayne(8) which showed that PTE resulted in an early breakdown of bone (the radioactivity of serum calcium rose to a maximum in 6 hours after administration of PTE) and Nichols(9) who found a PTE effect in 2 hours *in vitro* led us to the following experiment which we felt might reconcile the above observations. We assayed young rat bone at 2, 4, 6, 12, 24, and 72 hours after administering 1 unit PTE/g body weight for succinic dehydrogenase activity using the modified triphenyltetrazolium chloride method of Kun and Abood(10). The effect of parathormone on the level of succinic dehydrogenase in skin was also investigated.

Methods. Experimental animals. Male rats of the Holtzman strain 35 to 60 days old, weighing between 70 and 200 g and maintained on a natural stock diet‡ were used. Parathyroid extract (Lilly) was administered intraperitoneally, 1 unit/g body weight to the experimental group while the controls were injected a solution of 0.9% saline, 0.2% phenol, 1.6% glycerol. The animals were sacrificed at 2, 4, 6, 12, 24, and 72 hours after the injection and heart blood was collected for calcium determinations(11), as a check on the potency of the parathyroid extract.

Tissue preparation. The bone was removed and carefully cleaned of connective tissue, periosteum and bone marrow. The epiphyses and cartilage caps of long bones were discarded and the metaphyseal region separated from the diaphyses. The bones of each animal were divided into metaphyses, diaphyses and calvaria, pooled, and frozen in dry ice-ether mixture. They were then crushed in a frozen stainless steel mortar and pestle, and the entire bone sample, after weighing, was quantitatively transferred to the incubation

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vials with the aid of 1 ml of phosphate buffer, pH 7.4.

The skin was shaved and carefully cleaned of loose connective tissue and fat. Small buttons of skin 1.5 mm in diameter were punched from the skin with a dental rubber dam punch and weighed.

Incubation. The incubation mixture was added to the vials containing the tissue samples in 1 ml of phosphate buffer. It consisted of 0.5 ml of 0.1% triphenyltetrazolium chloride (TTC) in water and 0.5 ml of 0.2 M sodium succinate pH 7.4. After mixing, the vials were incubated in air at 37°C for one hour on a Dubnoff shaker at 120 osc./min. At the end of one hour 3.0 ml of acetone were added to the reaction mixture in order to stop the tissue enzyme reaction as well as to extract the TTC and each vial was shaken 5 minutes. The sample was then centrifuged and the supernatant was read at 475 mμ in the Beckman D.U. spectrophotometer. The enzyme activity of each sample was expressed as mg reduced/g wet wt tissue.

Results. There was a marked increase in succinic dehydrogenase activity as a result of PTE extract in the metaphyses at 2 hours. This reached a maximum at 4 hours and returned to normal by 24 hours, decreasing below normal by 72 hours. On the other hand, the diaphyses showed an initial slight decrease at 2 hours followed by a marked increase to a maximum at 6 hours, remaining elevated for 24 hours but returning to normal by 72 hours (Fig. 1).

The calvariae showed a slower and less marked increase, reaching a maximum at 6 hours and returning below normal by 24 hours.

The skin showed an immediate decrease in succinic dehydrogenase activity at 2, 4, and 6 hours and an elevated level at 12 and 24 hours returning below normal at 72 hours.

Discussion. Our results indicate that the effect of PTE on succinic dehydrogenase activity varies depending upon the interval of time elapsing after injection of the hormone. These observations may help to clarify some of the apparently divergent results reported in the literature(1-7).

Summary. Administration of PTE to young rats results in early stimulation of succinic dehydrogenase activity in bone followed by a return toward normal in 12 or 24 hours. This response differs in diaphyseal from the metaphyseal areas as well as in calvaria. The skin responds to PTE by an initial decreased activity and then an apparent compensatory rebound.

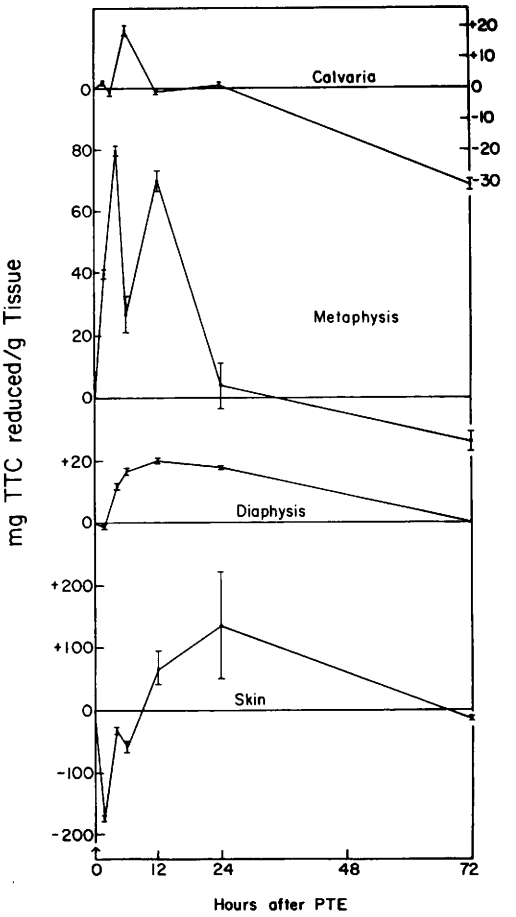

FIG. 1. Succinic dehydrogenase activity in rat bone and skin after treatment with parathyroid extract. Values represent the mean difference between control animals and treated animals and include standard error of the difference of the 2 means.

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Short-Term Caloric Regulation in the Adult Opossum (*Didelphis virginiana*).* (29818)

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A number of investigators have found food intake to be closely associated with the caloric values of the food(1-4). Metabolic consequences of the nutrients have been shown to be of greater importance in regulation of food intake than has the palatability of the foodstuff(5-7).

This study was designed to measure the ability of the opossum to adjust its food intake in accordance with alterations in the caloric concentration of its diet. The opossum was selected because it is feral and is considered to be a primitive mammal which has remained relatively unchanged since the Cretaceous period(8). Furthermore, there is considerable lore about the unusual appetite and feeding habits of the opossum(9).

Methods. Eight locally trapped adult opossum were individually caged in a battery with 15 × 30 × 36 inch units. Body weights at the outset of experiment averaged 2752 g. Standard deviation was ± 869 g. The experiment was carried out between 24 March and 27 June 1964. The average environmental temperature during the experiment was 23°C with standard deviation of ± 2°. The animals were given food and water *ad lib.* and intake was measured daily to the nearest gram or milliliter. The base diet was a ground dog food, consisting of 22.5% protein, 9.3% fat, 42.3% carbohydrate, 21.3% ash and 4.6% water (supplied by Quaker Oats, Barrington, Ill.). Caloric dilutions were obtained by adding nonnutritive cellulose. Caloric enrichments were obtained by adding commercial fat (lard) containing an anti-oxidant to retard spoilage.

A base line on food and water consumption was established over a 14-day period prior to altering the caloric content of the diet. The animals were then placed on the adulterated diets for a 7-day period. There were a total of 6 test periods, 3 dilution tests (10, 25 and 50%) and 3 enrichment tests (9.4, 25 and 50%). Each of these test periods was followed by a recovery period on the base diet for at least 7 days. The calculated caloric concentration of the base diet was 3.4 Kcal/g: the diluted diets were 3.1, 2.6 and 1.7. The enriched diets contained 3.8, 4.3 and 5.1 Kcal/g.

Results and discussion. The intake data were evaluated by comparing mean values of intake on the base diet with those obtained on the adulterated diets. Statistically significant changes in caloric intake were assessed by computing 95% confidence limits for the mean caloric intake on the base diet(10).

Fig. 1 illustrates graphically the caloric regulation of the opossum. Precise caloric

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