Effects of Parathyroid Extract on Sulfate Metabolism of Cartilage and Bone Matrix of Rachitic Rats.* (24343)

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A considerable body of evidence now indicates that, in addition to its effects in the kidney, parathyroid hormone exerts a direct action on bone, leading ultimately to an increase in the serum calcium concentration, but the mechanism of this action remains obscure. Carnes(1) noted that parathyroid extract affected not only mature bone, but also elicited the appearance of osteoclastic resorption in the cartilage matrix of rachitic rats. Following administration of parathyroid extract, Engel(2) observed depolymerization of the mucoprotein ground substance of bone and of the cartilage spicules at epiphyses associated with an increase in plasma and urine mucoprotein concentration(3). Dziewiatkowski(4,5) found that $S^{35}O_4^=$ given to rats is readily incorporated into the chondroitin sulfate of cartilage; the radioactivity level attained a maximum within 24 hrs after intraperitoneal administration and declined slowly thereafter. The plasma $S^{35}O_4^=$ concentration declined rapidly and 95% of injected $S^{35}O_4^=$ was excreted within 120 hrs (6). To determine whether parathyroid hormone influences the metabolism of mucopolysaccharide, it appeared desirable to ascertain the effect of the hormone on the incorporation and disappearance of $S^{35}O_4^=$ in chondroitin sulfate of cartilage and bone matrix.

Methods. Weanling, male rats of the Vanderbilt strain were fed a high calcium-low phosphorus diet (U. S. P. Rachitogenic Diet No. 2) for three weeks before use. The diet uniformly produced florid rickets in this time. The rats were given 37.5 μcuries of S^{35} as carrier-free $Na_2S^{35}O_4$ in saline, intraperitoneally. In independent experiments, parathyroid extract (200 units, Eli Lilly and Co., Indianapolis) was given, intramuscularly, either simultaneous with the $Na_2S^{35}O_4$, 24 hrs earlier or 24 hrs later. Only those rats which appeared well, continued to eat their rations and gained at least 1 g per day after parathyroid administration, were used for the subsequent analyses. At intervals thereafter, groups of 3 rats were sacrificed and the femurs and tibias were removed and cleaned of connective tissue and muscle. The cleaned bones were kept in the cold in distilled water until extraction of the bones for the mucopolysaccharide could be performed.

The method for preparation of mucopolysaccharides was a modification of Astrup's procedure(7). The cleaned bones were pulverized in a micro-Waring blendor until a smooth watery homogenate was obtained. The homogenate was diluted to 50 ml with distilled water and 25 ml of saturated benzidine hydrochloride was added. After stirring, 2 ml of 5N HCl was added, the mixture centrifuged and the supernatant was discarded. To the precipitate was added 10 ml of 10% NH_3 and 50 ml of ether and the mixture was transferred to a separatory funnel. The aqueous layer was filtered and the filter paper was rinsed with a little more 10% NH_3. The filtrate was collected and solid NaCl was added to a concentration of approximately 0.5%. Four volumes of absolute alcohol were then added and a white flocculent precipitate was obtained by centrifugation. The precipitate was dissolved in 10 to 15 ml of distilled water and dialyzed against 20-30 volumes of distilled water overnight in the cold. The dialysate was freeze-dried, the residue weighed and dissolved in distilled water. An aliquot was dried in a steel planchet and counted in a gas flow counter. Control experiments, in which $CaS^{35}O_4$ was added to the bone homogenate, indicated that only organically bound sulfate was determined by this procedure. Moreover, all radioactivity migrated with the mucopolysaccharide on paper electro-

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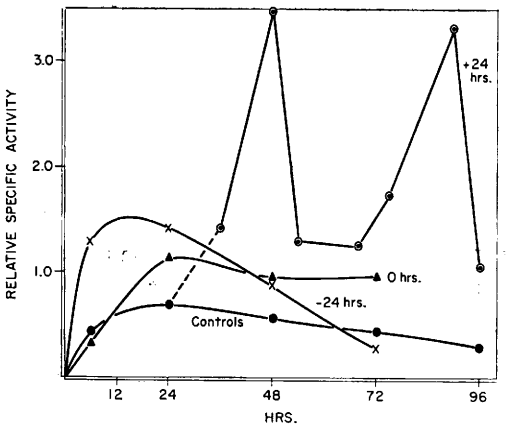

FIG. 1. Influence of parathyroid hormone on incorporation and release of sulfate-S^{35} in chondroitin sulfate of rachitic rat cartilage.

phoresis of the polysaccharide obtained after administration of $S^{35}O_4^=$ to the rats.

Results. Fig. 1 shows the data which were so obtained. In each case, parathyroid hormone markedly increased the rate of incorporation of $S^{35}O_4^=$ into chondroitin sulfate. The most striking response was seen when parathyroid extract was given 24 hrs after administration of $S^{35}O_4^=$. No explanation is apparent for the biphasic curve obtained, but there can be no doubt of the reality of this response since the experiment was performed, independently, 5 times, with 3 rats used for each point in each experiment, and with essentially identical data obtained in each run. It was thought possible that parathormone inhibition of renal sulfate excretion, with consequent maintenance of a higher extra- and intracellular concentration of $S^{35}O_4^=$, might account, in part, for these data. However, in separately conducted experiments, no significant influence of parathyroid hormone on renal sulfate excretion, in normal or rachitic rats, was observed. No observations were made of the influence of parathyroid hormone in normal rats since the method for obtaining the mucopolysaccharide is not satisfactory with normal bone.

The mechanism whereby parathyroid hormone effects the changes observed in these studies is unclear. However, it seems likely that they reflect a "direct" influence of this hormone on the metabolism of the mucopolysaccharide of bone and it may be assumed that these changes are related to the gross chemical and histological changes in bone after parathyroid hormone administration. Alteration of the configuration or charge of the mucopolysaccharide might well suffice to prevent it from binding calcium or from participating in the complex with collagen which initiates calcification as suggested by Sobel (8).

Summary. Incorporation of $S^{35}O_4^=$ into the mucopolysaccharide of rachitic cartilage, and its subsequent release, were enhanced by administration of parathyroid extract when given before, with, or after the labelled sulfate. The mechanism of operation of parathyroid hormone in this regard is unclear, but these observations reinforce the possibility that the action of the hormone on bone is mediated by its influence, direct or indirect, on the metabolism of bone mucopolysaccharide.

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