THE INDUCTION AND INHIBITION OF BONE RESORPTION IN VITRO

John J. Reynolds and John T. Dingle, Strangeways Research Laboratory, Cambridge, United Kingdom.

The effects of parathyroid hormone, vitamin A, and calcitonin on explanted mouse calvariae have been investigated. The essential details of our experimental system have been described previously (Reynolds, 1968). For quantitative studies we use Ca-45-labelled calvariae from six day old mice (Reynolds and Dingle, in press).

Either parathyroid hormone (2 units/ml) or vitamin A (22 international units/ml) induce bone resorption, and many multinucleate osteocalsts are seen histologically (Gaillard, 1961; Talmage et al., 1965; Reynolds, 1968). No morphological changes can be observed in treated bones viewed under a dissecting microscope until after about 30 hours; then small transparent resorption lacunae become evident, and these enlarge as resorption proceeds. However, an increased release of Ca-45 from the treated bones to the medium, compared with their controls, begins much sooner than this. We have been able to observe a significantly increased release of Ca-45 in 2 hours, whether we treat the bones with vitamin A or parathyroid hormone. A modified in vitro system should enable us to see changes earlier than this. Since an increased release of Ca-45 occurs quickly, we interpret the results as indicating that both vitamin A and parathyroid hormone act on pre-existing cells to stimulate an increased mobilization of bone mineral; the increased numbers of osteoclasts are a later event (Talmage (1967). A similar conclusion was reached by Raisz and Niemann (1967) who studied the early effects of parathyroid hormone on rat bone in vitro.

Previous in vitor experiments (Reynolds, 1968; Reynolds and Dingle, in press; Reynolds et al., in press) showed that calcitonin prevents both the formation of multinucleate osteoclasts and their ability to mobilize bone mineral. Recently we have shown that calcitonin acts in vitro in a similar time interval to that required for the action of calcitonin in vivo (Reynolds and Dingle, in press). Mouse calvariae were treated for 2 days in vitro with either parathyroid hormone (2 units/ml) or vitamin A (22 I.U./ml) to induce extensive bone resorption. Then either porcine calcitonin or chicken calcitonin was added to one of each pair of resorbing bones. Inhibition of release of Ca-45 from calcitonin treated bones was observed within 25 minutes. Calcitonin also has a marked effect on the release of Ca-45 from mouse calvariae when tested at the time of explanation. During the first six hours in vitro, clacitonin treated bones release only about 60% as much Ca-45 as untreated controls. We attribute this reduction to the suppression of the endogenous resorption that is in progress when the bones are explanted, and it can be used as an assay for "calcitonin-like" activity.

Several calcitonin preparations differ in their ability to inhibit resorption. The efficacy of these various calcitonin preparations, including human calcitonin, will be discussed in relation to differences in species response to calcitonins. In vivo studies (Parsons and Reynolds, 1968) have also shown that there is species discrimination between calcitonins.

Reynolds, J.J., Proc. Roy. Soc. B. 170:61 (1968).

Reynolds, J.J. and Dingle, J.T., Nature (in press).

Gaillard, P.J., The Parathyroids (ed. Greep, R.O. and Talmage, R.V.), p. 20, Springfield, Illinois: Charles C. Thomas (1961).

Talmage, R.V., Doty, S.B., Cooper, C.W., Yates, C. and Neuenschwander, J. The parathyroid glands: ultrastructure,secretion and function (ed. Gaillard, P.J., Talmage, R.V. and Budy, A.M.), p.107, Chicago: University Press, 1965.

Talmage, R.V., Clin. Orthop. 54:163 (1967)

Raisz, L.G. and Niemann, I., Nature 214:486 (1967).

Reynolds, J.J., Dingle, J.T., MacIntyre, I. and Gudmundsson, T.V., Calcitonin: Proceedings of Symposium on Thyrocalcitonin and the C Cells (ed. Taylor, S.) London: Heinemann Medical Books (in press).

Parsons, J.A. and Reynolds, J.J., Lancet I, 1067, (1968).