Direct Humoral Control of Parathyroid Function in the Dog.*† (26619)

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The primary function of the parathyroids is the control of blood calcium(1,2). It is generally agreed that this control is reciprocal. Rasmussen(3) states that: "the primary factor regulating the secretory activity of the parathyroid glands is the calcium ion activity ($A_{Ca^{++}}$) of the plasma." However, evidence for this has been largely indirect. Ham *et al.*(4) demonstrated hyperplasia of the parathyroids of calcium depleted rats. Stoerk and Carnes(5) showed that this enlargement correlated directly with the lowered serum calcium values of these animals, but had no definite relation to the inorganic phosphate level in the plasma. Toft and Talmage(6) have observed a quantitative increase in numbers of osteoclasts in the long bones of rats associated with the hypocalcemia produced by continuous peritoneal lavage with calcium-free rinse. Patt and Luckhardt(7) perfused calcium depleted blood through an isolated dog thyroid-parathyroid gland, and when this perfusate was subsequently injected into a second dog, they demonstrated a rise in blood calcium similar to that obtained with parathyroid extract. Their experiments provided the stimulus for the studies reported here in which a direct humoral control of parathyroid function has been demonstrated.

Procedures. Dogs were fasted overnight and anesthetized with nembutal, the right thyroid-parathyroid glands were removed, and the left glands were exposed, along with the carotid and superior thyroid arteries. The muscular branches of the latter were ligated. A dose of 0.5 mg/kg heparin was then injected *i.v.* to prevent clotting in the apparatus, and the carotid was cannulated proximally. Blood from the cannula was conducted in plastic tubing through a variable speed perfusion pump (Model TM-11, Sigmamotor, Inc., Middleport, N. Y.) to a second cannula inserted into the carotid well below the superior thyroid artery and directed towards it. The carotid was then tied above this branch, so that blood from the pump flowed only through the thyroid and parathyroids supplied by it. The tubing entering and leaving the animal was bound together in an insulated sheath, so that the returning blood was warmed by counter-current exchange. The experimental arrangement is shown diagrammatically in Fig. 1. Systemic blood pressure and perfusion pressure were observed directly.

The plasma calcium in the perfusing blood was raised approximately 4 mg % (1 mM/l) by addition of a solution containing 5 mM/l calcium chloride or calcium gluconate, or was lowered by addition of an equivalent amount of the calcium complexing agent disodiumethylenediaminetetraacetate (EDTA). Both were made up in physiological saline containing 50 mg/l heparin. They were pumped through tubes with approximately 10% of the capacity of the blood

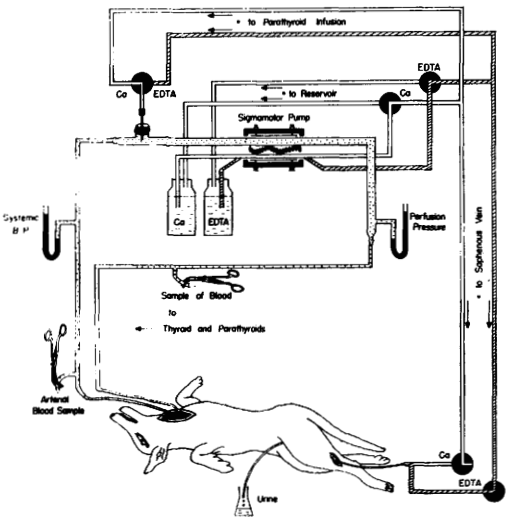

FIG. 1. Apparatus for perfusion of thyroid-parathyroid glands of the dog *in situ.*

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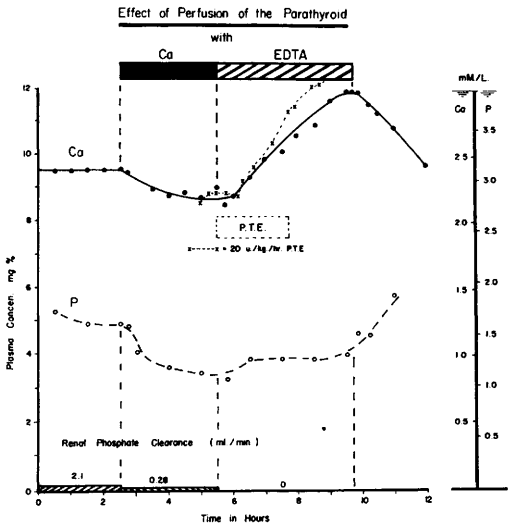

FIG. 2. Typical response of systemic plasma calcium and inorganic phosphate to perfusion of parathyroid with blood to which calcium or EDTA had been added. Dotted line shows changes in the same dog when parathyroid extract (20 u./kg/hr) was injected for a 3 hour period 6 hours after parathyroidectomy.

tube, using the same pump. In this way, the proportion of calcium or EDTA added to the blood remained constant despite the variations in pump speed which were required to maintain a perfusion pressure of 120-150 mm Hg. The flow through the thyroid-parathyroids varied from 5-20 ml/min. By using 3 way stopcocks, it was possible to direct Ca or EDTA into the saphenous vein, the blood perfusing the parathyroids, or the appropriate reservoir. During the control period (2 hr), both Ca and EDTA were perfused into the saphenous vein, and since they form a physiologically inert complex, there was essentially no change in calcium balance. Calcium was then switched to the parathyroid perfusate, while EDTA continued to flow into the saphenous vein. The animal remained in calcium balance, but the parathyroids were exposed to hypercalcemic blood. After 2-3 hours, the solutions were reversed, so that the parathyroids were exposed to blood low in calcium.

Samples of systemic arterial blood and parathyroid perfusion blood were collected at intervals of 15-30 minutes. Plasma calcium was determined by potentiometric titration with EDTA, using a modification of the method of Lehmann(8). Inorganic phosphate in plasma and urine was determined by the Taussky and Shorr(9) modification of the method of Fiske and Subbarow. Renal clearance of phosphate was determined over 2 hr clearance periods. Fifteen animals were studied.

Experimental results. A typical experiment is illustrated in Fig. 2. Plasma calcium remained constant during the control period. Shortly after beginning perfusion of the parathyroids with high calcium blood, there was a fall in systemic blood calcium which began to level off after 2 hours. When the glands were now perfused with low calcium blood, there was a delay of 30 minutes, followed by a sharp rise in blood calcium, reaching a level of 12 mg% within 4 hours. Perfusion of the gland was now stopped, and continuity of the carotid was restored. Almost immediately, blood calcium fell towards normal. Two days later, the animal was normocalcemic. The surviving parathyroid and the arterial graft were now removed, and 6 hours later, the animal received a continuous *i.v.* infusion of 20 units Parathyroid Extract (Lilly) per kg/hr for 3 hours. As shown by the dotted curve in Fig. 2, there was a rise in plasma calcium which corresponded to that obtained with hypocalcemic stimulation of the parathyroid.

There was no significant change in plasma inorganic phosphate during stimulation of the gland with low calcium blood. This was observed in 8 perfusions; in 4 the plasma phosphate rose during this phase; in 3 there was a fall associated with increased excretion of phosphate in the urine. In one experiment, illustrated in Fig. 3, inorganic phosphate was injected *i.v.* during this phase, doubling plasma phosphate concentration without preventing the rise in plasma calcium.

Discussion. These experiments show that the level of calcium in the blood perfusing the parathyroids has a direct effect on their function with respect to regulating blood calcium, and provide a basis for the "feedback" mechanism proposed by McLean(2). The effects are sufficiently sensitive and fast-acting to explain the acute homeostatic con-

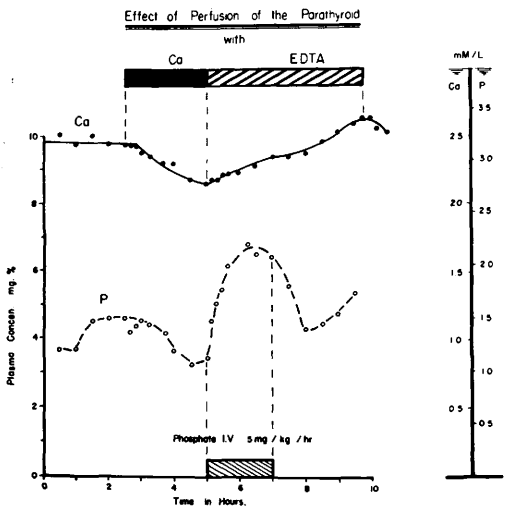

FIG. 3. Changes in plasma calcium and inorganic phosphate in a dog during perfusion of the parathyroids with high calcium (Ca) or low calcium (EDTA) blood. During the phase of hypocalcemic stimulation of the gland, isotonic phosphate solution (5 mg P/kg/hr) was injected *i.v.* for 2 hours.

trol of calcium in body fluids(10). Perfusion of the glands with low calcium blood caused a rise in systemic plasma calcium similar to that resulting from continuous *i.v.* infusion of parathyroid extract, so that this effect is probably due to liberation of parathyroid hormone.

Albright(11) has suggested that the rise in serum calcium resulting from injection of parathyroid hormone is secondary to a fall in blood phosphate. This was certainly not the case in our experiments. During this phase of "physiological stimulation" of parathyroid hormone production, the rise in plasma calcium occurred despite the fact that in most instances the blood phosphate remained unchanged or even increased. Patt and Luckhardt(12) also observed a rise in blood phosphate associated with the rise in calcium following injection of their parathyroid perfusate.

Summary. Direct humoral control of parathyroid function has been demonstrated in dogs in which the isolated thyroid-parathyroid glands were perfused with high calcium or low calcium blood. The latter appears to release parathyroid hormone or a substance with identical action. The resulting rise in systemic blood calcium does not depend on a fall in the inorganic phosphate of blood. These experiments indicate a "feedback" mechanism involving the parathyroids which is sufficiently sensitive and fast-acting to account for the acute homeostatic control of blood calcium.

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