Differential centrifugation of bovine parathyroid tissue

Although the exact role of the parathyroid glands in metabolism has not yet been established, there is general agreement among investigators of two well-demonstrated effects of the active secretion. One of these is a direct action on the kidney resulting in an inhibition of tubular reabsorption of phosphate and the second is a direct action on bone tissue, reflected in an increased osteoclastic activity. Controversy still continues as to which of these effects is primarily responsible for the cardinal role of the parathyroid glands in the regulation of serum calcium.

Recently published works raise the question whether or not these responses and the difficulties encountered in evaluating their relative primary importance may be due to the presence of more than one hormonal principle in the active secretion. STEWART AND BOWEN[1] have reported that preparations of parathyroid hormone after treatment with formaldehyde fail to raise the serum calcium in dogs but do increase the urinary excretion of phosphate. The differentiation of the calcium-mobilizing and the phosphate-excretion activities present in parathyroid gland extracts with the subsequent demonstration of the latter activity in similarly prepared extracts of non-parathyroid tissues led STEWART AND BOWEN to consider the phosphate-excretion factor as a pharmacologically-active degradation factor. DAVIES AND GORDON[2] have found that prolonged dialysis of parathyroid extracts can be employed to separate material which increases the serum calcium in parathyroidectomized rats from material which increases the urinary excretion of phosphate. They believe that the parathyroid gland, at least, secretes a factor necessary in some way for the maintenance of normal phosphate excretion. In this report are described experiments on the differential centrifugation of bovine parathyroid tissue homogenates. The findings suggest that this procedure may serve as a basis for the development of a technique applicable to the separation of the major parathyroid gland activities as well as for the intracellular localization of these activities. The parathyroid gland, to our knowledge, has not yet been the subject of such fractionation studies.

The procedure established by SCHNEIDER[3] for differential centrifugation of liver tissue was employed with some modification. Frozen bovine parathyroid glands§, cleaned of connective tissue and fat by trimming, were first homogenized with 0.25 *M* sucrose solution in the Waring blendor, and then with a teflon pestle in a glass tube. Four fractions were obtained from the homogenate (H) at the International refrigerated PR-1 centrifuge: Fraction (A) sedimented at

TABLE I

DISTRIBUTION OF ACTIVITIES IN SUBCELLULAR FRACTIONS OF BOVINE PARATHYROID TISSUE HOMOGENATES

Expt. No.	*Fraction*	*Dose mg nitrogen*	*Rise in serum Ca mg %*	*Decrease in serum P mg %*
60	H			
	A	1.00	0.0	
	A1	0.20	0.0*	
		0.52	1.0*	
	A2	0.20	0.7*	
		0.30	1.2*	
	S	1.00	—0.9*	
		0.10	0.0	
61	H	1.00	0.6	1.8
	A	0.90	0.0	0.5
	A1	0.30	0.0	1.4
	A2	0.20	0.8	1.4
	S	0.64	0.3	0.2
63	H	0.38	0.2	3.2
	A	0.50	0.2	1.0
	A1	0.35	0.2	1.1
	A2	0.25	1.5	2.0
	S	0.83	0.2	1.5

* Analysis on pooled sample from three animals.

§ We are indebted to Dr. IRBY BUNDING and The Armour Laboratories for the supply of frozen bovine parathyroid glands used in this work.

600 *g* for ten minutes at 1° C; Fraction (A1) at 8,500 *g* for twenty minutes at 1° C; Fraction (A2) at 24,000 *g* for sixty minutes at 1° C and Fraction (S), the supernatant. During the procedure Fractions A, A1 and A2 were washed twice with 0.25 *M* sucrose solutions, recentrifuged twice and the washings were added to the supernatant fluid. The fractions were extracted with 0.4 *M* HCl for 5–8 minutes at 75° C on a water bath and then shaken for two hours on a mechanical shaker at room temperature. The acid extracts were cleared by filtering with suction and dialyzed against three changes of distilled water during forty-eight hours and lyophilized. The fractions were assayed on the basis of changes in serum calcium and phosphorus six hours after the intraperitoneal injection of one ml of the solution of the test substance into fasted thyroparathyroidectomized rats. Blood samples were obtained from the tail vein and the analyses were performed on pooled samples of sera from six and in some instances three animals. Calcium was determined by the method of NATELSON AND PENNIALL[4] and inorganic phosphate by the method of FISKE AND SUBBAROW[5].

The rat assay data for the fractions obtained from the differential centrifugation experiments of the glandular homogenates is given in Table I. The distribution of calcium mobilizing activity in the various fractions for experiment 61 is given in Table II. The calcium activities were estimated from a dose-response relationship established with Lilly Parathyroid Extract.

These data indicate that material affecting the serum calcium is concentrated in fractions sedimented from the homogenate at 8,500 *g* and 24,000 *g*, whereas material affecting serum phosphorus is found in all four fractions from the homogenate. Although careful cytological examination has not been performed, the findings suggest that the calcium mobilizing principle is localized in the smaller subcellular structures of the cell and that a factor (or factors) effecting serum phosphorus changes is distributed throughout the cell. Further experiments are in progress to supplement these findings.

TABLE II

RECOVERY OF SERUM CALCIUM ACTIVITY

Fraction	*Amount of nitrogen (mg)*	*Activity per mg nitrogen (U.S.P. units)*	*Recovery of activity (U.S.P. units)*
61 H*	22.0	25	550
A	22.0	0	0
A1	8.0	0	0
A2	6.0	200	1200
S	25.0	20	500

* H was a 25% aliquot of the homogenate.

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