

The Influence of Plasma Magnesium Concentrations on Calcitonin Secretion in the Pig¹ (35415)

E. TRAVIS LITTLEDIKE AND C. D. ARNAUD
(Introduced by R. W. Dougherty)

National Animal Disease Laboratory, Veterinary Sciences Research Division,
Agricultural Research Service, U.S. Department of Agriculture, Ames,
Iowa 50010; and Mayo Clinic, Rochester, Minnesota 55901

The intravenous infusion of magnesium produces hypocalcemia in dogs (1, 2), man (3, 4), and cats (5). The reason for this hypocalcemic effect of magnesium has not been determined. Care *et al.* (6) reported that magnesium had no effect on the secretion rate of calcitonin of the pig (as determined using bioassay techniques with thyroid venous blood).

Using a sensitive radioimmunoassay for the measurement of porcine calcitonin in plasma (7), we have studied the factors involved in the regulation of the secretion of calcitonin in the pig (8). In the present report, we describe experiments which show that induced hypermagnesemia stimulates calcitonin secretion. Further, the results suggest that the hypocalcemia (both total and ionized calcium) and hypophosphatemia associated with increased plasma magnesium are due to increased plasma levels of immunoreactive calcitonin.

Materials and Methods. Plasma immunoreactive calcitonin (CT) levels were determined using radioimmunoassay methods (7). Plasma total calcium (Ca_T) and magnesium (Mg) were analyzed by atomic absorption spectrophotometry (9). Total inorganic phosphate (P_i) was determined by the method of Taussky and Shorr (10). Plasma ionic calcium (Ca_i) was determined using the series 99-20 calcium activity flow-thru system² of the Orion Research Company attached to a Beckman Expandomatic pH meter³ and Pho-

tovolt varicord recorder (Model 43)⁴ (1-100 mV). Total protein was determined with an autoanalyzer⁵ according to procedure N-14b as suggested by manufacturer.

One male and 7 female crossbred pigs, from 100 to 150 kg, were included in this study. They were fed the normal calcium diet previously used (11). All pigs had chronically implanted femoral artery and vein catheters (12).

The parathyroidectomy procedures used were previously reported (11). The thyro-parathyroidectomized pig was maintained on 4 g of USP thyroid powder/day following surgery. Femoral arterial catheters were used to obtain blood samples, and samples were drawn using heparinized syringes. The blood was cooled, centrifuged immediately, and the plasma was frozen (1-15°) within 15 min after collection.

Solutions of magnesium were made as 1.38 M $MgCl_2 \cdot 6H_2O$ in distilled water. A Harvard peristaltic pump⁶ was used to pump the infusate. All infusions were done through femoral vein catheters in conscious pigs which were restrained in 2 × 5 ft. cages.

Initially, 5 intact pigs were infused with magnesium at different rates. Next a thyro-parathyroidectomized pig was infused with magnesium. Then an intact and a parathyroidectomized pig were infused with magnesium at 3 different sequential infusion rates.

Results. The response of a 100-kg pig to an

¹ Supported in part by NIH Grant AM 12302.

² Orion Research Co., Inc., 11 Blackstone Street, Cambridge, Mass.

³ Beckman Instruments, Inc., 2500 Harbor Blvd., Fullerton, Calif.

⁴ Photovolt Corporation, 1115 Broadway, New York, N.Y.

⁵ AutoAnalyzer, Technicon Corporation, Ardsley, N.Y.

⁶ Model 600-1200 (VDC equipped), Harvard Apparatus Company, Inc., Dover, Mass.

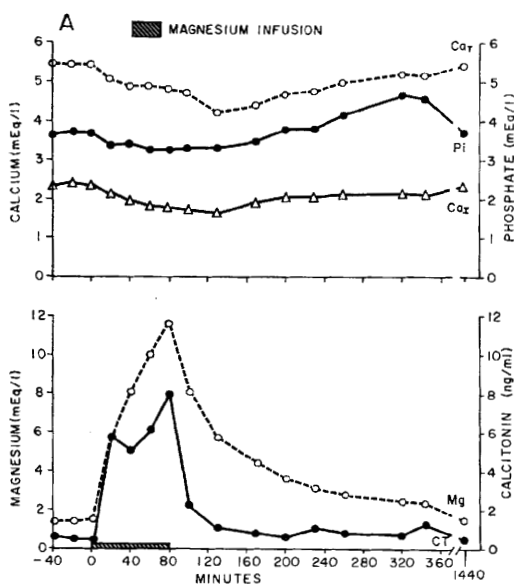


FIG. 1. Effect of intravenous infusion of 1.38 moles/liter of magnesium chloride ($27.6 \mu\text{moles/kg/min}$) on the concentrations of plasma total calcium (Ca_T), ionized calcium (Ca_I), total inorganic phosphate (P_i), and calcitonin (CT) levels of a 100-kg pig (pig A).

80-min intravenous infusion of magnesium chloride ($27.6 \mu\text{moles/kg/min}$) is shown in Fig. 1 (pig A).

Plasma magnesium concentrations (Mg) increased to 11.60 mEq/liter at the end of the infusion period. Narcosis occurred at this concentration of Mg. Plasma Mg decreased gradually; however, it was still 1 mEq/liter above the preinfusion level 4 hr after the magnesium infusion was stopped.

Calcitonin (CT) markedly increased during the magnesium infusion, and a maximum CT concentration of 8 ng/ml occurred at the end of the magnesium infusion period. Plasma CT levels remained above the preinfusion levels for several hours after magnesium infusion was stopped.

A marked hypocalcemic response occurred and plasma total calcium (Ca_T) and plasma ionizable calcium (Ca_I) did not return to preinfusion levels 340 min after the start of the Mg infusion. The Ca_T concentration decreased to 76% of the initial levels of Ca_T ; however, the Ca_I decreased to a minimum of 67% of the initial levels of Ca_I 50 min after

the infusion was stopped. When the total protein present in each sample was determined there was a gradual increase in total protein concentration during and shortly after the period of magnesium infusion from 7.2 to 8.2 g/100 ml.

Plasma total inorganic phosphate concentration (P_i) gradually decreased 11% during the magnesium infusion. However, the P_i returned to the preinfusion level much sooner than the Ca_T . This period of decreased P_i was followed by a marked increase in P_i that lasted for several hours.

Figure 2 shows the response of a 102-kg pig (pig B) to magnesium infused at $13.8 \mu\text{moles/kg/min}$ (one-half the rate infused into pig A). The response of pig B was similar to the response of the pig A. Plasma Mg increased to a maximum of 6.6 mEq/liter at the end of the infusion period and remained elevated for several hours. The increased plasma CT again appeared as a double-peaked curve, and the maximum CT concentration (3.6 ng/ml) in plasma at the end of the infusion period was only about one-half of that obtained in pig A. Again the CT level

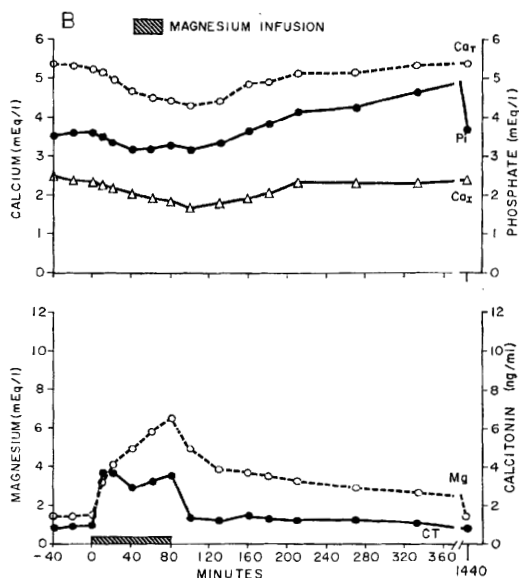


FIG. 2. Effect of intravenous infusion of 1.38 moles/liter of magnesium chloride ($13.8 \mu\text{moles/kg/min}$) on the concentrations of plasma total calcium (Ca_T), ionized calcium (Ca_I), total inorganic phosphate (P_i), and calcitonin (CT) levels of a 102-kg pig (pig B).

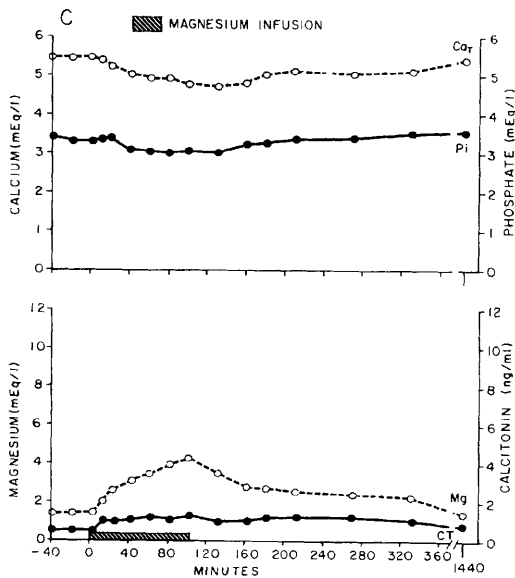


FIG. 3. Effect of intravenous infusion of 1.38 moles/liter of magnesium chloride ($5.52 \mu\text{moles/kg/min}$) on the concentrations of plasma total calcium (Ca_T), total inorganic phosphate (P_i), and calcitonin (CT) levels of a 105-kg pig (pig C).

in the plasma remained elevated for several hours.

Figure 3 shows the response of a 105-kg pig (pig C) to magnesium infused at $5.52 \mu\text{moles/kg/min}$ (one-fifth the rate of that infused into pig A). The plasma magnesium increased to 3.95 mEq/liter after 80 min and to 4.28 mEq/liter at 100 min. This increased level of plasma Mg was present for several hours. The CT was increased 0.4 to 0.8 ng/ml for several hours, suggesting a relatively small sustained release of CT occurred. Again there was a decrease in Ca_T , Ca_i , and P_i . The hypocalcemia was of lesser magnitude in this pig than the pigs receiving more magnesium (pigs A and B).

Infusion of magnesium at $2.76 \mu\text{moles/kg/min}$ in a 104-kg pig (pig D) evoked a very slight decrease (0.6 mEq/liter) in plasma Ca at the end of a 100-min infusion period. The peak magnesium level was 3.58 mEq/liter at the end of the 100-min infusion period. The increase in plasma CT concentration was barely detectable (0.3 to 0.4 ng/ml).

After infusion of $1.38 \mu\text{moles/kg/min}$ of magnesium to a 105-kg pig (pig E), the Ca_T

decreased less than 0.2 mEq/liter at the end of the 100-min infusion period. A change in CT was not detected.

In Figure 4, the response of a thyro-parathyroidectomized male 120-kg pig (pig F) to magnesium infusion ($27.6 \mu\text{moles/kg/min}$) is shown. A rise in total and ionized plasma calcium was present during the infusion period. No change in CT was detected. Little change in P_i occurred during the infusion period; however, there was a hyperphosphatemia several hours later.

The relationship between plasma Mg and plasma CT concentrations is shown in Fig. 5. The increase of plasma CT concentrations was plotted against the increase of plasma Mg concentrations present during the last 40 min of the 80-min magnesium infusion period of the pigs in Fig. 1 (pig A) and Fig. 2 (pig B) and during the last 60 min of the 100-min infusion of the pigs in Fig. 3 (pig C) and pig D (Fig. not included). A straight line was fitted by the method of least squares to describe the relationship between the concentration of CT and the concentration of Mg. The 95% confidence interval of

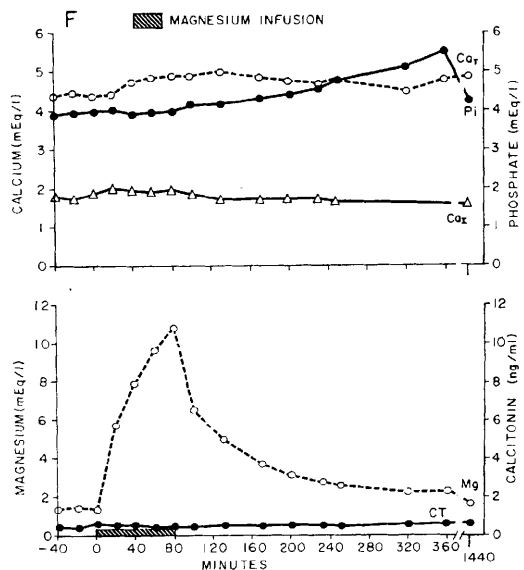


FIG. 4. Effect of intravenous infusion of 1.38 moles/liter of magnesium chloride ($27.6 \mu\text{moles/kg/min}$) on the concentrations of plasma total calcium (Ca_T), total inorganic phosphate (P_i), and calcitonin (CT) levels of a 120-kg male thyro-parathyroidectomized pig (pig F).

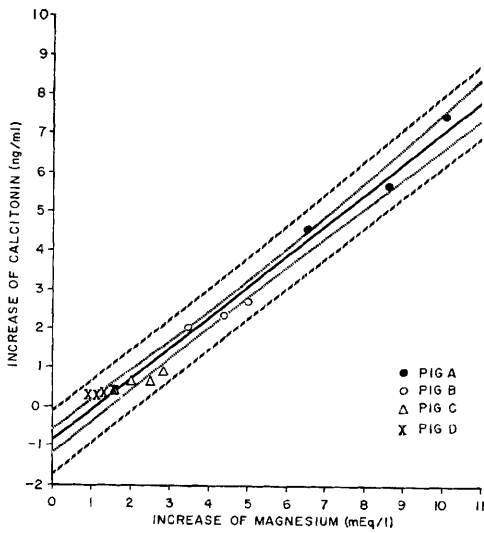


FIG. 5. The interrelationships between plasma magnesium concentrations and plasma calcitonin concentrations during the latter part of the infusions of pigs A, B, C, and D: the population line (—); the 95% confidence interval of the population line (---); as well as the 95% confidence interval for the predicted calcitonin values (diagonally broken line) are shown.

the population regression line is shown in Fig. 5, as well as the 95% confidence interval for the predicted calcitonin values.

There appears to be a definite proportional relationship between the peak concentrations of Mg and CT during the first part of the infusion period when the increase above the preinfusion levels of each are compared. As shown in Figs. 1, 2, and 3, the concentration of CT in the plasma relative to the plasma magnesium concentration during the first 40 min of magnesium infusion was higher than during the latter part of the infusions. The peak CT levels of pigs A, B, and C at 20 min were 5.8, 3.7, and 1.1 ng/ml, respectively, and the peak Mg levels at the corresponding time were 5.8, 4.1, and 2.1 mEq/ml, respectively.

The results of sequential infusion of an intact 120-kg pig (pig G) at 3 different infusion rates of magnesium are shown in Fig. 6. A definite decrease in plasma Ca_T , but no change in CT, was detected when magnesium was infused at the lowest rate (1.24 μ moles/kg/min). At increased rates of infusion

(3.62, 12.28 μ moles/kg/min) an increase in CT was detected, and this increase in CT was accompanied by a prolonged and marked hypocalcemia. The same sequence of infusions in a parathyroidectomized (PTX) male, 120-kg pig (pig H) is shown in Fig. 7. A decrease in Ca_T similar to that of pig G occurred in the PTX pig. The hypophosphatemia present paralleled the hypocalcemia present in the pigs shown in Figs. 6 and 7. The P_i levels were higher in the PTX pig (pig H) than the intact pig G during the entire experiment. The P_i did not increase above preinfusion levels in the PTX pig as it did in the intact pig toward the end of the experimental period.

Discussion. The results of this study indicate that an increase in magnesium concentration in the plasma will stimulate a release of calcitonin from the thyroid gland of the pig. The increased concentration of CT in the plasma was directly proportional to the increased concentration of magnesium ion present in plasma during the last part of the infusions.

During the first part of the infusion peri-

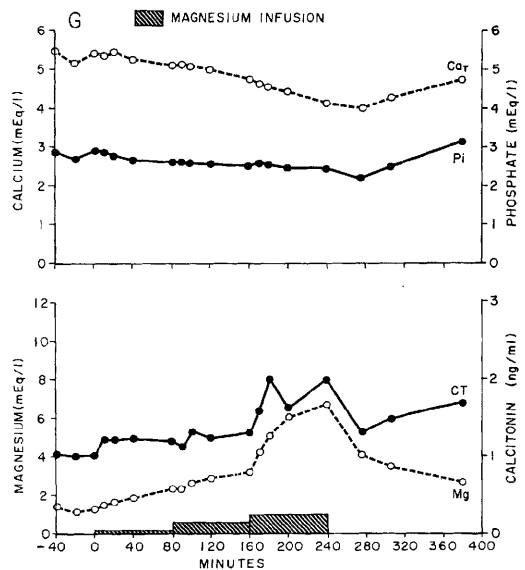


FIG. 6. Effect of the intravenous infusions of magnesium chloride at the three different rates (1.24, 3.63, 12.28 ml/kg/min) on the concentrations of plasma total calcium (Ca_T), total inorganic phosphate (P_i), and calcitonin (CT) in a 120-kg pig (pig G).

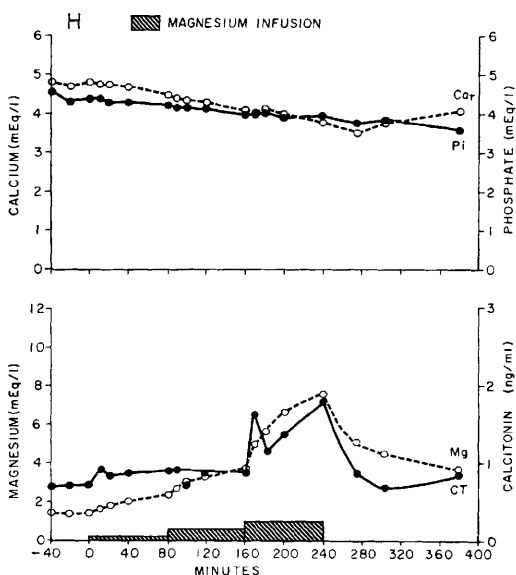


Fig. 7. Effect of the intravenous infusions of magnesium chloride at the three different rates (1.24, 3.63, 12.28 ml/kg/min) on the concentrations of plasma total calcium (Ca_T), total inorganic phosphate (P_i), and calcitonin (CT) in a 120-kg parathyroidectomized pig (pig H).

od, the increased plasma concentrations of CT were much higher in relation to the increase in Mg concentrations than those during the latter part of the infusions. A proportional relationship between plasma Mg and plasma CT was present during the first part of the infusion period, however the relationship is complex and further study will be required to adequately characterize it. A similar biphasic increase in CT concentrations was observed previously when relatively large amounts of calcium were infused into pigs (14). The observed pattern of CT secretion is consistent with the hypothesis that the initial release of CT represents the release of stored hormone, whereas subsequent secretion of CT represents the secretion of newly synthesized hormone. The relative amounts of CT released during prolonged infusion of magnesium are comparable to the amount of CT released during prolonged infusion of equimolar amounts of calcium (14).

The CT released during the magnesium infusions was associated with a marked hypocalcemia. The degree and duration of hypocalcemia was correlated with the amount of

Mg infused. The decrease in total calcium concentrations in the plasma was accompanied by a greater reduction in the ionized calcium concentrations. The difference may be explained by the increase in total protein concentration in the plasma. Magnesium infused at the low rates caused detectable decreases in Ca_T , even though changes in CT concentrations were not detected. This suggests that CT is being released which was not detected by the CT assay.

There are, however, alternative explanations of the hypocalcemic and hypophosphatemic effects of the magnesium infusions. The calciuric effects of parenterally administered magnesium salts were first described by Mendel and Benedict (15). The calciuric effects are usually thought of as competitive inhibition of the tubular calcium reabsorption. Jones and Fourman (16) thought that the urinary excretion of calcium could not account for the decrease in plasma calcium following the infusion of magnesium into hypoparathyroid patients, but that the urinary excretion of calcium could account for the decrease in plasma calcium following the infusion of magnesium into normal control patients. After 2 hr of magnesium chloride infusion into cats, Nielson (5) found that the calculated losses of calcium from the extracellular fluid compartment were much greater than the relatively small (less than 2%) losses of calcium in the urine. However, after nephrectomy magnesium chloride infusion produced no fall in plasma calcium. One explanation for this could have been the development of hyperparathyroidism following nephrectomy, thus calcitonin would be less effective in producing hypocalcemia. The lack of a hypocalcemic effect of magnesium in thyroparathyroidectomized pigs in the present report, and the lack of a hypocalcemic effect of magnesium in thyroparathyroidectomized cats (5) and thyroidectomized rats (17) would suggest that the major factor responsible for the hypocalcemic effect of magnesium is calcitonin.

Only small transient decreases in plasma phosphate concentration occurred during magnesium infusions. This could be due to the hypophosphatemic effect of calcitonin, since it did not occur in the thyroparathyroidecto-

mized pig. The rise in plasma phosphate that occurred in the intact and in the thyroparathyroidectomized pigs after receiving large amounts of magnesium could be due to a hypophosphaturic effect of magnesium (18, 19). However, studies of the changes in the urine following magnesium administration will be necessary to quantitate the influence of the urinary changes of calcium and phosphate on the changes in the plasma calcium and phosphate of the pig.

In the CT assay, as used in this laboratory, there is a certain nonspecific interference (0.4 to 0.6 m μ g/ml) in even plasma or serum from thyroparathyroidectomized pigs similar to that previously reported (13). Thus, small changes in CT in the plasma are difficult to detect and this is also the reason that the increase in CT was used in this report rather than the absolute values.

Concentrations of Mg similar to those shown to be effective in increasing CT concentrations in pigs are found in such conditions as parturient paresis of cows. These increased Mg concentrations could be expected to increase the CT concentrations in the plasma, thereby contributing to a prolonged hypocalcemia.

Magnesium has inhibited the release of parathyroid hormone both *in vivo* (20, 21) and *in vitro* (22) in a quantitatively similar manner to changes in calcium ion concentration. The influence of magnesium concentrations on parathyroid hormone secretion is currently being done in this laboratory. Magnesium may be inhibiting the secretion of parathyroid hormone, thus the action of TCT may be unopposed. This is supported in the present experiments by the similar response in the intact and PTX pigs.

It is concluded that an increase in CT may explain much of the hypocalcemic effects, noted previously by other (1, 2, 15), of parenterally administered magnesium.

Summary. The effect of Mg on the secretion of CT was studied in pigs. Infusion of magnesium was shown to increase the secretion of CT. During the latter part of sustained infusions, this relationship appears directly proportional up to very high concentrations of magnesium. Increased secretion of

CT occurred under conditions of decreasing plasma calcium levels. Both total and ionizable calcium decrease in response to the increased CT level elicited by the infusion of magnesium. Thus, the influence of Mg on CT secretion is an important factor to consider in evaluating CT secretion rates.

The skillful laboratory work of Mrs. Jeanette Winge, the photographic work of Mr. Ralph Glazier, and the statistical treatment of data by Mr. Gordon Booth are gratefully acknowledged.

1. Kemeny, A., Boldizsár, H., and Pethes, G. J., *Neurochemistry* 7, 218 (1961).
2. Knippers, R., and Hehl, U., *Z. Gesamte Exp. Med.* 139, 154 (1965).
3. Kelly, H. G., Cross, H. C., Turton, M. R., and Hatcher, J. D., *Can. Med. Ass. J.* 82, 866 (1960).
4. Jones, K. H., and Fourman, P., *Clin. Sci.* 30, 139 (1966).
5. Nielsen, S. P., *Acta Endocrinol.* 64, 150 (1970).
6. Care, A. D., Duncan, T., and Webster, D., *J. Endocrinol.* 37, 155 (1967).
7. Arnaud, C. D., Littledike, E. T., Tsao, H. S., and Kaplan, E. L., *Mayo Clinic Proc.* 43, 496 (1968).
8. Arnaud, C. D., Tsao, H. S., and Littledike, T., *Mayo Clin. Proc.* 45, 125 (1970).
9. Perkin-Elmer Corp., "Anal. Methods for Atomic Absorp. Spectrophotometry." Perkin-Elmer Corp., Norwalk, Connecticut (1965).
10. Taussky, H. H., and Shorr, E., *J. Biol. Chem.* 202, 675 (1953).
11. Littledike, E. T., St. Clair, L. E., and Notzold, R. A., *Amer. J. Vet. Res.* 29, 635 (1968).
12. Dougherty, R. W., Shuman, R. D., Mullenax, C. H., Witzel, D. A., Buck, W. B., Wood, R. L., and Cook, H. M., *Cornell Vet.* 55, 87 (1965).
13. Arnaud, C. D., Tsao, H. S., and Littledike, T., *in press.*
14. Arnaud, C. D., Littledike, E. T., and Tsao, H. S., *in "Calcitonin—Proceedings of the 2nd International Symposium"* (S. Taylor and G. Foster, eds.), p. 95. Spring-Verlag, New York (1970).
15. Mendel, L. B., and Benedict, S. R., *Amer. J. Physiol.* 25, 1 (1909-10).
16. Jones, K. H., and Fourman, P., *Clin. Sci.* 30, 139 (1966).
17. Radde, I. C., Wittermann, E. R., and Pen-suwan, S., *Endocrinology* 83, 1285 (1968).
18. Clark, I., and Belanger, L., *Calcif. Tissue Res.* 1, 205 (1967).
19. Clark, I., *Amer. J. Physiol.* 214 348 (1968).
20. Care, A. D., Sherwood, L. M., Potts, J. T.,

and Aurbach, G. D., *Nature (London)* **209**, 55 (1966).

21. Care, A. D., *in* "Proceedings of the Symposia Sessions of the 3rd International Congress of Endocrinology." Excerpta Med. Found., Amsterdam, to

be published.

22. Sherwood, L. M., and Bassett, C. A. L., *Int. Congr. Endocrinol.*, 3rd, Excerpta Med. Int. Ser. **157**, 89 (1968).

Received Sept. 1, 1970. P.S.E.B.M., 1971, Vol. 136.