Effects of Changes in Parathyroid Status and Calcium Equilibrium on Bone Matrix Metabolism.* (29902)

C. Conrad Johnston, Jr., William P. Deiss, Jr. and Richard S. French
(Introduced by D. E. Bowman)
Departments of Medicine and Biochemistry, Indiana University School of Medicine, Indianapolis

Parathyroid hormone-induced changes in bone matrix metabolism, as measured by incorporation of proline-C^{14} into hydroxyproline of matrix collagen and glucose-2-C^{14} into hexosamine of matrix mucoprotein, have previously been reported from this laboratory (1,2). It has also been shown that changes in the level of calcium bathing bone *in vivo* may alter the level of equilibration during later *in vitro* incubation(3). Thus, it is possible that the changes in local calcium ion concentration *in vivo* could affect matrix metabolism as well. In addition, acute changes in calcium homeostasis could release the recently proposed hormones, calcitonin(4) or thyrocalcitonin(5), which might also produce metabolic alterations in the bone. Consequently experiments were designed to determine the specificity of the metabolic changes produced by injection of Parathyroid Extract (PTE).

Methods. Male Wistar rats, weighing 90 to 110 g were utilized in all experiments. Each group of animals, experimental or control, consisted of 6 rats. Calcium and PTE† were administered as previously described, except in 2 experiments where a 20% solution of Neo-Calglucon (Sandoz) was utilized and the total dose was 9 mg of calcium rather than 13.5 mg of calcium. Animals receiving PTE were killed 15 hours after the last injection and those receiving calcium infusions were killed 3 hours after the first injection. Parathyroidectomy was performed by electrocautery 3 days before death and the success of the operation was confirmed by measurement of the serum calcium level. Thyro-parathyroidectomy was performed surgically 3 days before the experiment.

Calvaria were removed, cleaned, and cut longitudinally into sections which were incubated in 3 ml of Krebs Ringer bicarbonate medium containing no calcium. In all experiments, except those designed to determine the specific activity of matrix hexosamine, glucose was added to a final concentration of 100 mg per 100 ml. When indicated proline-C^{14} and glucose-2-C^{14} were added in the amounts of 1 μc per flask and the specific activity of matrix hydroxyproline or hexosamine was determined as previously described (2). At the end of the incubation aliquots of medium were removed for determination of calcium(6), glucose(7), and lactate(8). It was previously demonstrated that calcium equilibrium existed at 6 hours in incubation of dead bone(3), and this observation was confirmed in incubation of living bone.

Means were calculated and compared using Student's t test and the probability values are shown in the Tables. In Table I the abbreviations used are shown. The experiments presented were performed over the period of a year. Each experimental group is paired with its control. The variation in controls is due in part to the natural variation between different groups of animals; and also, in the case of hydroxyproline and hexosamine specific activities, to the utilization of isotope of different specific activity in different experiments.

Results. Matrix hydroxyproline and hexosamine. In Table II, the results of a number of different experiments are shown. It can be seen that there is a significant decrease in the specific activity of hydroxyproline whenever PTE had been administered. Calcium infusion produced no change. The removal of the parathyroid gland, either by electrocautery or thyro-parathyroidectomy, also produced a significant decrease in the

* This investigation was supported in part by a USPHS Research Career Program Award, Research Grant and Training Grant from Nat. Inst. of Arthritis and Metab. Dis.

† Kindly supplied by Eli Lilly & Co., Indianapolis, Ind.

TABLE I. Legend for Abbreviations Used in Following Tables.

Group	Control	Experimental
N-PTE	Normals injected with diluent	Normals injected with PTE
N-CA	Normals injected with saline	" " " calcium
SH-PTX	Sham operated parathyroidectomized	Parathyroidectomized
PTX-PTE	Parathyroidectomized injected with diluent	" injected with PTE
PTX-CA	Parathyroidectomized injected with saline	Parathyroidectomized injected with calcium
SH-TPTX	Sham operated thyro-parathyroidectomized	Thyro-parathyroidectomized
TPTX-PTE	Thyro-parathyroidectomized injected with diluent	" injected with PTE

TABLE II. Changes Found in Matrix Hydroxyproline Specific Activity and Hexosamine Specific Activity. All values in this and following tables are means ± standard error of mean.

	Hydroxyproline (cpm/μmole)			Hexosamine (cpm/μmole)		
Group	Control	Exp	P	Control	Exp	P
N-PTE	168 ± 26	23 ± 4	<.001	248 ± 18	441 ± 44	<.005
N-CA	168 ± 26	205 ± 32	NS	248 ± 18	224 ± 27	NS
SH-PTX	522 ± 24	292 ± 66	<.02	620 ± 126	497 ± 80	NS
PTX-PTE	192 ± 24	16 ± 6	<.001	367 ± 40	482 ± 24	<.05
PTX-CA	192 ± 24	214 ± 31	NS	367 ± 40	266 ± 26	NS
SH-TPTX	522 ± 24	251 ± 61	<.005	620 ± 126	548 ± 89	NS
TPTX-PTE	262 ± 31	139 ± 7	<.001	548 ± 89	984 ± 51	<.005

TABLE III. Changes Found in Glucose Utilization and Lactate Production.

	Glucose (μmoles utilized/100 mg bone)			Lactate (μmoles produced/100 mg bone)		
Group	Control	Exp	P	Control	Exp	P
N-PTE	3.3 ± .13	3.5 ± .08	NS	3.2 ± .17	4.2 ± .25	<.005
N-CA	3.3 ± .13	2.6 ± .26	<.02	3.2 ± .17	3.3 ± .37	NS
SH-PTX	3.2 ± .04	1.9 ± .20	<.02	6.4 ± .57	4.5 ± .03	<.02
PTX-PTE	1.7 ± .22	2.2 ± .29	NS	4.5 ± .27	6.3 ± .49	<.01
PTX-CA	1.9 ± .20	2.0 ± .20	NS	4.5 ± .30	5.1 ± .40	NS
SH-TPTX	5.8 ± .20	3.9 ± .30	<.005	5.4 ± .35	4.3 ± .21	<.05
TPTX-PTE	3.9 ± .30	4.4 ± .30	NS	4.3 ± .21	6.7 ± .28	<.001

specific activity of hydroxyproline, although the effect of injection of PTE was still apparent.

An increase in specific activity of hexosamine following PTE is demonstrated in all groups. No change is found after calcium infusion or removal of the parathyroid gland, although a trend toward decreased specific activity after parathyroidectomy is noted.

Thus, decreased specific activity of hydroxyproline and increased specific activity of hexosamine appear to be related to injection of PTE and not to an increased level of calcium ion or acute release of calcitonin or thyrocalcitonin.

Glucose or lactate. The changes produced in glucose utilization and lactate production are shown in Table III. A significant decrease in glucose uptake is produced both by removal of the parathyroid gland and by injection of calcium. When normal calvaria were incubated in medium with varying calcium concentrations (up to 15 mg per 100 ml) no change in glucose utilization of significance was produced. Thus, the change produced by infusion of calcium *in vivo* may be due to decreased output of parathyroid hormone. In all cases administration of PTE tended to increase glucose utilization, although the values did not reach a level of significance.

TABLE IV. Changes Found in Medium Calcium.

Group	Medium calcium (mg/100 ml) Control	Exp	P
N-PTE	3.6 – .04	4.3 – .04	<.001
N-CA	3.6 – .04	4.0 – .05	<.001
SH-PTX	4.0 – .09	3.4 – .06	<.001
PTX-PTE	3.4 – .14	4.2 – .24	<.02
PTX-CA	3.4 – .06	3.8 – .06	<.005
SH-TPTX	3.0 – .11	2.1 – .36	<.05
TPTX-PTE	2.1 – .36	3.0 – .23	NS

Lactate production was significantly increased by administration of PTE in all cases and was decreased by removal of the parathyroid gland.

These data confirm the previous observation by Borle, Nichols, and Nichols(9), and others, that parathyroid extract does increase lactate production, and also imply that parathyroid extract may increase glucose uptake, as has also been suggested. At least in the absence of the hormone, glucose utilization was decreased, although at the dose level of PTE given, uptake was not significantly increased.

Calcium. It can be seen by the results shown in Table IV that medium calcium is increased by infusion of calcium or injection of PTE and decreased by removal of the parathyroid gland. In the case of the thyroparathyroidectomized animals the increase after PTE injection did not reach a significant level in this experiment. Thus, medium calcium appears to reflect the level of calcium bathing bone at time of death.

Discussion. The changes in bone matrix metabolism noted after treatment with PTE: decreased incorporation of label from proline C^{14} into matrix hydroxyproline and increased incorporation of label from glucose-2-C^{14} into matrix hexosamine, are found in bone from normal, parathyroidectomized, and thyroparathyroidectomized animals, and cannot be reproduced by calcium infusion. Thus, it may be suggested that these changes are directly related to the osseous action of the extract. Since the extract is a crude preparation, other fractions than the hypercalcemic principle may be responsible for the effects noted. Rasmussen *et al*(10) have recently isolated fractions from the parathyroid gland which produce different metabolic effects, and one of these may be responsible for the changes. These questions can be answered only with the use of more purified preparations.

Parathyroidectomy does not produce an opposite metabolic effect to injection of PTE. Bone from parathyroidectomized and thyroparathyroidectomized animals incorporated less label from proline C^{14} into hydroxyproline, less label from glucose-2-C^{14} into hexosamine, utilized less glucose, and produced less lactate than corresponding controls. These changes may reflect a general decrease in the metabolic activity of this tissue.

It may be reasoned that parathyroid hormone induces cellular differentiation to cells responsible for bone lysis. Histologic evidence(11) and experimental data utilizing bone in tissue culture(12) support this view. In response to the increased lysis of bone, a secondary stimulation to proliferation of bone would possibly be elicited, and indeed an increased synthesis of collagen is found following the initial decrease after injection of PTE(1). When parathyroid hormone is removed, the rate of differentiation to lytic cells would decrease and as a secondary response the number of blastic cells would also decrease. Thus, the decreased rate of cellular activity shown by the data presented here would be expected.

These data in no way negate the proposed existence of calcitonin or thyrocalcitonin, although no positive evidence for their effect on metabolic parameters measured here is found. These hormones have a very rapid effect, and any changes produced could easily be missed by the techniques utilized here.

Others have shown that the metabolic activity of bone cells is in part responsible for the level at which calcium equilibration occurs *in vitro*(13,14), and that the higher level of equilibration attained by bones from animals treated with PTE may also be due to changes in cellular activity. The data presented here do not refute this position. However, it is shown that hypercalcemia can produce similar results, and it would be difficult to determine which factor played the major role in the increase noted following treatment with PTE. The hormone-induced lysis of bone would probably produce a local hyper-

calcemia and the change in medium calcium could be explained on this basis.

In the experiments presented here, animals were treated acutely and the changes produced in medium and bone were measured over a 6-hour incubation period. These conditions are not comparable to those obtained in tissue culture incubation. Thus, the results found by Mecca *et al*(15), *i.e.*, an increase in medium calcium after 2 days' incubation, cannot be compared.

Conclusion. It may be concluded that the metabolic changes noted in *in vitro* incubations of bone from animals treated with PTE probably reflect the osseous action of the hormone and are not secondary to changes in level of calcium ion or release of calcitonin or thyrocalcitonin. The specific activity of matrix hydroxyproline is decreased and the specific activity of matrix hexosamine is increased in incubation of bone shortly following injection of PTE. Lactate production is enhanced and glucose uptake may be increased by the hormone. The level of equilibration of medium calcium may reflect the level of calcium bathing the bones at death of the animal.

1. Johnston, C. C., Jr., Deiss, W. P., Jr., Miner, E. B., J. Biol. Chem., 1962, v237, 3560.

2. Deiss, W. P., Holmes, L. B., Johnston, C. C., Jr., ibid., 1962, v237, 3555.

3. Johnston, C. C., Jr., Miner, E. B., Smith, D. M., Deiss, W. P., Jr., J. Lab. & Clin. Med., 1962, v60, 689.

4. Copp, D. H., Cameron, E. C., Cheney, B. A., Davidson, A. G. F., Henze, K. G., Endocrinology, 1962, v70, 638.

5. Hirsch, F., Gauthier, G. F., Munson, P. L., ibid., 1963, v73, 244.

6. Kingsley, G. R., Robnett, O., Am. J. Clin. Path., 1957, v27, 223.

7. Huggett, A. St. G., Nixon, D. A., Lancet, 1957, v2, 368.

8. Barker, S. B., Summerson, W. H., J. Biol. Chem., 1941, v138, 535.

9. Borle, A. B., Nichols, N., Nichols, G., Jr., ibid., 1960, v235, 1121.

10. Rasmussen, H., Sze, Y. L., Young, R., ibid., 1964, v239, 2852.

11. Kroon, D. B., Acta Morphol., 1958, v2, 38.

12. Gaillard, P. J., The Parathyroids, 1961, Charles C Thomas Co., p20.

13. Raisz, G., Au, W. Y. W., Tepperman, J., Endocrinol., 1961, v68, 783.

14. Schartum, S., Nichols, G., Jr., J. Clin. Invest., 1961, v40, 2083.

15. Mecca, C. E., Martin, G. R., Goldhaber, P., Proc. Soc. Exp. Biol. and Med., 1963, v113, 538.

Received September 28, 1964. P.S.E.B.M., 1965, v118.

Suppression of the Primary Immune Response Induced by D-L Penicillamine.* (29903)

KURT ALTMAN† AND MARK S. TOBIN‡ (Introduced by K. Lange)
Department of Medicine, New York Medical College, Flower and Fifth Avenue Hospitals, New York City

In a previous communication(1) it was demonstrated that rabbits treated with D-L penicillamine (β,β-dimethyl cysteine) for 28 days prior to a single intravenous injection of I^{131} labeled human serum albumin, exhibited accelerated elimination of the antigen and a correspondingly earlier appearance of free circulating antibody when compared with control animals. The accelerated immune response was characterized by a shortening of the induction period preceding antibody synthesis. Prolonged pretreatment with the drug prior to immunization was found to be a prerequisite for eliciting the accelerating effect. The rate of antibody synthesis appeared to be equal in both treated as well as control animals, suggesting that the drug in-

* This work was supported by grants from USPHS and Health Research Council of City of New York (U1426).

† Postdoctorate Research Fellow, Arthritis and Rheumatism Foundation.

‡ Career Scientist, Health Research Council of City of New York (Contract I-286).