and tortuous blood vessels were observed regularly in the subcutaneous layer of mice that belonged to Groups II and III in contrast to those in Group I.

Discussion. It has been repeatedly shown that acquired immunological tolerance of homologous skin grafts can be induced in adult mice of the parent strain by means of parabiosis with the F_1 hybrid(2,3,4,7). In previous studies, investigators have reported that the duration of parabiosis is very critical in the development of tolerance. It is obvious that sufficient time must be allowed for the exchange or transfer of antigens and development of immunologic tolerance(7). The antigen-dosage time relationship has been adequately shown to be critical in determining whether immunologic tolerance will be obtained or not(7).

This study suggests that if the antigen dosage or transfer of transplantation antigens between parabionts can be increased, the duration of parabiosis necessary for development of tolerance can be significantly reduced. This was accomplished by methods which promoted vascular communication between parabionts.

Summary. Using C_3H and (Ax C_3H)F_1 mice in parabiosis, this study was undertaken to find means of reducing the duration of parabiosis necessary for development of immunologic tolerance. Parabiosis in the usual way maintained for 8-10 days did not result in development of tolerance. When talc powder was sprinkled and mechanical abrasion employed, 25% of the grafted C_3H mice became tolerant to the hybrid tissue. If talc powder was applied 6-7 days prior to parabiosis and the area of contact between parabiosis increased by creation of skin flaps, 85.7% of grafted C_3H mice became tolerant to (Ax C_3H)F_1 skin.

1. Rubin, B. A., *Nature,* 1959, v184, 205.
2. Aust, J. B., Martinez, C., Guttman, R. D., *Surgical Forum,* 1960, v10, 26.
3. Martinez, C., Shapiro, F., Kelman, H., Onstad, T., Good, R. A., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 266.
4. Mariani, T., Martinez, C., Smith, J. M., Good, R. A., *ibid.,* 1959, v101, 596.
5. Sauerbuch, F., Heyde, M., *Munch. med. Wohnschr.,* 1908, v55, 53.
6. Bunster, E., Meyer, R. K., *Anat. Rec.,* 1953, v57, 339.
7. Martinez, C., Shapiro, F., Good, R. A., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 256.
8. Martinez, C., Smith, J. M., Shapiro, F., Good, R. A., *ibid.,* 1959, v102, 413.

Received December 5, 1962. P.S.E.B.M., 1963, v112.

Effect of Parathyroid Hormone Administration on Bone Composition in Guinea Pigs.* (28193)

ALFRED JAY BOLLET, JOHN R. HANDY AND WILLIAM PARSON
Departments of Internal Medicine and Preventive Medicine, University of Virginia School of Medicine, Charlottesville

In view of the role of collagen in nucleating mineral deposition(1), and the influence of polysaccharide on mineralization(1,2), the metabolism of bone matrix components apparently plays a fundamental role in normal and abnormal bone physiology. Several studies have shown an effect of parathyroid hormone on bone matrix metabolism which might be responsible for mineral mobilization. Johnston and Deiss reported a decrease in bone hexosamine concentration after parathyroid hormone administration to rats, suggesting a fall in matrix polysaccharide concentration(3). Hexosamine, however, is present in many proteins and polysaccharides, and its concentration is not a reliable measure of changes in the primary polysaccharide component of bone, chondroitin sulfate(4). Johnston, Miner, and Deiss(5) reported a suppression of hydroxyproline synthesis and stimulation of hexosamine synthesis by parathyroid hormone, support-

* Supported by USPHS grants.

ing the possibility that alterations in matrix composition underlie the mechanism of local bone action by parathyroid hormone. Bernstein and Handler(6) noted an increase of radiosulfate incorporation into rachitic rat cartilage as a result of administration of parathyroid hormone, suggesting a stimulation of chondroitin sulfate synthesis. In contrast to such suggestions of a primary hormonal effect on matrix metabolism are the oft-quoted statements that mineral and matrix are mobilized simultaneously in normal and pathological bone removal(7), and the concept that mineral removal occurs as a result of local acid production(8).

The effect of parathyroid hormone on bone composition was investigated as an approach to the question of whether calcium removal during parathyroid administration results from a primary change in matrix composition, primary mineral mobilization, or simultaneous absorption of both mineral and matrix. Parathyroid hormone was administered to guinea pigs; bone calcium, polysaccharide (as uronic acid), total hexosamine, collagen (as hydroxyproline), and desoxyribosenucleic acid (DNA) concentrations were determined. The data suggest that mineral removal from bone precedes a detectable change in concentration of matrix components, resulting in decreased bone calcium concentration.

Methods. Parathyroid hormone† was administered intraperitoneally to 12 male adult guinea pigs, weighing 300-500 g, in doses of one unit per 5 g body weight given daily for 3 days. Controls were given a similar volume of vehicle. The animals were killed by a blow on the head 24 hours after the last injection. Each femur was dissected free of soft tissues including periosteum; using a bone-cutter, the shaft was cut near the epiphyses, discarding approximately 2 mm of bone with each epiphysis to be certain that no epiphyseal cartilage remained. The main portion of the shaft was then fractured into small pieces, washed carefully with 0.15 M NaCl to remove all visible marrow, cut into fine fragments, and extracted in at least 10 volumes of acetone for 18-24 hours.

† As Parathyroid, U.S.P., kindly supplied by Dr. Glenn W. Irwin, Eli Lilly and Co.

Aliquots of the dried diaphyseal bone were weighed on a Kahn electrobalance and were analyzed by the following procedures: (1) calcium concentration was determined following hydrolysis in 6 N HCl evaporated to dryness on a hot plate; the residue was dissolved in water and an aliquot titrated with EDTA using a calcein indicator, following the procedure described by Diehl and Ellingboe for limestone(9). (2) Hydroxyproline was determined following hydrolysis in 6 N HCl at 15 lb pressure for 6 hours, evaporation to dryness and analysis by the procedure of Kivirikko and Leismaa(10). (3) Total hexosamine was done on bone fragments hydrolyzed in 3 N HCl for 4 hours at 100°C, evaporated to dryness, dissolved in water and analyzed by the procedure of Neuhaus and Letzring (11). (4) DNA was determined by extraction with 10% TCA, using the indole method described by Ogur and Rosen(12). (5) Polysaccharide was determined after decalcification of weighed bone fragments in 8 to 10 volumes of 2 N HCl in 95% ethanol for 3 days. The residue was analyzed by the procedure of Bollet(13); data were expressed in terms of the uronic acid concentration found in the polysaccharide fraction. The completeness of the polysaccharide removal was tested by determining the hexosamine remaining in the residue after extraction of the polysaccharide from the decalcified fragments with sodium hydroxide; 90-95% of the hexosamine had been removed in each instance.

Results. The observations are summarized in the Table. Collagen and DNA concentrations remained unchanged in the parathyroid hormone treated animals. A small but statistically insignificant fall in hexosamine concentration was observed, and no significant change in polysaccharide concentration was detected. The only significant change in bone composition observed in the parathyroid hormone treated group was in the calcium concentration, which was consistently lower in both femora by an average of 7%. This observation was statistically significant ($p = 0.01$).

Discussion. These data show that parathyroid hormone altered bone composition by removal of mineral without significant altera-

TABLE I. Means of Observations on Both Femora of 12 Control and 12 Parathyroid Hormone Guinea Pigs.†

	Control	Treated	
Calcium (g%)	27.5	25.3	$p < .01$
S.E.M.	± .26	± .45	
Hydroxyproline	1.29	1.32	n.s.
(g%) S.E.M.	± .04	± .03	
Hexosamine (mg%)	313*	307	"
S.E.M.	± 3.9	± 9.5	
Desoxyribosenucleic	1.93	1.89	"
acid (g%) S.E.M.	± .14	± .15	
Polysaccharide*	62.0	60.2	"
(uronic acid, mg%)	± 6.3	± 6.0	

* Based on 9 animals.

† Serum calcium levels averaged 9.5 mg % in controls and 10.5 mg % in treated animals.

(n.s. = not significant.)

(S.E.M. = standard error of mean.)

tions in concentration of the major components of the matrix during the period of observation. The authors are aware of 2 previous studies showing mineral mobilization in excess of matrix removal. Shelling, Esher, and Jackson(14) reported a decrease in calcium, phosphorus, and total ash concentration in the femora of rats given 10 or 20 units of parathyroid hormone daily for 3 weeks. Gillespie(15) showed a significant decrease in ash concentration of the tibia of denervated osteoporotic limbs of cats. The removal of mineral and matrix did not proceed at the same rate in these studies. Apparently such a phenomenon can occur in contrast to oft-quoted statements in the literature, based on histologic observations, that bone mineral and matrix are absorbed simultaneously(7).

The mechanism by which parathyroid hormone caused calcium removal from bone remains unclear. Our data favor the older concept of "halisteresis," perhaps as a result of local acid production as suggested by Neumann(8), rather than an initial effect of parathyroid hormone on bone matrix composition, as suggested by others(3,5). There undoubtedly are changes in metabolism of the organic extracellular components of bone, but these effects were not detectable in this study as changes in concentration of these substances, whereas alterations in mineral distribution caused by the hormone had produced changes in bone mineral concentration. It must be kept in mind, however, that the change in hydroxyproline synthesis following parathyroid hormone demonstrated by Johnston, Miner and Deiss(5), would be expected to influence the more rapidly metabolized, salt-soluble collagen, or tropocollagen. Since this fraction is a small percentage of total collagen, a change in total hydroxyproline concentration would not be expected, particularly in view of the incomplete suppression of hydroxyproline synthesis reported. The role of the tropocollagen fraction in influencing mineral deposition in bone is not clear; suppression of collagen formation would be expected to decrease mineral deposition, allowing bone removal to proceed unopposed. If such a mechanism occurred in this study, the data still permit the conclusion that mineral mobilization occurred faster than matrix removal during the period of observation.

Summary. Administration of parathyroid hormone to guinea pigs caused a consistent significant decrease in the femoral concentration of calcium, but no change in concentration of collagen (measured as hydroxyproline), DNA, or polysaccharide (measured as uronic acid). A small but not significant decrease in bone hexosamine concentration occurred. These data indicate that mineral can be mobilized from bone without a concomitant decrease in concentration of matrix constituents.

1. Glimcher, M. J., in *Calcification in Biological Systems,* Ed. Sognnaes, AAAS, Washington, D. C., 1960.

2. Sobel, A. E., Burger, M., PROC. SOC. EXP. BIOL. AND MED., 1954, v78, 7.

3. Johnston, C. C., Jr., Deiss, W. P., Jr., Holmes, L. B., *Endocrinology,* 1961, v68, 484.

4. Meyer, K., Davidson, E., Linker, A., Hoffman, P., *Biochim. Biophys. Acta,* 1956, v21, 506.

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

6. Bernstein, D. S., Handler, P., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 339.

7. McLean, F. C., Urist, M. R., *Bone,* 2 Ed., Univ. Chicago Press, Chicago, Ill., 1961.

8. Neuman, W. F., Neuman, M. W., *Chemical Dynamics of Bone Mineral,* Univ. Chicago Press, Chicago, Ill., 1958.

9. Diehl, H., Ellingboe, J. L., *Analyt. Chem.,* 1956, v28, 882.

10. Kivirikko, K. I., Leismaa, M., *Scand. J. Clin. and Lab. Invest.*, 1959, v11, 128.

11. Neuhaus, O. W., Letzring, M., *Anal. Chem.*, 1957, v29, 1230.

12. Ogur, M., Rosen, G., *Arch. Biochem.*, 1950, v25, 262.

13. Bollet, A. J., *J. Clin. Invest.*, 1958, v37, 854.

14. Shelling, D. H., Asher, D. E., Jackson, D. A., *Bull. Johns Hopkins Hosp.*, 1933, v53, 348.

15. Gillespie, J. A., *J. Bone and Joint Surg.*, 1954, v36B, 464.

Received January 3, 1963. P.S.E.B.M., 1963, v112.

Mucopolysaccharides of Renal Collecting Tubule Cells in Potassium Deficient Rats.* (28194)

GABRIEL GASIC† AND ASHTON B. MORRISON

Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia and Department of Pathology, University of Rochester School of Medicine and Dentistry, Rochester

Striking cytoplasmic granules appear in the cells of the renal collecting tubules in potassium deficient rats. These granules are PAS positive(1), and there is evidence that some components of the serum proteins which pass through the glomerular filter enter into their composition(2). This report is concerned with further investigations dealing with the nature of the amino-sugar component of these granules. The Hale iron method(3) was employed as well as the well-known PAS technique. This Hale procedure involved exposure of the tissue to a colloidal ferric hydroxide. When acid groups are present in the mucopolysaccharide, a colorless compound is formed, which becomes blue after potassium ferrocyanide treatment.

Methods. Male Wistar rats, about 200 g in weight, were made potassium deficient by feeding a commercial low-potassium diet (Nutritional Biochemicals Corp., Cleveland). Control animals were pair-fed this diet adequately supplemented with potassium. After 4 weeks of feeding, blood was withdrawn by aortic puncture, the rats were killed, and portions of the renal papillae were fixed with a formalin-sublimate solution(4) and embedded in paraffin for sectioning at 3 μ. Sections were stained with the Hale iron technique(3), the PAS method, Azure A at low pH(5), and with hematoxylin and eosin. Other procedures used before applying the Hale or PAS method included mild methylation(5), extraction with hot chloroform-methanol(5), or incubation at 37°C in the presence of one of the following enzymes, according to the directions given in Pearse(6): amylase-alpha, malt diastase, crystalline pepsin, crystalline trypsin, chymotrypsin (Nutritional Biochemicals Corp.), cathepsin (Bios Laboratories, Inc.), testis hyaluronidase (Mann Research Laboratories), receptor destroying enzyme (Beringwerke Ag., Marburg-Lahn, Germany), or a highly purified neuraminidase preparation (1.1 mg of protein/ml), kindly supplied by Dr. Leonard Warren, Nat. Inst. Health. Analyses of the serum for potassium were made by flame photometry.

Results. Rats fed the low potassium diet failed to gain in weight during the 4 weeks of the experiment. The mean serum-potassium of the experimental rats was 3.1 $\pm$ 0.2 meq/l compared to 4.9 $\pm$ 0.1 meq/l in the controls. These findings are consistent with a satisfactory induction of potassium deficiency in the experimental animals.

Characteristic PAS positive granules were seen in the collecting tubule cells of the potassium deficient rats. Since chloroform-methanol extraction or pretreatment of the sections with 1% amylase-alpha in saline at 37°C for one hour did not affect the staining capacity of the granules with the PAS, the granules presumably contain a polysaccharide other than glycogen. The granules were also positive with the Hale iron method which

* This work was supported by grants from Nat. Inst. Health.

† On leave of absence from Faculty of Medicine, Dept. of Oncology, Univ. of Chile, Santiago, Chile.