precipitate gave 17 mg. (20%) of orange-red crystals of 5,6-dihydrosempervirine perchlorate (XVII), m.p. 308–309°, on crystallization in methanol.

Dissolution of the perchlorate in a small amount of methanol and 5% aqueous acetic acid and addition of saturated aqueous ammonium nitrate solution led to the nitrate. Several crystallizations in methanol yielded red needles of 5,6-dihydrosempervirine nitrate (XVII), m.p. 305–306°, identical in m.p., mixed m.p. and ultraviolet and infrared spectra with a sample from the catalytic dehydrogenation reaction.

Reduction of 5,6-Dihydrosempervirine Nitrate (XVII).—When a catalytic hydrogenation was carried out on 410 mg. of XVII by the method described above, 135 mg. (40%) of crude alloyohimbane was obtained. Recrystallization in methanol yielded crystalline *d,l*-alloyohimbane, m.p. 144–145°, identical in m.p., mixed m.p. and infrared spectrum with an authentic specimen.

When a sodium borohydride reduction was carried out on 210 mg. of XVII by the method described above, 65 mg. of pure *d,l*-$\Delta^{15(20)}$-yohimbene (XVI), m.p. 194–195°, identified by mixed m.p. and infrared spectrum, was obtained.

Qualitative Data of the Rate of Catalytic Dehydrogenation.—Aqueous fumaric acid solutions (0.44%) of 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100 yohimbine–tetradehydroyohimbine perchlorate mixtures were prepared and their ultraviolet spectra (220–370 mμ) determined. A graph of the resulting six curves was used as standard for determining the contents of dehydrogenation mixtures.

In a typical run, used to diagnose the extent of dehydrogenation, 0.05 mole of the amine was dissolved in 8 ml. of 0.44% aqueous maleic acid solution, 15 mg. of palladium black added and the mixture stirred and refluxed. After filtration the solution was diluted to 10 ml. with water and a 0.4-ml. aliquot diluted to 100 ml. The ultraviolet spectra were plotted and the curves compared with standards. The intensity of the 248, 305 and 365 mμ peaks determined the degree of dehydrogenation.

Four-hour runs with one fairly active batch of palladium black led to the following results: I, (1) yohimbine 95% dehydrogenated, (2) pseudoyohimbine 95%, (3) rauwolscine 95% and (4) 3-epi-α-yohimbine 20%; II, (1) pseudoyohimbyl alcohol 90% and (2) 3-epi-α-yohimbyl alcohol 70%; III (1) pseudoyohimbane 50% and (2) *d,l*-epialloyohimbane 45%. Eight-hour runs with less active catalyst: I, (1) yohimbine 90%, (2) pseudoyohimbine 90%, (3) rauwolscine 90% and (4) 3-epi-α-yohimbine 80%; II, (1) pseudoyohimbyl alcohol 90% and (2) 3-epi-α-yohimbyl alcohol 65%; III, (1) apoyohimbine 80%, (2) aporauwolscine 80%, (3) apo-3-epi-α-yohimbine 30% and (4) *d,l*-epialloyohimbane 40%; IV, (1) ajmalicine 80%, (2) 3-isoajmalicine 0% and (3) akuammigine 90%.

AMES, IOWA

[CONTRIBUTION FROM THE DIVISIONS OF PHARMACOLOGY AND BIOCHEMISTRY, DEPARTMENT OF RADIATION BIOLOGY, SCHOOL OF MEDICINE AND DENTISTRY, THE UNIVERSITY OF ROCHESTER]

Concerning the Mechanism of Action of Parathyroid Hormone I Ion-Gradients[1]

BY H. FIRSCHEIN, G. MARTIN, B. J. MULRYAN, B. STRATES AND W. F. NEUMAN

RECEIVED OCTOBER 11, 1957

Citrate ion has been shown to exert a powerful solubilizing action on hydroxy apatite and on bone mineral. A net production of citrate by bone and its transfer to the circulation has been demonstrated by arteriovenous differences coupled with radiostrontium clearances. The general effects of parathyroid activity on citrate metabolism has been confirmed. Following intravenous administration of parathyroid extracts, increased citrate levels (also increased phosphate levels, by exchange-displacement with citrate?) in serum *preceded* the rise in serum calcium. In keeping with recent data on the solubility of bone, a general hypothesis of the possible mechanism of the action of the parathyroid secretions on bone is given.

Ever since Dickens first demonstrated that bone contains relatively large quantities of citrate,[2,3] there has been a continuing interest in the possibility that this organic anion is of importance in calcium metabolism. Two recent reviews document this interest[4,5] which has prompted numerous suggestions concerning the role of citrate in the homeostatic regulation of calcium levels in serum by the actions of vitamin D and of parathyroid secretions. Reported here are a series of studies designed to clarify the importance of citrate metabolism in the mediation of the action of the parathyroid secretions on bone.

Confirmation of the Correlation between Parathyroid Activity and Serum Citrate Levels.—Mongrel dogs, six months old, were used in this experiment. Three animals were thyroparathyroidectomized, under Nembutal anesthesia, and then maintained for three days on lean hamburger and water *ad libitum*. Two animals served as controls (no sham operation) while two additional dogs received subcutaneous injections of parathyroid extract (Eli Lilly Co.) as follows: 200 units 48 hr., 100 units 36 hr. and 500 units 24 hr. prior to sacrifice. All animals were anesthetized with Nembutal for the withdrawal of samples of venous blood from the jugular vein. Serum was analyzed for calcium[6] and citrate.[7] In addition, the serum samples were ultrafiltered[8,9] to estimate the levels of diffusible calcium, citrate and phosphate.[10] These results are summarized in Table I.

Grossly, these data show that parathyroidectomy decreases the amount of circulating citrate, while injections of parathyroid extracts increase serum citrate. This correlation between serum citrate

(1) This paper is based in part on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York, and in part on work supported by a grant, A1209, from the National Institutes of Arthritis and Metabolic Diseases, U. S. Public Health Service. The authors gratefully acknowledge the assistance of V. DiStefano and D. Leary in developing the surgical procedures.

(2) F. Dickens, *Biochem. J.*, **35**, 1011 (1941).

(3) F. Dickens, *Chem. and Ind.*, **59**, 135 (1940).

(4) T. F. Dixon and H. R. Perkins, "The Biochemistry and Physiology of Bone," edited by G. H. Bourne, Academic Press, Inc., New York, N. Y., 1956, Chapter 11.

(5) H. E. Harrison, *Am. J. Med.*, **20**, 1 (1956).

(6) T. Y. Toribara, P. A. Dewey and H. Warner, *Anal. Chem.*, **29**, 540 (1957).

(7) R. H. Ettinger, L. R. Goldbaum and L. H. Smith, Jr., *J. Biol. Chem.*, **199**, 531 (1952).

(8) T. Y. Toribara, *Anal. Chem.*, **25**, 1286 (1953).

(9) T. Y. Toribara, A. R. Terepka and P. A. Dewey, *J. Clin. Invest.*, **36**, 738 (1957).

(10) P. S. Chen, Jr., T. Y. Toribara and H. Warner, *Anal. Chem.*, **28**, 1756 (1956).

TABLE I

THE EFFECT OF PARATHYROID ACTIVITY ON SERUM LEVELS OF Ca, INORGANIC PO_4 AND CITRATE[a]

Treatment	No. of dogs	Serum Citrate	Serum Calcium	Serum ultrafiltrate Citrate	Serum ultrafiltrate Calcium	Serum ultrafiltrate P
Parathyroidectomized	3	4.2 ∓ 0.1	6.0 ∓ 0.4	3.3 ∓ 0.4	3.6 ∓ 0.4	7.4 ∓ 0.3
Controls	2	5.7 ∓ 0.4	12 ∓ 0.1	4.2 ∓ 0.2	5.8 ∓ 0.2	7.2 ∓ 0.2
Parathyroid extract-injected	2	10 ∓ 0.2	31 ∓ 3	4.9 ∓ 1.7	7.0 ∓ 2.5	5.0 ∓ 0.3

[a] All values expressed as mg. % ∓ av. deviation.

levels and the levels of parathyroid activity has been shown many times.[4,5,11-14]

There also seemed to be an indication that some of the citrate in dog serum is non-diffusible since the ultrafiltered samples were always lower in citrate content than was whole serum. A more exhaustive study, however, of the ultrafilterability in human serum has failed to confirm this finding.[15] Nonetheless, in the parathyroid-injected animals, there was a sharp decrease in the percentage of total calcium that was ultrafilterable. In this case, it seems reasonable to suppose that non-filterable colloidal calcium phosphate[15,16] (which can also bind citrate)[17-19] was formed as a result of the massive dosage given.

The Hypothesized Relation between Citrate Ion and Parathyroid Action on Bone.—The commonly observed correlation between citrate levels and parathyroid activity led to the postulation[20] of a direct metabolic action of the hormone on bone cells: "It was hypothesized that cellular elements of bone normally secrete citrate (or citric acid) in response to parathyroid activity. This citrate—(and)—a local *p*H gradient contribute to the transport of ionized calcium to serum." Direct, though preliminary, evidence of the production of citrate by bone cells was given.[20] The question arises: is such a mechanism workable; is the solubility of bone mineral markedly sensitive to variations in citrate and hydrogen ion concentrations?

The Effect of Citrate on the Solubility of the Calcium Phosphate System.—Because bone mineral is thought to be an impure member of the hydroxy apatite series of minerals,[21,22] a series of equilibrations were made using inorganic salt solutions of varying citrate content and a well-studied, synthetic hydroxy apatite preparation (L-apatite[23-25]) These results are summarized in Table II and Fig. 1.

TABLE II

THE INFLUENCE OF CITRATE ON THE SOLUBILITY OF APATITE[a]

Citrate initial	Citrate final	Calcium final	PO_4 final	*p*H final	$a_{Ca^{++}} \cdot a_{HPO_4^{=}} \times 10^{-9}$
0	0	3.1	1.5	7.39	3.1
2.6	0.47	2.4	2.5	7.42	4.1
5.2	1.9	2.4	3.1	7.44	5.1
7.8	4.0	2.6	3.6	7.45	6.4
10	5.9	2.8	4.1	7.45	8.0
13	8.8	3.1	4.5	7.46	9.2
15	6.8	3.1	4.7	7.42	9.9
39	28	5.6	6.8	7.48	26
79	68	8.5	9.7	7.51	58
156	139	13.6	13.5	7.54	131

[a] 5-g. samples of L-apatite were equilibrated 10 days at 25 ∓ 2° with 1 liter of solutions of approximately 0.145 *M* KCl containing 0.02 *M* veronal buffer and varying amounts of citric acid (+KOH). Initially, *p*H 7.4 and $\mu = 0.165$. All ionic concentrations expressed as $M \times 10^{-4}$. The activity product in the last column was calculated according to Levinskas[23,33] ($\gamma_{Ca^{++}} = 0.36$, $\gamma_{HPO_4^{=}} = 0.23$).

It is evident that the solubility of apatite increases directly (though not linearly) with increasing levels of citrate throughout the range of concentrations investigated. This is true whether solubility is expressed in terms of phosphate or the thermodynamic product, $a_{Ca^{++}} \cdot a_{HPO_4^{=}}$. Calculations, using the currently accepted dissociation constants[26,27] for the calcium citrate chelate, were performed to determine whether chelation of calcium by citrate was an important reaction in the system employed. In all instances, less than 5% of the dissolved calcium was present in complexed form. Clearly, the primary action of citrate is on the solid

TABLE III

INFLUENCE OF CITRATE ON SOLUBILITY OF BONE IN SERUM ULTRAFILTRATE[a]

	Citrate initial	Citrate final	Ca final	PO_4 final	$a_{Ca^{++}} \cdot a_{HPO_4^{=}}$ final $\times 10^{-7}$
No added	0.78	1.1	5.8	13	0.57
Citrate	0.78	1.5	5.8	13	.56
	3.4	2.7	6.5	14	.69
	3.4	2.7	5.8	14	.62
	6.0	4.5	7.3	14	.74
	8.6	6.9	8.0	14	.88
	8.6	6.9	7.3	14	.78
	11.2	8.9	10.9	15	1.2
	11.2	9.9	10.2	15	1.1
No bone added	0.78	0.78	16.5	21	2.3

[a] 5 g. of powdered bone per liter of serum ultrafiltrate. Initial *p*H (adjusted with 5% CO_2), 7.4; final *p*H 7.7 ∓ 0.05. All concentrations are expressed as $M \times 10^{-4}$.

(11) T. S. Chang and S. Freeman, *Am. J. Physiol.*, **160**, 341 (1950).
(12) M. L'Heureaux and G. Roth, *Proc. Soc. Exp. Biol. and Med.*, **84**, 7 (1953).
(13) N. Alwall, *Acta Med. Scand.*, **116**, 447 (1944).
(14) R. V. Talmage and J. R. Elliott, *Endocrinology*, **59**, 27 (1956).
(15) T. J. Hopkins, J. E. Howard and H. Eisenberg, *Bull. Johns Hopkins Hosp.*, **91**, 1 (1952).
(16) M. Laskowski, *Biochem. Z*, **265**, 401 (1933).
(17) A. C. Kuyper, *J. Biol. Chem.*, **159**, 411, 417 (1945).
(18) W. D. Armstrong and L. Singer, "Bone Structure and Metabolism," edited by G. E. W. Wolstenholme and C. M. O'Connor, Little, Brown, and Co., Boston, Mass., 1956, p. 103.
(19) M. Thompson and A. B. Hastings (unpublished results).
(20) W. F. Neuman, H. Firschein, P. S. Chen, Jr., B. J. Mulryan and V. DiStefano, THIS JOURNAL, **78**, 3863 (1956).
(21) W. F. Neuman and M. W. Neuman, *Chem. Revs.*, **53**, 1 (1953).
(22) W. F. Neuman and M. W. Neuman, *Am. J. Med.*, **22**, 123 (1957).
(23) G. J. Levinskas, Thesis, Univ. Rochester, 1953.
(24) W. F. Neuman, T. Y. Toribara and B. J. Mulryan, THIS JOURNAL, **75**, 4239 (1953).
(25) J. H. Weikel, Jr., W. F. Neuman and I. Feldman, *ibid.*, **76**, 5202 (1954).
(26) F. McLean and A. B. Hastings, *J. Biol. Chem.*, **108**, 285 (1935).
(27) J. Schubert and A. Lindenbaum, THIS JOURNAL, **74**, 3529 (1952).

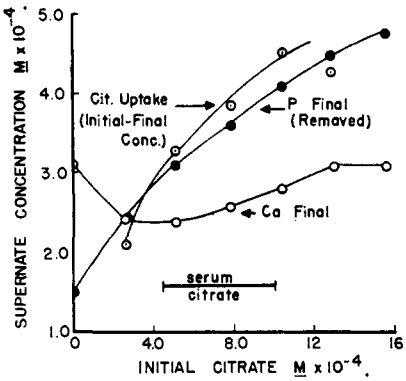

Fig. 1.—The influence of citrate ion on the solubility of apatite. The bar labelled serum citrate represents the range in *final* citrate concentrations which corresponds to the extreme range seen in serum, from 2 to 10 mg. %. Note that in this range, increasing citrate levels increase *both* calcium and phosphate values. Note also the close, 1:1, correspondence between citrate uptake and phosphate dissolved from the solid, P final removed.

phase. Citrate ion enters the solid phase by an exchange-displacement of surface phosphate groups[18,19] and the resulting "citrated-surface" is more soluble than the surface of a pure crystalline apatite. In a closed simple system such as employed in these tests, the phosphate displaced by citrate appeared to repress somewhat the amounts of calcium dissolved at low levels of citrate (below physiological concentrations, 10–20 $\times$ 10^{-4} M).

While these results are clear-cut and decisive, they were obtained under rather idealized conditions. The experiment was repeated, therefore, using powdered (60 mesh), fat-free, cortical veal bone equilibrated with an ultrafiltrate[9] of veal serum to which potassium citrate was added in varying amounts. These results are presented in Table III.

These results again illustrate the powerful, solubilizing action of citrate on a hydroxy apatite mineral even though the system employed was quite complex. Two other points are noteworthy. The serum ultrafiltrate was, by itself, stable and did not undergo spontaneous precipitation. In the presence of powdered bone, however, both calcium and phosphate invariably left the solution *despite the solubilizing action of the added citrate.* This seeming paradox will be discussed more fully subsequently.

That bone mineral is soluble in acid is an observation almost as old as the field of chemistry itself. The increasing solubility of bone mineral with decreasing *p*H is so well documented in the literature[21,28] that only one special aspect deserves mention here. Working with the same apatite preparation employed in the previous solubility study, Table I, Levinskas[23] found that the acid-effect was most pronounced in the region of near-neutrality where a *p*H change of less than 0.1 *p*H unit caused the solubility, expressed as the thermodynamic product, $a_{Ca^{++}}a_{HPO_4^{=}}$, to vary ten-fold. This is illustrated in Fig. 2.

The combined effect of organic acid-production

(28) H. C. Hodge, "Trans. Second Conf. on Metab. Interrelations," 1950, p. 73.

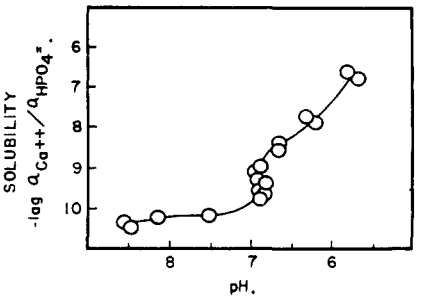

Fig. 2.—The relation between *p*H and the solubility of L-apatite, taken from Levinskas (ref. 23).

(decreased local *p*H) and increased citrate levels could easily explain the apparent solubilizing action of parathormone if such were its metabolic action on bone cells.

The Production of Citrate in Bone and the Effects of Parathyroid Extract.—An attempt was made to determine, by arteriovenous differences, the net transfer of citrate from bone to the circulation in normal and parathyroid extract-injected dogs. By means of the technique described earlier[20] for the cannulation of a small hole drilled into the spongiosa of the metaphysis of the femur of an anesthetized (Dial) dog, mixed arteriovenous blood was collected for comparison with simultaneously drawn arterial samples. Twenty-three such experiments have been performed, nine control dogs and fourteen injected with 500 to 1000 units of parathyroid extract, Eli Lilly. Six experiments have been summarized in Fig. 3.

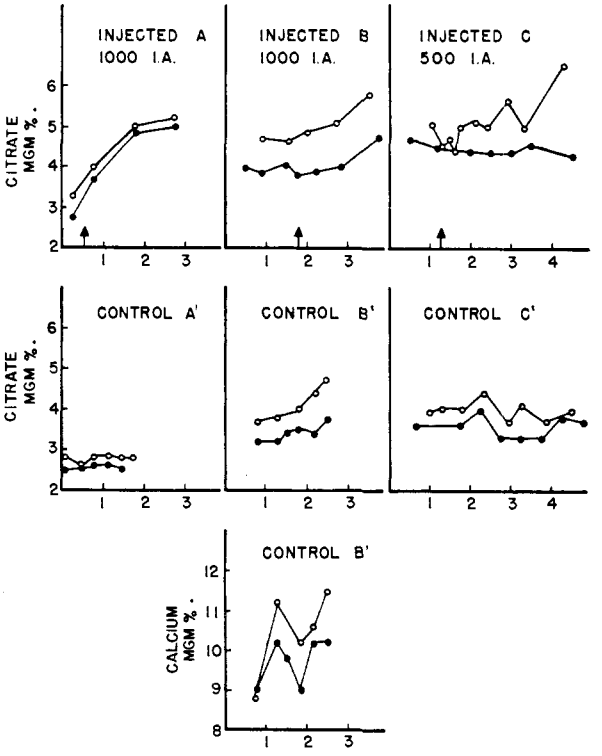

Fig. 3.—The time response in citrate levels to injections of parathyroid extract. Open circles denote catheter blood from bone, solid circles denote arterial blood. Time is given in hours. For explanation, see text.

Simultaneous measurements of radiostrontium, Sr-89, gave a crude measure of the proportion of collected sample which was actually venous flow from bone as distinguished from venous flow from marrow and arterial blood. Radiostrontium, like Ca^{45}, deposits almost exclusively (99%) in bone and, immediately after injection, it is almost completely cleared from the blood flowing through bone.[29] From the average clearance, 23%, of 17 such experiments, it can be presumed that any concentration difference between venous blood coming from bone and its arterial supply is probably about 4 to 5 times that actually observed. Furthermore, since the previous data (Tables II and III) show that bone mineral adsorbs citrate from solution, the bone mineral must act as an enormous "buffer system" resisting changes in the concentration of citrate in the fluids bathing the crystals. It was to be expected, then, that arteriovenous differences and changes in these differences are minimized by the experimental techniques employed.

Under these circumstances, it is especially significant that in all animals, controls and injected, the blood from the bone catheter invariably evinced a positive citrate gradient, *i.e.*, contained more citrate than was found in the arterial supply. By contrast, a comparison of arterial citrate levels with those in the mixed venous blood (jugular) showed no significant difference (8 negative, 5 positive, 4 equal; mean arterial 4.01 mg. %, mean venous 3.94 mg. %). A variety of responses to injections of parathyroid extract was observed. In some instances (A), the citrate levels in both arterial and bone blood rose promptly after injection with little or no change in the gradient. In other cases (B), the level in the bone outflow rose more promptly than that of the arterial supply. In some dogs (C), the arterial supply remained comparatively steady while the citrate gradient of bone rose sharply.

Most control dogs gave consistent results, *i.e.*, as in A^1 and C^1, there was a small, positive citrate gradient seen in the catheter blood from bone, but the citrate levels were steady within analytical error. Occasionally, however, aberrant results were obtained, as in B^1, where the increasing citrate levels and gradients were disturbingly similar to those obtained after injections of extract. In this dog, fortunately, sufficient blood was available for calcium analyses. These data, in Fig. 3, indicate that the dog was slightly hypocalcemic at the start of the experiment and, more importantly, there was a rising and positive calcium-gradient in the bone blood. This suggests that dog B^1, rather than being a true control, was rather a self-activated, temporarily hyperparathyroid animal.

At this stage of the study, it became self-evident that analyses for citrate only could not adequately develop the sequence of events following parathyroid injection. Therefore, a time sequence study was begun with analyses for calcium and phosphate, as well as citrate.

The Sequential Changes following Parathyroid Injection.—From the hypothesized metabolic action of the parathyroid secretions, it follows that changes in citrate metabolism should occur *before* a measurable change in serum calcium can result from a sudden (I.V. injection) increase in circulating hormone. To test this idea, intact healthy dogs were maintained under light anesthesia with Dial and venous samples withdrawn from the jugular vein at least hourly for from 7 to 10 hr. These results are presented graphically in Fig. 4.

The earliest responses to injection of parathyroid extract were changes in the serum levels of citrate accompanied in nearly parallel fashion by changes in phosphate concentrations. Only after the concentration of these two ions had risen sharply and declined again did serum calcium show a significant increase. In the control dogs, there were no marked changes in any of the blood constituents analyzed, though disturbing, small variations were observed. This has been our general experience even in unoperated dogs, but it is uncertain at present whether these small variations in controls are physiological, pathological (the animals were stray mongrels) or analytical in origin.

Discussion

The actions of parathyroid hormone(s) cannot be discussed intelligently without reference to the solubility of bone mineral. Two reviews[30,31] have stressed this point recently: the levels of calcium and phosphate in serum seem to reflect in a direct manner the level of parathyroid activity. This suggests strongly an effect of the hormone on bone solubilities. The difficulties posed by this conclusion have been twofold: (a) the subject of the solubility of bone mineral has, through the years, remained controversial and unclear and (b) it is difficult to see how a hormone is able, in catalytically small amounts, to affect the solubility of an inorganic, crystalline salt which is distributed extracellularly.

Fortunately, in recent years, our understanding of the underlying solubility relationships has been clarified considerably. We now realize that bone salt does not and cannot exhibit a fixed solubility or K_{sp}.[21,23,32] Even in the purest and simplest inorganic solutions, synthetic hydroxy apatite crystals show widely varying solubilities *under equilibrium conditions*.[32] Bone mineral is an impure apatite of varying composition, containing varying quantities of impurities ($CO_3^=$, $Cit^{\equiv}$, Mg^{++}, etc.) and exposed to solutions of varying composition. It is doubtful, too, that a true equilibrium between bone mineral and extracellular fluid is ever achieved *in vivo*. Under these circumstances, we can hardly expect bone mineral to exhibit a fixed solubility or K_{sp}.

We now realize, too, that serum is normally quite supersaturated with respect to bone mineral.[21,33] This is a very old observation[34] confirmed quite recently[33] and indicated clearly in the data presented in Table III. Bone powder added to serum ultra-

(29) D. H. Copp (personal communication).

(30) F. C. McLean and M. R. Urist, "Bone," Univ. Chicago Press, Chicago, Ill., 1955, p. 72.

(31) J. E. Howard, "Bone Structure and Metabolism," edited by G. E. W. Wolstenholme and C. M. O'Connor, Little, Brown and Co., Boston, Mass., 1956, p. 206.

(32) G. J. Levinskas and W. F. Neuman, *J. Phys. Chem.*, **59**, 164 (1955).

(33) B. S. Strates, W. F. Neuman and G. J. Levinskas, *ibid.*, **61**, 279 (1957).

(34) M. A. Logan, *Physiol. Rev.*, **20**, 522 (1940).

filtrate removed calcium and phosphate from solution.

It has proven difficult for investigators to accept the idea that serum is supersaturated because of the simple observation that bone mineral does not spontaneously precipitate from normal sera or even from sera with moderately elevated calcium and phosphate products. The explanation of this seeming paradox is quite simple: it is a rare chemical experience to observe precipitation at or near the dissolution ion-product; some degree of supersaturation invariably is required to induce solid formation. There are also special reasons why this old truism applies to the calcium phosphate system.[33]

There still remains a stubborn question. How can serum *in vivo* remain in a supersaturated condition in the presence of the enormous quantities of solid phase (bone mineral) to which, as isotopic tracers prove, it has ready access? At present, the answer to this question cannot be categorically given but rather can only be hypothesized. To develop this hypothesis was the fundamental purpose of the present investigations.

The hypothesis can be stated briefly. While the $a_{Ca^{++}}a_{HPO_4^-}$ of normal serum is demonstrably supersaturated *under serum conditions*, this same product may be just saturated or even undersaturated *under slightly altered conditions* (lower *p*H, higher citrate level, etc.). On the basis of this chemical argument, it seems reasonable to propose that the normal fluid environment of the bone crystals differs slightly, because of local cellular activities, from the composition of serum itself. Further, it follows that the parathyroid principle responsible for the mobilization of bone mineral may act by causing the bone cells to increase the composition differences between bone fluid and the general circulation.

The present experiments demonstrate the existence of such an ion-gradient (between bone and serum) in the concentration of one important solubilizing ion, citrate. They also demonstrate the influence of parathyroid level on this gradient and the over-all citrate levels in the general circulation. Even the time sequence of events seems compatible with the hypothesis.

Besides the citrate-gradient, there may be bone-blood gradients of other organic acids. The release by or production in bone of any organic acid would, perforce, cause a local *p*H-gradient, in addition to possible specific solubilizing action on bone mineral. This point is currently under investigation.[35] It is likewise too early to decide whether the apparent increase in the net transfer of citrate from bone to blood after the administration of parathyroid extract is resulting from increased synthesis, decreased catabolism or release of citrate stores. Finally, the importance of extraskeletal tissues in the parathyroid-response is not yet clear.

It might be also argued that the demonstration of a citrate gradient between catheter blood from bone and arterial blood does not prove that the citrate is derived from bone. It is a point difficult to prove,

(35) H. Firschein, G. Martin, B. J. Mulryan and W. F. Neuman (unpublished results).

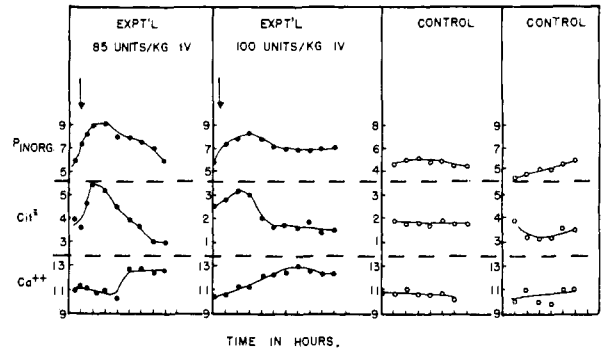

Fig. 4.—The sequence of serum changes in dogs following intravenous administration of parathyroid extract.

but against this argument are the following facts: (a) as shown earlier, the citrate gradient is proportional to the degree of clearance of radiostrontium,[20] (b) in one dog (not included above) no Sr*-clearance was observed and no citrate gradient was seen (c) in other normal animals[35] arteriovenous differences in citrate were observed only in bone (positive) and kidney (negative) and (d) the only tissue other than bone which could supply the gradient in citrate is marrow. Physiologically and histologically parathyroid exerts its primary effects on bone and kidney *not* on marrow.

Finally, the hypothesis presented here is consistent with observations in the recent literature. The citrogenase system, the enzyme complex responsible for synthesis of citrate has been shown to be present in bone and cartilage.[4] On the other hand, the isocitric dehydrogenase system, required for citrate catabolism, has been found to be absent or in low concentration.[4] A number of recent papers have conclusively demonstrated a direct connection between the parathyroid glands and the metabolism of citrate,[14,36–38] but the nature of the hormonal action has not been clearly defined. The postulated[30] "dual mechanism with feedback control" is in no way contradictory to the more specific mechanism of parathryoid action hypothesized here. Also, the suggestion that the bony elements possess a "membrane" or "barrier"[31] is semantically different from but fundamentally similar to the present proposal of cellularly-controlled ion-gradients. Finally, a study of the solubility of powdered bone mineral has led one investigator to conclude recently that the "tissue fluid concentrations of calcium and phosphate could represent an equilibrium with the bone salt if the *p*H on the surface of the bone crystal was about 6.6 to 6.8."[39]

In this development, the renal effects of the parathyroid secretions have been ignored. This problem is beyond the scope of the present data. It is also evident that the *final* decisions regarding the biochemical actions of the parathyroid hormone(s?)[40] must await the isolation of pure components.

ROCHESTER, N. Y.

(36) J. R. Elliott and S. Freeman, *Endocrinology*, **59**, 181 (1956).
(37) J. R. Elliott and S. Freeman, *ibid.*, **59**, 196 (1956).
(38) S. Freeman and J. R. Elliott, *ibid.*, **59**, 190 (1956).
(39) B. E. C. Nordin, *J. Biol. Chem.*, **227**, 551 (1957).
(40) P. L. Munson, *Ann. N. Y. Acad. Sci.*, **60**, 776 (1955).