stoichiometric point. The authors have found this to be the case also when an amino acid is similarly titrated, and recently the interferometer method of following simple acid-base neutralizations has been reported elsewhere.[2]

The data for a gelatin titration are given in Table I and are shown graphically in Figure I. The gelatin used was a pure specimen obtained from the Eastman Kodak Company. Its ash content was 0.03%.

The 2 branches of the curve intersect at the point equal to about 7.9 cc. of acid. Consequently the equivalent weight of this specimen of gelatin is about 1111. For the benefit of the reader interested in a description of the instrument used by the authors, the excellent paper of Adams[3] is recommended.

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Rickets in Rats. VI.* Effect of Phosphate Added to the Diet of Non-Ricketic Rats.

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Phosphate added to the diet causes rapid healing of rats made ricketic on a high calcium-low phosphorus regimen which has not been irradiated and is poor in vitamin D. This is evidenced by histological sections of the bones, blood serum analyses, bone analyses and studies of the metabolism of calcium and phosphorus.[1] As a result of the alteration in diet not only is the rickets cured but a transient tetany is also produced. The blood serum phosphate becomes extremely high—the calcium low. If the animals survive the tetany the blood serum calcium and phosphorus return to values considered normal in 2 weeks. Similarly, the feeding of phosphate to normal dogs is known to produce tetany (Salvesen, Hastings and McIntosh[2]).

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[2] Berl, E., and Ranis, L., *Berichte* (B), 1928, lxi, 92.

[3] Adams, L. H., *J. Am. Chem. Soc.*, 1915, xxxvii, 1181.

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[1] Karelitz, S., and Shohl, A. T., *J. Biol. Chem.*, 1927, lxxiii, 665.

[2] Salvesen, H. A., Hastings, A. B., and McIntosh, J. F., *J. Biol. Chem.*, 1924, lx, 311.

When the diet for rats was altered by the addition of phosphate it was not considered a high phosphorus diet. It was known from the studies of McCollum *et al.*[3] to produce normal rats. The question accordingly arises whether non-ricketic animals shifted from a diet of low phosphorus to one of moderate phosphorus content would develop tetany. If so, would this be of short duration like that observed in the cure of rickets? Or is there something in the condition called rickets which predisposes to an exaggerated effect of phosphate?

Plan—Rats fed with diets of a high calcium low phosphorus content and a liberal supply of anti-ricketic agents were compared to those fed on the same diet without vitamins. After 3 weeks on this diet all were given enough phosphate to make the Ca/P ratio 1/1. Seven and 14 days later the blood serum was analyzed for calcium and inorganic phosphorus, histological preparations of the bones were made, and the percentage ash in the fat-free bones was determined.

Procedure—The basal diet, which produces marked rickets, was Steenbock and Black's diet No. 2965. It consists of 76% of yellow corn, 20% of wheat gluten, 1% of NaCl and 3% of $CaCO_3$. 10% of lard was added. This diet was fed to the group which served as a rickets control. The ratio of Ca/P was 4.25. Diet I was prepared from fine ground yellow corn which had been exposed to ultraviolet rays from a Hanovia lamp for 20 minutes at a distance of 24 inches. To 100 gm. of diet were added 8 gm. of lard and 2 gm. of cod liver oil, which had been previously tested and was rich in vitamins A and D. The ratio of Ca/P, as in the original diet, was 4.25. Diet II was the same as Diet I, except that enough sodium acid phosphate was added to make the ratio of Ca/P 2.0. Diet III was the same as Diet II, except that the corn was not irradiated.

The rats were fed on Sherman's normal diet B during pregnancy and lactation. The young were separated from their mother at 21 days and continued on Sherman's normal diet for 1 week only when they were given the experimental diets. Each group of animals was fed one of these experimental diets for 21 days. All were then changed to a diet similar in all respects to that which they had previously received but with the addition of enough sodium acid phosphate in every case to make the phosphorus content equal to that of the calcium. After 7 and 14 days on the altered diet, they were

[3] McCollum, E. V., Simmonds, N., Shipley, P. G., and Park, E. A., *Bull. Johns Hopkins Hosp.*, 1922, xxxiii, 296.

anesthetized lightly by ether and killed by bleeding. The blood serum of 3 or 4 rats was pooled and analyzed for calcium and inorganic phosphate. The bones were freed from tissue, dried, extracted with alcohol and ether and the ash determined. The methods have been described previously.[1]

TABLE I.

Effect of Moderate Phosphate Additions to the Diet of Ricketic and Non-ricketic Rats.

DIET				SERUM		BONES
	Vitamin added	Ca/P	Days on added P	Ca	P	Ash of fat-free bones
Sherman B				mg. %	mg. %	%
Normal control	None	0.85	0	10.6	9.8	57.4
I	C.L.O.+ irrad.	4.25	7	10.6	9.0	53.0
			14	10.9	10.4	58.0
II	C.L.O.+ irrad.	2.0	7	10.9	9.0	58.0
			14	10.9	9.3	58.4
III	C.L.O.	2.0	7	10.6	9.8	57.6
			14	10.3	10.2	58.0
Basal	None	4.25	7	7.0	13.0	—
			14	8.2	7.6	46.2
Basal Rickets control	None	4.25	0	12.1	1.5	33.5

The high calcium-low phosphorus diets were fed for 21 days and then altered by the addition of sufficient NaH_2PO_4 to make the calcium and phosphorus equal in amount.

Results: The values for the serum calcium and phosphorus and for bone ash are given in Table I. As a standard of comparison values obtained from animals fed Sherman's normal diet are included. The rickets control group showed low serum phosphorus and low ash values characteristic of rickets produced with this diet. The ricketic animals which received added phosphate had serum which was high in phosphate and low in calcium. The bones increased considerably in ash content. The blood serum analyses for the animals on Diets I, II and III show neither a rise in phosphorus nor a drop in calcium and are essentially normal. The histological preparations showed normal bones. The ash content of the bones is normal also.

Ricketic rats develop tetany and have high phosphorus and low calcium in the blood serum when moderate amounts of phosphate are added to the diet. Rats fed with the same diet, plus anti-ricketic vitamins, show neither tetany nor an alteration in blood calcium and phosphorus and their bones yield a normal amount of ash.