

probably occluded and is not an integral part of the protein molecule.

The portion of the purified protein set aside for test purpose assayed 12.6 mgm. protein per cc. and gave a positive Biuret, Xanthoproteic, Millon and Molisch but a negative sulphur and Hopkins-Cole test. It contained a small amount of phosphorus. The protein solution was diluted and tested on tuberculous guinea-pigs and humans. It gave a positive typical skin reaction in doses as low as 0.00005 mgm. on humans. In comparison with O.T. and tuberculin made by Seibert's ultrafiltration method, this protein was relatively less toxic.

4906

The Relation of Vitamin D to Deposition of Calcium in Bone.*

H. C. SHERMAN AND H. K. STIEBELING.

From the Department of Chemistry, Columbia University, New York.

Quantitative studies in our laboratory¹ have shown that when the food of the growing animal (rat) includes a liberal allowance of vitamin D and a constant supply of phosphorus (0.42%), the calcium content of the bodies as determined at intervals during the period of growth is markedly influenced by the calcium content of the food, as varied in these experiments from 0.16 to 0.50% of calcium in the dry food mixture.

In the present experiments we find further, that in rats receiving a basal diet containing a generous allowance of calcium and phosphorus in good proportions (calcium, 0.74%; phosphorus, 0.58%) but with the vitamin D supply restricted practically to a bodily store acquired by 21 to 28 days of age, the growing body has at a given age about the same calcium content as has been acquired by similar animals which received 0.32% calcium, 0.42% phosphorus, and a liberal supply of vitamin D.

The calcium content of the femurs of rats which had been kept from the age of 21 or 28 days to the age of 56 days on the vitamin-D-deficient diet which we have previously described² is about twice as high as in otherwise similar rats fed the high-calcium rickets-producing diet of Steenbock (No. 2965) for the same length of time.

* Published as Contribution No. 624, Department of Chemistry, Columbia University.

¹ Booher, L. E., Dissertation, Columbia University, 1928.

² Sherman, H. C., and Stiebeling, H. K., *J. Biol. Chem.*, 1929, lxxxiii, 497.

Our rats have not had rickets according to the criterion of the "line" test, yet the deposition of calcium in their growing bones has been influenced by the vitamin D content of the food, even though the percentages of calcium and phosphorus in the dry food mixtures were favorable and uniform. Graded amounts of vitamin D supplied in the form of whole (summer) milk powder induced graded increments in calcification up to what appears to be the maximum potential level. In this particular series of experiments the favorable effect of the milk powder upon the normal calcification of the developing bone appears to be specifically due to the vitamin D which the milk contained and not to its mineral elements, because the largest amounts of milk powder fed to these rats did not significantly change either the ratio or the percentage of calcium or phosphorus in the total food, our basal diet being amply reenforced with a salt mixture approximating the composition of milk ash. Nor can the calcifying effect of the milk powder be attributed to fat (as such) or to lactose, for a few parallel experiments showed that the effect was not induced by the feeding of a mixture of milk ash, lactose, (commercial vegetable) fat, and purified casein, in the proportions found in milk solids.

From these new experiments, interpreted in the light of earlier work, it now seems clear that the interdependence of calcium, phosphorus, and vitamin D in bone development is such that under properly controlled conditions any one of the three may be made the limiting factor. In the work of Sherman and Pappenheimer³ rats regularly became rachitic on a certain high-calcium, low-phosphorus diet and were regularly protected by the simple addition of a phosphate to their food. Here the diet was practically devoid of vitamin D but, the diet being such as to inhibit growth, the bodily store of vitamin D which the animals had previously acquired presumably remained as an available but subnormal supply during the experimental period. McCollum and his coworkers,⁴ using diets similarly lacking in vitamin D and of constant phosphorus content, found marked differences to result from shifts in the calcium:phosphorus ratio. Our results also confirm and extend the finding of Steenbock and Black⁵ and of Soames and Leigh-Clare⁶ that vitamin D may be the limiting factor when the mineral content of the diet is good; and likewise

³ Sherman, H. C., and Pappenheimer, A. M., *J. Exp. Med.*, 1921, xxxiv, 189.

⁴ McCollum, E. V., Simmonds, N., Shipley, P. G., and Park, E. A., *Johns Hopkins Hosp. Bull.*, 1922, xxxiii, 296.

⁵ Steenbock, H., and Black, A., *J. Biol. Chem.*, 1924, lxii, 275.

⁶ Soames, K. M., and Leigh-Clare, J. C., *Biochem. J.*, 1928, xxi, 522.

the later work of Steenbock and his associates, of Fairhall, and of Sherman and MacLeod, which shows clearly that vitamin D can not take the place of the calcium needed for optimal bone development. Vitamin D appears to have influence upon the rate of normal calcification, even at levels well above those associated with rickets; but the supplying of vitamin D in abundance does not justify any lack of care in providing the growing organism with the liberal calcium intake which has also been shown to be essential to the optimal development of bone.

4907

Some Differences in Action Between Irradiated Ergosterol and Cod Liver Oil.

A. F. HESS, M. WEINSTOCK AND H. RIVKIN.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

In a recent communication¹ it was reported that chickens require a much greater amount of irradiated ergosterol than its cod liver oil equivalent in order to protect them against leg-weakness. The potency of the irradiated ergosterol preparation used in these experiments has been standardized on rats on the basis of cod liver oil, as is the custom in the United States. In another communication² from this laboratory, it was shown that irradiated ergosterol, when given to rats in inadequate amounts, acted differently from cod liver oil, in that it raised the inorganic phosphorus of the blood to its normal concentration without healing the rachitic lesion.

The action of these two specific antirachitic agents is likewise not identical in relation to infantile rickets. This winter, in the course of an extended series of observations, it was found that 20 drops of a standard preparation of irradiated ergosterol (viosterol) in some instances failed to fully protect infants from rickets, although the inorganic phosphorus concentration of the blood was maintained. According to the standard biological test, 20 drops of this preparation is equivalent to 10 teaspoonfuls of cod liver oil. In one in-

¹ Hess, A. F., and Supplee, G. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, xxvii, 609.

² Hess, A. F., Lewis, J. M., and Rivkin, H., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, xxvii, 611.