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A Biologic Assay of Blood and Feces of Infants Receiving Various Antirachitics.

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It has been shown that the standard method of estimating and establishing the unitage of antirachitic agents by means of biologic assays on rats, does not conform to clinical experience.[1] For example, 40 units of irradiated milk, in other words 650 cc. daily, suffice to protect an infant from rickets whereas approximately 600 units of irradiated ergosterol (viosterol) or 240 units of cod liver oil are required to accomplish a similar result.

In an investigation of this problem an attempt was made to ascertain the antirachitic potency of the blood and the feces of infants who had been given these and other specific antirachitics. It was found possible to carry out satisfactory titrations of the blood when as little as 0.1 cc. of citrated blood was fed daily, or 0.8 for the period of 8 days in the course of the regular 10-day test. Usually twice this amount was fed and in some instances a total of 3.2 cc. of blood. Even less may be used if serum or plasma is fed. In an extended series of assays conducted in this way it developed that the relatively high potency of, for example, irradiated milk as compared to viosterol, could not be accounted for by the relative amounts of antirachitic factor in the blood. When protective doses were given, notwithstanding the fact that the clinical effect was about the same, a definitely larger number of units were found in the blood of the infants which had received viosterol. Evidently antirachitic effect clinically does not parallel the antirachitic content of the blood, as tested on rats. Possibly the explanation of the difference in activity when, for example, irradiated milk or irradiated ergosterol are given, can be ascribed to the production of dissimilar forms of the vitamin.

Assays of the feces served to confirm these results. Although differences in regard to absorption were found, viosterol, cod liver oil, irradiated milk, "yeast milk" were all well absorbed from the intestine. In general, the absorption was relatively the same; where the antirachitic unitage was large, more was recovered from the

[1] Hess, A. F., and Lewis, J. M., *J. Am. Med. Assn.*, 1932, **99**, 647.

feces, and where a small number of units was fed the absolute amount in the feces was small. Direct irradiation of the infant resulted in a moderate amount of antirachitic in the blood and no excretion of vitamin in the feces.

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Comparative Analysis of Forms of Calcium and Inorganic Phosphorus in Human and Cow's Milk.

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It is a common observation that rickets occurs less frequently in breast-fed infants than in those which are artificially fed. The antirachitic superiority of human milk is not due to a greater content of vitamin D.[1] Moreover, cow's milk contains more Ca and P than does breast milk and the ratio of Ca:P is lower in the former. It was thought that, in spite of its lower content of total Ca and P, human milk might contain a larger amount of the most effective forms of these elements and that therein might lie an explanation for the difference in antirachitic value of the 2 kinds of milk.

In a recent communication,[2] a technic was described which rendered it possible to separate the Ca of serum into 4 forms and the P into 2. The technic involved removal, by means of dry $BaSO_4$, of the adsorbable forms of Ca and of inorganic P from the serum and from the corresponding ultrafiltrate. It was shown[3] further that one of the forms, namely the filtrable, adsorbable Ca-P complex, was the one primarily involved in bone formation. Gyorgy,[4] as well as others, have suggested that the Ca and P of milk exist in the form of complex salts and Klinke[5] has demonstrated that more than half of the Ca of cow's milk can be adsorbed with $Al(OH)_3$. There is, thus, some indication that milk may contain a fraction similar to the filtrable, adsorbable Ca-P complex of serum. Accordingly, the above

[1] Hess, A. F., and Weinstock, M., *Am. J. Dis. Child.*, 1927, **34**, 845. Outhouse, J., Macy, I. G., and Brekke, V., *J. Biol. Chem.*, 1928, **78**, 129.

[2] Benjamin, H. R., and Hess, A. F., *J. Biol. Chem.*, 1933, **100**, 27.

[3] Benjamin, H. R., *J. Biol. Chem.*, 1933, **100**, 57.

[4] Gyorgy, P., *Biochem. Z.*, 1923, **142**, 1.

[5] Klinke, K., *Ergeb. d. Physiol.*, 1928, **26**, 235.