ERRATUM

We much regret a printer's error in the pagination of two articles in the October issue of these Proceedings. The second and third pages of the article by Blunt et al. were locked up for press in a position preceding the last page of the article by Pinnell and Martin. As may be apparent from the authors' names, which are correctly given in the page headings, the article by Pinnell and Martin does not include pages 714–5. The pages of the article by Blunt et al. must be read in the following order: 717, 714–5, 718. We appreciate the kind acceptance of our apologies to these authors and the Academy members who communicated these articles.
THE BIOLOGICAL ACTIVITY OF 25-HYDROXYCHOLECALCIFEROL,
A METABOLITE OF VITAMIN D₃*

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In 1966 Lund and DeLuca¹ demonstrated unequivocally the existence of a major polar metabolite fraction of vitamin D in many tissues of rats given ³H-vitamin D₃. This metabolite, which is able to cure rickets in rats at least as well as vitamin D, has since been demonstrated in human plasma,² porcine plasma,³ and chicks.⁴ Further study revealed that this metabolite fraction, like vitamin D, initiates bone mobilization and intestinal transport of calcium,⁵ and its effect on calcium transport is more rapid than that of vitamin D. Stohs and DeLuca⁶ demonstrated that this metabolite fraction is virtually the exclusive form of vitamin D in intestinal nuclei prior to the initiation of biochemical events leading to the rise in calcium transport.

Recently, the major if not the sole biologically active component of this fraction was isolated in pure form from the plasma of hogs fed vitamin D₃, and its structure was unequivocally established as 25-hydroxycholecalciferol (25-OH D₃).⁷ The biological properties of this pure compound have now been examined in a number of systems, and the results are reported in this communication.

Materials and Methods.—Vitamin D: The crystalline vitamin D₃ was obtained from General Biochemicals, Inc., Chagrin Falls, Ohio. 25-Hydroxycholecalciferol (25-OH D₃) was isolated in pure form from the plasma of hogs, as described previously.³ The exact concentrations of these substances were determined by UV absorption at 265 mμ with a molar extinction coefficient of 18,200. The substances were dissolved in cottonseed oil for oral administration or in ethanol for intravenous administration.

Preparation of rats: Weanling male albino rats were obtained from the Sprague-Dawley Co., or from the Badger Research Corporation, both of Madison, Wisconsin. They were housed individually in hanging wire cages and given food and water ad libitum.

For the rickets cure test, we used the rachitogenic diet of Steenbock and Black⁸ as modified by the addition of crystalline vitamins at the level described by DeLuca et al.⁹ After the rats had been on this diet for 21 days, they were given either 25-OH D₃ or vitamin D₃ orally in 0.1 ml cottonseed oil or intrajugularly in 0.02 ml ethanol. Controls were given the appropriate carrier alone. The rats were killed 7 days later, their radii removed and stained with AgNO₃ solution, and the degree of new calcification was scored visually as described in the U.S. Pharmacopoeia.¹⁰

In intestinal transport experiments, rats were fed for 5 weeks the adequate Ca and P diet described earlier,⁹ after which the animals exhibited a serum calcium concentration of 4.0–4.5 mg/100 ml. The 25-OH D₃ or vitamin D₃ was administered intravenously in 0.02 ml ethanol. At the indicated times, the ability of the duodenal section of intestine to transport calcium against a concentration gradient was assessed by the everted gut sac technique described earlier,¹¹ except that the medium consisted of 125 mM NaCl, 10 mM fructose, 0.25 mM CaCl₂ containing ⁴⁴Ca, and 30 mM tris(hydroxymethyl)aminomethane buffer (Tris buffer), pH 7.4.¹² The incubations were at 37°C under a stream of O₂. Samples from inside and outside the sacs were taken after 1.5 hr and counted in a model 3000 Packard Tri-Carb liquid scintillation counter. The scintillator solution¹³ consisted of 3 liters dioxane, 300 gm naphthalene, 14 gm 2,5-diphenyloxazole, 600 mg 1,4-bis-(4-methyl-5-phenyloxazolyl) benzene, to which was added a solution of 36 mg Na₂ EDTA in

717
various D vitamins of different side-chain structure. Previous modifications of the side-chain structure have been shown to decrease antirachitic potency in one or both species.

Of great interest is the high probability that the 25-OH D₃ represents the metabolically active form of vitamin D₃. Not only is this metabolite more active than vitamin D₃, but it acts much more rapidly in both intestine and bone, the prime targets of vitamin D action. As little as 0.25 µg of 25-OH D₃ elicits an intestinal calcium transport response within three hours, while about eight to ten hours is required for the same amount of vitamin D₃ to exert its action. Thus, much of the lag in vitamin D action on intestinal calcium transport and on bone mobilization can be accounted for by its conversion to 25-OH D₃. For a 0.25-µg dose, approximately five to six hours of the lag in vitamin D action probably can be attributed to the conversion of D₃ to 25-OH D₃. Although the present results provide strong evidence that 25-OH D₃ is the metabolically active form of vitamin D, final proof must rest with isolated systems incapable of converting vitamin D₃ to 25-OH D₃, which respond to 25-OH D₃ and not to vitamin D₃ itself. Investigations with 25-OH D₃ are thus continuing to clarify this point.

Summary.—The biological activity of 25-hydroxycholecalciferol, a metabolite of vitamin D₃ isolated from porcine plasma, is established. The metabolite is approximately 1.4 times more active than vitamin D₃ in curing rickets in rats, and is also more active than the vitamin in chicks as determined by the bone ash assay. In vitamin D-deficient rats, intravenous administration of 0.25 µg of the metabolite initiates calcium transport across the intestine much earlier than a similar dose of the vitamin. A 2.5-µg dose of the metabolite administered intravenously to deficient rats also causes an earlier rise in serum calcium concentration resulting from bone resorption than does a similar dose of vitamin D₃. These data, together with those obtained earlier with impure preparations of the metabolite, suggest that 25-hydroxycholecalciferol is the metabolically active form of vitamin D₃.

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6 Stohs, S. J., and H. F. DeLuca, Biochemistry, 6, 3338 (1967).
8 Steenbock, H., and A. Black, J. Biol. Chem., 64, 263 (1925).