Influence of reproductive status on in-vitro renal production of 1,24,25-trihydroxyvitamin D₃ in Japanese quail

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Vitamin D₃ is metabolized first in the liver, producing 25-hydroxyvitamin D₃ which is then further hydroxylated at the 1-position in the kidney, producing the hormonal form 1,25-dihydroxyvitamin D₃ (Holick & DeLuca, 1974). Kenny (1976) has demonstrated that when kidney homogenates of Japanese quail were incubated with 25-hydroxyvitamin D₃ birds with a calcified egg in the shell gland produce predominantly 1,25-dihydroxyvitamin D₃, whereas those without an egg produce mainly 24,25-dihydroxyvitamin D₃.

Text-fig. 1. Chromatographic profiles of the enzymatically generated tritiated substrates using a 0.7 × 18 cm column of Sephadex LH-20, and either a chloroform:Skellysolve B (65:35 v/v) or a chloroform:Skellysolve B:methanol (75:23:2 by vol.) solvent system and 125-drop (0.92 ml) fractions. (a) Enzymatic generation of [³H]1,25-dihydroxyvitamin D₃ from 25-[26, 27-³H]hydroxyvitamin D₃ and an homogenate of kidney removed from a female quail with an egg; fractions 27–40, containing the 1,25-dihydroxy metabolite, were combined for purification (b) using the 75:23:2 solvent system; fractions 12–23 were combined for use as a substrate. (c) Enzymatic generation of [³H]24,25-dihydroxyvitamin D₃ from 25-[26, 27-³H]hydroxyvitamin D₃ and an homogenate of kidney removed from a female quail without an egg; fractions 17–27 containing the 24,25-dihydroxy metabolite were combined for purification (d) using the 75:23:2 solvent system; fractions 7–17 were combined for use as a substrate.

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Text-fig. 2. Chromatographic profiles using a 0.7×18 cm column of Sephadex LH-20 and the chloroform: Skellysolve B:methanol (75:23:2 by vol.) solvent system and 125-drop (0.92 ml) fractions. The 20-min incubations used 10 pmol substrate and a kidney homogenate prepared from birds of different reproductive condition. (a) Substrate = $[24,25\text{-}^3\text{H}]$ dihydroxyvitamin D_3 and kidney homogenate from female with calcified egg in the oviduct; (b, c) Substrate = $[1,25\text{-}^3\text{H}]$ dihydroxyvitamin D_3 and kidney homogenate from female without an egg (b) and a mature male (c). (d, e, f) Substrate = $25\text{-}[26,27\text{-}^3\text{H}]$ hydroxyvitamin D_3 and kidney homogenate was from a female with egg (d), a female without egg (e) and an adult male (f).

Kleiner & DeLuca (1974) and Friedlander & Norman (1975) have reported that the kidney of the immature chicken is capable of producing 1,24,25-trihydroxyvitamin D_3 *in vitro* from the 1,25- or 24,25- form of dihydroxyvitamin D_3 , depending on the dietary conditions. The precise physiological role of 1,24,25-trihydroxyvitamin D_3 is unclear, but it has been shown to mobilize calcium from the intestine (Holick *et al.*, 1973).

The present investigation is concerned with the *in-vitro* production of 1,24,25-trihydroxyvitamin D_3 by kidneys removed from 3 groups (3 birds/group) of adult Japanese quail: (A) females with a calcified egg in the shell gland; (B) females without an egg in the oviduct; and (C) males. All birds were raised on Game Bird Breeder Layena diet (Ralston Purina, St Louis, Missouri) containing adequate calcium (2.3–3.3%) and phosphorus (>0.8%). Three tritiated substrates were used for the kidney homogenate incubations for each group: (a) $25\text{-}[26,27\text{-}^3\text{H}]$ hydroxyvitamin D_3 (sp. act. 2.9 Ci/mmol: Amersham Searle); (b) $[^3\text{H}]1,25\text{-dihydroxyvitamin } \text{D}_3$; and (c) $[^3\text{H}]24,25\text{-dihydroxyvitamin } \text{D}_3$, the dihydroxy substrates being generated enzymatically from the tritiated 25-hydroxy compound using kidneys removed from an adult female quail with or without a calcified egg respectively. The metabolites to be used as substrates were repurified using Sephadex LH-20 chromatography and a chloroform: Skellysolve B (1:1 v/v) solvent system as described by Kenny (1976). Each of the three substrates (approximately 10 pmol in 25 μl 95% ethanol) was incubated with 0.25 ml of a 10% kidney homogenate and 4.75 ml phosphate buffer, pH 7.4, with the necessary co-factors

(Kenny, 1976). The incubation was carried out for 20 min at 37°C in a metabolic shaking bath. Three controls (with 0.25 ml 0.3 M-sucrose instead of the homogenate) were also incubated with each of the three substrates. All 5.0 ml of the incubation mixture were extracted and the metabolites were separated by Sephadex LH-20 using a chloroform: Skellysolve: methanol (75:23:2 by vol.) solvent system as described by Holick *et al.* (1973) but using a smaller (0.7 × 18 cm) column and collecting 50 125-drop (0.92 ml) fractions. One profile of each substrate alone was also chromatographed. The fractions were air dried and counted by liquid scintillation. The chromatographic profiles of the enzymatically generated substrates are presented in Text-fig. 1.

The results (Text-fig. 2) show that when [³H]1,25-dihydroxyvitamin D₃ was used as a substrate, only birds in Groups B (no egg) and C (male) produced 1,24,25-trihydroxyvitamin D₃. When [³H]-24,25-dihydroxyvitamin D₃ was used as the substrate, only Group-A birds (with egg) produced the trihydroxy metabolite. None of the groups produced 1,24,25-trihydroxyvitamin D₃ when 25-[26,27-³H]hydroxyvitamin D₃ was used as a substrate: Groups B and C produced the 24,25- and Group A the 1,25-dihydroxy metabolite (Text-fig. 2). There was no metabolism of any of the substrates in the control incubations without added kidney homogenate.

Our study indicates that the reproductive status in Japanese quail can influence the production of 1,24,25-trihydroxyvitamin D₃ *in vitro* without prior dietary manipulation. In females with a calcified egg in the shell gland the renal 1-hydroxylase is more active than the 24-hydroxylase, confirming the report by Kenny (1976).

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