On the interaction between vitamin D and parathyroid hormone in the domestic fowl

It has recently been reported by HARRISON, HARRISON AND PARK[1] that the typical hypercalcaemic response of a normal animal to parathyroid hormone does not occur in young rats maintained on a diet deficient in vitamin D but adequate in all other nutrients, including calcium and phosphorus. These authors interpreted their results as indicating that vitamin D is required for the action of PTH. Similar findings have been reported by other workers[2].

The object of the present investigation was to study whether or not vitamin D is necessary for parathyroid action in the domestic fowl. Since it is the ionic calcium which is regarded as the fraction of the total plasma calcium primarily influenced by the parathyroid gland, diffusible as well as total calcium levels were studied, and, in view of the probable relationship between citrate metabolism and vitamin D and PTH action, plasma citric acid was also investigated.

The birds were kept in separate metabolism cages from the onset of reproduction and fed a standard laying ration containing 2.3 % Ca and 1.1 % P. The basal diet was virtually devoid of vitamin D, though adequate in all other respects, and the control birds received this diet supplemented with vitamin D_3 at a level of 200 i.u./100 g diet. As a result of the deficient diet, the shells of the eggs laid by the birds on the experimental diet became progressively thinner and the rate of lay declined, until after 6 months egg production ceased almost entirely, with the birds laying 1 egg/month on the average. Virtual cessation of lay was in fact the criterion used to indicate that a serious deficiency of vitamin D had been induced.

Approx. 10 ml blood (providing about 7.0 ml plasma) were required for each set of determinations, and since it was not desirable to withdraw two samples of this magnitude from a hen within a few hours, the first sample was taken 24 h before injection of PTH. However, a small sample of blood (0.5 ml) was taken immediately prior to PTH injection to check that the level of total calcium had not altered materially since the previous day. In the hens deficient in vitamin D no changes were observed but small differences were found in some of the control birds. Previous experiments (unpublished) had shown that the diffusible calcium of the plasma of laying hens is very constant from day to day at any particular stage of the laying cycle, and the level of total calcium just before PTH injection, rather than that observed the previous day, was used to compare with the level after injection. The control birds were bled from 1–4 h after oviposition, *i.e.*, when egg-shell calcification was not in progress.

PTH ("Para-thor-mone", Lilly) was injected subcutaneously at a rate of 75 units/kg live weight and the blood samples were taken 2–2.5 h afterwards, when the maximum response to the hormone is observed (unpublished observations). A plasma ultrafiltrate, on which diffusible calcium was determined, was obtained using the apparatus described by ROSE[3]. The pH of the plasma was controlled throughout the ultrafiltration by circulating a mixture of 5 % CO_2 in oxygen around the collodion filtration shell. Determinations of total and diffusible calcium were made by a photometric titration with EDTA at pH 12–13 using murexide as indicator,

Abbreviations: PTH, parathyroid hormone; EDTA, ethylenediaminetetraacetate.

TABLE I

EFFECT OF PARATHYROID EXTRACT ON THE MEAN CONCENTRATIONS OF TOTAL, DIFFUSIBLE AND NON-DIFFUSIBLE CALCIUM AND CITRIC ACID OF THE PLASMA OF VITAMIN-D-DEFICIENT AND NORMAL HENS

Values are means (in mg/100 ml) and standard errors of mean.

Treatment	*No. of animals*	*Total*		*Diffusible*		*Non-diffusible*		*Citric acid*	
		Before PTH	*After PTH*	*Before PTH*	*After PTH*	*Before PTH*	*After PTH*	*Before PTH*	*After PTH*
Vitamin D deficien	7	13.4 ± 0.7	13.4 ± 0.7 (N.S.)	6.4 ± 0.2	7.6 ± 0.3*	6.9 ± 1.0	5.8 ± 0.7*	3.37 ± 0.44***	3.91 ± 0.44*** (N.S.)
Control (laying)	7	22.7 ± 1.7	26.1 ± 3.3**	8.2 ± 0.4	10.1 ± 0.8*	14.5 ± 1.4	16.0 ± 2.4 (N.S.)	2.64 ± 0.12	2.58 ± 0.18 (N.S.)

* Significantly different ($P < 0.05$) from the level before PTH injection.
** Significantly different ($P < 0.01$) from the level before PTH injection.
*** Data available for 4 birds only.
N.S. Not significantly different from the level before PTH injection.

and citric acid was determined on trichloroacetic acid filtrates of plasma by the method of TAYLOR[4].

The levels of citric acid, total, diffusible and non-diffusible calcium in the plasma after PTH injection were compared statistically with the corresponding levels before injection, using the paired "*t*" test, and the results are shown in Table I. In the control group, PTH exerted a typical effect on the blood calcium, elevating both total and diffusible levels significantly, though the increase in the non-diffusible calcium was not significant. The mean plasma citric acid level was not affected by the PTH and it seems probable that the high level of oestrogen in the circulation prevented the expected rise.

PTH treatment had no effect on the total calcium level of the plasma of the birds deficient in vitamin D. The diffusible calcium and the citric acid levels on the other hand were increased (significantly only in the former), and it would appear, therefore, that vitamin D is not necessary for the action of PTH in the hen. Associated with the rise in diffusible calcium was a significant fall in the non-diffusible calcium level after PTH injection. It seems most unlikely that this can have been due to a reduction in the protein concentration of the plasma since the main bleedings were only 24 h apart and a fall in total calcium would have been expected at the time of the bleeding immediately before the PTH injection if a fall in plasma proteins had occurred. LLOYD AND ROSE[5], as a result of investigations on 12 hyperparathyroid patients before and after operation, and on a normal subject treated with PTH, have produced evidence that PTH reduces the calcium-binding power of the plasma proteins, a conclusion also reached by FANCONI AND ROSE[6]. The results of the present experiments are consistent with this suggestion.

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[1] H. C. HARRISON, H. E. HARRISON AND E. A. PARK, *Am. J. Physiol.*, 192 (1958) 432.
[2] C. MARNAY AND Y. RAOUL, *Comp. rend. soc. biol.*, 153 (1959) 1949.
[3] G. A. ROSE, *Clin. Chim. Acta*, 2 (1957) 227.
[4] T. G. TAYLOR, *Biochem. J.*, 54 (1953) 48.
[5] H. M. LLOYD AND G. A. ROSE, *Lancet*, (1958 II), 1258.
[6] A. FANCONI AND G. A. ROSE, *Quart. J. Med.*, 27 (1958) 463.

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Cytochrome 556 and electron-transport system in snail hepatopancreas

The occurrence of a cytochrome-like haemoprotein called enterochrome 556 in the gut-fluid of garden snails has recently been reported and some of its properties have been described[1]. A cytochrome resembling enterochrome 556 has now been found to occur in the hepatopancreatic cells of garden snails, *Euhadra amaliae* and *E. sandai*.

Abbreviation: DPNH, reduced diphosphopyridine nucleotide.