of rats 1 week after irradiation[9,10]. Melatonin synthesis is also elevated from 4–19 days after irradiation[12] and melatonin can elevate MAO activity in vitro[13] and in vivo[13,14]. Both melatonin synthesis and MAO activity were elevated by feed deprivation in rats[14], while androgen synthesis and testicular weights were depressed. Our data suggest that the increase in testicular MAO activity noted after irradiation could be due to an increase in melatonin production.

The decline in MAO activity noted for the seminiferous tubules of the 126–137-day-old control animals from 4 through 153 days after treatment is consistent with the decline in MAO activity of rat testes associated with senescence of this organ[6]. The increase in MAO activity noted on day 80 and the return of this activity to control levels is thought to be due to the repair process and repopulation of the germinal epithelium that was evidenced by an increased testicular weight.

These data offer additional evidence for a possible direct effect of melatonin on the testis and for a functional role of the pineal gland in the radiation syndrome confirming earlier observations from our laboratory that the pineal gland may be important in determining the extent of damage to the testis after irradiation and the influence of light and dark schedules on this phenomenon[12].

Résumé. Chez le rat l'activité de l'oxydase testiculaire monoaminée est augmentée d'une manière significative 7 à 10 jours après une irradiation-X de 250 R du corps entier. Elle s'est abaissée au-dessous de la valeur du contrôle pendant les 153 jours qui suivirent le traitement.

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Lag Period of Action of 25-Hydroxycholecalciferol on Bone Collagen Metabolism in Vitamin D Deficient Rats

Vitamin D undergoes various transformations within the body. Among its known metabolites, two compounds exhibit considerable biological activity: 25-hydroxycholecalciferol (25-HCC) and 1,25-dihydroxycholecalciferol (1,25-DHCC)[1]. Intestinal calcium absorption is more rapidly stimulated by 25-HCC than by D_3[2]. The lag of action of 1,25 DHCC on the gut is even shorter than that of 25-HCC, and 1,25-DHCC is more effective in lesser dose than either D_3 or 25-HCC[3,4]. It is less certain whether similar differences exist in Vitamin D dependant metabolic processes of bone. In bone tissue culture, the calcium mobilization of bone was unchanged when D_3 was added to the medium; it was increased following 25-HCC administration, and the effect of 1,25-DHCC exceeded that of 25-HCC[5,6]. The latter, however, was more than twice as active as the former in curing rickets in the rat[4]. To elucidate the biological significance of 25-HCC and 1,25-DHCC in bone metabolism, further studies on their effects on different metabolic processes of bone seem to be necessary.

The cholecalciferol status of the organism has an appreciable effect on bone collagen metabolism[7–9]. The total hydroxyproline release from rachitic rat bone in vitro was found to be higher than that of normal bone, and cholecalciferol supplementation, given 1 day before sacrifice, resulted in a further significant increase of of hydroxyproline release[10]. In a previous study we observed that 25-HCC given 9 h before sacrifice to rachitic rats influenced bone collagen metabolism, while vitamin D_3 was ineffective[11].

In this report we provide evidence that the lag period of action of 25-HCC on collagen metabolism of rachitic rat bone is significantly shorter than that of cholecalciferol.

Materials and methods. 102 inbred rats (R Amsterdam) of both sexes, 23 days old, were used; 8 rats received a semisynthetic normal diet (0,8% Ca, 0.5% P, 1 µg/100 g vitamin D_3) for 28 days and served as controls. The others received a vitamin D deficient, high Ca low P rachitogenic diet (1.2% Ca, 0.1 P) for 25 days, then a diet low in Ca and P, and lacking vitamin D (0.2% Ca, 0.1 P) for the 3 days previous to killing as reported earlier[11]. They were fasted for the last night. All the rats consuming the rachitogenic diet had widened metaphyses and hypocalcemia when killed. On the 29th day, the rachitic rats were divided into 3 groups: group D_3 received 2.5 µg cholecalciferol, group 25-HCC received 2.5 µg 25-HCC, group R received the solvent only. The supplements were given i.v. 4, 6, 10, 13, 15, 19, and 24 h after the injection 4–6 rats of each group were killed by decapitation and their bones were used for the in vitro study. The normal controls were killed together with the '10 h supplement' groups.

25-HCC (kindly donated by Dr. E. KODICEK and P. BELL of the Dunn Nutritional Laboratory, Cambridge) and cholecalciferol (Philips Duphar, Amsterdam) were dissolved in ethanol and further diluted before injection with the solution used for the incubation of bone.

Immediately after killing, pieces of proximal tibia metaphysis and of distal femur metaphysis 4 mm long were taken, split into 2 halves and cleaned of soft tissue and epiphyseal cartilage. Bone fragments of each rat were incubated in a separate Warburg vessel at 37 °C, for 4 h

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In vitro hydroxyproline release from bone of rachitic rats killed 4–24 h after the i.v. administration of 2.5 μg 25-HCC or 2.5 μg D_3, or the solvent

Time lag between injection and killing (h)	Hydroxyproline release (ng/mg dry bone)		
	Group R (solvent)	Group 25-HCC	Group D_3
4	340 ± 99 (4)	355 ± 181 (5)	299 ± 86 (4)
6	374 ± 105 (5)	629 ± 173 (5)	360 ± 139 (5)
10	381 ± 70 (5)	790 ± 242 (4)	329 ± 73 (4)
13	321 ± 45 (5)	705 ± 138 (5)	379 ± 39 (6)
15	366 ± 68 (4)	998 ± 142 (4)	540 ± 180 (4)
19	340 ± 73 (4)	1065 ± 220 (4)	874 ± 153 (4)
24	329 ± 54 (4)	993 ± 212 (4)	982 ± 129 (5)

Means and standard deviations. The number of rats is given in brackets.

in 3 ml of modified Krebs-Ringer bicarbonate solution. It contained in 100 ml: 2.6 mg Ca, 10 mg inorganic P, 100 mg glucose, 15 mg ascorbic acid, 500 IU Penicillin, 0.1 mg Streptomycin and 6 ml serum from vitamin D deficient rats. The vessels were gassed with 95 % O_2 + 5% CO_2 and shaken in a Warburg apparatus. The hydroxyproline content of the incubation medium at the end of the incubation period was considered as hydroxyproline release from bone. The samples were hydrolysed in 6 *N* HCl at 120 °C for 3 h, and their hydroxyproline content was estimated[12].

Results and discussion. The mean hydroxyproline release of normal bone during the incubation period was 97 ± 27 ng/mg dry bone tissue. The other results are summarized in the Table. The hydroxyproline release of rachitic rat bone (R group) was more than 200 % higher than that of the normal control group. Compared to the R groups an increase of hydroxyproline release was found in all the groups killed 6–24 h after the 25-HCC supplementation and 13–24 h after the injection of vitamin D_3. For the groups killed 6, 10 and 13 h after the injection, the hydroxyproline liberation of the 25-HCC groups was significantly greater than that of the D_3 groups. At 24 h this difference had disappeared.

The observed 7 h difference in the lag period of action on bone collagen metabolism between 25-HCC and D_3 is similar to the findings of BLUNT et al.[2] for intestinal Ca transport and for serum Ca level. According to BLUNT et al. 25-HCC exhibits 140 % of the antirachitic activity of cholecalciferol[13]. KODICEK et al.[14] did not observe such difference for the intestinal ^{45}Ca transfer into the blood of rachitic chick. We administered larger doses, compared with the aforementioned studies, and observed a stronger effect of 25-HCC than that of D_3 at 13 and 15 h after the supplementation, but 24 h after the injection, when full effect of D_3 had developed, 25-HCC was not more active than D_3. Similar results were published earlier on intestinal transport of calcium[15].

Zusammenfassung. Nachweis, dass die Freisetzung von Hydroxyprolin bei Knochenfragmenten bereits 6 h nach i.v. Verabreichung von 2,5 μg 25-Hydroxycalciferol signifikant erhöht war, während eine entsprechend zugeführte Dosis von Vitamin D_3 erst 13 h später deutlich wirkte.

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Determination of the Binding Constant of Thiamine Diphosphate in Transketolase from Baker's Yeast by Circular Dichroism Titration

Transketolase from baker's yeast utilizes thiamine diphosphate and Mg^{++} as cofactors[1]. In order to understand the nature of coenzyme binding, transketolase reconstitution has been studied by activity and fluorescence quenching measurements[2]. The involvement of a tryptophan residue at the thiamine diphosphate binding site could be demonstrated by its chemical modification[3]. Furthermore, the appearance of a new circular dichroism band around 320 nm upon addition of thiamine diphosphate to apotransketolase was regarded as the result of a charge transfer interaction between the indole ring system of a tryptophan residue and the positively charged thiazolium ring system of thiamine diphosphate[4]. It is

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