Cholesterol Reduces and Corticosteroids Enhance the Toxicity of Vitamin D in Rats

Masaru KUNITOMO, Yoshiko FUTAGAWA, Yoko TANAKA,
Yu YAMAGUCHI and Yoshio BANDÔ

Department of Pharmacology, Faculty of Pharmaceutical Sciences,
Mukogawa Women's University, Nishinomiya 663, Japan

Accepted December 8, 1988

Abstract—The effect of a high-cholesterol (CHOL) diet and corticosteroids on the toxicity of vitamin D₂ (VD₂) in rats was studied. VD₂ was administered orally at the dosage of 5–60×10⁴ IU/kg, once daily for 4 days. Animals fed CHOL showed a decrease in mortality due to VD₂ treatment. Dietary CHOL inhibited toxic responses such as a diminished growth rate following anorexia, elevated serum calcium level and calcium deposition in tissues, which were produced by a sublethal dose of VD₂ (20×10⁴ IU/kg, once daily for 4 days). Animals pretreated with the high-CHOL diet from 2 weeks before the first VD₂ administration showed much more symptomatic relief than those given this diet after the first VD₂ administration. On the other hand, dexamethasone (DEX) as well as corticosterone remarkably increased the mortality due to VD₂. The degree of VD₂ toxicity, enhanced by DEX, was correlated with the degree of hypercalcemia and tissue calcification. Therefore, the inhibitory effect of CHOL is not likely to be due to activation of the CHOL-corticosterone system in the adrenal gland.

Large doses of vitamin D (VD) evoke arterial damage with severe calcification in the media and cause the development of atherosclerotic lesions in experimental animals (1–3) and humans (4). Many investigators have attempted to produce a convenient model in rats, which are considered to be resistant to the development of atherosclerosis, by administering excess VD with a cholesterol (CHOL) diet (5–8). Some of the experimental models have been used for studies on the pathogenesis of atherosclerosis (2) and the reactivity of the vessel to vasoactive substances (9), and also for screening of anti-atherosclerotic drugs (10, 11). However, the procedures used for the model have considerably varied with the VD dosage, the experimental duration and the rat strain. Moreover, obtaining the anticipated lesions seems to be difficult even if an experiment is carried out as described in the literature because toxic doses of VD are used (12). The increase in the number of animals dying of VD intoxication, which appears

within few days after its administration, is not adequate for drug evaluation. Most papers have not reported the mortality rate of such animals during the period in which atherosclerosis is occurring and have not determined the interaction between VD and CHOL intake.

In the present study, we examined in detail the subacute toxicity of vitamin D₂ (VD₂) and the effects of dietary CHOL on it in rats. Our results suggest that VD₂ toxicity can be markedly reduced by CHOL feeding. Therefore, we further examined whether the inhibitory effects of CHOL can be exerted through the actions of glucocorticoids, because serum CHOL is the major substrate for the production of glucocorticoids in adrenal glands (13), which can antagonize the effect of VD (14).

Materials and Methods

Animals and diet: Male Sprague Dawley strain rats (Shizuoka Laboratory Animal Center, Hamamatsu), housed in an aircon-

ditioned room ($23 \pm 2^\circ\text{C}$ and $60 \pm 10\%$ humidity), were used in this study. In the experiment using VD_2 and CHOL feeding, four-week-old rats were maintained on a purified basal diet or a high-CHOL diet. The basal diet contained 20% casein, 63.2% sucrose, 10% corn oil, 2% agar, 0.8% vitamin mixture and 4% salt mixture. The high-CHOL diet consisted of the basal diet with 1.5% CHOL and 0.5% cholic acid in place of an equal amount of sucrose. In the experiment using VD_2 and corticosteroids, six-week-old rats were used, and they received a commercial chow (Oriental Yeast Co., Tokyo). Each animal was given 15 g of the respective diet at 5 p.m. every day, and all food not consumed by the next day was weighed and then discarded. Water was freely available. The rats were weighed every day.

VD_2 toxicity: The VD_2 solutions were given orally in graded doses ($5\text{--}60 \times 10^4$ IU/kg, once daily) for four consecutive days, at 11 a.m. every day. The mortality was determined from the total number of animals that died by the 11th day after the first VD_2 administration.

Cholesterol feeding and corticosteroid treatments: Rats given the high-CHOL diet were divided into two groups by duration of feeding: continuously from 2 weeks before (Pre-CHOL) and from the time after (Post-CHOL) the first VD_2 administration. In some experiments, the Pre-CHOL group received the basal diet instead of the high-CHOL diet only from the night before the first VD_2 administration to the last day of it, in order to examine the effect of dietary CHOL on the absorption of VD_2 in the intestine.

DEX (0.2 mg/kg) and corticosterone (10 mg/kg) were subcutaneously injected once daily immediately after the administration of VD_2 for 4 days.

Sampling procedure: During the experiment, blood (approx. 200 μl) was drawn from the tail vein for the determination of serum calcium and CHOL. At the end of the experimental period (on the 11th day after the first VD_2 administration), the rats were killed under ether anesthesia by bleeding from a cannula inserted into the abdominal aorta. The organs were excised and weighed. The aorta, heart and kidney were freeze-dried to a constant weight and hydrolyzed with 6

N HCl for 24 hr at 105°C . Hydrolyzates were evaporated under vacuum and used to determine the calcium and phosphorus contents.

Analytical methods: Serum CHOL was fluoroenzymatically determined as described previously (15). Calcium was determined with an absorption spectrophotometer (Model 180-60, Hitachi). Phosphorus was determined by the method of McClare (16).

Drugs: VD_2 , dexamethasone (DEX) and corticosterone were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), CHOL from Nakarai Chemicals, Ltd. (Kyoto) and cholic acid from Wako Pure Chemical Industries, Ltd. (Osaka). VD_2 was dissolved in olive oil to obtain solutions of various concentrations and administered to animals in a constant volume of olive oil. DEX was dissolved in 0.9% saline.

Statistics: The results obtained were expressed as the mean $\pm$ S.E. Student's *t*-test for paired observations was used to test for significance.

Results

Dose-mortality curves: The effects of CHOL and DEX on the subacute toxicity of VD_2 are

Fig. 1. Dosage-mortality curves. Rats received VD_2 orally once daily for 4 consecutive days (VD_2 group). DEX was injected subcutaneously at 0.2 mg/kg simultaneously with the VD_2 administration ($\text{VD}_2 + \text{DEX}$ group). A high-CHOL diet was given continuously from 2 weeks before ($\text{VD}_2 + \text{Pre-CHOL}$ group) and from the time after ($\text{VD}_2 + \text{Post-CHOL}$ group) the first VD_2 administration. The number of rats used at each dose of VD_2 was 8 to 15.

Fig. 2. Effects of dietary CHOL on growth rate (A) and food consumption (B) in VD₂-treated rats. The subtoxic dose (20×10^4 IU/kg, once daily for 4 days) of VD₂ was given orally to a group of rats (VD₂ group) on a basal diet. A high-CHOL diet was given continuously from 2 weeks before (VD₂ + Pre-CHOL group) and from the time after (VD₂ + Post-CHOL group) the first VD₂ administration (on day 0). A control group was given the basal diet alone. Each point represents the mean of 8 animals.

shown in Fig. 1. The LD₅₀ value of VD₂ was 32×10^4 IU/kg, when it was administered orally once daily for 4 days. The dosage-mortality curve shifted to the right (LD₅₀ value: $> 50 \times 10^4$ IU/kg) with treatment with CHOL diet and to the left (LD₅₀ value: 15×10^4 IU/kg) with treatment with DEX; that is, the toxicity of VD₂ was markedly reduced by CHOL and markedly enhanced by DEX. CHOL-pretreatment (Pre-CHOL) seemed to be more effective than CHOL-post treatment (Post-CHOL). VD₂ had almost the same toxicity when commercial chow was used instead of the purified basal diet.

Effects of CHOL feeding on the toxic effect of VD₂: A sublethal dose of VD₂ (20×10^4 IU/kg, once daily for 4 days) causing 0% mortality was given to CHOL-fed and control rats. The VD₂-treated animals showed rapid loss of body weight together with an extremely lessened intake of food after the last administration (on day 3). The maximum loss (approx. 20 g) of weight was on day 6, after which the body weight rapidly recovered as food consumption increased. These changes were alleviated by CHOL feeding; animals in the Pre-CHOL group showed milder symptoms than those in the Post-CHOL group (Fig. 2).

Fig. 3. Effects of dietary CHOL on serum calcium level in VD₂-treated rats. Each point represents the mean $\pm$ S.E. of 8 animals. ** $P < 0.01$, as compared with the VD₂ group. For other references, see the legend to Fig. 2.

Figure 3 shows the time course of the serum calcium level in rats treated with VD₂ and the high-CHOL diet. The serum calcium level of the VD₂-treated animals gradually increased as the administration of VD₂ was repeated, and it peaked on the next day after the last administration of VD₂ (on day 4), after which it rapidly decreased. This elevation of the calcium level was significantly inhibited by the

CHOL diet, especially in the Pre-CHOL group.

The time course of the serum total CHOL level is shown in Fig. 4. The CHOL level of animals given VD₂ alone was roughly the same as that of the control. The Pre-CHOL group maintained a high level of CHOL that was 2.5–3 times the control level. The Post-CHOL group showed a mild but significant increase in the serum CHOL level, although its level temporarily decreased with the loss of appetite.

On day 11 (the end of the experiment),

Fig. 4. Effects of dietary CHOL on serum total CHOL level in VD₂-treated rats. Each point represents the mean±S.E. of 8 animals. *P<0.05, **P<0.01, as compared with the control. For other references, see the legend to Fig. 2.

VD₂-treated animals showed a marked increase in the calcium levels of the aorta, heart and kidney compared to those of the normal control animals (Table 1). Pretreatment with CHOL diet significantly eliminated calcium deposition in these tissues and almost completely restored the calcium contents in the aorta and heart to the normal values. However, in the animals of the Post-CHOL group, there was little effect on the calcium deposition induced by VD₂.

Effect of hypercholesterolemia on the calcium deposition in the aorta: Pre-CHOL rats, which had been hypercholesterolemic until the first VD₂ administration (Fig. 4), were switched to the basal diet only for 4 days during the VD₂ administration period. As shown in Fig. 5, such treated animals (group II) showed a significant (P<0.05) decrease in the aorta calcium content (1.77 ± 0.52 mg/g dry weight) compared to that (4.70 ± 1.10 mg/kg dry weight) of the control animals (group I), which were maintained on the basal diet from 2 weeks before the VD₂ administration to the end of the experiment.

Effects of corticosteroids on the toxic effect of VD₂: Relatively lower doses of VD₂ ($5\text{--}20 \times 10^4$ IU/kg, p.o., once daily) were administered with DEX (0.2 mg/kg, s.c., once daily) for 4 consecutive days. On days 4 and 5, the serum calcium level in animals treated with VD₂ and DEX was significantly higher than that in animals treated with VD₂ alone, although DEX treatment had little influence on it (Fig. 6). At the end of the experiment (on day 11), the body weight of animals

Table 1. Effects of dietary CHOL on tissue calcium deposition in VD₂-treated rats

Group	Mortality	Aorta	Heart	Kidney
			(mg/g dry weight)	
Control	0/8	0.288±0.021	0.101±0.011	0.298±0.009
VD ₂	0/8	11.18±2.33**	0.287±0.071*	4.77±1.41**
VD ₂ +Pre-CHOL	0/8	0.314±0.030 [#]	0.112±0.010 [#]	1.22±0.37** [#]
VD ₂ +Post-CHOL	0/8	7.14±2.89*	0.264±0.080*	10.69±2.76**

A subtoxic dose (20×10^4 IU/kg, once daily for 4 days) of VD₂ was given orally to a group (VD₂ group) of rats on a basal diet and two groups of rats on a high-CHOL diet. The high-CHOL diet was given continuously from 2 weeks before (VD₂+Pre-CHOL group) and from the time after (VD₂+Post-CHOL group) the first VD₂ administration. A control group was given the basal diet alone. Rats were killed on the 11th day after the first VD₂ administration. Each value represents the mean±S.E. *P<0.05, **P<0.01, as compared with the control group. #P<0.05, ##P<0.01, as compared with the VD₂ group.

given both DEX and VD₂ significantly decreased compared to those given VD₂ alone. DEX caused a greater increase in the elevated aorta calcium and phosphorus contents in animals treated with the higher doses of VD₂

Fig. 5. Effects of hypercholesterolemia on aorta calcium content in VD₂-treated rats. Animals received oral administration of VD₂ (20×10^4 IU/kg, once daily for 4 days) under two dietary conditions. Animals of group I (column I) received the basal diet throughout the experiment. Animals of group II (column II), which had been given a high-CHOL diet from 2 weeks before the first VD₂ administration, were switched to the basal diet for the 4 days of the VD₂ treatment, and then were returned to the high-CHOL diet. Rats were killed on the 11th day after the first VD₂ administration. Each column represents the mean $\pm$ S.E. of 9 animals.

(10 and 15×10^4 IU/kg), although it had no such potentiating effect at the lower dose of VD₂ (5×10^4 IU/kg) (Table 2). Similar findings were obtained when corticosterone (10 mg/kg, s.c., once daily) was used instead of DEX. DEX also caused a marked decrease in the thymus weight, which was larger at higher doses of VD₂.

Discussion

The results of this study with rats demon-

Fig. 6. Effects of DEX on serum calcium level in VD₂-treated rats. Animals received oral administration of VD₂ (20×10^4 IU/kg) and subcutaneous injection of DEX (0.2 mg/kg) once daily for 4 days. Each point represents the mean $\pm$ S.E. of 9 animals. ** $P < 0.01$, as compared with the VD₂ group.

Table 2. Effects of DEX on the mortality and the aorta calcium and phosphorus contents in rats treated with VD₂ at sublethal doses

Treatment		Mortality	Aorta	
VD ₂ ($\times 10^4$ IU/kg)	DEX (0.2 mg/kg)		Calcium (mg/g dry weight)	Phosphorus (mg/g dry weight)
0 (Control)	—	0/8	0.267 $\pm$ 0.025	1.47 $\pm$ 0.03
0	+	0/8	0.250 $\pm$ 0.017	1.46 $\pm$ 0.03
5	—	0/8	0.336 $\pm$ 0.073	1.39 $\pm$ 0.04
5	+	0/8	0.279 $\pm$ 0.035	1.36 $\pm$ 0.02*
10	—	0/8	0.382 $\pm$ 0.044*	1.54 $\pm$ 0.02
10	+	1/8	8.60 $\pm$ 3.63***	6.12 $\pm$ 2.13*#
15	—	0/8	1.88 $\pm$ 0.63**	2.17 $\pm$ 0.35
15	+	3/8	13.28 $\pm$ 4.42***	11.61 $\pm$ 4.69***

Animals received oral administration of VD₂ and subcutaneous injection of DEX once daily for 4 days and were killed on the 11th day after the first administration of these compounds. Each value represents the mean $\pm$ S.E. * $P < 0.05$, ** $P < 0.01$, as compared with the control group. # $P < 0.05$, ## $P < 0.01$, as compared with the respective control group without DEX treatment.

strate that the systemic toxicity of VD_2 could be reduced by CHOL feeding and the resulting hypercholesterolemia, and also could be enhanced by DEX administration. The toxic responses changed concomitantly with the degree of hypercalcemia and tissue calcification. The mechanism of these effects is not clear.

The principal causes of death following excessive doses of VD are hypercalcemia and metastatic calcification. VD is hydroxylated to become 25-hydroxy-VD in the liver and then is converted to its most biologically active form, 1,25-dihydroxy-VD, by a renal 1-hydroxylase, which is thought to be tightly regulated by homeostatic mechanisms. Actually, large doses of VD in rats cause a decrease or no change in circulating concentrations of 1,25-dihydroxy-VD (17). On the other hand, the concentration of 25-hydroxy-VD is markedly increased in rats (17) and humans (18) when the dietary intake of VD is elevated. Therefore, the development of hypercalcemia in VD intoxication may be related to the increased 25-hydroxy-VD level, although it is less biologically active than 1,25-dihydroxy-VD. Toda et al. (19) reported that 25-hydroxy-VD is incorporated into aortic smooth muscle cell membranes and enhances the membrane permeability to Ca^{2+} , leading to cell necrosis. Tissues other than the aorta may also cause calcification by similar mechanisms.

Inhibition of the systemic toxicity of VD_2 by CHOL feeding is apparently not due to the inhibition of VD_2 absorption by competition with CHOL from the intestine, since VD_2 was administered when the intestine was sufficiently empty, and also since a significant inhibition of the toxic effect of VD_2 by CHOL was observed in the hypercholesterolemic rats, which had been given the high-CHOL diet for 2 weeks and then were switched to the basal diet during the VD_2 administration period (Fig. 5).

CHOL is essential to the structure of all cell membranes and stabilizes them against exciting states. Hence, CHOL seems to play some nonspecific role as a homeostatic control factor under some severe pathologic conditions. Cohen et al. (20) have reported that dietary CHOL retards colon carcinogenesis.

Ramachandran et al. (21) also reported evidence for an inhibitory effect of CHOL in the metastasis of tumor cells to endothelial cells. In previous studies, we observed that CHOL exerts a suppressive effect on adjuvant arthritis, a disease of cell-mediated immunity (22).

CHOL also serves as a precursor of glucocorticoids. Serum lipoprotein CHOL has been known as the major substrate in the production of steroid hormones in the adrenal glands (13, 23). Adrenal corticosteroids can prevent and eliminate the elevated calcium concentration found in hypervitaminosis D by a mechanism associated with reduced intestinal calcium absorption (24). Glucocorticoids are actually used to treat hypervitaminosis D. Although the toxicity of VD is closely related to the activation of the complement system (25), glucocorticoids display some anticomplement action (26). Thus, glucocorticoids can be expected to offer protection against VD toxicity. However, we obtained the incompatible results that DEX as well as corticosterone had an aggravating effect on the VD_2 -induced toxic responses. Therefore, it is unlikely that the inhibitory effect of CHOL on the VD_2 toxic response is due to the activation of the CHOL-corticosterone system in the adrenal gland. Such a synergism between glucocorticoids and overdose of VD has been observed in histological studies carried out for a long period (27), but not over a short term (28). This is a subject for further study.

Recent studies have shown that glucocorticoids increase the population of the 1,25-dihydroxy-VD receptor in bone cells (29) and stimulate bone resorption by this metabolite of VD (30). Moreover, there is evidence that one action of glucocorticoids is the stimulation of renal 1-hydroxylase activity (31, 32). Therefore, in the present experiment in which rats were treated with glucocorticoids plus VD_2 , the synergistic effect on the release of calcium from the skeleton may have overcome the antagonistic effect on calcium transport in the intestine and renal tubules, resulting in the induction of hypercalcemia.

References

- 1 Taura, S., Taura, M., Kamio, A. and Kummerow,

- F.A.: Vitamin D-induced coronary atherosclerosis in normolipemic swine: comparison with human disease. *Tohoku J. Exp. Med.* **129**, 9–16 (1979)
- 2 Kamio, A., Taguchi, T., Shiraishi, M., Shitama, K., Fukushima, K. and Takebayashi, S.: Vitamin D sclerosis in rats. *Acta Pathol. Japon.* **29**, 545–562 (1979)
- 3 Toda, T., Ito, M., Toda, Y., Smith, T. and Kummerow, F.A.: Angiotoxicity in swine of a moderate excess of dietary vitamin D₃. *Food Chem. Toxicol.* **23**, 585–592 (1985)
- 4 Seeling, M.S.: Vitamin D and cardiovascular, renal and brain damage in infancy and childhood. *Ann. N.Y. Acad. Sci.* **147**, 537–582 (1969)
- 5 Testa, R., Canestrini, C. and Oldani, C.: Experimental atherosclerosis in the rat: biochemical evaluation. *J. Pharm. Pharmacol.* **27**, 699–700 (1975)
- 6 Altman, R.F.A.: A simple method for the rapid production of atherosclerosis in rats. *Experientia* **29**, 256 (1973)
- 7 Bajwa, G.S., Morrison, L.M. and Ershoff, B.H.: Induction of aortic and coronary atherosclerosis in rats fed a hypervitaminosis D, cholesterol-containing diet. *Proc. Soc. Exp. Biol. Med.* **138**, 975–982 (1971)
- 8 Kunitomo, M., Kinoshita, K. and Bandô, Y.: Experimental atherosclerosis in rats fed a vitamin D, cholesterol-rich diet. *J. Pharmacobiodyn.* **4**, 718–723 (1981)
- 9 Baraïa, Y.M. and Bekemeier, H.: Drug actions in rats with arteriosclerosis induced by toxic doses of vitamin D₂. *Arch. Toxicol. [Suppl.]* **2**, 431–435 (1979)
- 10 Iwanowska, J., Starzyński, S. and Kalinowska, A.: The effects of piridinol carbamate on hypercholesteremia and aortal damage produced by 5 days' application of Altman's diet to the rat. *Pol. J. Pharm. Pharmacol.* **32**, 333–341 (1980)
- 11 Katsunuma, H., Ebihara, T., Shimizu, K., Iwamoto, T. and Kiyokawa, M.: Antiatherosclerotic action of elastase: with special reference to its effect on elastic fibers. *Ketsugo Soshiki* **15**, 89–100 (1983)
- 12 Parke, D.V., Sacra, P.J. and Thornton, P.C.: Experimental atherosclerosis in the Wistar rat. *Br. J. Pharmacol.* **63**, 346P–347P (1978)
- 13 Andersen, J.M. and Dietschy, J.M.: Relative importance of high and low density lipoproteins in the regulation of cholesterol synthesis in the adrenal gland, ovary, and testis of the rat. *J. Biol. Chem.* **253**, 9024–9032 (1978)
- 14 Kimberg, D.V., Baerg, R.D., Gershon, E. and Graudusius, R.T.: Effect of cortisone treatment on the active transport of calcium by the small intestine. *J. Clin. Invest.* **50**, 1309–1321 (1971)
- 15 Kunitomo, M., Yamaguchi, Y., Matsushima, K. and Bandô, Y.: Cholesterol metabolism in serum and aorta of inbred mice fed a high-cholesterol diet. *Japan. J. Pharmacol.* **34**, 153–158 (1984)
- 16 McClare, C.W.F.: An accurate and convenient organic phosphorus assay. *Anal. Biochem.* **39**, 527–530 (1971)
- 17 Hughes, M.R., Baylink, D.J., Gonnerman, W.A., Toverud, S.U., Ramp, W.K. and Haussler, M.R.: Influence of dietary vitamin D₃ on the circulating concentration of its active metabolites in the chick and rat. *Endocrinology* **100**, 799–806 (1977)
- 18 Haddad, J.G., Jr., Chyu, K.J., Hahn, T.J. and Stamp, T.C.B.: Serum concentrations of 25-hydroxyvitamin D in sex-linked hypophosphatemic vitamin D-resistant rickets. *J. Lab. Clin. Med.* **81**, 22–27 (1973)
- 19 Toda, T., Leszczynski, D.E. and Kummerow, F.A.: The role of 25-hydroxy-vitamin D₃ in the induction of atherosclerosis in swine and rabbit by hypervitaminosis D. *Acta Pathol. Japon.* **33**, 37–44 (1983)
- 20 Cohen, B.I., Raicht, R.F. and Fazzini, E.: Reduction of N-methyl-N-nitrosourea-induced colon tumors in the rat by cholesterol. *Cancer Res.* **42**, 5050–5052 (1982)
- 21 Ramachandran, C.K., Sanders, K. and Melnykovich, G.: Enhancement in the adhesion of tumor cells to endothelial cells by decreased cholesterol synthesis. *Cancer Res.* **46**, 2520–2525 (1986)
- 22 Kunitomo, M., Yamaguchi, Y., Futagawa, Y., Hamaguchi, Y. and Bandô, Y.: Suppression of adjuvant arthritis in rats by cholesterol. *Japan. J. Pharmacol.* **44**, 15–22 (1987)
- 23 Carr, B.R., Parker, C.R., Jr., Milewich, L., Porter, J.C., MacDonald, P.C. and Simpson, E.R.: The role of low density, high density, and very low density lipoproteins in steroidogenesis by the human fetal adrenal gland. *Endocrinology* **106**, 1854–1860 (1980)
- 24 Caniggia, A., Nuti, R., Lore, F. and Vattimo, A.: Pathophysiology of the adverse effects of glucoactive corticosteroids on calcium metabolism in man. *J. Steroid Biochem.* **15**, 153–161 (1981)
- 25 Pang, A.S.D. and Minta, J.O.: Inhibition of vitamin D₂-induced arteriosclerosis in rats by depletion of complement with cobra venom factor. *Artery* **7**, 109–122 (1980)
- 26 Gewurz, H., Wernick, P.R., Quie, P.G. and Good, R.A.: Effects of hydrocortisone succinate on the complement system. *Nature* **208**, 755–757

- (1965)
- 27 **Vanha-Perttula, T.P.J. and Näätänen, E.K.:** The effect of cortisone on cardiovascular sclerosis induced in rats with cholesterol and vitamin D. *Acta Endocrinol.* **35**, 20–33 (1960)
- 28 **Gillman, J. and Gilbert, C.:** Periarthritis and other forms of necrotising arteritis produced by vitamin D in thyroxinised rats with an assessment of the aetiology of those vascular lesions. *Br. J. Exp. Pathol.* **37**, 584–596 (1956)
- 29 **Manolagas, S.C., Anderson, D.C. and Lumb, G.A.:** Glucocorticoids regulate the concentration of 1,25-dihydroxycholecalciferol receptors in bone. *Nature* **277**, 314–315 (1979)
- 30 **Chen, T.L., Hauschka, P.V. and Feldman, D.:** Dexamethasone increases 1,25-dihydroxy-vitamin D₃ receptor levels and augments bio-responses in rat osteoblast-like cells. *Endocrinology* **118**, 1119–1126 (1986)
- 31 **Lukert, B.P., Stanbury, S.W. and Mawer, E.B.:** Vitamin D and intestinal transport of calcium: effects of prednisolone. *Endocrinology* **93**, 718–722 (1973)
- 32 **Spanos, E. and MacIntyre, I.:** Effect of glucocorticoids on vitamin D-metabolism. *In* Vitamin D: Biochemical, Chemical and Clinical Aspects Related to Calcium Metabolism, Edited by Normal, A.W., Schaefer, K., Coburn, J.W., DeLuca, H.F., Fraser, D., Grigoleit, H.G. and Herrath, D.V., p. 191–195, Walter de Gruyter, Berlin and New York (1977)