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Vitamin D – Soltriol
The heliogenic steroid hormone: Somatotrophic activator and modulator
Discoveries from histochemical studies lead to new concepts

W.E. Stumpf
Department of Cell Biology and Anatomy, University of North Carolina, Chapel Hill, NC 27599, USA

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Summary. Evidence from autoradiographic studies with ^3H 1,25(OH)$_2$ vitamin D$_3$ (soltriol) about its many sites of nuclear binding and multiple actions suggests that the traditional view of "vitamin D and calcium" is too limited and requires modification. A new concept has been developed which proposes that the skin-derived hormone of sunshine, soltriol, is a somatotrophic activator and modulator that affects all vital systems. Regulation of calcium homeostasis is only one of its many actions. Target tissues for soltriol include not only bone, intestine and kidney, but also brain, spinal cord, pituitary, thyroid, endocrine pancreas, adrenal medulla, enteroendocrine cells, thymus, and male and female reproductive organs. Accordingly, actions of soltriol involve effects on autonomic and endocrine regulation with changes in tissue and blood hormone levels, innervation of skeletal muscle, immune and stress response, digestion, blood formation, fertility, pregnancy and lactation, general energy metabolism, mental processes and mood, and others. The skin-mediated transduction of short-wave sunlight induces a purposeful modulation of growth, reproduction and other biological activities in tune with the conditions of the sun cycle and season. Synthesis and actions of vitamin D$_3$-soltriol are dependent not only on the amount of sunlight, but also on the availability of precursor in the skin and access of sunlight, the rate of hydroxylation in liver and kidney, and the modulation of these events by the endocrine status, in particular growth and reproduction. A concept of a five-level control of soltriol synthesis is proposed, in which the hydroxylation steps provide for a sensitive tuning. Relationships between the heliogenic skin-derived hormonal system and the helioprivic pineal-derived hormonal system are recognized and a comprehensive concept of the "endocrinology of sunlight and darkness" is pointed out.

Introduction

The new and integrated concept of vitamin D action holds that vitamin D is a heliogenic somatotrophic activator and that regulation of plasma calcium is only one part of its complex actions. This concept has evolved from accumulated new evidence from the results of our autoradiographic studies, in particular those related to the multiple cellular sites of action of 1,25(OH)$_2$ vitamin D$_3$ (1,25D$_3$, soltriol) in the pituitary and in the brain (Stumpf et al. 1987a; Stumpf and O'Brien 1987b), in conjunction with those of the spinal cord, the thyroid, the pancreas B-cells, the adrenal medulla, the pyloric stomach, the testis and ductuli efferentes, the skin, and many others. The new evidence suggests that involvement of vitamin D target tissues in the regulation of plasma calcium is limited and that there are many functions of 1,25D$_3$ not primarily associated with or linked to calcium homeostasis. It appears that there are effects of vitamin D that can not be forced into the Procrustean bed of "vitamin D and bone" or considered only under the restrictive view of a calcium homeostasis-linked "vitamin D endocrine system". Conceptualization and design of experiments, as well as the interpretation of data from studies of the "calcium homeostatic steroid hormone" (Norman 1979) were and still are dominated by the premise that any of the functions of vitamin D are somehow connected with the regulation of calcium levels. The presence or absence of specific calcium binding proteins is viewed by many as indicative of the presence or absence of 1,25D$_3$ receptors, and the localization of antibodies to specific calcium binding protein has been advocated in the search for and identification of vitamin D target tissues (Norman 1979; Jande et al. 1981), even though the use of the radiolabeled hormone in our autoradiographic binding studies with ^3H 1,25D$_3$ frequently provided different results.

A vitamin D endocrine system has become increasingly recognized. But, it remained related to the traditional concept of "vitamin D and calcium metabolism". An erosion of the cliche "vitamin D and calcium" started in 1979 and 1980, when the existence of nuclear receptors for 1,25D$_3$ was discovered in pituitary and brain (Stumpf et al. 1979, 1980a; Sar et al. 1980), in organs that had been excluded in the literature from the regulation of calcium levels and of vitamin D actions. Evidence for receptors in several other endocrine tissues followed, including, B-cells in islets of Langerhans (Clark et al. 1980, 1981), epinephrine and norepinephrine producing cells in the adrenal medulla (Clark et al. 1986c), thyroid follicle epithelium (Stumpf et al. 1981a; Stumpf and O'Brien 1987a) and parathyroid parenchymal cells (Stumpf et al. 1979; Narbaitz et al. 1980; Stumpf et al. 1981a), enteroendocrine cells in the pyloric stomach (Stumpf et al. 1979; 1988b), and Sertoli cells in the testis (Stumpf et al. 1987b). While some investigators

responded to such unexpected information with the characteristic scepticism of "experts" (with unfortunate consequences in anonymous "peer" reviews of related research proposals), the functional significance of some of the discoveries soon became apparent.

The cellular localization provided important new information and clues for the understanding of the functional significance, which was effectively pursued through the combined use of autoradiography and immunohistochemistry with antibodies to cellular products, as well as through physiological studies in our laboratory (Sar et al. 1981; Clark et al. 1981) and in other laboratories. Thus, it became recognized very early in our experience that "modifications must be made in the present models for calcium homeostatic mechanisms" (Stumpf et al. 1979). Brain and pituitary were suggested to be implicated not only in the control of calcium homeostasis (Stumpf et al. 1980a), but also in the existence of a vitamin D-regulated brain-pituitary-thyroid axis (Sar et al. 1980; Stumpf et al. 1982) and a brain-pituitary-intestinal axis (Stumpf et al. 1982). Because of this and other accumulating evidence, reviews of the field recognized that the vitamin D endocrine system "embraces many more target tissues than simply the intestine, bone, and kidney" (Norman et al. 1982), and that "these observations collectively demonstrate the widespread involvement of vitamin D in both cellular as well as whole animal calcium metabolism" (Norman et al. 1982). More recently, it has been noted that the vitamin D-endocrine system is "important not only in the regulation of bone and mineral metabolism but in modulation of other systems as well" (Bell 1985), and "as a nutritional principle, vitamin D has assumed a pivotal position as the precursor of a biological modifier of major significance" (Haussler 1986). While there appears to be recognition of "new functions" for vitamin D, these new functions remained associated with calcium metabolism and were not viewed as part of an independent and comprehensive endocrine system. It is of interest to note that one generation ago Steenbock and Herting (1955) had suggested that vitamin D_3 has metabolic effects and that effects on tissues other than bone should be investigated.

The new concept, formulated first in 1987 (Stumpf and O'Brien 1987b; Stumpf et al. 1987a) and detailed here and elsewhere (Stumpf 1988a, b) considers the regulation of systemic and cellular calcium homeostasis as only one of the many effects of $1,25D_3$. The new concept recognizes $1,25D_3$ as a comprehensive somatotrophic activator and modulator with actions on all somatic and endocrine systems. In addition, an interrelationship between the skin-vitamin D and the eye-pineal hormonal systems has been recognized and the concept of "the endocrinology of light and darkness" developed (Stumpf et al. 1987a; Stumpf 1988a, b). Support for the new concepts is provided here through a brief synopsis of nuclear targets for $1,25D_3$ and related functions:

Brain and spinal cord

In the telencephalon, the nervous circuit of the stria terminalis shows the strongest and most consistent representation of nuclear binding of ^3H $1,25D_3$ under variable experimental conditions, with neuronal targets in the bed nucleus (its dorsolateral, lateral and thalamic-anterior hypothalamic components) and the central nucleus of the amygdala. $1,25D_3$ probably affects manufacture and secretion of cholecystokinin (CCK) and neurotensin in these neurons. Other cell groups with a subpopulation of $1,25D_3$ target neurons include the piriform cortex, the adjacent periallocortex and isocortex, the entorhinal cortex, the ventral hippocampus area CA4, and the presubiculum, that is, the transition area between the ventral subiculum and the entorhinal cortex. There is strong nuclear binding of ^3H $1,25D_3$ in the reticular nucleus of the thalamus, an important structure that controls thalamic sensory outflow to the neocortex. Target neurons in the hypothalamic periventricular nucleus and its extensions, the parvocellular paraventricular and the infundibular nucleus, suggest modulatory influences on somatostatin, TRH, CRF, CCK and dopamine secretion.

In the lower brain stem, target neurons in the parabrachial nuclei suggest involvement of $1,25D_3$ in the regulation of gustatory, respiratory and cardiovasculary mechanisms. The parabrachial nuclei are a component of major circuits that extend to the hypothalamus, amygdala and limbic cortex, as well as to the medulla oblongata within the allocortex-brainstem-core (ABC) circuitry of autonomic-endocrine integration (Stumpf and Jennes 1984). In the caudal midbrain, neurons in the nucleus raphe dorsalis show a distinct nuclear labeling, which suggests effects on serotonin secretion. The locus ceruleus also contains scattered target cells for $1,25D_3$, therefore probably influencing catecholamine turnover. Target neurons in the substantia gelatinosa of the spinal nucleus of the nervus trigeminus and of the spinal cord, in conjunction with target neurons in spinal ganglion, suggest effects of $1,25D_3$ on perception of touch and pain, perhaps involving substance P-producing cells. The extensive nuclear uptake in neurons of cranial motor nerve nuclei and ventral horn neurons throughout the spinal cord (Stumpf et al. 1980a, 1988a) as well as in motor relay nuclei in the mid- and hindbrain indicates extensive effects on skeletal muscle physiology. Effects of $1,25D_3$ on the activity of choline acetyl transferase in the ventral forebrain have already been reported (Sonnenberg et al. 1986).

Pituitary

The extensive nuclear binding of ^3H $1,25D_3$ to certain pituitary cells (Stumpf et al. 1979), characterized as thyrotropes (Sar et al. 1980), is linked to the stimulation of TSH secretion in vivo (Sar et al. 1981). The latter was confirmed in vivo (Törnquist and Lamberg-Allardt 1985) and in vitro (Rose and Holick 1985). In studies with intact mice and with a higher dose of ^3H $1,25D_3$, additional nuclear binding, but less than to thyrotropes, has been observed in small subpopulations of lactotropes, somatotropes and gonadotropes (Stumpf et al. 1987a). This suggests that $1,25D_3$ also exerts direct effects on the secretion of hormones that are produced by these cells. Elevation of serum LH and testosterone levels (Sonnenberg et al. 1986) after $1,25D_3$ treatment may be mediated through effects on gonadotropes as well as on target neurons in the basal hypothalamus. Vitamin D-related effects on body growth were noted in earlier studies published in the literature that could not be explained by corrections of dietary deficiencies alone (Steenbock and Herting 1955). The promotion of 1-alpha hydroxylation of $25D_3$ by prolactin (Spanos et al. 1976) and by growth hormone (Spencer and Tobiasson 1977) may imply a positive feedback relationship in which rising estradiol blood levels in particular but also those of $1,25D_3$

stimulate the secretion of prolactin, which in turn stimulates the manufacture of $1,25D_3$ in the kidney.

The promotion of TSH secretion by $1,25D_3$ is probably the most important event in the vitamin D pituitary interaction, which results in a heliogenic stimulation of thyroid hormone secretion and subsequent activation of general protein synthesis and turnover. This activation of the pituitary-thyroid axis is elemental to the promotion of development and growth, and seasonal activation of life and reproduction.

While results from biochemical studies with homogenized pituitaries (Haussler et al. 1980) and tumor cell lines (Haussler et al. 1982; Gelbard et al. 1980) confirmed the presences of receptors for $1,25D_3$, no biochemical data on individual non-transformed cell types have been furnished to date.

The nuclear uptake in pituicytes of the posterior pituitary (Stumpf et al. 1979) remains puzzling, as does the earlier demonstrated binding to pituicytes of ^{3}H estradiol and ^{3}H dihydrotestosterone (Stumpf and Sar 1976). These results indicate or strongly suggest that pituicytes play an important role in posterior pituitary physiology.

B-Cells of pancreatic islands

Nuclear binding of ^{3}H $1,25D^3$ to B-cells of the islands of Langerhans (Clark et al. 1980) corresponds to the stimulation of insulin secretion by $1,25D_3$ (Clark et al. 1981) and by vitamin D (Norman et al. 1980) in vivo as well as to direct effects of vitamin D deficiency on insulin secretion in vivo and in vitro, independent of extracellular calcium levels (Clark et al. 1981). In addition, $1,25D_3$ probably plays a role in the maturation of pancreatic islets since nuclear binding exists before B-cells are morphologically and functionally mature (Clark et al. 1987).

The functional and clinical significance of the findings, still under investigation, is apparent from several studies reviewed elsewhere (Clark et al. 1981). A positive feedback relationship between pancreatic B-cells and kidney proximal tubule cells has been considered: insulin affects the 25(OH)-1a-hydroxylase enzyme; the $1,25D_3$ produced by this enzyme directly affects kidney distal tubule cells and intestinal absorptive epithelium which results in elevated blood calcium and also acts directly on pancreas B-cells to affect insulin release (Clark et al. 1981)

Adrenal medulla

The adrenal was studied since target cells for $1,25D_3$ had been identified in other endocrine organs, such as the pituitary, pancreatic islets and intestinal endocrine cells. Evidence for nuclear uptake and retention of ^{3}H $1,25D_3$ was obtained and found to exist in both epinephrine and norepinephrine producing cells (Clark et al. 1986c). Epinephrine cells were identified by colocalization with antibodies to the enzyme phenylethanolamine-*N*-methyltransferase by combined autoradiography-immunohistochemistry. Additional immunohistochemical studies with antiserum to 28000 D-calcium-binding protein (D-CaBP) revealed that the epinephrine-producing cells do not contain D-CaBP.

The results of these studies indicate the presence of nuclear receptors for $1,25D_3$ in adrenal medullary cells and suggest direct effects of $1,25D_3$ on certain functions of these cells. These effects may involve a sensitation of the stress response through stimulation of the manufacture of epinephrine and norepinephrine as an adaptation to the needs of the solar environmental changes and as suggested in the "endocrinology of sunshine and darkness", that is, the relationships between the skin-vitamin D and eye-pineal hormone endocrine systems (Stumpf et al. 1987a; Stumpf 1988a).

Enteroendocrine cells

In the pyloric stomach, certain cells in the epithelium of antral glands concentrate ^{3}H $1,25D_3$. Because of their location and the immunostaining of autoradiograms with antibodies to gastrin, these cells are probably gastrin producing cells (Stumpf et al. 1979, 1988b). These data, together with the known gastrin-mediated paracrine effects on the stimulation of the secretion of pepsinogen and hydrochloric acid, and endocrine effects on calcitonin secretion (Cooper et al. 1971), suggest that $1,25D_3$ directly affects gastrin secretion as a nutritional regulator of digestion and growth. Low gastric acidity may explain the low food efficiency of vitamin D-deficient diet (Clark et al. 1986b). The present data correspond well to observations published earlier in the literature, in which vitamin D was found to increase the volume and acidity of gastric secretion (Herting and Steenbock 1955) and a reduced gastric acidity in rachitic infants (Wills et al. 1926).

It is possible that not only gastrin-producing cells are targets for $1,25D_3$, but also CCK cells and other enteroendocrine cells.

Thyroid follicle epithelium and parafollicular cells, parathyroid parenchymal cells

As earlier shown with sex steroids, central regulatory and peripheral effector target organs are apparently coordinated and integrated by direct actions of the related steroid hormone on all of these structures (Stumpf and Sar 1976). Such can be expected to be the case with $1,25D_3$ for pituitary thyrotropes (Sar et al. 1980), possibly brain TRH neurons (Stumpf and O'Brien 1987b), and thyroid follicle epithelium. Nuclear uptake, although weak, has been shown to exist in certain epithelial cells of certain follicles as well as in certain parafollicular cells (Stumpf et al. 1981a; Stumpf and O'Brien 1987a). In addition, in the lumina of many of the larger thyroid follicles radioactivity was accumulated after injection of ^{3}H $1,25D_3$ and ^{3}H $25D_3$. This suggests binding of $1,25D_3$ and of $25D_3$ to thyroglobulin and storage in follicular colloid (Stumpf anc O'Brien 1987a) or uptake of a radiolabeled portion of the metabolized secosteroid.

In these studies C-cells identified by staining with antibodies to calcitonin could not be shown to concentrate ^{3}H $1,25D_3$, even though cells of ultimobranchial remnants and certain unidentified parafollicular cells concentrated radioactivity after ^{3}H $1,25D_3$ injection.

In the parathyroid, nuclear concentration of ^{3}H $1,25D_3$ in parenchymal cells is generally strong, but varies with age and endocrine status, and exceeds several times the nuclear uptake in follicle epithelium. Nuclear labeling of follicle epithelium may be absent at low tracer doses or relatively short autoradiographic exposure times (Stumpf et al. 1979, 1981b). In biochemical homogenates of whole parathyroid organs, the presence of $1,25D_3$ specific binding

to protein has been reported in bird (Brumbaugh et al. 1975; Henry and Norman 1975; Wecksler et al. 1977; Hughes and Haussler 1978). It must be noted that these biochemical studies did not distinguish cell types. In the parathyroid there are nuclear receptors also for ^{3}H estradiol. However, they are in stromal cells rather than in parenchymal cells (Stumpf and Sar 1976). With homogenized tissue one would not know the difference.

Bone, teeth, cartilage

With biochemical techniques, cytosolic (Kream et al. 1977; Manolagas et al. 1979) and nuclear (Weber et al. 1971) binding of $1,25D_3$ to bone cells has been reported without identifying specific cell types. With our thaw-mount autoradiographic technique, osteoblasts and osteoprogenitor cells were identified in undecalcified perinatal bone to concentrate ^{3}H $1,25D_3$ in their nuclei (Stumpf et al. 1981a; Narbaitz et al. 1983). No nuclear concentration in these cells was observed with ^{3}H $25D_3$ or ^{3}H $24,25D_3$ (Stumpf et al. 1985).

In undecalcified rodent incisor and molar teeth in late fetal and early neonatal life, nuclear accumulation of ^{3}H $1,25D_3$ was shown in vivo to exist predominantly in pulp cells, less in cells of the stratum intermedium, and comparatively weak or absent in differentiated odontoblasts and ameloblasts (Kim et al. 1983, 1985; Clark et al. 1985). Nuclear concentration in pulp cells could also be demonstrated in human molars in vitro (Clark et al. 1985). The results from these studies suggest that $1,25D_3$ action in teeth differs from that in bone at birth. In teeth, the predominant action appears to be on differentiation of odontoblast precursor or supportive cells, while in bone, mainly developed osteoblasts appear to be affected.

Nuclear binding of ^{3}H $1,25D_3$ in cartilage has been demonstrated (Stumpf et al. 1981a) and reported to be nonuniform and restricted to certain maturational states of chondroblasts and chondrocytes that includes perichondrial and hyaline cartilage. A detailed assessment of the relationships between nuclear binding and type and maturational state of cartilage remains to be published.

Muscle

Skeletal muscle weakness is a characteristic accompaniment of vitamin D deficiency and muscle physiology is severely affected (Steenbock and Herting 1955). However, under the conditions of our experiments, in skeletal muscle no evidence for direct effects of $1,25D_3$ was obtained since no nuclear concentration of ^{3}H $1,25D_3$ could be observed. Smooth muscle of the intestine also does not display nuclear binding, with the exception of the pyloric sphincter (Stumpf et al. 1981a, 1988b).

For heart muscle, there are some indications for the presence of nuclear receptors for $1,25D_3$ (Stumpf et al. 1981a; Walters et al. 1986). However, this needs to be confirmed since our studies showed nuclear binding to be comparatively weak, borderline, or absent.

Muscle, in general, may be affected by changes in serum calcium levels, and skeletal muscle, secondarily, by the impaired conditions of the skeleton in vitamin D deficiency. However, strong nuclear binding of $1,25D_3$ to motor neurons in the spinal cord and lower brain stem (Stumpf et al. 1988a; Stumpf and O'Brien 1987b), similar to androgen and adrenal steroids, has been interpreted as $1,25D_3$ acting on skeletal muscle through neurotrophic effects (Stumpf et al. 1980a, 1988a). This could explain the effects of $1,25D_3$ on muscles in the absence of binding to myocyte nuclei.

Myoepithelial cells in tracheal glands and in the submandibular gland have been recognized to concentrate ^{3}H $1,25D_3$ in their nuclei (Stumpf and Downs 1987), while myoepithelial cells in the mammary gland did not show nuclear concentration in the presence of nuclear labeling of alveolar and duct cells (Narbaitz et al. 1981).

Alimentary tract

The epithelium throughout the alimentary tract, perhaps with the exception of the stomach and some specialized cells such as goblet cells, appears to be directly affected by $1,25D_3$ (Stumpf et al. 1979). The lower esophagus and most parts of the stomach remain to be studied, and not all of the other parts of the alimentary tract are published in detail. The oral epithelium and the epithelium of the pharynx and upper esophagus show nuclear concentration of ^{3}H $1,25D_3$, predominantly in the basal layers, similar to the skin (Stumpf et al. 1985). In the small and large intestine, results from our autoradiographic studies indicate nuclear concentration of ^{3}H $1,25D_3$ with variable intensity throughout the absorptive epithelium. In certain of the experimental animals, the nuclear labeling of epithelium is highest in crypts of Lieberkühn, while in other animals, it is highest in intestinal villi.

Liver

While nuclear receptors for $1,25D_3$ are expected to exist in hepatocytes, no evidence for their presence has been reported. The latter would suggest that there is no direct feedback regulation of the 25-hydroxylation step of the prohormone in contrast to the 1-hydroxylation step in the kidney proximal tubule. Since the nuclear concentration of ^{3}H $1,25D_3$ in the epithelium of the kidney proximal tubule cells is weak or may not be detectable under certain experimental conditions when distinct nuclear labeling of distal tubule epithelium is present (Stumpf et al. 1980b), a situation of low-level receptors must be considered also for liver cells. A transcriptional or posttranscriptional regulation of 25-hydroxylation of vitamin D_3 by $1,25D_3$, estradiol, and other hormones is likely and requires clarification. Under the conditions of our autoradiographic studies, radioactivity levels are generally high in the cytoplasm of hepatocytes without recognizable nuclear concentration. This cytoplasmic radioactivity may reperesent metabolites, or sites of action for ^{3}H $1,25D_3$, or both.

Accumulation of radioactivity is present in biliary duct lumena and, on occasion, in the cytoplasm of certain littoral cells. These cells are probably lipocytes as can be derived from their distribution and appearance (Stumpf et al. 1979). Accordingly, lipocytes may be the sites of storage not only for vitamin A, but also for vitamin D.

Kidney

The kidney is perceived as one of the difficult tissues for biochemical studies of nuclear localization of $1,25D_3$ since

"there is a plethora of cell types, which may tend to 'average out' any specialized subcellular localization occuring in only one of the cell types" (Norman 1979). Results from our autoradiographic studies demonstrate that epithelial cells of renal tubules are targets for $1,25D_3$, however, with variations in the nuclear uptake of ^3H $1,25D_3$ among the various segments. After a single injection of ^3H $1,25\,D_3$, strongest nuclear uptake is seen in portions of the distal tubule, including the macula densa (Stumpf et al. 1980b; Narbaitz et al. 1982). Epithelial nuclear uptake is also present in the thick and thin limbs of the loop of Henle and in the proximal tubule, but less intense than in the distal tubule. Podocytes in the glomerulus that regulate capillary filtration, and perhaps produce erythropoietin, show a distinct nuclear labeling (Stumpf et al. 1981a, 1982). Which particular functions of podocytes are influenced by $1,25D_3$, and whether this includes endocrine or paracrine mechanisms, remains to be studied.

When results obtained with ^3H $1,25D_3$ are compared with those of ^3H estradiol, there is overlap of nuclear labeling in proximal tubule cells. This may reflect synergistic effects of both steroid hormones on the hydroxylation of $25D_3$ to $1,25D_3$.

When ^3H $25D_3$ or ^3H $24,25D_3$ is used, no such nuclear labeling occurs in the various tissues of the kidney as is seen with ^3H $1,25D_3$ (Stumpf et al. 1985). With ^3H $25D_3$, concentration of radioactivity is, however, observed in the cytoplasm of the epithelium of the proximal tubule (Stumpf et al. 1985) where the prohormone is known to be hydroxylated (Fraser and Kodicek 1970). The extensive and diffential nuclear localization of $1,25D_3$ in different tissues of the kidney indicate diverse effects with corresponding differential levels of activation that may include reabsorption of calcium and perhaps other ions, regulation of podocyte-governed filtration, modulation of 25-hydroxyvitamin D_3-1a-hydroxylase and 24-hydroxylase activities, production of calcium-binding protein and possibly erythropoietin, and probably others.

Thymus and other lymphatic organs

Results from biochemical studies with whole lymphatic organs are interpreted to indicate that lymphocytes contain receptors for $1,25D_3$ and that the action on lymphocytes mediates effects of $1,25D_3$ on the immune system (Manolagas 1985; Ohsugi et al. 1985). In our autoradiographic studies no nuclear concentration of ^3H $1,25D_3$ was observed in lymphocytes of thymus and intestinal lymph nodules or in diffusely occuring lymphocytes under conditions that produced detectable nuclear accumulation of ^3H $1,25D_3$ in intestinal epithelium. This does not exclude the possibility of nuclear uptake and a direct $1,25D_3$ action under different experimental conditions. In the autoradiograms, nuclear concentration of ^3H $1,25D_3$ was observed, however, in reticular cells in the thymus (Stumpf et al. 1981a; Stumpf and Downs 1987). These data suggest that $1,25D_3$, similar to estradiol (Stumpf and Sar 1976), exerts its effects on lymphatic tissues mainly through actions on reticular cells.

Individual cells in the dermis with nuclear labeling appeared to be macrophages (Stumpf et al. 1984). More histochemical evidence needs to be gathered, before conclusions can be drawn regarding macrophages and their differential appearance as antigen-providing cells in lymphatic tissues, lung supraalveolar cells, skin Langerhans cells, microglia, osteoclasts, and others.

Reproductive organs

Evidence from whole organ homogenates suggests that receptors for $1,25D_3$ are present in ovary (Dokoh et al. 1983) and uterus (Walters 1981) as well as in the testis (Walters et al. 1983; Merke et al. 1983; Levy et al. 1985). In the testis, the target cells were identified by autoradiography as Sertoli cells, and probably epithelium of the rete testis, in addition to certain epithelial cells in the ductuli efferentes (Stumpf et al. 1987b). Nuclear concentration of ^3H $1,25D_3$ is also observed in male accessory sex organs, such as, prostatic epithelium (unpublished). In autoradiographic studies of female reproductive organs, nuclear labeling with ^3H $1,25D_3$ is present in alveolar and ductal epithelium of mammary gland (Stumpf et al. 1981a; Narbaitz et al. 1983), and in certain cells in the uterus, oviduct and ovary (unpublished). Furthermore, nuclear labeling has been observed in epithelial cells of the vomeronasal organ in rats (unpublished), in a subpopulation of pituitary gonadotropes (Stumpf et al. 1987a), and in neurons of hypothalamic regions (Stumpf and O'Brien 1987b) associated with the regulation of gonadotropin secretion. These data together provide strong indications for direct and extensive effects of $1,25D_3$ on female and male reproduction at different levels of regulation.

A close relationship between reproduction and the vitamin D endocrine system is further suggested from several other observations such as the high $1,25D_3$ blood levels during puberty in humans (Aksnes and Aarskog 1982) and rats (Clark et al. 1986a). The serum concentration of vitamin D metabolites is related to the stage of pubic hair development in boys and breast development in girls, rather than to the chronological age (Aksnes and Aarskog 1982). Changes of $1,25D_3$ plasma levels during pregnancy and lactation (Halloran et al. 1979), sex differences in plasma $1,25D_3$ levels that disappear after castration, and estradiol induced dose-related lowering (Gray 1981) or elevation (Baksi and Kenney 1978) of $1,25D_3$ plasma levels, all indicate relationships between reproduction and the vitamin D endocrine system.

Existence of a bidirectional positive feedback interaction between the sex steroid system and the vitamin D system is likely. The supporting evidence presented here is extensive and includes $1,25D_3$ targets in peripheral and central reproductive tissues to elevate serum LH and testosterone (Sonnenberg et al. 1986) and probably others, and the localization of ^3H estradiol in kidney proximal tubule epithelium (Stumpf et al. 1980b) to promote the hydroxylation of the prohormone $25D_3$ (Tanaka et al. 1976 and thus to elevate serum $1,25D_3$. Cooperative interactions between soltriol and estradiol are also suggested in the physiology of skin and hair growth and in the calcification of bone, and are likely to exist elsewhere. A close endocrine systems link between the heliogenic soltriol and gonadal estradiol and other sex steroids is postulated and is an essential element in the new concept of comprehensive somatotrophic vitamin D action.

Pineal

Whether there are pineal receptors and direct effects of $1,25D_3$ on pineal function remains to be studied. Nuclear

receptors for $1,25D_3$ can be expected to exist in certain pineal cells in a fashion similar to estradiol (Stumpf et al. 1976).

There is evidence for interrelationships between pineal hormone and vitamin D actions. Derived from the new concept of comprehensive somatotrophic seasonal activation by $1,25D_3$ is the concept of complementary relationship between activational effects by the heliogenic $1,25D_3$ and inhibitory effects by the helioprivic nyctogenic pineal hormones (Stumpf et al. 1987a; Stumpf 1988a). The decrease of sunlight incurs concurrent active and passive regulations of biological events. The passive component relates to the decline of skin hormone-soltriol production, the active component relates to the increased pineal hormone-melatonin production. The decline of soltriol levels is followed by a reduction of physiological activities and gives way to a depression of reproductive and general activities that may result in hibernation in the extreme. Difficulties of pinealogists to explain and clarify certain seasonal changes, or lack thereof, from data of light effects on pineal function alone may reside in the involvement of skin in the perception and transduction of sunlight into somatotrophic and reproductive effects. As vitamin D is not only involved in calcium metabolism, so is pineal hormone(s) not only involved in reproduction. In the past, both hormonal systems have received inadequate appreciation of their extensive regulation of the seasonal adjustment of vertebrate physiology.

The notion may be entertained that the pineal is the "third eye" or, phylogenetically, the first eye. Phylogenetically, the bilateral eyes may not be first and second but rather the second and third eyes, or there may be justification to view the lateral eyes rather as the second and third pineal (Menaker 1985; Reiter 1986). Now, there is good evidence and recognition that some functions of these three eyes, namely the transduction of light into biological parameters, are shared by the skin. The skin, now recognized as the most important endocrine transducer of light, is therefore viewed as the first and primary somatic eye of great significance (Stumpf 1988b).

Skin

Skin has been recognized two generations ago as a site of production of the prohormone vitamin D_3. It was demonstrated in 1979 (Stumpf et al.) to be a site of action for the hormone $1,25(OH)_2$ vitamin D_3.

Evidence to support some of our autoradiographic findings was quick to follow by the demonstration of the presence of specific binding protein in homogenized skin (Colston et al. 1980; Esvelt et al. 1980; Feldman et al. 1980). Assays of human skin biopsies were utilized and introduced for diagnostic purposes (Eil and Marx 1981).

A histochemical analysis of the individual tissues in the skin indicates that the strongest nuclear concentration of ^3H $1,25D_3$ occurs in the epithelium of the outer hair sheaths, followed by the basal layers of the epidermis (Stumpf et al. 1979). Nuclear binding is also present in other epithelial layers of hair sheaths and epidermis, in sebaceous glands, in fibroblasts in the outer connective tissue sheath of certain hair, in basal cells of hair, and in certain dispersed cells in the dermis, probably macrophages (Stumpf et al. 1984). Interestingly, in the dermal papilla of hair, no evidence for nuclear binding was obtained for ^3H $1,25D_3$, which had been reported for ^3H estradiol (Stumpf et al. 1974). Similarly, fibroblasts in the dermis show a distinct nuclear uptake of ^3H estradiol. Such nuclear uptake in fibroblasts is not observed with ^3H $1,25D_3$, although some nuclear concentration has been noted in fibroblasts of the infundibular portion of the connective tissue sheath of the hair shaft of vibrissae. It should be noted that the nuclear binding of ^3H $1,25D_3$ is not uniform and that there are regional differences, It is therefore likely that the potential to produce vitamin D_3 similarly varies in different regions. The autoradiographic data suggest that $1,25D_3$ affects proliferation or differentiation, or both, of epidermis and hair and of some cells in the dermis. Experimental evidence for such effects has come from studies with epidermal cell cultures (Hosomi et al. 1983) and from clinical observations (Marx 1984). Since ^3H estradiol localizes predominantly in cells of the dermal papilla and ^3H $1,25D_3$ in cells of the hair sheath and bulb, a cooperative effect of these two hormones on hair growth is suggested by the different target sites. Whether or not skin melanocytes are targets for $1,25D_3$ remains to be studied. Enhancement of skin pigmentation through sunlight may be mediated by direct or indirect actions of $1,25D_3$ on melanocytes. Stimulation of melanogenesis by cholecalciferol in cultured human melanocytes has been reported (Tomita et al. 1986). Pigmentation, as well as keratinocyte and hair cell proliferation and differentiation are probably protective "negative feedback" responses against excessive irradiation. As "positive feedback" regulation may be viewed the stimulatory effect of $1,25D_3$ on the enzymatic conversion of cholesterol to 7-dehydrocholesterol (Esvelt et al. 1980). A positive effect on vitamin D_3 production is assumed by pineal melatonin (Fig. 1), since it incurs a retraction of skin melanin (Lerner et al. 1958). Accordingly, the skin appears to play an important regulatory role. Positive and negative mechanisms occur as a consequence of radiation and related dose and duration of $1,25D_3$ action together with an interplay with the pineal hormones of darkness.

Placenta and fetal membranes

Evidence for the presence of specific binding protein for $1,25D_3$ was found in homogenized placenta (Pike et al. 1980; Christakos and Norman 1980) without clarification which of the different placental tissues are involved. Results from autoradiographic studies (Stumpf et al. 1981a, 1983b) demonstrated nuclear uptake of ^3H $1,25D_3$ in epithelial cells of the visceral yolk sac and amnion and in certain trophoblast cells in the basal and labyrinthine parts of the placenta. Since these observations stem from late term rat placentae, more studies are required at different stages of development and in different species, so that the functional significance of $1,25D_3$ can be clarified. Although involvement of $1,25D_3$ target cells in calcium transport is likely, many other actions should be considered, including production of messenger peptides, local sex steroid effects, and placental steroid hormone production.

Hematopoetic system

Clinical observations in vitamin D deficient children, such as anemia and extramedullary hematopoesis (Yetgin and Oszoylu 1982), and impaired antiinflammatory response (Ströder and Franzen 1975; Lorente et al. 1976), implicate the red and white blood cell system as direct or indirect effector sites for $1,25D_3$.

Nuclear accumulation of ^3H 1,25D_3 in certain cells in bone marrow and fetal liver, believed to be mesenchymal reticular cells, was demonstrated in our autoradiographic in vivo studies (Stumpf et al. 1981a).

Since kidney podocytes or other structures in the kidney which are targets for 1,25D_3 and are suspected to produce erythropoietin, it is likely that vitamin D effects on hematopoesis are mediated through stimulation of erythropoietin synthesis and secretion.

Conclusions

From this synopsis of new 1,25$(OH)_2$ vitamin D_3 target tissues and functions it is evident that a new concept on vitamin D action had to be developed (Stumpf et al. 1979, 1987a; Stumpf and O'Brien 1987b). According to the new information that we have been able to accumulate, the vitamin D effector system is of much wider significance than is implied in the concept of "the calcium homeostatic steroid hormone" and the cliche "vitamin D-calcium metabolism".

Rather: 1,25$(OH)_2$ vitamin D_3 is a comprehensive somatotrophic activator and modulator for environmental (seasonal, cosmic) tuning of the organism toward optimal adaptation for development, maintenance and propagation of life. None of the bodily functions appears to be excluded from 1,25D_3 effects. Regulation of calcium homeostatis is but one of the many functions of soltriol, the heliogenic steroid hormone.

Interpretation of 1,25D_3 effects from aspects of calcium homeostasis alone is too limited. The widely assumed close relationship between 1,25D_3 action and production of specific calcium-binding proteins is often not existent when our autoradiographic localization of ^3H 1,25D_3 is compared with the immunohistochemical localization of antibodies to vitamin D-dependent calcium-binding proteins. A mismatch between the two has been noted even in the intestine, a classical target tissue for 1,25D_3, where vitamin D-dependent calcium-binding protein is absent (Marche et al. 1980) or low in crypt cells that display high nuclear uptake of ^3H 1,25D_3 (Stumpf et al. 1979). The recommended use of antibodies to specific calcium-binding protein for monitoring 1,25D_3 target cells (Norman 1979; Jande et al. 1981), therefore, needs reassessment. While vitamin D-dependent calcium-binding protein may be one endproduct for certain cells (Roth et al. 1981), many 1,25D_3-target cells appear to have different tissue-specific endproducts, such as, TSH, gastrin, insulin, parathyroid hormone, epinephrine, serotonin, cholecystokinin, cell proliferation, cell differentiation, and others. Multiple endproducts are probably the rule. Colocalization of radiolabeled hormone by autoradiography with antibodies to cellular product (Sar and Stumpf 1981) or receptor (Stumpf et al. 1982) by immunohistochemistry is suited to clarify cellular relationships between ligand binding, receptor, and product.

For many of the newly discovered target tissues, the full spectrum of 1,25D_3-related functions remains to be studied. This requires research with manifold approaches in different fields for years to come.

Histochemical information from autoradiography [that "nicely complemented" biochemical data (Norman et al. 1982)] with over 130 rodents has been gathered in our laboratory. This includes studies with different metabolites, such as, ^3H 1,25D_3, ^3H 25D_3 and ^3H 24, 25D_3, different doses and different time intervals after a single injection or multiple injections. No evidence for nuclear receptors for 25D_3 nor for 24,25D_3 has been obtained (Stumpf et al. 1985).

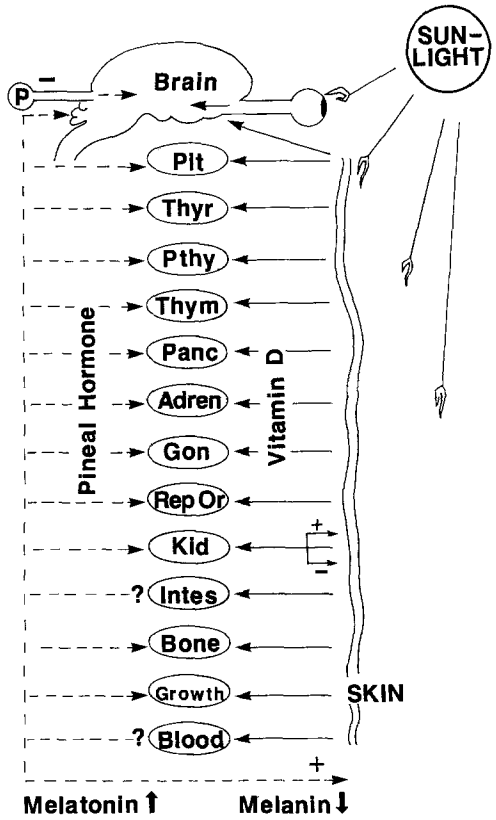

Fig. 1. The actions of vitamin D-soltriol can be understood as a heliogenic somatotrophic "activating"-modulating system. Regulation of calcium levels is a part of it. Similarly, the actions of the helioprivic-nyctogenic pineal hormone(s) constitute a somatotrophic "inactivating"-modulating system. Control of reproduction is a part of it. Sunlight's invisible shortware component controls the production of vitamin D_3-soltriol, while sunlight's visible component controls the production of pineal hormones. The heliogenic (and nutritional) skin-vitamin D_3 messenger system and the helioprivic eye-pineal messenger system provide for a complementary and purposeful adaptation to the solar conditions in order to facilitate development and procreation of life (Stumpf 1988a).

Another important observation relates to the average number of radiolabeled 1,25D_3 molecules per nucleus of a given target tissue. With a computer program that permits conversion of the number of silver grains to the number of molecules (Stumpf et al. 1981c), considerable differences have been noted among individual cells of identical tissues and among cell populations of different target tissues (Clark et al. 1987; Stumpf and O'Brien 1987a, b). Such differences appear to be related not only to development and age but also to the endocrine status. These observations require follow-up studies and further clarification. It has been suggested that there is a differential activation of target cells that depends on the number of receptors in particular cells. According to this concept of MAHS (Multiple activation of heterogeneous systems, Stumpf and Sar 1981), cells with high receptor numbers may respond at low hormone blood levels, while cells with low receptor numbers show no measurable response. Changes in hormone blood levels or in

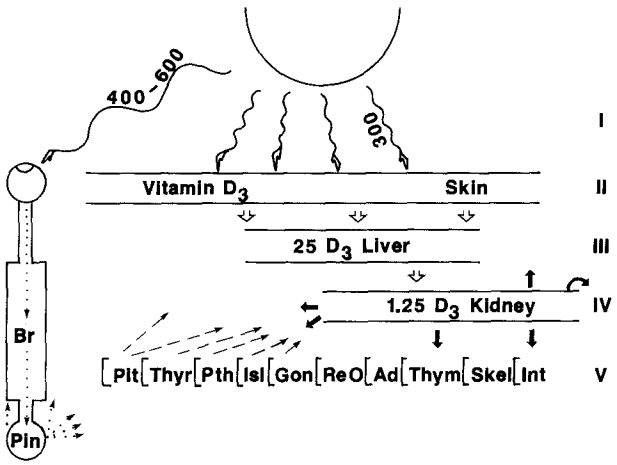

Fig. 2. The synthesis of $1,25(OH)_2$ vitamin D_3 (soltriol) appears to be controlled at five levels with increasing fine tuning: *I*, the amount of daily and seasonal sunshine (or artificial equivalent?); *II*, the skin transformation of cholesterol to 7-dehydrocholesterol and to the prohormone vitamin D_3; *III*, the 25-hydroxylation in the liver; *IV*, the 1-hydroxylation in the kidney; *V*, the receptor levels in target tissues and related hormone levels in the blood (including gonadal steroid hormones, insulin, parathyroid hormone, thyroid hormone, pituitary hormones, soltriol) which affect hydroxylation of vitamin D_3 or formation of 7-dehydrocholesterol.

This multilevel regulation of soltriol production permits the sun exposure-linked production of vitamin D_3-soltriol to be adjusted to the developmental and reproductive status of the individual. In particular, the control of the rate of hydroxylation in the liver and kidney permits a fine tuning and adaptation to correlate the blood levles of $1,25D_3$ with the varying needs during growth, puberty, and pregnancy as well as during periods of reduced endocrine and reproductive activity. Liver and kidney, which are regulated in tune with the changing endocrine conditions (level V), are thus considered endocrine and metabolic messenger-controlled regulators of $1,25$ D_3 synthesis.

The heliogenic vitamin D_3-soltriol then activates and modulates many tissues with receptors for soltriol, including brain (*Br*), pituitary (*Pit*), thyroid (*Thyr*), gonads (*Gon*), reproductive organs (*ReO*), parathyroids (*Pth*), pancreatic islands (*Isl*), adrenals (*Ad*), thymus (*Thym*), skeleton (*Skel*), intestine (*Int*), skin, and kidney. Note the inhibitory effect of visible light on pineal hormone production. The helioprivic (nyctogenic) pineal homone exerts effects that appear antagonistic to soltriol activation in pituitary, thyroid, gonads and reproductive organs, parathryoid, and others, except in skin, where it facilitates access of sun light and related production of vitamin D_3 (Stumpf 1988a, b).

nuclear receptor levels may result in corresponding changes in the activation of specific cell populations, which then may lead to variations in the intensity and possibly quality of biological response.

The new concept of general somatotrophic vitamin action on vital systems, including the brain, is likely to lead to a better understanding of the effects of heliotherapy on mood and seasonal affective disorder (Wehr et al. 1986), which can not be explained with concepts that lack awareness of skin-vitamin D production and actions.

An important wide open field is the relationships between soltriol and estradiol effects. The skin-vitamin D system and the gonadal sex steroid system seem to be closely interrelated and mutually promotional in the maintenance and propagation of life. The insights that come from the newly developed concept on vitamin D actions influence our understanding of the pineal hormonal system, which can now be viewed in a different perspective (Fig. 1). The interrelationships between pineal hormones induced by the decline of light (yin) and vitamin D induced by the rise of the sun (yang) can now be studied in more meaningful ways.

Perhaps, the sun-related activation of the skin-vitamin D system has some analogy to the leaf-chlorophyll transduction of sunlight in plants. While such analogy may seem far-fetched since animals do not have chlorophyll and photosynthesis to convert sunshine into chemical energy, the equivalent may be provided by the skin-vitamin D mediated DNA activation of vital systems. In animals, the energy has to come from plant products. The purpose of the plant and animal systems seems to be similar: to convert and utilize sunlight energy for growth, maintenance of life and procreation.

Suggestions for several positive and negative "feedback" relationships have been made in this review. Much more information needs to be gathered, before a full understanding of the physiology of "soltriol" can be obtained. There is accumulating evidence that suggests the existence of positive as well as negative feedback regulations in a fashion similar to sex and adrenal steroid relationships to a "brain-pituitary-axis". An obvious vitamin D related negative feedback can be seen in the degrees of human skin pigmentation (Loomis 1967; Holick 1984), which corresponds to the latitude of the global habitat, permitting more or less of the short wave sun light to penetrate. Regulation of production of $1,25D_3$ is multifactorial and occurs at several levels (Fig. 2). Sunlight exposure of the skin provides the prohormone vitamin D_3, as does nutrition. Sunlight exposure is enhanced through melanin retraction when melatonin levels rise during darkness (Lerner et al. 1958), in turn, rising cholecalciferol stimulates melanogenesis (Tomita et al. 1986). Hydroxylation and production of $1,25D_3$ is influenced by gonadal activity, pituitary hormones, parathyroid hormone, insulin, $1,25D_3$, plasma minerals, and others. Thus, a careful adjustment of $1,25D_3$ production and effects can be achieved and correlated with conditions and needs of the individual.

References

Aksnes L, Aarskog D (1982) Plasma concentrations of vitamin D metabolites in puberty: effect of sexual maturation and implications for growth. J Clin Endocrinol Metab 55:94–101

Baksi SN, Kenney AD (1978) Does estradiol stimulate in vivo production of 1,25-dihydrovitamin D_3 in the rat? Life Sci 22:787–791

Bastillo L, Tanaka Y, Wineland MJ, DeLuca HF (1979) Synergistic effect of progesterone, testosterone and estradiol in the stimulation of chick renal 25-hydroxy-vitamin D-1a-hydroxylase. Endocrinology 104:1598–1601

Bell NH (1985) Vitamin-D-endocrine system. J Clin Invest 76:1–6

Brumbaugh PF, Hughes MR, Haussler MR (1975) Cytoplasmic and nuclear binding components for 1a,25-dihydroxyvitamin D_3 in chick parathyroid glands. Proc Natl Acad Sci USA 72:4871–4875

Christakos S, Norman AW (1980) Specific receptor/binding proteins for $1,25(OH)_2$-vitamin D_3 in rat and human placenta. Fed Proc 39:560

Clark SA, Stumpf WE, Sar M, DeLuca HF, Tanaka Y (1980) Target cells for 1,25 dihydroxyvitamin D_3 in the pancreas. Cell Tissue Res 209:515–520

Clark SA, Stumpf WE, Sar M (1981) Effects of 1,25 dihydroxyvitamin D_3 on insulin secretion. Diabetes 30:382–386

Clark SA, Dame MC, Kim YS, Stumpf WE, DeLuca HF (1985) 1,25-Dihydroxyvitamin D_3 in teeth of rats and humans: receptors and nuclear localization. Anat Rec 212:250–254

Clark SA, Boass A, Toverud SU (1986a) Development-related regulation of plasma $1,25(OH)_2D_3$ concentration by calcium intake in rat pups. Bone Mineral 1:193–203

Clark SA, D'Ercole AJ, Toverud SU (1986b) Somatomedin-C/insulin-like growth factor I and vitamin-induced growth. Endocrinology 119:1660–1665

Clark SA, Stumpf WE, Bishop CW, DeLuca HF, Park DH, Joh TH (1986c) The adrenal: a new target organ of the calciotropic hormone 1,25 dihydroxyvitamin D_3. Cell Tissue Res 234:299–302

Clark SA, Stumpf WE, Sar M, DeLuca HF (1987) 1,25-Dihydroxyvitamin D_3 target cells in immature pancreatic islets. Am J Physiol 253:E99–E105

Colston K, Hirst M, Feldman D (1980) Organ distribution of the cytoplasmic 1,25 dihydroxycholecalciferol receptor in various mouse tissues. Endocrinology 107:1916–1922

Cooper CW, Schwesinger WH, Mahgoub AM, Ontjes DA (1971) Thyrocalcitonin: stimulation of secretion by pentagastrin. Science 172:1238–1240

Dokoh S, Donaldson CA, Marion SL, Pike JW, Haussler MR (1983) The ovary: a target for 1,25-dihydroxyvitamin D_3. Endocrinology 112:200–206

Eil C, Marx SJ (1981) Nucleaer uptake of 1,25 dihydroxy[^{3}H]cholecalciferol in dispersed fibroblasts cultured from normal human skin. Proc Natl Acad Sci USA 78:2562–2566

Esvelt RP, DeLuca HF, Wichman JK, Yishizawa S, Zurcher J, Sar M, Stumpf WE (1980) 1,25-Dihydroxyvitamin D_3 stimulated increase of 7,8-didehydrocholesterol levels in rat skin. Biochemistry 19:6158–6161

Feldman D, Chen T, Hirst M, Colston K, Karasek M, Cone C (1980) Demonstration of 1,25-dihydroxyvitamin D_3 receptors in human skin biopsies. J Clin Endocrinol Metab 51:1463–1465

Fraser DR, Kodicek E (1970) Unique biosynthesis by kidney of a biologically active vitamin D metabolite. Nature 228:764–766

Gelbard HA, Stern PH, U'Prichard DC (1980) 1,25-Dihydroxyvitamin D_3 nuclear receptors in pituitary. Science 209:1247–1249

Gray RW (1981) Effects of age and sex on the regulation of plasma $1,25\text{-}(OH)_2$-D by phosphorus in the rat. Calcif Tissue Int 33:477–484

Halloran BP, Barthell EN, DeLuca HF (1979) Vitamin D metabolism during pregnancy and lactation in the rat. Proc Natl Acad Sci USA 76:5549–5553

Haussler MR (1986) Vitamin D receptors: nature and function. Annu Rev Nutr 6:527–562

Haussler MR, Manolagas SC, Deftos LJ (1980) Evidence for a 1,25-dihydroxyvitamin D_3 receptor-like macromolecule in rat pituitary. J Biol Chem 225:5007–5010

Haussler MR, Manolagas SC, Deftos LJ (1982) Receptor for 1,25-dihydroxyvitamin D_3 in GH3 pituitary cells. J Steroid Biochem 16:15–19

Henry HL, Norman AW (1975) Studies on the mechanism of action of calciferol. VII. Localization of 1,25-dihydroxy-vitamin D_3 in chick parathyroid glands. Biochem Biophys Res Commun 62:781–788

Herting DC, Steenbock H (1955) Vitamin D and gastric secretion. J Nutrit 57:469–482

Holick MF (1984) The photobiology of vitamin D_3 in man. In: Kumar R (ed) Vitamin D. Basic and clinical aspects. Martinus Nijhoff, Boston, pp 197–216

Hosomi J, Hosoi J, Abe E, Suda T, Kuroki T (1983) Regulation of terminal differentiation of cultured mouse epidermal cells by 1a,25-dihydroxyvitamin D_3. Endocrinology 113:1950–1957

Hughes MR, Haussler MR (1978) 1,25-Dihydroxyvitamin D_3 receptors in parathyroid glands: preliminary characterization of cytoplasmic and nuclear binding components. J Biol Chem 253:1065–1073

Jande SS, Maler L, Lawson DEM (1981) Immunohistochemical mapping of vitamin D-dependent calcium-binding protein in brain. Nature 294:765–767

Kim YS, Stumpf WE, Clark SA, Sar M, DeLuca HF (1983) Target cells for 1,25-dihydroxyvitamin D_3 in developing rat incisor teeth. J Dent Res 62:58–59

Kim YS, Clark SA, Stumpf WE, DeLuca HF (1985) Nuclear uptake of 1,25-dihydroxyvitamin D_3 in developing rodent teeth: an autoradiographic study. Anat Rec 212:301–306

Kream BE, Jose M, Yamada S, DeLuca HF (1977) A specific high-affinity binding macromolecule for 1,25-dihydroxyvitamin D_3 in fetal bone. Science 197:1086–1088

Lerner AB, Case JD, Takahashi Y, Lee TH, Mori W (1958) Isolation of melatonin, the pineal gland factor that lightens melanocytes. J Am Chem Soc 80:2587

Levy FO, Eikvar L, Jute NHPM, Cervenka J, Yoganathan T, Hansson V (1985) Properties and compartmentalization of the testicular receptor for 1,25-dihydroxyvitamin D_3. J Steroid Biochem 22:453–460

Loomis WF (1967) Skin-pigment regulation of vitamin-D biosynthesis in man. Science 157:501–506

Lorente F, Fontan G, Para P, Casas C, Garcia-Rodriguez MC, Ojeda JA (1976) Defective neutrophil motility in hypovitaminosis D rickets. Acta Paediatr Scand 65:695–699

Manolagas SC (1985) Role of 1,25 dihydroxyvitamin D_3 in the immune system. In: Norman AW, Schaefer K, Grigoleit H-G, Herrath vD (eds) Vitamin D. A chemical, biochemical and clinical update. Walter de Gruyter, Berlin, pp 199–208

Manolagas SC, Taylor CM, Anderson DC (1979) High specific binding of 1,25-dihydroxycholecalciferol in bone cytosol. J Endocrinol 80:35–40

Marche P, Cassier P, Mathieu H (1980) Intestinal calcium-binding protein. Cell Tissue Res 212:63–72

Marx SJ (1984) Resistance to vitamin D. In: Kumar R (ed) Vitamin D. Basic and clinical aspects. Martinus Nijhoff, Boston, pp 721–745

Menaker M (1985) Eyes-the second (and third) pineal glands? In: Evered D, Clark S (eds) Photoperiodism, melatonin and the pineal. Ciba Foundation Symposium 117. Pitman, London, pp 78–87

Merke J, Kreussler W, Bier B, Ritz E (1983) Demonstration and characterization of a testicular receptor for 1,25-dihydroxycholecalciferol in the rat. J Biochem 130:303–308

Narbaitz R, Stumpf WE, Sar M, DeLuca HF, Tanaka Y (1980) Autoradiographic demonstration of target cells for 1,25 dihydroxycholecalciferol in the chick embryo chorioallantoic membrane, duodenum and parathyroid glands. Gen Comp Endocrinol 42:283–289

Narbaitz R, Sar M, Stumpf WE, Huang S, DeLuca HF (1981) 1,25-Dihydroxyvitamin D_3 target cells in rat mammary gland. Horm Res 15:263–270

Narbaitz R, Stumpf WE, Sar M, DeLuca HF (1982) The distal nephron in the chick embryo as a target tissue for 1-alpha, 25-dihydroxycholecalciferol. Acta Anatomica 112:208–216

Narbaitz R, Stumpf WE, Sar M, Huang S, DeLuca HF (1983) 1-alpha,25-dihydroxyvitamin D_3 in bones from fetal rat. Calcif Tissue Int 35:177–182

Norman AW (1979) Vitamin D. The calcium homeostatic steroid hormone. Academic Press, New York

Norman AW, Frankel BJ, Heldt AM, Grodsky GM (1980) Vitamin D deficiency inhibits pancreatic secretion of insulin. Science 209:823–825

Norman AW, Roth J, Orci L (1982) The vitamin D endocrine system: steroid metabolism, hormone receptors, and biologial response (calcium binding protein). Endocrine Rev 3:331–366

Ohsugi Y, Nakano T, Komori T, Ueno K, Sugawara Y, Fukushima M, Yamamoto T, Nishii Y, Masuda T, Matsuno M (1985) Effects of 1a-hydroxyvitamin D_3 on the immune response. In: Norman AW, Schaefer K, Grigoleit H-G, Herrath vD (eds) Vitamin D. A chemical, biochemical and clinical update. Walter de Gruyter, Berlin, pp 209–218

Pike J, Gooze L, Haussler M (1980) Biochemical evidence for 1,25-

dihydroxyvitamin D receptor macromolecules in parathyroid, pancreatic, pituitary and placental tissues. Life Sci 26:407–414

Reiter RJ (1986) The first, the second, and the third pineal gland. Neuroendocrinol Lett 8:1–4

Rose SD, Holick MF (1985) Effects of 1,25-dihydroxyvitamin D_3 on the function of rat anterior pituitary cells in primary culture. In: Norman AW, Schaefer K, Grigoleit H-G, Herrath vD (eds) Vitamin D. A chemical, biochemical and clinical update. Walter de Gruyter, Berlin, pp 253–254

Roth J, Thorens B, Hunziger W, Norman AW, Orci L (1981) Vitamin D-dependent Calcium binding protein: immunochemical localization in chick kidney. Science 214:197–199

Sar M, Stumpf WE (1981) Combined autoradiography and immunohistochemistry for simultaneous localization of radioactively labeled steroid hormone and antibodies in the brain. J Histochem Cytochem 29, 1A:201–206

Sar M, Stumpf WE, DeLuca HF (1980) Thyrotropes in the pituitary are target cells for 1,25$(OH)_2$ vitamin D_3. Cell Tissue Res 209:161–166

Sar M, Miller WE, Stumpf WE (1981) Effects of 1,25$(OH)_2$ vitamin D_3 on thyrotropin secretion in vitamin D deficient male rats. Physiologist 24:70

Sonnenberg J, Luine VN, Krey CR, Christakos S (1986) 1,25-Dihydroxyvitamin D_3 treatment results in increased choline acetyltransferase activity in specific brain nuclei. Endocrinology 118:1433–1439

Spanos E, Colston KW, Evans IMS, Galante LS, MacAuley SJ, MacIntyre I (1976) Effects of prolactin on vitamin D metabolism. Mol Cell Endocrinol 5:163–167

Spencer EM, Tobiasson O (1977) The effect of hypophysectomy on 25-hydroxyvitamin D metabolism in the rat. In: Norman AW, Schaefer K, Coburn JW, DeLuca HF, Fraser D, Grigoleit H-G, Herrath vD (eds) Vitamin D: biochemical, chemical, and clinical aspects related to calcium metabolism. Walter de Gruyter, Berlin, p 197

Steenbock H, Herting DC (1955) Vitamin D and growth. J Nutr 57:449–468

Ströder J, Franzen CH (1975) Die unspezifische Entzündungsreaktion bei Vitamin-D-Mangel-Rachitis. Klin Pädiatr 187:461–467

Stumpf WE (1988a) The endocrinology of sunlight and darkness. Complementary roles of vitamin D and pineal hormones. Naturwissenschaften 75:247–251

Stumpf WE (1988b) The first eye; and the second third and fourth eyes. Relationships between skin, pineal and lateral eyes .Neuroendocrinol Lett (in press)

Stumpf WE, Downs TW (1987) Nuclear receptors for 1,25$(OH)_2$ vitamin D_3 in thymus reticular cells studied by autoradiography. Histochemistry 87:367–369

Stumpf WE, Jennes L (1984) The A-B-C (Allocortex-Brainstem-Core) circuitry of endocrine-autonomic integration and regulation. Relationships between estradiol sites of action and peptidergic-aminergic neural systems. Peptides 5(Suppl 1):221–226

Stumpf WE, O'Brien LP (1987a) Autoradiographic studies with ^3H 1,25 dihydroxyvitamin D_3 in thyroid and associated tissues in the neck region. Histochemistry 87:53–58

Stumpf WE, O'Brien LP (1987b) 1,25$(OH)_2$ Vitamin D_3 sites of action in the brain: an autoradiographic study. Histochemistry 87:393–406

Stumpf WE, Sar M (1976) Autoradiographic localization of estrogen, androgen, progestin and glucocorticosteroid in "target tissues" and "non-target tissues". In: Pasqualini J (ed) Receptors and mechanism of action of steroid hormones. Marcel Dekker, New York, pp 41–84

Stumpf WE, Sar M (1981) Anatomical relationships between estrogen target sites and peptidergic-aminergic neurons: Multiple activation of heterogeneous systems (MAHS). Exp Brain Res (Suppl) 3:18–28

Stumpf WE, Sar M, Joshi SG (1974) Estrogen target cells in the skin. Experientia 30:196–198

Stumpf WE, Sar M, Keefer DA, Martinez-Vargas MC (1976) The anatomical substrate of neuroendocrine regulation as defined by autoradiography with ^3H-estradiol, ^3H-testosterone, ^3H-dihydrotestosterone and ^3H-progesterone. In: Anand Kumar TC (ed) Neuroendocrine regulation of fertility. Karger, Basel, pp 46–56

Stumpf WE, Sar M, Reid FA, Tanaka Y, DeLuca HF (1979) Target cells for 1,25-dihydroxyvitamin D_3 in intestinal tract, stomach, kidney, skin, pituitary and parathyroid. Science 206:1188–1190

Stumpf WE, Sar M, Clark SA, Lieth E, DeLuca HF (1980a) Target neurons for 1,25$(OH)_2$ vitamin D_3 in brain and spinal cord. Neuroendocrinol Lett 2:297–301

Stumpf WE, Sar M, Narbaitz R, Reid FA, DeLuca HF, Tanaka Y (1980b) Cellular and subcellular localization of 1,25 $(OH)_2$ vitamin D_3 in rat kidney: comparison with that of parathyroid hormone and estradiol. Proc Natl Acad Sci USA 77:1149–1153

Stumpf WE, Sar M, DeLuca HF (1981a) Sites of action of 1,25$(OH)_2$ vitamin D_3 identified by thaw-mount autoradiography. In: Cohn CV, Talmage RV, Matthews JrVL (eds) Hormonal control of calcium metabolism. Excerpta Medica, Amsterdam, pp 222–229

Stumpf WE, Sar M, Reid FA, Huang S, Narbaitz R, DeLuca HF (1981b) Autoradiographic studies with ^3H 1,25$(OH)_2$ vitamin D_3 and ^3H 25(OH) vitamin D_3 in rat parathyroid glands. Cell Tissue Res 221:333–338

Stumpf WE, Sar M, Zuber TJ, Soini E, Tuohimaa P (1981c) Quantitative assessment of steroid hormone binding sites by thaw-mount autoradiography. J Histochem Cytochem 29(1A):201–206

Stumpf WE, Sar M, Clark SA, DeLuca HF (1982) Brain target sites for 1,25-dihydroxyvitamin D_3. Science 215:1404–1405

Stumpf WE, Gasc JM, Baulieu EE (1983a) Progestin receptors in pituitary and brain: combined autoradiography-immunohistochemistry with tritium-labeled ligand and receptor antibodies. Mikroskopie 40:359–363

Stumpf WE, Sar M, Narbaitz R, Huang S, DeLuca HF (1983b) Autoradiographic localization of 1,25-dihydroxyvitamin D_3 in rat rat placenta and yolk sac. Horm Res 18:215–220

Stumpf WE, Clark SA, Sar M, DeLuca HF (1984) Topographical and developmental studies on 1,25$(OH)_2$ vitamin D_3 target sites in skin. Cell Tissue Res 238:489–496

Stumpf WE, Clark SA, Kim YS, DeLuca HF (1985) Comparison of cellular and subcellular distribution of vitamin D metabolites [1,25$(OH)_2$ vitamin D_3, 24,25$(OH)_2$ vitamin D_3, 25(OH) vitamin D_3] in target tissues. In: Norman AW, Schaefer K, Grigoleit HG, Herrath vD (eds) Vitamin D: a biochemical and clinical update. Walter de Gruyter, Berlin, pp 119–120

Stumpf WE, Sar M, O'Brien LP (1987a) Vitamin D sites of action in the pituitary studied by combined autoradiography-immunohistochemistry. Histochemistry 88:11–16

Stumpf WE, Sar M, Chen K, Morin J, DeLuca HF (1987b) Sertoli cells in the testis and epithelium of the ductuli efferentes are targets for ^3H 1,25$(OH)_2$ vitamin D_3: an autoradiographic study. Cell Tissue Res 247:453–455

Stumpf WE, O'Brien LP, Clark SA, Reid FA (1988a) 1,25$(OH)_2$ Vitamin D_3 sites of action in spinal cord and sensory ganglion. Anat Embryol 177:307–310

Stumpf WE, Sar M, O'Brien LP, Morin J (1988b) Pyloric gastrin-producing cells and pyloric sphincter muscle cells are nuclear targets for ^3H 1,25$(OH)_2$ vitamin D_3. Studied by autoradiography and immunohistochemistry. Histochemistry (in press)

Tanaka Y, Castillo L, DeLuca HF (1976) Control of renal vitamin D hydroxylase in birds by sex hormones. Proc Natl Acad Sci USA 73:2701–2705

Tomita Y, Fukushima M, Tagami H (1986) Stimulation of melanogenesis by cholecalciferol in cultured human melanocytes: a possible mechanism underlying pigmentation after ultraviolet irradiation. Tohoku J Exp Med 149:451–452

Törnquist K, Lamberg-Allardt C (1985) Effects of 1,25$(OH)_2D_3$, Verapamil and EDTA-infusion on the thyroliberin-induced thyrotropin release. In: Norman AW, Schaefer K, Grigoleit HG, Herrath vD (eds) Vitamin D. A chemical, biochemical and clinical update. Walter de Gruyter, Berlin, pp 363–364

Walters MR (1981) An estrogen-stimulated 1,25-dihydroxyvitamin D_3 receptor in rat uterus. Biochem Biophys Res Commun 103:721–726

Walters MR, Cuneo DL, Jamison AP (1983) Possible significance of new target tissues for 1,25-dihydroxyvitamin D_3. J Steroid Biochem 19:913–920

Walters MR, Wicker DC, Riggle PC (1986) 1,25-Dihydroxyvitamin D_3 receptors identified in the rat heart. J Mol Cell Cardiol 18:67–72

Weber JC, Pons V, Kodicek E (1971) The localization of 1,25-dihydroxycholecalciferol in bone cell nuclei of rachitic chicks. Biochem J 125:147–153

Wecksler WR, Henry HL, Norman AW (1977) Studies on the mode of action of calciferol. Subcellular localization of 1,25-dihydroxyvitamin D_3 in chicken parathyroid glands. Arch Biochem Biophys 183:168–175

Wehr TA, Jacobsen FM, Sack DA, Arendt J, Tamarkin L, Rosenthal NE (1986) Phototherapy of seasonal affective disorder. Arch Gen Psychiatry 43:870–875

Wills L, Sanderson P, Paterson D (1926) Calcium absorption in relation to gastric acidity (A study of rickets). Arch Dis Child 1:245–254

Yetgin S, Ozsoylu S (1982) Myeloid metaplasia in vitamin D deficiency rickets. Scand J Haematol 28:180–185