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Vitamin D and the digestive system

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SUMMARY

Target tissues of in vivo receptor binding and deposition of $1,25(OH)_2$ vitamin D_3 and its oxygen analog OCT are reviewed in rats, mice, hamsters and zebra finch, identified with high-resolution microscopic autoradiography. Throughout the digestive system numerous sites with nuclear receptor binding of ^{3}H-$1,25(OH)_2$ vitamin D_3 and ^{3}H-OCT exist: in the oral region, epithelial cells of the oral cavity, tongue and gingiva, teeth odontoblast and ameloblast precursor pulp and stratum intermedium cells; in the parotid, submandibular and sublingual salivary glands, epithelial cells of striated ducts and granular convoluted tubules, intercalated ducts and acinar cells, as well as myoepithelial cells; in the stomach, neck mucous cells of gastric glands, endocrine cells of the antrum, and muscle cells of the pyloric sphincter; in the small and large intestine, absorptive and crypt epithelial cells; in the pancreas, predominantly islet B-cells. Perisinusoidal stellate (Ito) cells in the liver concentrate and retain variable amounts of radiolabeled compound in regions of their cytoplasm after administration of ^{3}H-$1,25(OH)_2$ vitamin D_3 and ^{3}H-25(OH) vitamin D_3, probably sites of specific storage, similar to vitamin A. Submucosa in stomach and intestine also retain variable amounts of radiolabel, however unspecific with all compounds studied. In pilot studies with ^{3}H-$25(OH)_2$ vitamin D_3 and ^{3}H-$24,25(OH)_2$ vitamin D_3, no nuclear concentration was detectable. The reviewed data for vitamin D and its oxygen analogue OCT indicate genomic effects on multiple target tissues of the digestive system that involve cell proliferation and differentiation, endo- and exocrine secretion, digestion and absorption for maintaining optimal functions, with potentials for health prophylaxis and therapies.

INTRODUCTION

Vitamin D target tissues in the digestive system are extensive. Their presence and distribution indicate an important role of vitamin D for digestion. Recognition of many of the targets has evaded biochemical homogenates (1) because of embeddedness in non-targets and low signals. This review is based on results from histochemical studies with receptor microscopic autoradiography. Related experiments were conducted between 1979 and 1995 (1). In these studies the primary focus was not calcium regulation, but unbiased identification and characterization of vitamin D target sites. Therefore, through negative *peer* reviews funding of research proposals became difficult. Research was disrupted after new data challenged the concept of calcium regulation as the primary function of vitamin D (1-3). Discoveries of previously unexpected targets - that include brain, spinal cord, pituitary, skin, heart atrium, prostate, epididymis, ductus deferens, adrenal medulla, thymus reticular cells, teeth, esophagus, stomach, pyloric muscle, and others, slowly led to an appreciation of previously unexpected targets and related actions.

Considering our earlier reported *over 50 target tissues* (1) with their functions mostly unrelated to systemic calcium metabolism, we proposed a holistic concept for the main biological role of vitamin D:

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seasonal adaptation and regulation of vital functions (4,5). This view is not readily accepted, but has rather been ignored or resisted, still competing with the entrenched narrow primacy of *systemic calcium regulation*. Effects on calcium metabolism are a part of vitamin D's important actions on growth and maintenance of the musculo-skeletal system. Calcium metabolism is one component only of the multiple functions of the hormone of sunshine.

METHODS

The histochemical data are derived from experiments with male and female animals of different age: rats, mice, hamster, and zebra finch. Tritium-labeled $1,25(OH)_2$ vitamin D_3 (vitamin D, soltriol), $1,25(OH)_2$-22-oxa-vitamin D_3 (OCT), 25(OH) vitamin D_3 or $24,25(OH)_2$ vitamin D_3 with a specific activity of 160Ci/mM (a few animals with 100 or 90 Ci/mM), dissolved in alcohol-isotonic saline, was administered iv, subcutaneously, or intraperitonealy at a near physiological dose for young adults between 0.2 to 0.4 µg per 100g bw. Most animals were fed a normal chow, some a vitamin D-deficient diet. Target tissues recognized from animals fed a normal chow did not seem to differ from that of animals fed a vitamin D-deficient diet. However, no quantification through silver grain counting was made. For quantification, type and length of diet with a statistical number of animals would need to be considered and would require specifically designed studies. The total number of rodents studied in the author's laboratory with tritiated vitamin D compounds over a twenty-year period exceeds one hundert, including at least two or three animals for each specific condition. The main focus of the reviewed studies was aimed at the topographical identification and characterization of target cell populations in different age groups and species to provide an overview of vitamin D sites of action and to establish a basis for follow-up experiments.

All animals were kept under a regular lighting schedule and sacrificed by decapitation between 1 to 3 hours after the injection, when specifically bound hormone is retained at the receptor. Tissues were processed according to the receptor microscopic autoradiography procedure, dissected, freeze-mounted on tissue holders, frozen sections cut at 4 µm and thaw-mounted on nuclear emulsion-coated slides, exposed in desiccator boxes at -20 ºC for different lengths of time, then photographically processed, stained, and cover-slipped for microscopic examination.

Autoradiograms were evaluated at different times of exposure, after one to three months, some overexposed up to one year or longer. Long exposure times were used for easy recognition of radioactive labeling at low magnification, also for minimizing oversight of low receptor binding sites. Controls against artifacts included O-day exposure, competition with excess amount of unlabeled compound, and comparisons of results obtained with different compounds.

The pattern of ^{3}H-thymidin labeling of the cell cycle S-phase was compared with that of ^{3}H-vitamin D nuclear labeling of duodenal crypt epithelium: Two rats were injected intraperitonealy with ^{3}H-thymidin, specific activity 60 Ci/mM, 1 µC/g bw, and sacrificed one hour afterwards. Autoradiograms of duodenum were prepared as above, developed and processed after 3-week exposure time and evaluated.

Receptor microscopic autoradiography was developed in our laboratories and has been described in detail (6).

RESULTS

General

Figures 1-6 provide samples auf autoradiograms with evidence for concentration of developed silver grains over nuclei of specific cell populations (labeled cells), reviewed schematically in Fig. 7. No accumulation of silver grains over or near plasma membranes has been observed. Comparisons of nuclear concentration of radioactivity (nuclear labeling) in target tissues of the same animal, processed under identical conditions, reveal quantitative differences (7) attributable to properties of individual target cell populations. Target tissues with nuclear concentration of radioactivity appear to be identical for ^{3}H-$(OH)_2$ vitamin D_3 and ^{3}H-OCT. Apparent quantitative differences of concentration and retention of radiolabeled compound between ^{3}H-$(OH)_2$ vitamin D_3 and ^{3}H-OCT are possibly due to different blood and target pharmacokinetics, different target affinities and metabolism. The need for further quantitative studies is indicated.

In all animals, variable concentration and retention of radiolabeled compound is visible in lumina of blood vessels and in certain extracellular spaces, especially in the submucosa (Figs. 2C,D,E,F and 5D,E).

After injection of radiolabeled $24,25(OH)_2$ vitamin D_3 (Fig. 5F) or 25(OH) vitamin D_3 (Fig. 5E) no nuclear concentration of radioactivity has been noted, while diffuse radioactivity was present in extracellular spaces.

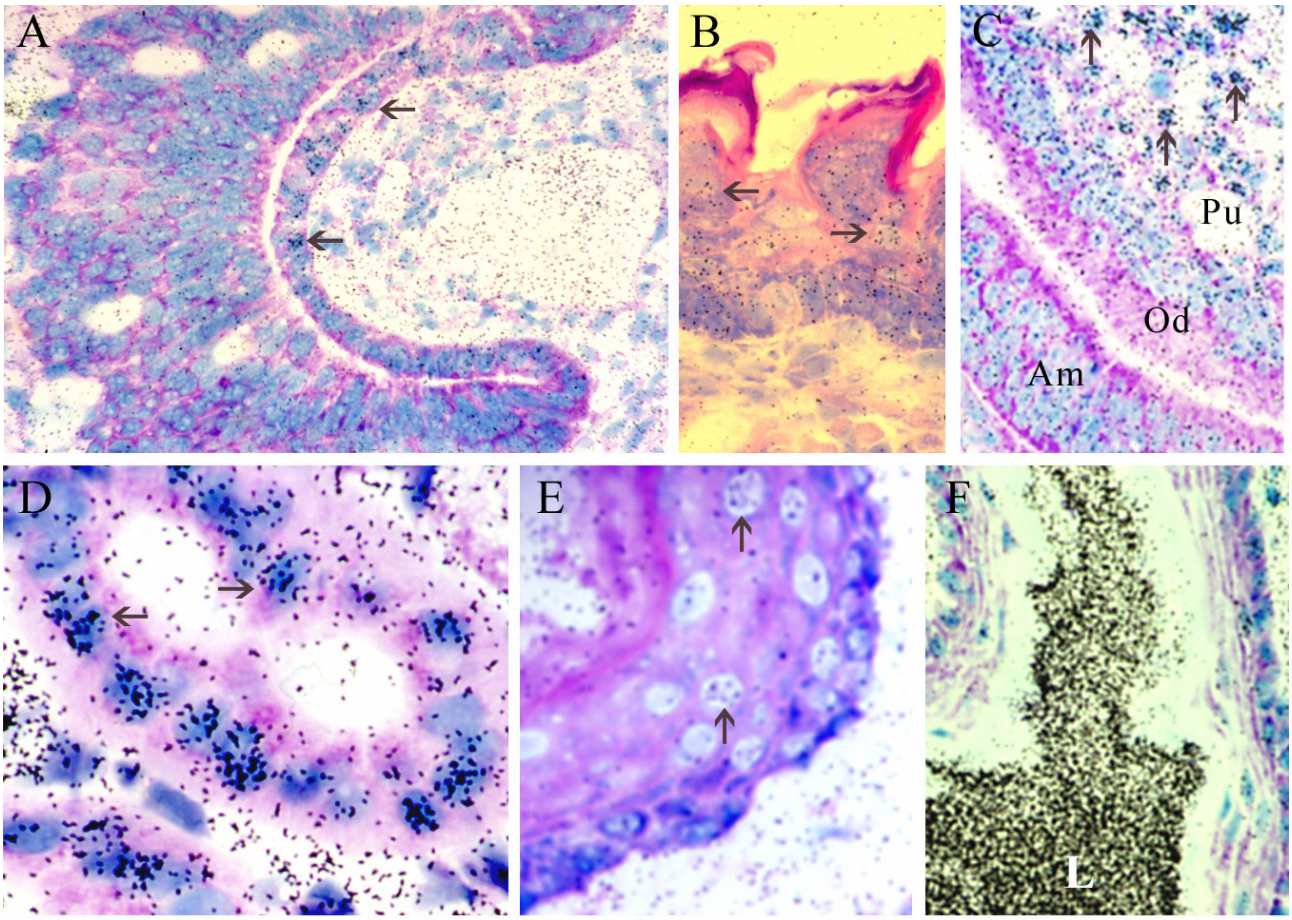

Figure 1. Autoradiograms after injection of ^{3}H-1,25(OH)$_2$ vitamin D$_3$ (Figs. A,C-F) or ^{3}H-OCT (Fig. B) to adult rat showing nuclear concentration (arrows) of radiolabeled compound in epithelial cells associated with the vomeronasal organ (Fig. A), in epithelium of the tongue and filiform papillae (Fig. B), incisor tooth of neonatal rat (Fig. C) with strongly labeled pulp cells (Pu), unlabeled or weakly labeled odontoblasts (Od) and ameloblasts (Am) (10). Labeled striated duct epithelium of submandibular gland (Fig. D) (8). Esophagus epithelium with nuclear labeling (Fig. E). Radiolabeled compound in lumen (L) of esophagus with apparent epithelial barrier (Fig. F).

In competition experiments with excess unlabeled 1,25(OH)$_2$ vitamin D$_3$ (Fig. 5D), nuclear concentration of radioactivity after injection of ^{3}H-1,25(OH)$_2$ vitamin D$_3$ or ^{3}H-OCT was abolished or diminished. Control autoradiograms with long exposure times prepared from tissues of animals without radiolabeled compound and with O-day exposure time from tissues of animals with radiolabeled compound did not show concentrations of silver grains.

Oral region (Fig. 1)

After administration of ^{3}H-1,25(OH)$_2$ vitamin D$_3$ or ^{3}H-OCT, nuclear concentration exists in the epithelium of oral and nasal cavities, tongue and esophagus; in cell populations of major salivary glands, especially epithelium of striated ducts and granular convoluted tubules, as well as in myoepithelial cells (8). Incisor teeth and molars of neonatal rats and adult human molars (9) contain strongly labeled cells in the dental pulp, especially those located closest to odontoblasts. In the layers of odontoblasts und ameloblasts of rats only scattered and comparatively weakly labeled cells are seen.

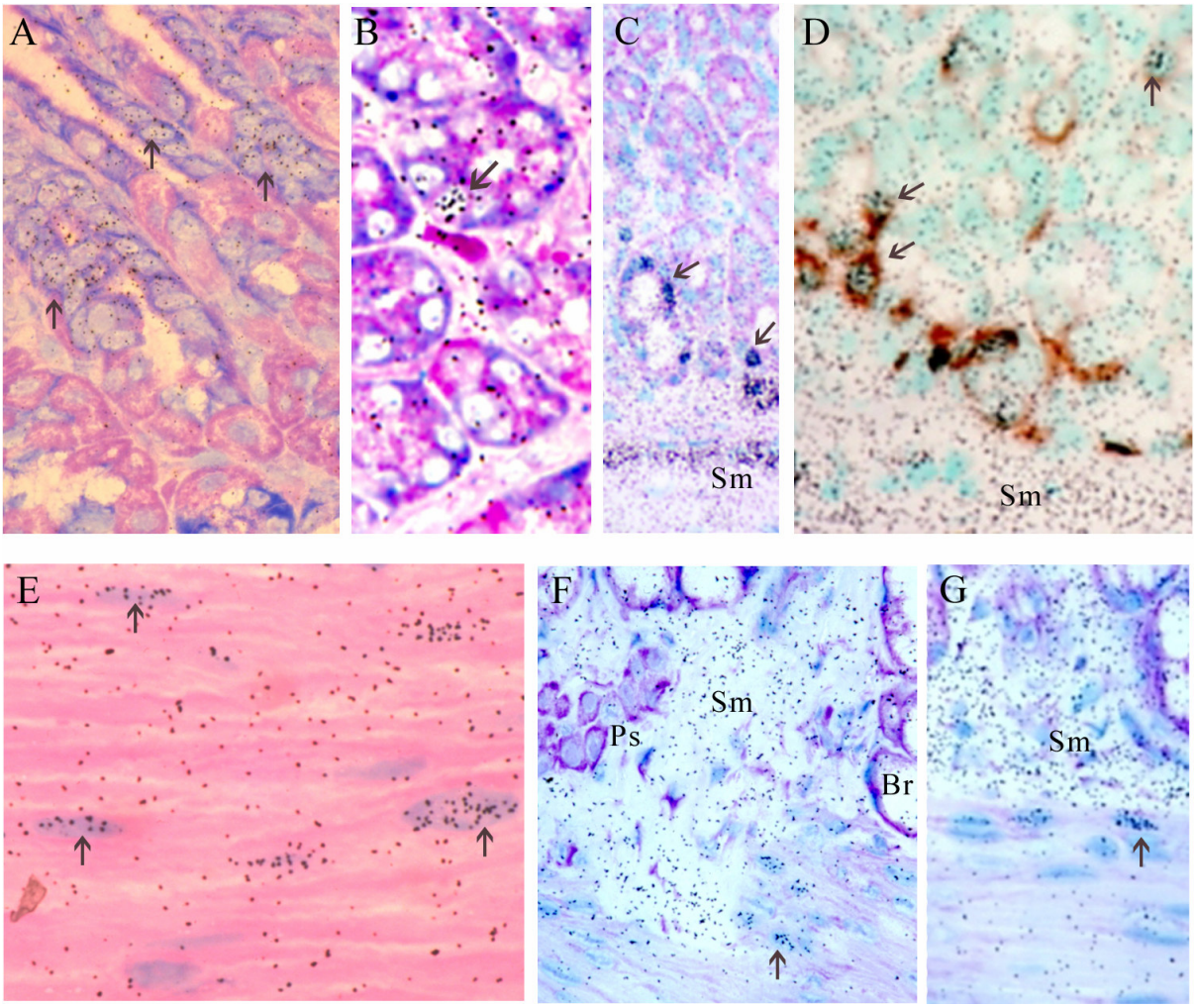

Figure 2. Autoradiograms of stomach after injection of ^{3}H-1,25(OH)$_2$ vitamin D$_3$ showing nuclear concentration (arrows) of radiolabel in rat gastric gland (Fig. A), entero-endocrine cells (Figs. B-D), and pyloric muscle (Figs. E-G). Nuclear labeling of mucous neck cells, but not of parietal cells (pink cytoplasm), chief cells (dark blue cytoplasm), and surface epithelial cells at top left (Fig. A). In the antrum, unlabeled or weakly labeled gland epithelium with singular labeled endocrine cell oriented toward a capillary (Fig. B, high magnification), several labeled endocrine cells near submucosa (Sm, Figs. C and D, low magnification), immunostained with antibodies to gastrin (Fig. D). Labeled pyloric muscle (Fig. E), interdigitating with unlabeled smooth muscle cells of the duodenum below Brunner's gland (Figs. F and G). Cells of Brunner's gland (Br, Figs. F and G) and neurons of the plexus submucosus Meissner (Ps, Fig. F) are also unlabeled. In submucosa (Sm) variable extracellular accumulation radioactivity (Figs. C, D, F, G). Figures A, B, D, E (from 11,12).

Stomach (Fig. 2)

In the stomach fundus, cells in the isthmus region of gastric glands, probably mucous neck cells, are selectively and weakly labeled, while no nuclear labeling has been observed in surface epithelium, parietals cell and chief cells (Fig. 2a). In the pyloric antrum, strong nuclear radioactivity exists in scattered cells located in unlabeled or weakly labeled antral gland epithelium (Figs. 2a-c). Antibodies to gastrin are co-localized in the cytoplasm of many, but not all, of the singular radiolabeled cells (Fig. 2c). In the pyloric sphincter muscle, nuclear concentration exists in many but not all muscle cells. Pyloric muscle cells with nuclear labeling can be followed to interdigitate with the unlabeled muscularis of the duodenum below the Brunner gland (Figs. 2e-g).

Small intestine (Figs. 3 - 5)

The transition between the unlabeled surface epithelium of the stomach and the labeled epithelium of the duodenum is abrupt (Fig. 3A), as is the transition from the unlabeled epithelium of the Brunner gland

to the labeled crypt epithelium of the duodenum (Fig. 3B). Throughout the duodenum, ileum and jejunum, epithelial cells show strong nuclear concentration of radiolabeled 1,25(OH)$_2$ vitamin D$_3$, while goblet cells are unlabeled. Migratory lymphocytes in the epithelium also appear unlabeled, as are most of the connective tissue cells in the mucosa. In the core of villi, occasional labeled cells are seen; their identity is unknown. In the same experiments, smooth muscle cells of the muscularis interna and externa and nerve cells of the plexus submucosus of Meissner and plexus muscularis of Auerbach are unlabeled. In the extratracellular space of the submucosa frequently and variably radiolabeld compound is accumulated.

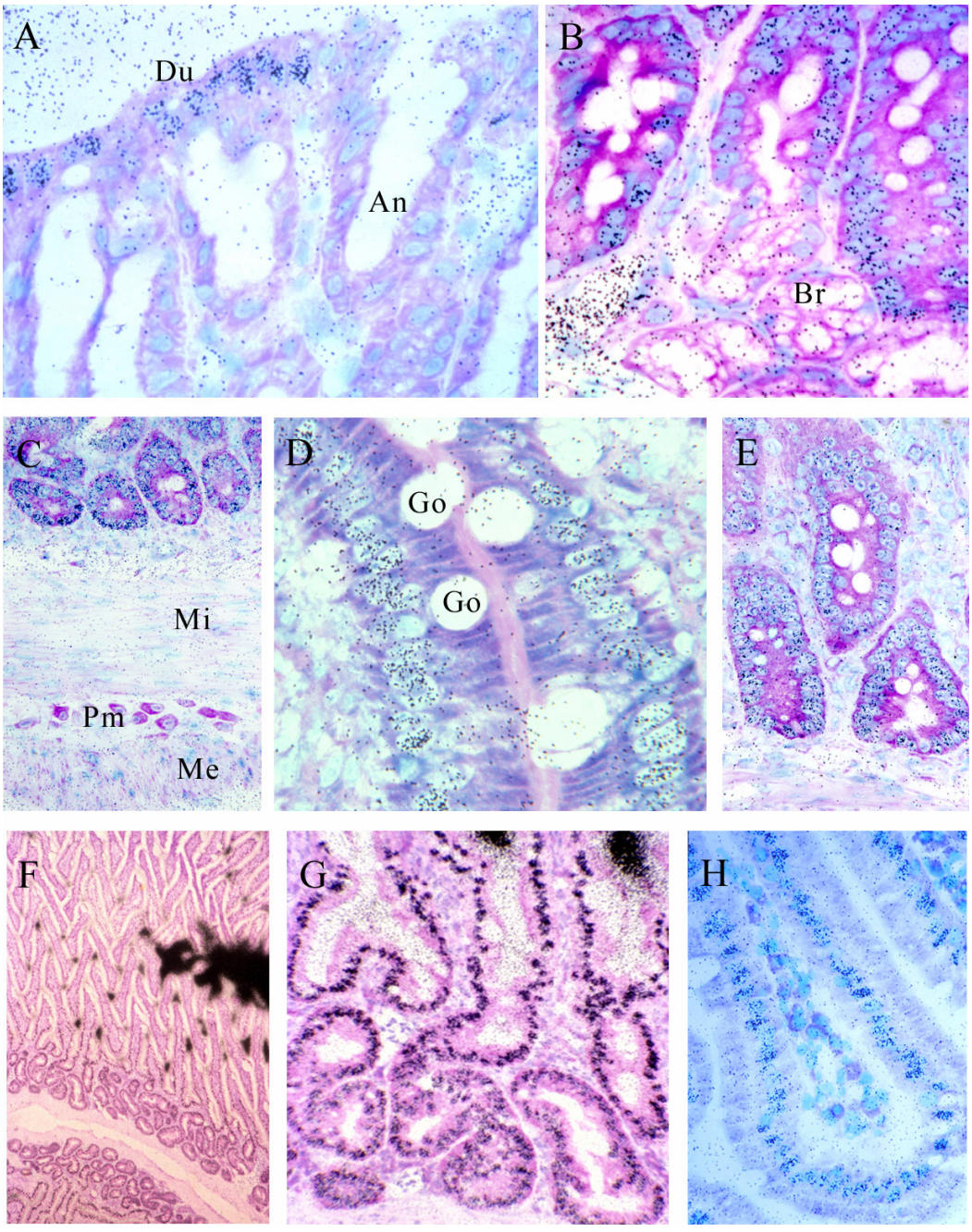

Figure 3 Autoradiograms of small intestine after injection of ^{3}H-1,25(OH)$_2$ vitamin D$_3$ showing nuclear concentration in epithelial cells of rat in the transition from stomach antrum (An) to duodenum (Du) with abrupt change from unlabeled (An antrum) to labeled

(Du duodenum) epithelial cells (Fig. A). Similarly, in Fig. B, unlabeled cells of Brunner gland (Br) below connect to labeled cells of duodenal crypts above. In Fig. C, muscle cells of muscularis interna (Mi) and externa (Me), neurons of plexus myentericus of Auerbach (Pm), and connective tissue cells of the mucosa and submucosa do not display nuclear labeling, in contrast to crypt cells above with strong nuclear labeling. High magnification of segments of rat duodenal villi (Fig. D, high magnification) with labeled absorptive epithelial and unlabeled goblet cells (Go). Epithelial nuclear labeling in crypt cells of rat ileum (Fig.E). Duodenum of Zebra finch (from 13) showing high amounts of radiolabeled compound in the lumen (Fig. F, low magnification) and strong nuclear labeling in absorptive epithelium, highest in villi above and slightly lower in crypt epithelium below (Fig. G, high magnification). Hamster duodenum villi with strongly labeled epithelium and some weakly labeled cells in the core of villus (Fig. H, high magnification).

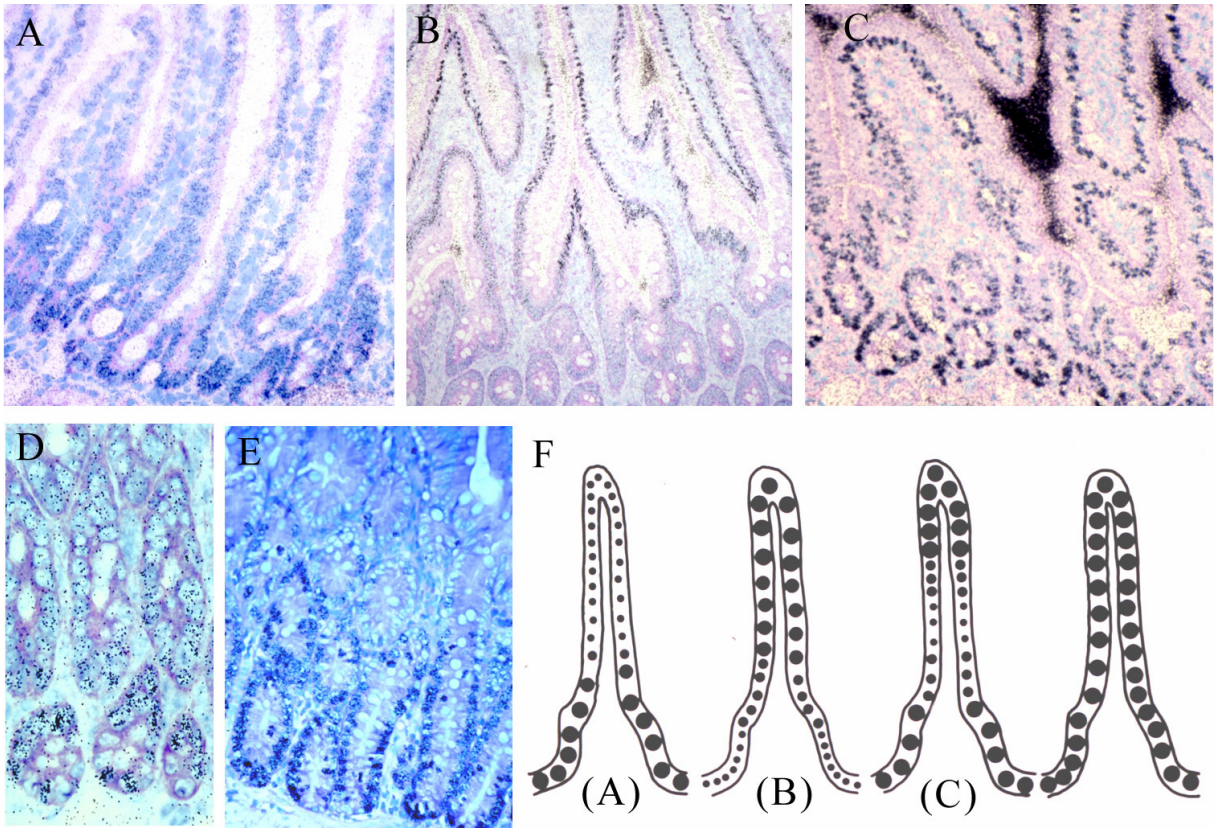

Figure 4. Autoradiograms after injection of ^{3}H-1,25(OH)$_2$ vitamin D$_3$ reveal different patterns of nuclear concentration in epithelium of intestinal crypts (at lower end of pictures) and villi. In some animals nuclear labeling is predominant in duodenum crypts as in adult rat (Fig. A) and adult mouse (Fig. D) or in villi as in 15-day old rat (Fig. B), or similar in crypts and villi but diminished between as in jejunum of adult rat (Fig. C), or uniform along crypts and villi (Fig F, schematic at right). Fig. F reviews patterns of vitamin D nuclear epithelial labeling of crypts and villi in small intestine (size of dots reflects different degrees of labeling). The pattern of labeling of crypt cells with ^{3}H-thymidin (Fig. E, one hour after injection) corresponds to that of vitamin D labeling in Figs A and D. High levels of radioactivity in intestinal lumen contrast with low or absent radioactivity in cytoplasm of epithelium of villi (Fig. 4C; also Figs. 3F and 3G), suggesting a penetration barrier to excreted metabolites.

In the intestinal epithelium, the degree of nuclear labeling can vary between the crypts of Lieberkuehn and along individual villi, in the same animal and among different animals. While in some animals the epithelial labeling of villi and crypts is fairly uniform, in others, labeling of epithelium in crypts is strong and toward the tip of villi gradually diminished (Figs. 4A and 4D). In some animals this is reversed, with strong nuclear labeling at the tip of individual villi and gradually diminished toward crypts (Fig. 4B). In other animals, cryptal and villous epithelial labeling is both strong but weak between at the base of villi (Fig. 4C). In young animals, strong labeling of crypt epithelium appears to prevail. This pattern of strong vitamin D crypt labeling corresponds to that of S-phase labeling with ^{3}H-thymidine (Fig. 4E). In both cases not all crypt cells are labeled (Fig. 4D).

The intestinal nuclear binding of vitamin D appears similar in different vertebrate species as demonstrated in the Zebra finch (Figs. 3F and 3G).

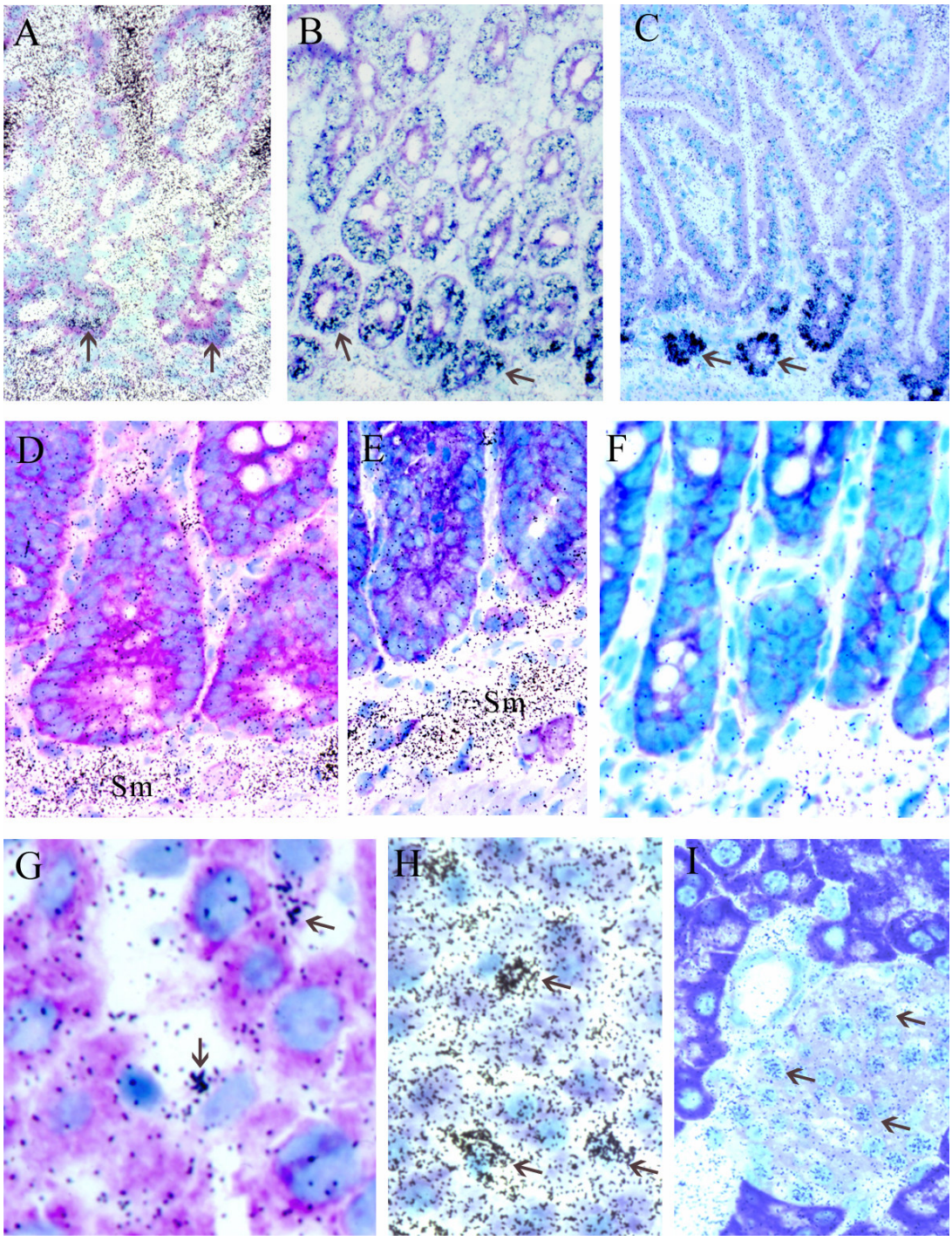

Figure 5. Autoradiograms after injection of ^{3}H-1,25(OH)$_2$ vitamin D$_3$ to neonatal rats, 9-hour old (Fig. A), 2-day old (Fig. B), and 10-day old (Fig. C), showing nuclear concentration of radiolabeled hormone in duodenal epithelium, strong in crypts (bottom) and weak in villi. 9-hour after birth nuclear labeling is already present but restricted to basal crypt cells (arrows); radioactivity in the intestinal lumen. In the 10-day old animal, a long exposure time renders crypt cells black through overexposure, while villous epithelium is weakly labeled or unlabeled. - In competition experiments with excess of unlabeled 1,25(OH)$_2$ vitamin D$_3$, nuclear concentration of

radiolabeled hormone is abolished (Fig. D, duodenum crypts) or diminished. One hour after injection of the precursor ^{3}H-(OH) vitamin D_3, no nuclear concentration can be seen in duodenal epithelium (Fig. E). Two hours after subcutaneous injection of ^{3}H-24,25$(OH)_2$ vitamin D_3, no nuclear concentration of radiolabeled compound is visible (Fig. F). Accumulation of radiolabeled compound exists in submucosa (Figs. A, D, E). - In the liver, after injection of ^{3}H-1,25$(OH)_2$ vitamin D_3 accumulation and retention of radioactivity is seen in cytoplasmic compartments of certain perisinusoidal cells, probably fat-storage (Ito) cells (Fig. G, arrows) that differ in position, size and staining properties from nearby hepatocytes. Similar accumulation and retention of radiolabeled compound in perisinusoidal cells exist after injection of ^{3}H-(OH) vitamin D_3 (Fig. H, long exposure time). - In the pancreas (Fig. I), after injection of ^{3}H-1,25$(OH)_2$ vitamin D_3, nuclear concentration is distinct in islet B-cells (14), while under the same experimental conditions cells of the exocrine pancreas generally appear unlabeled. A low presence of nuclear radioactivity can be noted occasionally in acinar cells and in epithelial cells of intercalated ducts (center above islet).

In competition controls, when excess unlabeled hormone is co-administered with radiolabeled hormone, nuclear uptake is suppressed. In pilot studies, after administration of ^{3}H-25(OH) vitamin D_3 (Fig. 5E) or ^{3}H-24,25$(OH)_2$ vitamin D_3, (Fig. 5F) no nuclear labeling is observed. Similarly, in O-day exposure autoradiograms from tissues of experimental animals that received radiolabeled compound, no nuclear accumulation of radioactivity is seen.

Liver (Figs. 5G,H) and Pancreas (Fig. 5I)

In the *liver* high levels of diffuse radioactivity with variations, depending on dose and time interveral between administration and sacrifice, can obscure recognition of possible selective cellular or subcellular specific concentration. Under the conditions of the experiments, no clear nuclear concentration in hepatocytes is observed, while in lumina of bile ducts, blood vessels (esp. veins) and sinusoids, radioactivity is accumulated. In some animals, in various regions of the liver, cells with pale cytoplasm, different in appearance from the larger hepatocytes, display strong cytoplasmic concentration of radioactivity (Fig. 5G,H). These cells are located at sinusoids and appear to correspond to perisinusoidal stellate cells, known also as fat-storing Ito cells. This select but variable and sometimes strong cytoplasmic concentration of radiolabeled compound is conspicuous not only after administration of ^{3}H-1,25$(OH)_2$ vitamin D_3 (Fig. 5G), but also after ^{3}H-25(OH) vitamin D_3 (Fig. 5H).

In the *pancreas*, distinct nuclear labeling exists in cells of islets, predominantly located in their center. Epithelial cells of intercalated ducts and acinar cells of the exocrine pancreas appear weakly labeled or unlabeled (Fig. 5I). Epithelial cells of excretory ducts and connective tissue cells are unlabeled.

Colon (Fig. 6)

Similar to the small intestine, strong nuclear uptake and retention of labeled vitamin D exists in epithelial cells, sometimes with regional variations of intensity between cells located at the bottom and luminal end of crypts. Connective tissue cells in general and muscle cells are unlabeled. In lymph nodules, lymphocytes are without nuclear labeling, but dispersed single labeled cells can occasionally be seen.

DISCUSSION

Vitamin D target sites of nuclear receptor binding and action exist at all levels of the digestive system in select cell populations. Nuclear uptake and retention of hormone varies in cells of the same organ and among different organs, suggesting a hierarchy of receptor expression with differential target responses. Since nuclear concentrations of hormone vary greatly, further quantitative studies are needed. In general, nuclear uptake and retention in target tissues depend on hormone blood levels, that is, blood *and* target bioavailability. Target bioavailability and target pharmacokinetics are relevant for action. Among the multiple factors that influence receptor expression and hormone binding are genetic aspects, age and endocrine status, time, dose, route of delivery, and conditions of the experimental procedure. The reviewed histochemical data reveal and emphasize the importance of information about in vivo targets. Studies of low-receptor targets and low-dose effects are a special challenge. Detailed information and at once integrative overviews provided by the histochemical approach are highly relevant for drug research and development (5,15).

Many of the data available on vitamin D targets are based on discoveries with tritium-labeled hormone of high specific activity, thin frozen sections without fixation-embedding, and nuclear emulsion for signal detection. The numerous target tissues thus discovered and characterized point to significance beyond calcium homeostasis. Systemic calcium regulation, related especially to skeletal growth and repair, is only part of vitamin D's many actions as '*seasonal regulator of vital functions'* (5).

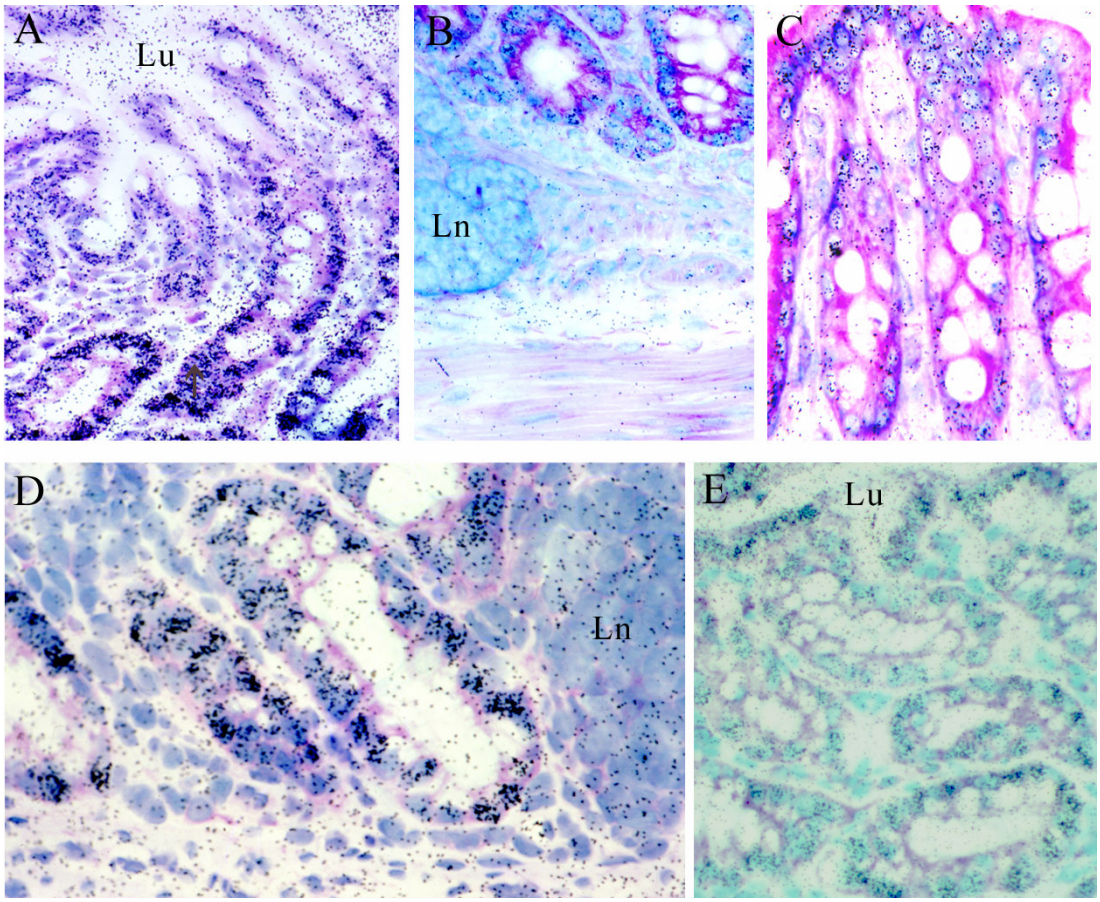

Figure 6. Autoradiograms of rat colon after injection of ^{3}H-1,25(OH)$_2$ vitamin D$_3$ showing nuclear concentration of radiolabeled hormone in epithelial cells (Figs. A-E) with variations along the length of crypts (Figs A,E). Goblet cell nuclei are unlabeled (Figs. C, D). Cells in lymph nodules (Ln, Figs. B,D) are generally unlabeled, except for occasional single labeled cells. Cells of the muscularis (Fig. B) and connective tissue are unlabeled. Lu lumen.

For effects on calcium absorption in the digestive system, ample experimental and clinical data exist. Other effects barely have been considered. Concept bias is one reason, another is methodology. Tissue homogenization has impaired recognition of target cell populations embedded in non-target tissue. Biochemical procedures need to be complemented and sometimes guided by appropriate histochemical approaches with cellular resolution and preserved tissue structure. Immunohistochemistry, while useful for identifying receptor proteins, does not inform about drug distribution, that is, tissue localization of non-covalently bound low molecular weight compounds. Therefore, immunohistochemistry cannot replace autoradiography. Receptor microautoradiography and immunocytochemistry provide different and complimentary information, optimal for target characterization when used together (1,6).

Oral region

Epithelial tissues related to the *oral cavity*, *nasal cavity*, *gingiva*, *tongue*, and *esophagus* display a pattern of nuclear labeling similar to that observed in the epidermis, with differences related to different cell layers (11). In these tissues, like in the epidermis, actions of vitamin D on cell renewal and differentiation seem to prevail.

In *teeth*, the strongest nuclear labeling exists in odontoblast precursor cells in the pulp of incisors and molars, less in mature osteoblasts (9,10). In the same studies, in the stratum intermedium, labeled ameloblast precursor cells were noted, while there was little or no concentration of labeled hormone in ameloblasts. Experiments with ^{14}C-vitamin D$_3$ and a different autoradiographic method failed to find evidence for vitamin D uptake in developing teeth (16). Supportive evidence for vitamin D sites of action in teeth was provided from immunohistochemical stud-

ies with antibodies to vitamin D receptor protein that was localized in precursor cells, differentiated ameloblasts, and odontoblasts, together with calbindin-D 28K antibodies in odontoblasts (17). Incubation of mouse embryonic molars with 1,25$(OH)_2$ vitamin D_3 resulted in an increase of the mitotic index of inner dental epithelium, indicating effects on cell proliferation (18). Similarly, in vitamin D-deficient rats, in postnatal molar tooth germ development, vitamin D treatment enhanced cytodifferentiation of pulp cells as well as enamel and dentin mineralization (19). The presence of calcium binding protein has been interpreted as supportive evidence for the presence of vitamin D receptors and action (20). While the latter conclusion may apply, "definitive evidence that calbindins are not required for active calcium transport" contradicts the dogma by showing that teeth and bones were produced normally in null mutant mice lacking calbindin (28kDa) (21). These latter data correlate with our observation that calcium binding protein cannot be used as a general guide for the existence of vitamin D targets since in several target tissues, e.g. brain and adrenal, there is no correspondence between vitamin D receptor binding and calcium binding protein (1). Further comparative studies are required for clarification.

The *vomeronasal organ*, albeit marginal to the topic of this review, contains vitamin D target cells. It is still present in humans and considered to "regulate reproductive, defensive and ingestive behavior" (22). There is an apparent nervous connection via the olfactory bulb to the central amygdala that has been shown to contain vitamin D target neurons (23), probably components of neural circuits that influence gastrointestinal functions.

In *salivary glands*, the strong nuclear concentration of vitamin D in epithelial cells of striated ducts and granular convoluted tubules, as well as in myoepithelial cells, indicates involvement of vitamin D in the regulation of secretion, probably exocrine and endocrine. Also, effects on cell proliferation and differentiation need to be considered The autoradiographic data provide important new information on salivary glands (8), extending results from experiments with homogenized parotid, in which "all of the metabolites of the cholecalciferol were present which normally occur in known target tissues of vitamin D" (24). In another study, vitamin D receptors were assayed in isolated rat parotid gland acinar cells, but were reported absent in the submandibular gland (25), while in both glands salivary flow was stimulated after treatment with 1,25$(OH)_2$ vitamin D_3 in vitamin D-deficient rats (26).

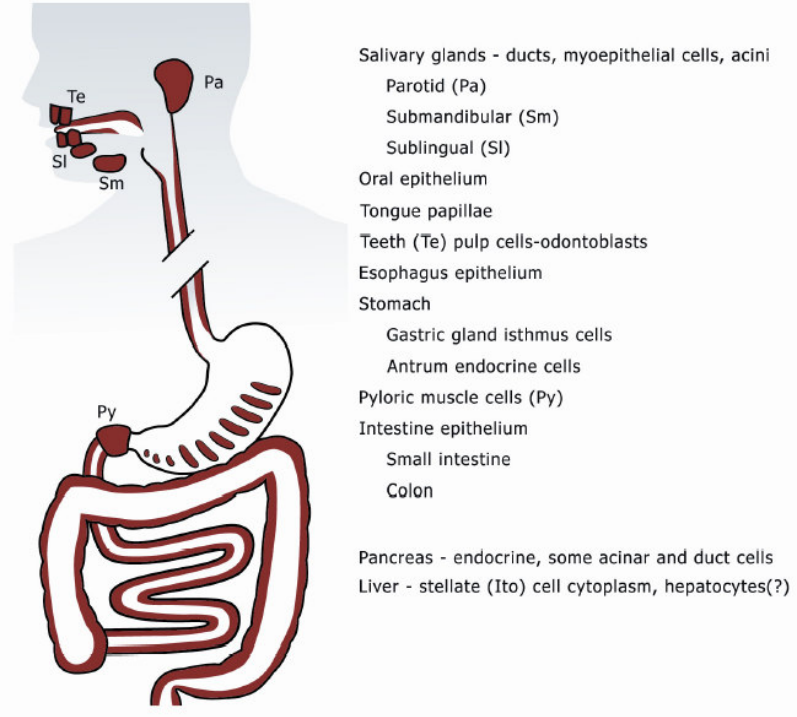

Figure 7. Vitamin D target tissues (red) in the digestive system (schematic overview).

Stomach

Vitamin D applied after the feeding of rachitogenic diet, increased the volume and acidity of gastric secretion (27). These early observations have not been followed. Radioassays provided no evidence for the presence of vitamin D sites of action in the stomach. This contrasts with our autoradiographic demonstration of several distinct target cell populations in gastric glands, antrum, and pylorus (2,12).

The selective nuclear concentration of radiolabeled vitamin D in cells of the isthmus region of gastric glands – albeit weaker and with different pharmacokinetics than that in duodenal epithelium, argues for selective effects on cell renewal and differentiation.

Mucous neck cells have been identified as providing the generative pool for surface epithelium, parietal cells and chief cells, even entero-endocrine cells, in autoradiographic studies with ^{3}H-thymidin (29,30, 31). Mucous neck cells received attention in earlier autoradiographic studies with macromolecular precursor molecules, and their special behavior was recognized. There was no or little uptake of ^{35}S-labeled amino acids in contrast to chief cells and surface epithelium (32). Evidence of the regenerative power of mucous neck cells and their involvement in wound healing has been demonstrated (33-35). Therefore, vitamin D appears to have high preventive and therapeutic potentials for injuries of gastric mucosa by stimulating cell proliferation and differentiation, similar to the promotion of wound healing in the epidermis (36) demonstrated after the discovery of vitamin target cells in the generating and differentiating stratum Malpighi of the skin (2,28).

The nuclear concentration in *gastrin cells* and other endocrine cells in the antrum (2,11,12) indicates vitamin D regulation of gastrin endocrine and paracrine secretion with secondary effects, e.g. on parietal cell HCL and chief cell pepsinogen secretion. In isolated perfused rat stomach of vitamin D-deficient rats, gastrin and gastric somatostatin secretion is impaired (37). Antrectomy in rats is followed by hypogatrinemia and a decrease in bone density and bone dry weight, a condition designated *postantrectomy osteopenia* (38). Extensive gastric surgery is known to cause bone disorder, as measured in experiments with gastrectomy or fundectomy that resulted in lowering of blood calcium, indicating that the stomach plays an important role in calcium homeostasis (39).

In *pyloric muscle* fibers, nuclear concentration of vitamin D exists adjacent to negative stomach and intestinal smooth muscle cells. The doctrine of 'the calcium homeostatic hormone' suggests direct involvement of vitamin D in any calcium-handling tissue. Unexpectedly, in skeletal and smooth muscle in rodents, no nuclear concentration and retention of vitamin D has been found (1). The action of vitamin D on skeletal muscle, however, can be attributed primarily to neurotrophic effects through strong nuclear receptor occupation in spinal and cranial motor neurons (40). Like pyloric muscle, atrial muscle is a vitamin D genomic target, the latter related to the secretion of the heart hormone ANF (41). In atrial muscle cells, it correlates with a large Golgi apparatus and secretory vesicles. For pyloric muscle, no specific ultrastructural features have been reported. One wonders what specific pyloric functions vitamin D has and whether there are pyloric conditions treatable with vitamin D. A report of a few cases indicates a relationship between some skin conditions (epidermolysis), impaired bone mineralization, dental caries, and pyloric atresia (42), suggesting further studies and therapeutic considerations with vitamin D. Evidence for hormonal regulation of pyloric sphincter function implicates secretin, cholecystokinin and gastrin (43), to which vitamin D needs to be added. Pyloric muscle fibers with vitamin D nuclear labeling that are recognized in the autoradiograms to extend into the region of the duodenum below the Brunner's gland (Figs. 2F and 2G) may help - associated with pyloric contractions, to provide abundant alkaline mucus to neutralize acid stomach secretions.

Small intestine

Calcium uptake and transport in the intestine has long been considered a major effect of vitamin D. There is abundant literature to support vitamin D effects on calcium absorption (44-46). Other effects of vitamin D, however, may be equally important, but are less well studied, such as amino acid absorption (47), protection against environmental toxins (48), and cell proliferation and differentiation.

The results of the autoradiographic studies reveal that nuclear receptor binding can differ quantitatively along villi, between villi and crypts, within the same animal and among different animals. In postnatal animals, epithelial nuclear receptor binding of vitamin D is much stronger in crypts compared to that in villi, although calcium transport through villi and related vitamin D effects on villous absorptive epithelium would seem to be very important during growth of the skeleton at postnatal age. In animals with prevailing vitamin D crypt labeling, the pattern corresponds to that of ^{3}H-thymidin labeling of the S-phase of the

cell cycle. This supports a primary role of vitamin D on cell proliferation in these animals. With ^{3}H-thymidine and ^{3}H-1,25(OH)$_2$ vitamin D$_3$, not all crypt cells are labeled. Unlabeled cells may belong to a resting stem cells population, but also may include goblet cell precursors or Paneth cells, which requires further studies.

The vitamin D receptor expression in intestinal epithelium and the size of villi change.

Semiquantitative immunocytochemical studies with antibodies to vitamin D receptor noted differences in vitamin D receptor staining in goat intestine in segment- and location-specific patterns (49). Changes of enterocyte expression of receptor and function are likely to occur during their migration from crypts to the tip of villi and to hormonal effects during sexual maturation as observed in connection to altered calcium transport in chickens (50). Vitamin D treatment increased mucousal weight in vitamin D-depleted rats, predominantly in the duodenum and, to a lower degree, in the ileum (51). In experiments with vitamin D-deficient rats, after a single dose of vitamin D$_3$, ^{3}H-thymidin incorporation and the migration rate of epithelial cells enhanced and the villous hight increased (52). Similarly, the villous length in normal chicken was 30 % longer than that of vitamin D-deficient chicks (53), although these authors concluded that "1,25-dihydroxyvitamin D can exert its action only on cells well advanced in their migration up the villus."

In the reviewed autoradiographic studies, considerable variations in the crypt-villous pattern of nuclear uptake and retention of radiolabeled vitamin D have been noted among different animals. Causes and functional significance require further quantitative analyses under consideration of age, hormonal status, time and dose of treatment. It is also conceivable that differences exist in receptor binding and related functions among different segments of the small intestine, although at present there is no evidence to support it.

A type of absorption barrier is suggested in autoradiograms of the small intestine with high radioactivity in the lumen versus absent or very low radioactivity in adjacent cytoplasm of absorptive epithelium. The barrier may exist against excreted liver metabolites. A barrier has been noted also in the esophagus, however with stratified epithelium, thus constituting a barrier of a different kind.

In the experiments with ^{3}H-(OH) vitamin D$_3$ and ^{3}H-24,25(OH)$_2$ vitamin D$_3$, the absence of nuclear concentration in the autoradiograms indicates that these compounds do not have noticeable affinities to nuclear receptors and are not genomically direct active.

None of the vitamin D compounds studied by microscopic autoradiography provide support for plasma membrane associated receptor existence and binding.

Liver

Depending on dose and time interval after administration of radiolabeled compound, large amounts of radiolabeled compound are present in blood vessels, bile ducts, and cellular-extracellular compartments. At early time intervals, tissue levels of diffuse radioactivity are high but not uniform. Differences related to perivenous compared to periportal lobular distribution of labeled compound can be noted. High amounts of diffuse radioactivity obscure specific associations and render cellular attribution difficult. It is therefore important to study different time intervals after the administration and different dose levels to be able to identify sites of select distribution of labeled compound as related to metabolism, receptor binding, storage, and biliary excretion.

In autoradiographic studies with ^{3}H-1,25(OH)$_2$ vitamin D$_3$ and ^{3}H-25(OH) vitamin D$_3$, a high accumulation of radiolabeled compound has been noted in the cytoplasm in scattered littoral cells located at sinusoidal margins, described first by Kupffer as stellate cells and later by Ito as fat-storing cells (54). These perisinusoidal stellate cells have been recognized as vitamin A-storing cells (55). Based on our autoradiographic evidence, these same cells probably are also vitamin D-storing for both the precursor and the active hormone. Liver stellate cells are implicated in human liver diseases and play an active role in fibrotic liver disorders (56). Whether and to which degree vitamin D, like vitamin A, is involved in liver pathology, remains to be studied.

Pancreas

Evidence for the presence of vitamin D binding protein has been obtained in cytosol from homogenized whole chick pancreas (57). Specific nuclear binding sites in B-cells of islets were first demonstrated with receptor microautoradiography (14,58). Effects of vitamin D on blood levels of glucose and insulin have been reported (58-60). Vitamin D deficiency has been shown to impair insulin synthesis and secretion in humans and in animal models of diabetes and may be involved in the pathogenesis of both forms of

diabetes (61). In cell lines derived from human pancreatic cancers, vitamin D receptor is expressed and increased three-fold compared to normal pancreas, responding with a decrease in cell numbers to treatment with high concentrations of vitamin D_3 analogue (62). In xenografts of human pancreas tumor, heterogeneous cell populations concentrate the radiolabeled vitamin D analogue OCT (63). Vitamin D receptor expression and function in cells of the exocrine pancreas, the potential role for vitamin D in the pathogenesis and prevention of pancreatic cancer (64) require further studies.

Colon

Uptake and retention of ^{3}H-1,25$(OH)_2$ vitamin D_3 in nuclei of epithelial cells in the colon resembles that in the small intestine. Nuclear labeling occasionally varies between the bottom and the luminal surface of crypts. It may be highest at one end, bottom or top or high at both ends and weak between. The radio-labeled cells are absorptive (principal) cells, not goblet cells. Whether caveolated cells, entero-endocrine and undifferentiated stem cells are also vitamin D targets, remains to be studied. While there is no conspicuous labeling of lymphocytes, except for dispersed single cells in lymph nodules, vitamin D regulation of the intestinal immune system and their cellular elements is expected and requires further investigation.

Epithelial cell proliferation in the distal colon apparently is not as simple as that in the small intestine. Studies in rats, mice and humans determined that proliferation is maximal in the basal thirds of crypts, but the zone of proliferation may reach as high as 80% of the crypt height. In the rabbit distal colon, a major proliferation zone was detected in the upper third of the crypt column and *mitotic figures were noted at all levels of the crypt column. In the rabbit distal colon, therefore, populations of proliferative cells are not limited to the crypt base, but extend into the upper third of the crypt column* (65). Differentiated vacuolated cell proliferation was detected at three crypt sites: base, middle and top of the crypt, while columnar cells arose from a population of dividing cells at the top of the crypt. Goblet cells exhibited maximal proliferation at the crypt base and migrated at a slower rate than the other cell types (65).

Which cell types are involved in tumor genesis, prevention and therapy? Cell proliferation, studied with ^{3}H-thymidine autoradiography, is abundant in the lower half of the crypts (29), and it changes with age (65). Similar as in the small intestine, vitamin D may be involved in the regulation of cell proliferation. Human colon adenocarcinomas contain vitamin D binding sites (66) that also play a crucial role in inflammatory bowel diseases (67). What determines expression of vitamin D receptors, vitamin D binding affinity and metabolism at the target? Other steroids, e.g. estrogens (68), adrenal steroids, retinoic acid, thyroid hormone are likely modulators. Their blood levels may play a significant role in tumor pathoclisis and therapeutic responses interactive with vitamin D (69,70).

Conclusions

This review testifies to the importance of high-resolution identification of in vivo targets and the related recognition of vitamin D's involvement in the physiology and pathology of the digestive system. According to the histochemical evidence of vitamin D receptor binding, vitamin D-*soltriol* effects on digestive processes are extensive and involve all levels of the digestive system selectively. From the extensive presence of vitamin D-specific sites of action it is apparent that vitamin D is needed to assure - at the right dose (71) - optimal functions for development, growth, maintainance and repair, with high therapeutic potentials. Vitamin D deficiencies are likely to predispose for multiple chronic diseases, presenting a challenge for preventive medicine (69).

A near century concentration on vitamin D and bone by focus-expedient but gestalt-scotomized experts has put researchers in a Procrustes' calcium bed (1), inhibiting advancement of research and recognition of vitamin D's main biological role as a seasonal regulator steroid hormone (3-5). Vitamin D actions need to be viewed in context and concert with other steroid hormones (Fig. 8), including also retinoic acid, thyroid hormone, and melatonin (4,5). These relationships remain to be studied. Functional *connections between vitamin D and melatonin* have been pointed out (72,73) and are considered relevant for harmonious regulations in a natural environment. Evidence exists in the literature for melatonin actions on the digestive system as well, such as protective effects against gastric ulceration (74,75) and periodontal inflammatory disease (76), uptake of melatonin-related compound in submandibular gland (77) and secretion of nerve growth factor (78). Identification of melatonin targets is still lacking but would be indispensable for clarification and assessment of functional relationships between these hormonal regulators and solar zeitgebers (72).

After tritium-labeled vitamin D became available, with the sensitive method of receptor microscopic autoradiography, over 50 vitamin D targets have been identified and characterized following the first demonstration of non-calcitropic target tissues in 1979 (1,2). Comparisons of nuclear receptor binding of vitamin D with the localization of antibodies to specific calcium-binding protein revealed that there is only occasional correspondence and that calcium-binding proteins are no general guide posts for vitamin D sites of action as had been assumed. Now, after two decades, the existence "in over 30 tissue/organs of man" of vitamin D receptors has been endorsed with biochemical approaches (79). The general correspondence of preclinical animal and clinical human targets (80,81) is noteworthy. Many of the vitamin D target tissues discovered and reported earlier (1), including those of the digestive system reviewed, remain to be further studied and their significance for health and disease evaluated.

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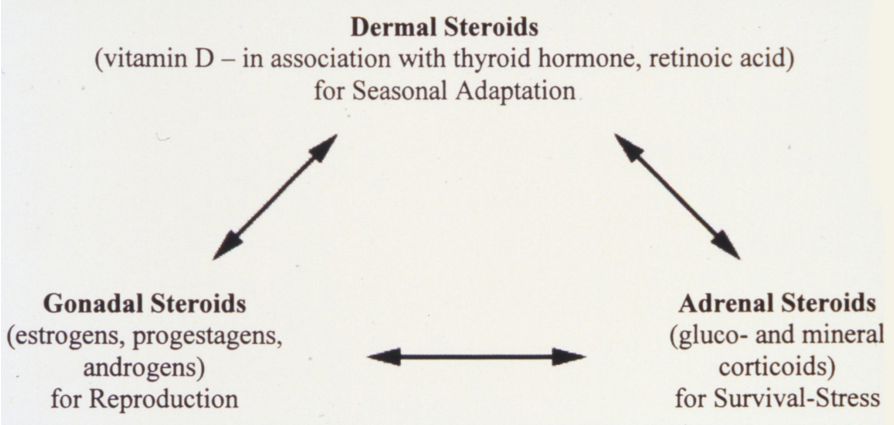

Figure 8. Vitamin D (soltriol) steroid hormone for seasonal adaptation of growth, procreation and maintainance-survival. Interactions with gonadal and adrenal steroid hormones (incl. retinoic acid, thyroid hormone, melatonin).

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