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Immunocytochemical localization of vitamin D-dependent calcium-binding protein in renal tubules of rabbit, rat, and chick

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Immunocytochemical localization of vitamin D-dependent calcium-binding protein in renal tubules of rabbit, rat, and chick. Vitamin D-dependent calcium-binding protein (CaBP) was localized in tissue sections of kidneys from rabbits, rats, and chicks using antiserum specific for chick intestinal CaBP. In rabbit kidney, CaBP was present in all cells of the distal convoluted tubule and most cells of the connecting tubule. Fewer, but still a majority, of the cells of cortical collecting ducts contained CaBP. The intensity of immunochemical staining and the number of stained cells decreased markedly in medullary collecting ducts, and only a few collecting duct cells contained CaBP at the junction of the inner and outer medulla. In the rat kidney, CaBP was present in all distal convoluted tubule cells, but the immunochemical staining was less intense than in the rabbit. The protein also was found in most connecting tubule cells of the rat; however, only a few collecting duct cells in the superficial cortex of the rat contained CaBP. CaBP was essentially absent from mid- to deep-cortical collecting duct cells, while a very few collecting duct cells always contained CaBP at the junction of the inner and outer stripes of the outer medulla. In the chick, CaBP was present in distal convoluted tubule cells as the distal convoluted tubule coursed adjacent to the central vein. CaBP was absent from chick collecting duct cells. In all three species CaBP was not detected in the other portions of the nephron.

Localisation immunocytochimique de la protéine-de-liaison-du-calcium vitamine D-dépendante dans les tubes rénaux de lapin, de rat et de poulet. La protéine de liaison du calcium vitamine D dépendante (CaBP) a été localisée dans des coupes de reins de lapin, de rat et de poulet à l'aide d'un antisérum spécifique pour la CaBP intestinale de poulet. Dans le rein de lapin, la CaBP était présente dans toutes les cellules du tube contourné distal, et dans la plupart des cellules du tubule connecteur. Un moins grand nombre, mais toutefois la majorité, des cellules des canaux collecteurs corticaux contenait de la CaBP. L'intensité de la coloration immunochimique et le nombre de cellules colorées diminuait considérablement dans les tubes collecteurs de la médullaire, et à la jonction entre la médullaire interne et externe quelques cellules du tube collecteur seulement contenaient de la CaBP. Dans le rein de rat, la CaBP était présente dans toutes les cellules du tube contourné distal, mais le marquage immuno-chimique était moins intense que chez le lapin. La protéine était également trouvée dans la plupart des cellules du tubule connecteur du rat; cependant seules quelques cellules du tube collecteur du cortex superficiel du rat contenaient de la CaBP. La CaBP était pratiquement absente du tube collecteur de la corticale moyenne et profonde, bien que quelques cellules du tube collecteur de la jonction entre les couches interne et externe de la médullaire externe contiennent toujours de la CaBP. Chez le poulet, la CaBP était présente dans les cellules du tube contourné distal, tant que le tube contourné distal restait adjacent à la veine centrale. La CaBP était absente des cellules du tube collecteur du poulet. Dans les trois espèces étudiées, la CaBP n'a été détectée dans aucune autre portion du néphron.

The vitamin D-induced calcium-binding protein (CaBP), which was first discovered in the chick intestine [1], has been proposed as being involved in vitamin D-dependent intestinal calcium transport on the basis of several nutritional and physiological studies [2–5]. One study provided direct evidence for the mediation of embryonic intestinal calcium transport by CaBP [6]. CaBP is also present in several other tissues [4, 7], including the kidney [8], and it has been suggested that the protein may play a role in renal calcium handling [9]. Because of the potential significance of CaBP in renal tubular calcium transport, the aim of the present study was to localize CaBP precisely along the nephron in the rabbit, the rat, and the chick.

Results

Animals. One female (3.6 kg) and three male (1.3 to 1.8 kg) New Zealand White rabbits, maintained on a standard laboratory chow (complete blend, Ralston Purina®) were sacrificed by an intravenous injection of euthanasia solution (T-61, National Laboratories Corp.). Both kidneys were removed rapidly, and 1 to 2 mm slices were cut perpendicular to the long axis of the kidney and frozen as described below. Six female Sprague-Dawley rats (Outbred, Tex-SD, Timco Breeding, Houston, Texas) maintained on a standard rat chow (#5012, Ralston Purina®, St. Louis, Missouri) were anesthetized with ether and then decapitated. The kidneys were removed, and 1 to 2 mm slices were rapidly cut and frozen as described below. Three- to five-week old chicks, raised on a rachitogenic diet [10] and injected 72 hr prior to sacrifice with 500 I.U. of vitamin D_3, were decapitated; 1 to 2 mm slices of chick kidney were frozen rapidly and processed identically to the rabbit and rat kidney slices.

Tissue processing. The slices were frozen rapidly in isopentane maintained at its melting point (−160° C) in liquid nitrogen. Then they were transferred to ethanol containing 0.5% glutaraldehyde (−75° C) and were fixed by freeze-substi-

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tution for 2 weeks [10]. After fixation the tissue slices were brought to room temperature and then immersed successively in 100% ethanol (1 hr), acetone containing 8 g Drierite/liter (2 changes, .5 hr each), paraffin at 60° C (1hr), and paraffin at 60° C (2 hr). Then they were embedded in paraffin. Microscopic sections (6 μm) were cut, floated briefly on warm water, picked up on microscope slides, and dried. Prior to the immunocytochemical localization of CaBP, the slides were deparaffinized by successive immersion in xylene (3 changes, 20 sec each), 100% ethanol (2 changes, 20 sec each), and 95% ethanol (2 changes, 20 sec each). Finally, the sections were rinsed with phosphate buffered saline (PBS) (3 changes, 1 min each).

Immunohistochemistry. The primary antiserum (sheep anti-chick intestinal CaBP) is monospecific for vitamin D-induced CaBP [10, 11] and is known to cross-react with CaBP having the same molecular weight (28,000 daltons) in rat kidney [4, 12]. It does not cross-react with the 11,000-dalton CaBP present in kidneys of guinea pig, pig, and cow [4]. Prior to this study, it had not been tested with rabbit kidneys. Homogenates of rabbit kidney demonstrated a single precipitin line when reacted against sheep anti-chick intestinal CaBP (anti-CaBP) by the micro-Ouchterlony method.

The peroxidase-antiperoxidase (PAP) method [13] was used to localize CaBP in cells of the renal tubule. Kidney sections were incubated sequentially at room temperature in a humid chamber with the following solutions: 3% normal rabbit serum in PBS (30 min), sheep anti-CaBP diluted 1:1,000 with 1% normal rabbit serum in PBS (30 min), PBS wash (3 × 2 min), rabbit anti-sheep IgG diluted 1:20 with PBS (30 min), PBS wash (3 × 2 min), goat peroxidase-antiperoxidase complex diluted 1:100 with 1% normal rabbit serum in PBS (30 min), PBS wash (2 min), 0.05 M tris buffer (pH 7.6) wash (2 × 2 min), 0.05% 3,3′-diaminobenzidine·hydrochloric acid, and 0.01% H_2O_2 in tris buffer (5 min). Immunologic control specimens were kidney sections incubated with nonimmune sheep serum in place of sheep anti-CaBP. After the immunohistochemical incubations, the sections were counterstained with periodic acid-Schiff reagent and hematoxylin.

Results

Cells containing CaBP were labeled with the dark deposits of the reaction product 3,3′-diaminobenzidine (DAB) as shown in Figures 1 and 2. The deposits of DAB demonstrate the specific presence of CaBP because DAB was absent from the same cells of adjacent immunocontrol sections (compare Figs. 2 and 3). Usually, the deposits of DAB were very concentrated in the cells, thus obscuring cell structure. Identification of cell type and tubular segment was facilitated greatly by observation of an adjacent section processed as an immunocontrol.

Along the rabbit nephron CaBP is first seen a short distance beyond the macula densa in cells of the distal convoluted tubule (Fig. 2). From the transition of the cortical thick ascending limb to the distal convoluted tubule CaBP is present uniformly in the cytoplasm of all distal convoluted tubule cells. The transition from the distal convoluted tubule to the connecting tubule is shown in Figure 4. A few connecting tubule cells lack CaBP. However, most cells contain CaBP and they exhibit the same staining intensity as distal convoluted tubule cells. The presence of a few CaBP-free connecting tubule cells is shown also in the arcade in Figure 5. Generally, as the connecting tubule joins the cortical collecting duct, the number of CaBP-containing cells decreases (Fig. 5). However, in a few instances the connecting tubule joined the cortical collecting duct without apparent decrease in CaBP-containing cells (compare indicated connecting tubules in Fig. 1).

The collecting ducts of rabbit kidney contained cells with CaBP from the superficial cortex to the junction between the inner and outer medulla. The number of cortical collecting duct cells containing CaBP was less than noted in connecting tubules (Figs. 1 and 5). Generally, the intensity of the DAB reaction product in cells with CaBP was as great as in distal convoluted tubule and connecting tubule cells. However, both the number of CaBP-containing collecting duct cells and the intensity of the DAB reaction product decreased in the medulla. Figure 6 illustrates collecting ducts in the outer stripe of the outer medulla where fewer CaBP-containing cells are present. Figure 7 shows collecting ducts in the inner stripe of the outer medulla near the junction between the inner and outer medulla. Very few cells with CaBP are seen, and the DAB reaction product is much less concentrated. DAB reaction product was absent, indicating the absence of detectable CaBP, in the renal corpuscle, proximal tubule, Henle's loop, and collecting ducts of the inner medulla. There was no difference in the distribution of CaBP in female versus male rabbit kidneys.

Comparison of rat kidney (Fig. 8) with rabbit kidney (Fig. 1) illustrates differences in CaBP content and distribution between the two species. Immunocontrols (not shown) clearly indicated that the deposits of DAB in sections of rat kidney represented sites of CaBP localization. Figure 8 shows the nearly complete absence of CaBP in the cells of rat collecting ducts. It also demonstrates the presence of CaBP at two different staining intensities, that is, cells lightly stained with DAB reaction product and other cells more darkly stained.

Similar to the rabbit, CaBP is first observed in the rat nephron in cells of the distal convoluted tubule at a short distance beyond the macula densa (Fig. 9). All distal convoluted tubule cells contain CaBP, but the apparent concentration of CaBP is less than in the rabbit or the CaBP present may be less accessible to the anti-CaBP, because the DAB reaction product is uniformly lighter (Figs. 8, 9, 10). The transition from distal convoluted tubule to connecting tubule in the rat apparently was less abrupt than in the rabbit. Often, darker DAB-staining cells characteristic of the connecting tubule were noted along with the lighter staining distal convoluted tubule cells (Fig. 10). Connecting tubule cells lacking CaBP were more frequent in the rat than in the rabbit kidney (Figs. 10, 11). On some occasions CaBP-containing cells of the connecting tubule appeared to extend into the epithelium of the cortical collecting duct (Fig. 11). In the rat it frequently was difficult to distinguish the transition from the connecting tubule to the cortical collecting duct. Only the initial portion of the collecting ducts in the superficial cortex demonstrated CaBP-containing cells. CaBP was absent in collecting duct cells deeper in the cortex. Medullary collecting duct cells were virtually devoid of CaBP. Only at the junction of the inner and outer stripes of the outer medulla were CaBP-containing collecting duct cells observed. They were present in all sections of rat kidney, but they were very few in number. CaBP was not detected in renal corpuscles, proximal tubules, or segments of Henle's loop.

In the chick kidney, CaBP was present in distal convoluted tubule cells as the distal convoluted tubule coursed near the central vein (Fig. 12). In birds the nephrons are situated radially

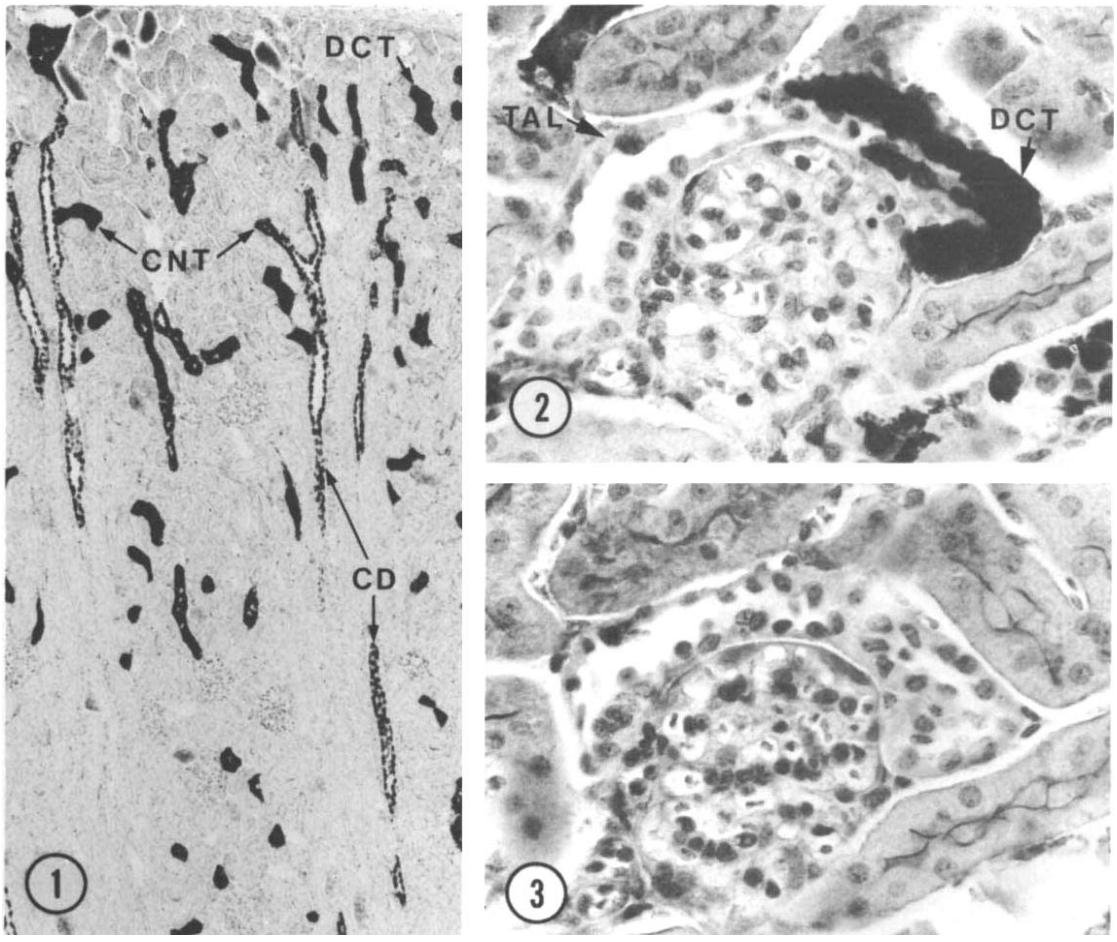

Fig. 1. *Rabbit kidney cortex* (capsule at top) *showing immunocytochemical distribution of CaBP* (dark deposits of DAB) *in cells of distal convoluted tubule* (DCT), *connecting tubule* (CNT), *and collecting ducts* (CD), *(× 60).*

Fig. 2. *Rabbit cortical thick ascending limb* (TAL) *in transition to distal convoluted tubule* (DCT). CaBP is absent in cells of macula densa and cortical thick ascending limb, but CaBP is present in cells of distal convoluted tubule (× 370).

Fig. 3. *Immunocontrol.* Adjacent serial section to the one illustrated in Fig. 2 reacted with non-immune serum instead of anti-CaBP. It shows that the deposits of DAB in the other figures are specific for the presence of CaBP (× 370).

around the central vein of each kidney lobule. As distal tubules course from the central vein toward the collecting ducts at the lobule periphery, the cells containing CaBP decrease in number. Figure 13 shows CaBP-containing distal convoluted tubule cells near the central vein. The number of cells with CaBP decreases rapidly to zero as the distal convoluted tubule cells approach the renal corpuscle. CaBP was not detected in renal corpuscles, proximal tubules, medullary loops of the mammalian-type nephrons, cortical intermediate segments, connecting tubules, or collecting ducts in the chick kidney.

The results of the immunocytochemical localization of CaBP along the nephron of the three species studied are summarized in Figure 14.

Discussion

1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) induces CaBP synthesis in the intestine at the same sites at which it increases calcium absorption [8, 14]. How CaBP is involved in the physiologic mechanism by which $1,25(OH)_2D_3$ promotes intestinal calcium transport is unknown, but the spacial correlation of the two parameters suggests that there is a relationship. Because of the correlation between the localization of CaBP in the intestine and the action of $1,25(OH)_2D_3$ to increase intestinal calcium transport, we believe it is of interest to analyze this relationship along the renal tubule.

Studies on calcium transport across rabbit kidney tubules have been done by perfusing individual segments in vitro [15–23]. These findings are shown in relationship to the localization of CaBP in Table 1.

CaBP was absent from cells of the rabbit proximal tubule. The only proximal tubule segment across which calcium transport has been studied is the S2 (or pars recta) portion of superficial nephrons. It was concluded that there is active calcium absorption at this site [19]. Because fluid normally coursing through the lumen of this nephron segment results in a lumen-positive transepithelial voltage [24], it is possible that under physiologic conditions passive calcium ion absorption may be driven by the lumen-positive voltage.

CaBP was absent from cells of both the thin descending and thin ascending limbs of Henle's loop. Likewise, there is no measurable calcium transport across these renal tubule segments [15, 19].

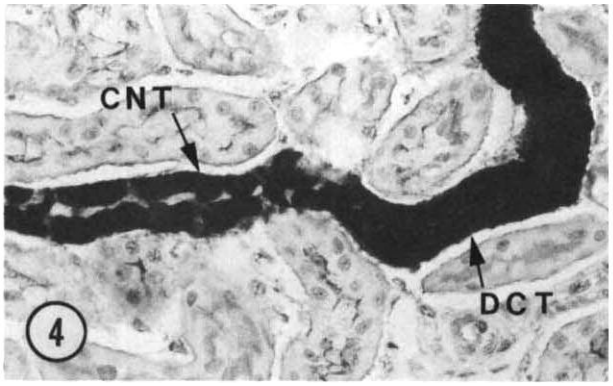

Fig. 4. *Rabbit distal convoluted tubule* (DCT) *with CaBP-containing cells in transition to connecting tubule* (CNT) *with CaBP-containing cells (× 250).*

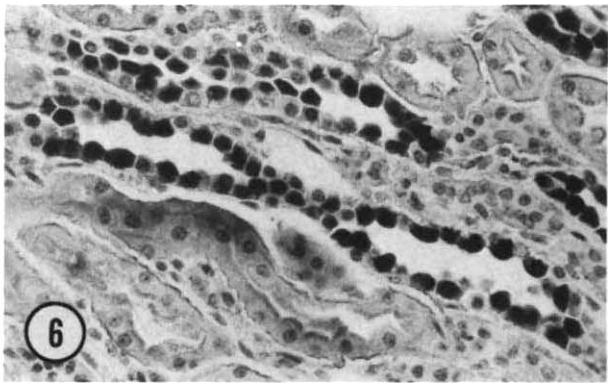

Fig. 6. *CaBP-containing cells in rabbit medullary collecting duct in outer stripe of outer medulla (× 250).*

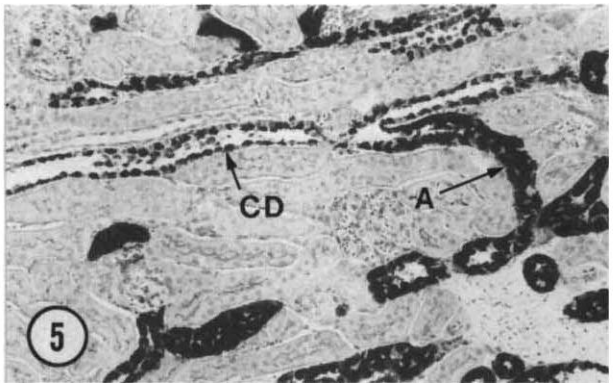

Fig. 5. *Rabbit connecting tubule arcade* (A) *joining cortical collecting duct* (CD): *CaBP-containing cells are present in both segments.* Kidney cortical surface is to the right (× 100).

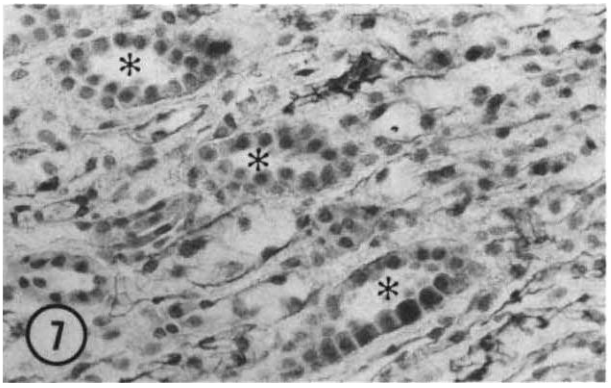

Fig. 7. *Very light DAB staining (that is, CaBP) in a few cells of rabbit medullary collecting ducts* (*) *in inner stripe of outer medulla near junction of inner and outer medulla (× 250).*

CaBP was absent from the cells of the medullary and cortical thick ascending limbs of Henle's loop. Passive calcium absorption driven by the transepithelial voltage exists in both segments [16–18, 25]. In addition, several investigators have concluded that there is an active component of calcium absorption across the cortical thick ascending limb [15, 17, 21]. The issue of the relative importance of active versus passive calcium absorption across the cortical thick ascending limb is unresolved. The parathyroid hormone, in high concentrations, acts on the peritubular cell membrane to increase calcium absorption across the cortical thick ascending limb [20–22]. Although the parathyroid hormone has been shown to increase calcium absorption at this site by stimulation of adenylate cyclase, the physiologic mechanism by which calcium absorption is increased is unknown [26].

CaBP appeared abruptly in cells of the distal convoluted tubule at the junction between the end of the cortical thick ascending limb and the beginning of the distal convoluted tubule. Shareghi and Stoner [16] have reported the presence of active calcium transport across this segment. A calcitonin-sensitive adenylate cyclase is present in this portion of the nephron, but parathyroid hormone-sensitive adenylate cyclase is absent [27]. A perplexing aspect of Shareghi and Stoner's results is that they found that physiologic concentrations of the parathyroid hormone increased calcium absorption across the distal convoluted tubule [16]. Although these results are possible, they raise the question of whether or not connecting tubule segments may have been attached to the distal convoluted tubules perfused by Shareghi and Stoner. The former have a parathyroid hormone-sensitive adenylate cyclase [27] and actively transport calcium [16, 22]. In this regard, Imai was technically unable to measure calcium transport across the distal convoluted tubule of the rabbit because of its short length [22].

The majority of the cells in the connecting tubule contained CaBP. This tubule segment possesses a lumen-negative voltage [16, 22]. It actively absorbs calcium against an electrochemical gradient [16, 22]. Physiologic concentrations of the parathyroid hormone stimulate adenylate cyclase here, resulting in increased calcium absorption [16, 22]. Therefore, this tubule segment appears to be important in the control of the renal excretion of calcium.

Cells of the cortical collecting duct also contained CaBP, although less frequently than in either the distal convoluted

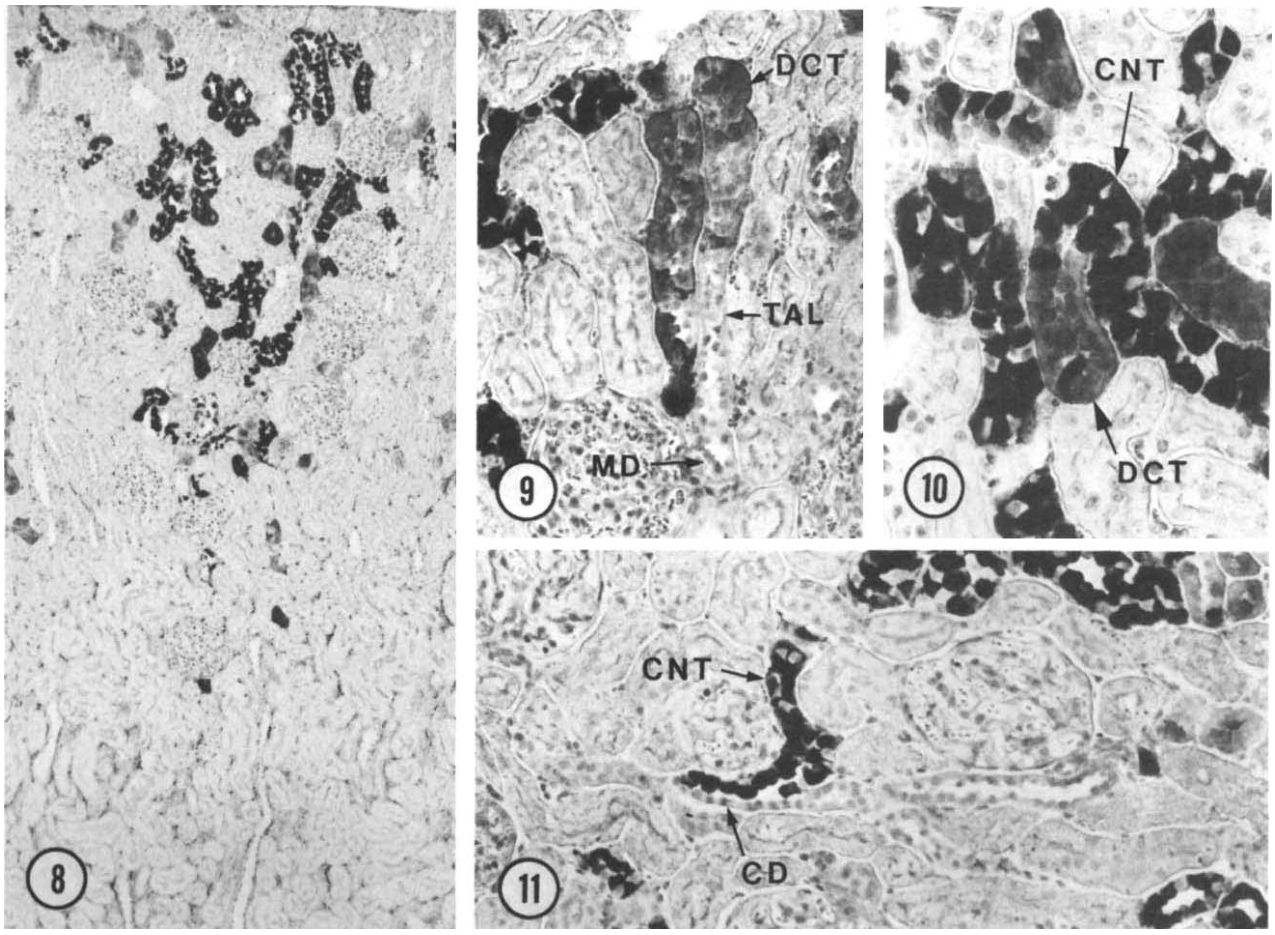

Fig. 8. *Rat kidney cortex* (capsule at top) *showing immunocytochemical distribution of CaBP in cells of distal convoluted tubules* (light deposits) *and connecting tubules* (darker deposits). Infrequent cells with CaBP in collecting ducts (× 60).

Fig. 9. *Rat cortical thick ascending limb* (TAL) *in transition to distal convoluted tubule* (DCT). CaBP is absent in thick ascending limb and macula densa cells *(MD)*, but appears in cells of distal convoluted tubule (× 170).

Fig. 10. *Rat distal convoluted tubule* (DCT) *with CaBP in transition to connecting tubule* (CNT) *with CaBP-containing cells (× 240).*

Fig. 11. *Rat connecting tubule* (CNT) *with CaBP-containing cells joining superficial cortical collecting duct* (CD). Only a few CaBP-containing cells in cortical collecting duct at the junction with connecting tubule (× 170).

tubule or connecting tubule. Shareghi and Stoner found no measurable calcium transport across this segment using helium glow photometry [16]. They concluded that the low intrinsic permeability of the cortical collecting duct to calcium prevented calcium secretion when the voltage was lumen-negative. Bourdeau and Hellstrom-Stein measured calcium movement across the cortical collecting duct using ^{45}Ca [23]. They found a very low intrinsic permeability of the cortical collecting duct to calcium as well. However, they observed a small, but measurable, component of passive net calcium flux driven by the transepithelial voltage. Neither study presents evidence to indicate that the cortical collecting duct of the rabbit actively absorbs calcium or is important in the control of urinary calcium excretion. However, it has been reported that the intracellular calcium ion activity in cells of the cortical collecting duct may be important in the regulation of sodium and water absorption by this portion of the nephron [28]. Therefore, although there is no correlation between the presence of calcium-binding protein and active calcium transport across the cortical collecting duct, CaBP may be important in the regulation of intracellular calcium ion activity here.

Studies on calcium transport across rat kidney tubules have been done by micropuncture and microperfusion techniques in vivo [30–36]. These findings are summarized and shown in relationship to the localization of CaBP in Table 2. Because micropuncture techniques in vivo are limited in the sites along the nephron which can be studied, the conclusions regarding localization of calcium transport cannot be made as discretely as for the segments of the rabbit nephron studied in vitro.

Evidence supporting passive and/or active calcium transport across the rat proximal tubule has been reported [30, 34]. The questions of whether or not both mechanisms are operative and their relative importance remain controversial. Although the rat proximal tubule absorbs the majority of calcium filtered at the glomerulus, it is not believed to be the site where urinary calcium excretion is finely controlled [30].

Calcium is absorbed in the loop of Henle [30]. Although calcium absorption is presumed to occur in the thick ascending

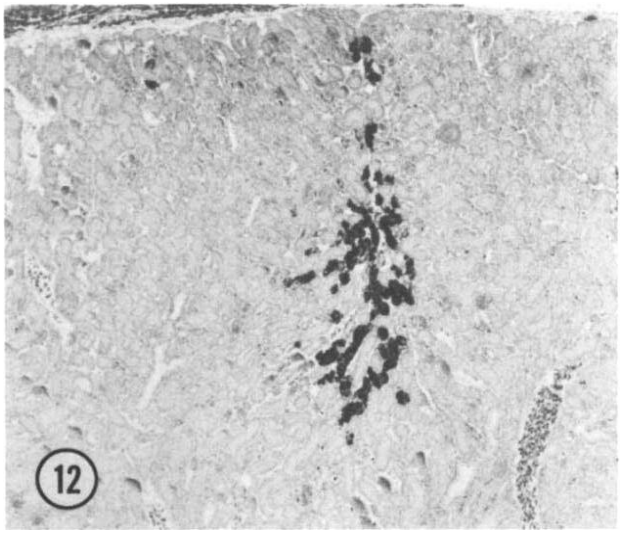

Fig. 12. *Chick kidney* (capsule at top) *showing immunocytochemical distribution of CaBP.* CaBP is present in cells of distal convoluted tubule near the central vein which is situated in the middle of the kidney lobule (× 60).

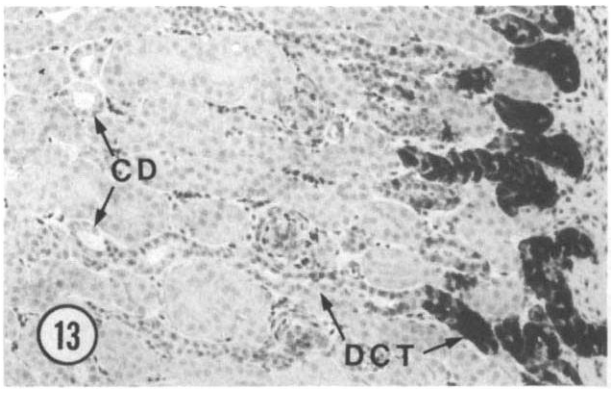

Fig. 13. *CaBP in cells of distal convoluted tubule* (DCT) *near central vein* (right of photo). Fewer cells contain CaBP as distal tubule extends toward periphery of kidney lobule. CaBP is absent in distal tubule cells near the renal corpuscle and in cells of the connecting tubule and collecting ducts *(CD)* (× 150).

limb, the evidence is circumstantial. There is no direct information regarding the mechanism of calcium transport in the loop of Henle in the rat. Parathyroid hormone-sensitive adenylate cyclase is present in the rat cortical thick ascending limb [27], but its role in regulating tubular calcium absorption is unknown.

Constanzo and Windhager [31, 32] provide clearcut evidence of active calcium absorption, which is increased by the parathyroid hormone, across the distal convoluted tubule of the rat. They speculated that the variability noted in the effect of parathyroid hormone on calcium transport among individual tubules in their study might result from the heterogeneity of cell types in the distal convoluted tubule of the rat and from the possibility that parathyroid hormone might not affect every cell type to the same extent [32]. Based on the observations of the present study, it is tempting to speculate that the heterogeneity of localization of CaBP might also relate to the heterogeneity of cell types in the distal convoluted tubule.

In the rat the transition of the connecting tubule to the cortical collecting duct is gradual, and there may be intermingling of cell types [37]. The localization of calcium-binding protein to cells of the initial portion of the cortical collecting duct, but not to later portions, correlates with parathyroid hormone-sensitive adenylate cyclase [27] and parathyroid hormone-sensitive ^{45}Ca efflux [33] from this region of the rat nephron.

CaBP was present in less than 2% of medullary collecting duct cells at the junction of the inner and outer stripes of the outer medulla. CaBP was not detected in collecting duct cells of the inner medulla where parathyroid hormone-insensitive calcium absorption occurs [36]. The magnitude of this transport is low, and the mechanism by which calcium absorption occurs is unknown.

Because $1,25(OH)_2D_3$ is the hormonal (that is, tissue active) form of vitamin D_3 and because the synthesis of CaBP is dependent on vitamin D, it is of interest to compare the cellular localization of $1,25(OH)_2D_3$ and CaBP. By thaw-mount autoradiography Stumpf et al [38] found the highest concentrations of ^3H-$1,25(OH)_2D_3$ in the nuclei of podocytes and the cells of distal tubules. Labeled cells in the latter included those of the thick ascending limb of Henle's loop, the macula densa, and the pars convoluta in rat kidney. In the present study CaBP was not found in association with the renal corpuscle or the thick ascending limb, as might have been expected on the basis of the nuclear localization of ^3H-$1,25(OH)_2D_3$. However, the presence of CaBP in distal convoluted and connecting tubule cells is consistent with the nuclear localization of ^3H-$1,25(OH)_2D_3$ there.

In the chick kidney, CaBP was present in cells of that portion of the distal tubule adjacent to the central vein, confirming previous observations [39, 40]. The localization of CaBP in cells of chick kidney tubules by immunocytochemical techniques was consistent with quantitative measurements of CaBP in microdissected chick nephron segments [41]. The presence of CaBP in cells of both the rabbit and rat connecting tubules raised the question of the presence of CaBP in the chick connecting tubule. The question is complicated by inconsistent information on the length of avian connecting tubules [42–44] and remains unanswered. There are no calcium transport studies in individual chick kidney tubules to our knowledge.

The role of vitamin D-dependent CaBP in cells of the rabbit, rat, and chick renal tubule is unknown. Comparison of the location of CaBP in the renal tubule with present knowledge about calcium transport across various segments of the nephron suggests that CaBP is localized at sites either where active calcium transport occurs or where intracellular calcium ion activity appears to be important in regulating other transport processes. That vitamin D-induced CaBP might be involved in the renal tubular absorption of calcium is suggested by the work of Costanzo, Sheehe, and Weiner [45]. They found that calcium clearance ratios (C_{Ca}/GFR), at comparable sodium clearance ratios, were significantly lower in vitamin D-repleted than in vitamin D-depleted rats. Their results were consistent with a direct effect of vitamin D to enhance calcium reabsorption relative to sodium reabsorption. Whether renal CaBP is involved in transcellular calcium translocation and/or is secondarily involved in regulating the transport of calcium or of other solutes by controlling intracellular calcium ion activity remains to be investigated.

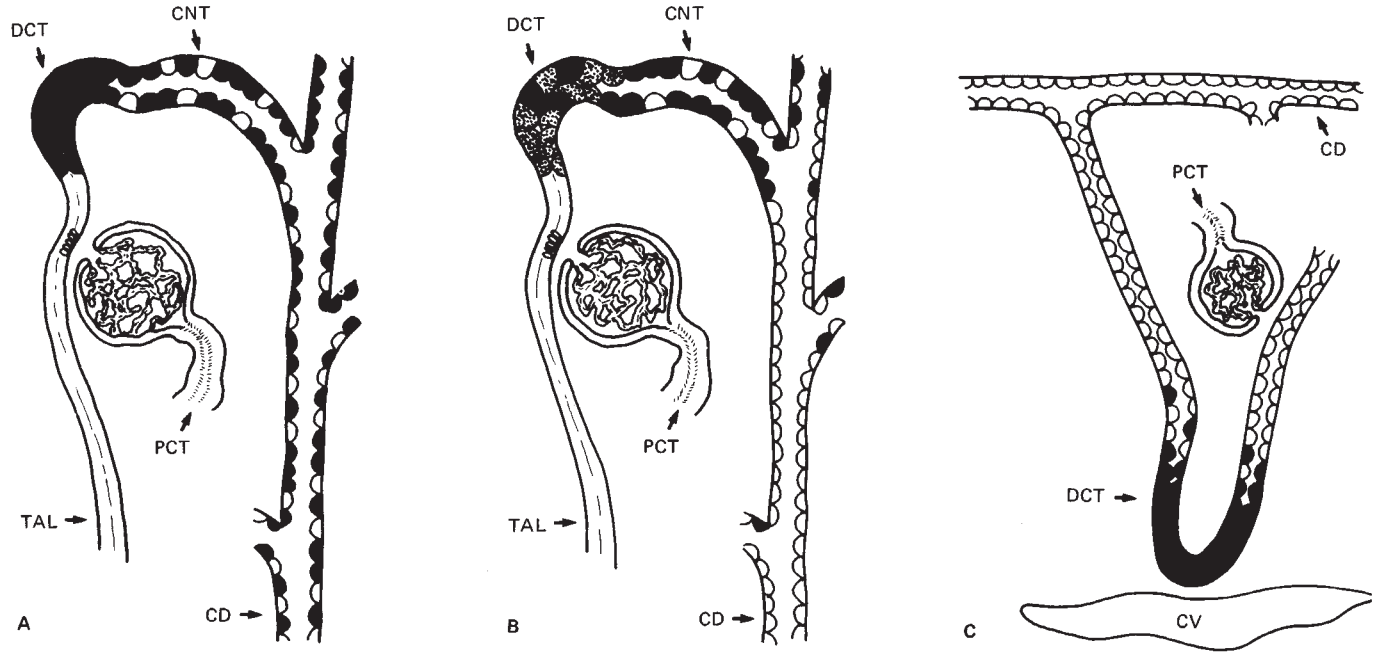

Fig. 14. *Schematic representation of CaBP distribution along nephrons from the cortex of the rabbit* (A), *the rat* (B), *and the chick* (C). CaBP-containing cells with the strongest DAB staining reaction are indicated in *black* and those with a lighter DAB reaction are *stippled*. Abbreviations are: proximal convoluted tubule *(PCT)*, thick ascending limb *(TAL)*, distal convoluted tubule *(DCT)*, connecting tubule *(CNT)*, collecting duct *(CD)*, and central vein *(CV)*.

Table 1. CaBP and calcium transport across the rabbit kidney tubule[a]

Tubule segment	CaBP	Net calcium transport	Mechanism(s)	Reference
Proximal				
S1	0	?		
S2	0	Absorption	Active (?)	19
S3	0	?		
Thin limbs				
Descending	0	0		15, 19
Ascending	0	0		15
Distal				
Thick ascending limb				
Medullary	0	Absorption	Passive	21
Cortical	0	Absorption	Passive/active (?)	16 to 18/15, 17, 21
Convoluted	+	Absorption (?)	?	16
Collecting duct				
Connecting tubule	+	Absorption	Active	16, 22
Cortical	+	Secretion[b]	Passive	16, 23
Outer medullary	+	?		
Inner medullary	0	?		

[a] The nomenclature of the tubule segments is that of Kaissling and Kriz [29]. Symbols and abbreviations are: 0, looked for, but absent; +, present; ?, not yet investigated (unknown); (?), controversial; CaBP, calcium-binding protein.
[b] Of very low magnitude.

Table 2. Calcium-binding protein (CaBP) and calcium transport across the rat kidney tubule

Tubule segment	CaBP	Net calcium transport	Mechanism(s)	Reference
Proximal	0	Absorption	Passive (?)/active (?)	30/34
Loop of Henle	0	Absorption[a]	?	30
Distal convoluted tubule[b]	+	Absorption	Active	31, 32
Collecting duct				
Cortical	+	Absorption[c]	Active (?)	33, 35
Medullary	+[d]	?		
Papillary	0	Absorption	?	36

Symbols are: 0, looked for, but absent; +, present; ?, not yet investigated (unknown); (?), controversial.
[a] Absorption is presumed to occur in the thick ascending limb of Henle's loop.
[b] The distal convoluted tubule is defined as those cells comprising the renal tubule between the macula densa and the first junction with the cortical collecting duct.
[c] Absorption is presumed to occur in the initial "granular" portion of the cortical collecting duct.
[d] Although CaBP was observed, it was present in very few cells (<2%).

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