Vitamin A Uptake Cells in the Endocrine Organs of Japanese Quails, *Coturnix coturnix japonica*

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ABSTRACT

The vitamin A uptake cells in the anterior pituitary, thyroid gland, and pancreas of Japanese quails, *coturnix coturnix japonica*, were examined by the use of Sudan III staining, toluidine blue staining, fluorescence microscopy and electron microscopy. After excess vitamin A administration, most of interstitial cells, corresponding to fibroblasts, increased markedly the size and number of lipid droplets in the cytoplasm which emitted vitamin A fluorescence intensively. The reactions to vitamin A administration were in parallel with the vitamin A dosage. In particular, the cells of the thyroid gland and pancreas were comparable to the Ito cells of the liver in their vitamin A uptake capacity. The present study demonstrates that interstitial cells either identical with or closely related to the Ito cells of the liver are widely distributed in the connective tissue of these endocrine organs. The possible physiological roles of the vitamin A uptake cells and also the species difference in the vitamin A uptake capacity of these cells are discussed.

Key words: Vitamin A, Cell, Pituitary, Thyroid, Pancreas

Since Ito (1951) discovered the "fat-storing cells" in the space of Disse as a new cellular constituent of the liver[7], their constant localization and independent nature have been fully established by him and his collaborators[21]. Nakane (1963) first revealed via fluorescence microscopy that Ito cells in the mouse liver store vitamin A in the cytoplasmic lipid droplets[15]. Thereafter, the universal property was confirmed by several investigators[3,5,21]. Ito cells in the liver, hepatic vitamin A uptake cells, were recognized as specific to the liver, and their occurrence in other tissues and organs was not known. However, after a while, research groups discovered independently similar vitamin A uptake cells in other tissues and organs by means of autoradiography using ³H-vitamin A[23] and other histological methods[9,21,24]. Consequently the concept of a "vitamin A-storing cell system" was proposed[23]. In phylogenetic studies on the system[2,11,21], no reports of vitamin A uptake cells in avian organs are available outside our preliminary reports[10,11]. The present study clearly demonstrates vitamin A uptake cells in the endocrine organs of Japanese quails: The anterior pituitary, thyroid gland and pancreas. So far there have been no studies on the occurrence of vitamin A uptake cells in the anterior pituitary and thyroid gland of any other species. The pancreatic vitamin A uptake cells were previously identified using mouse, rat and human specimens[6,22], but the cells examined ex-perimentally were very low in vitamin A uptake and storage capacity, regardless of a very high dosage of vitamin A. Furthermore, only a few studies on the effects of vitamin A on the functions of these organs have been performed[4,14,20].

MATERIALS AND METHODS

Japanese quails of both sexes (n=15), weighing 80 to 100 g, were subcutaneously administered an aqueous solution of vitamin A palmitate ("Chocola A", Eisai Co. Ltd., Tokyo) 4 times, once every other day, with a total dose of 200,000, 400,000 and 800,000 I.U./kg. The control animals were injected with a 4% aqueous solution of HCO 60 (the surfactant contained in "Chocola A"). From one to three weeks after the last administration, the anterior pituitary, thyroid gland and pancreas were taken from the birds under nembutal anesthesia. These organs were examined by the following procedures. For vitamin A detection by fluorescence microscopy and staining of lipid droplets, frozen sections, 20–30 μm thick, were prepared after fixation in 10% formaldehyde. Some sections were mounted in physiological saline and observed under the fluorescence microscope. When excited by ultraviolet light, vitamin A is identified as a quickly fading green fluorescence[16]. Other sections were dipped for 30 min in 100 ml 70% alcohol containing 1 g of Sudan III at 37 °C and later with counterstaining by Mayer's hematoxylin. For ultrastructural observation,

the organs were fixed by perfusion via the left ventricle using a syringe fitted with a needle, serving 2.5% phosphate buffered glutaraldehyde. Postfixation of the small blocks was carried out
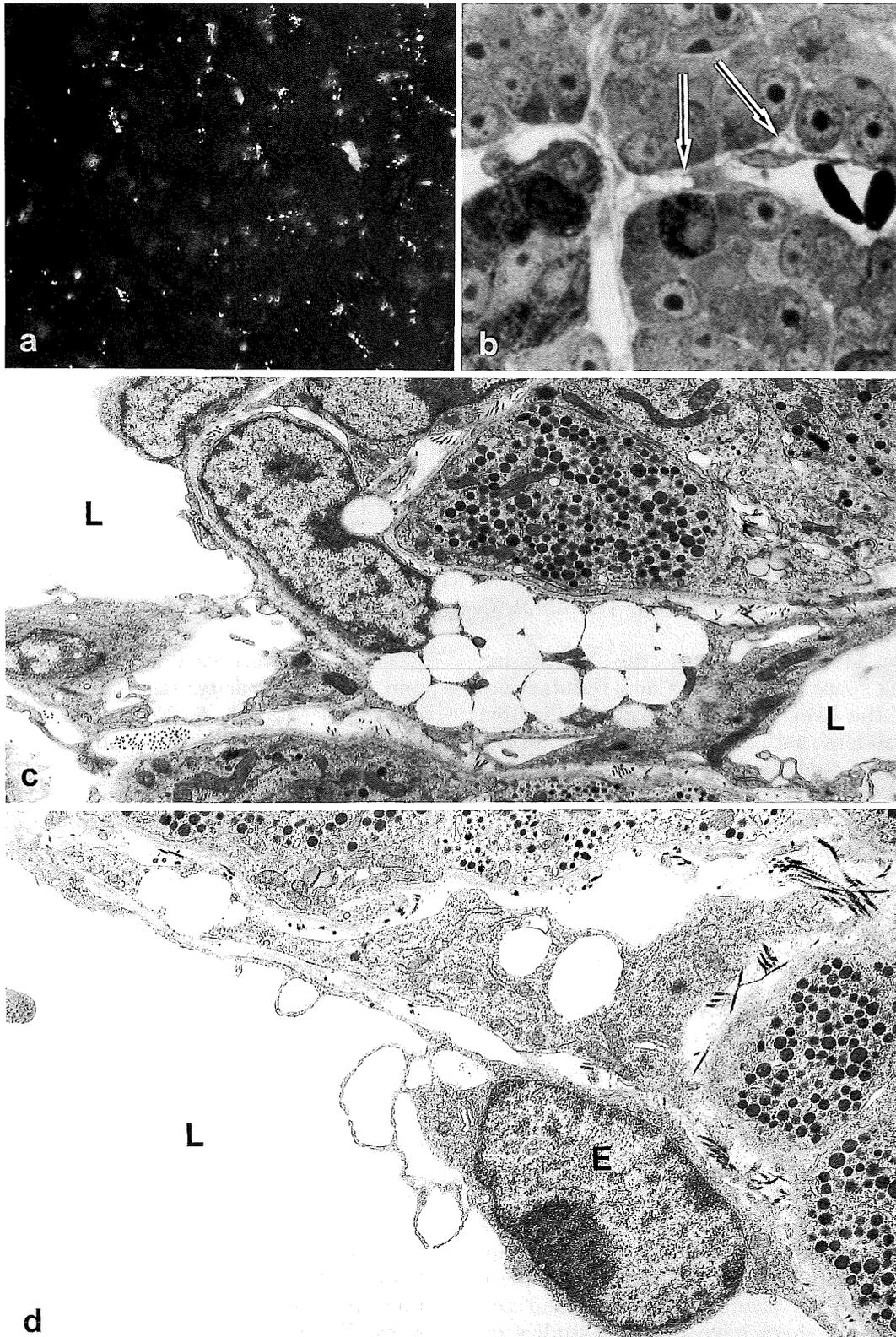
Fig. 1. Anterior pituitary after vitamin A administration with a total dose of 800,000 I.U./kg.
Vitamin A uptake cells demonstrated by fluorescence microscopy (**a**), toluidine blue staining (**b, arrows**) and electron microscopy (**c, d**). The cytoplasm is filled with lipid droplets, partly fused (**b, c**). **d** shows the cytoplasmic process observed between the endocrine cells and a peculiarly shaped endothelial cell (**E**). **L**: blood capillary lumen, **a**: ×350, **b**: ×1,300, **c**: ×10,400, **d**: ×12,500

in 1% osmium tetroxide in phosphate buffer saline (pH 7.4), dehydrated and embedded in Araldite resin. Ultrathin sections were stained with uranium acetate and lead citrate, and examined using an electron microscope. Semithin sections, 1 μm thick, were stained with toluidine blue for light microscopic observation.

RESULTS

In the organs of the control animals, the interstitial cells emitted no or only faint vitamin A fluorescence under fluorescence microscopy and it
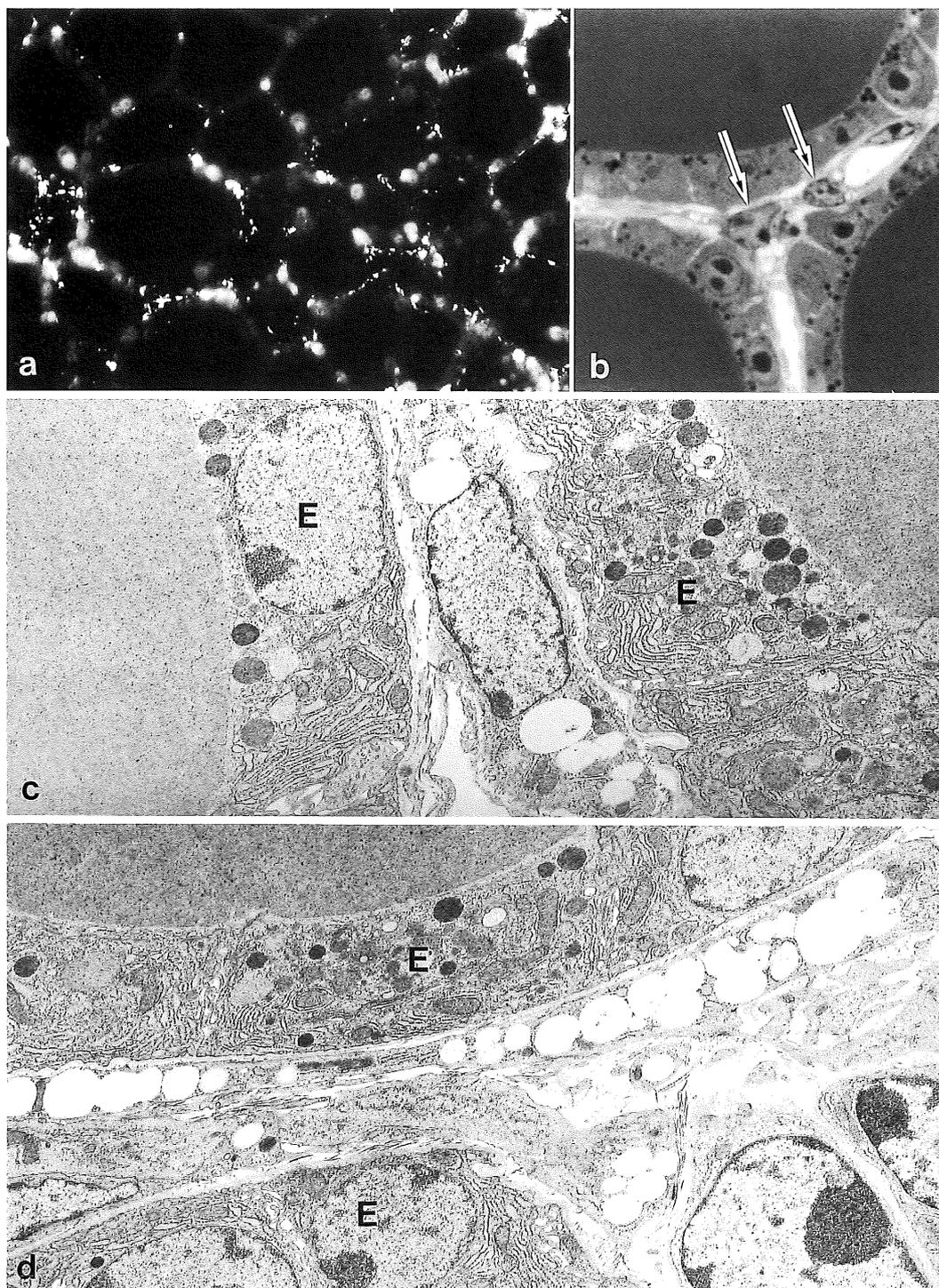
Fig. 2. Thyroid gland after vitamin A administration with a total dose of 800,000 I.U./kg.
Vitamin A uptake cells demonstrated by fluorescence microscopy and toluidine blue staining are shown in **a** and **b (arrows)**, respectively. Electron micrographs show one vitamin A uptake cell occurring between two follicles **(c)** and two long cytoplasmic processes of the cells extending along the base of the follicular epithelium **(d)**. **E:** follicular epithelium, **a:** ×350, **b:** ×1,300, **c:** ×8,700, **d:** ×8,700

was also impossible to find cells containing suda-
nophilic lipid droplets in the connective tissue
space of the organs under a light microscope. Af-
ter excess vitamin A administration the intersti-
tial cells demonstrated, in proportion to the
dosage, lipid droplets remarkably increased in
size and number, emitting an intensive vitamin A
fluorescence which characteristically fades quick-
ly under ultraviolet light. Such cells were widely
distributed in the connective tissue space of the
organs. Ultrastructurally, the interstitial cells
were observed to possess multilocular lipid drop-
lets which were partly fused, and had rough en-
doplasmic reticula, numerous free ribosomes,
microfilaments and a Golgi complex. Their nuclei
were often indented by lipid droplets in the cyto-
plasm. No basement membrane was observed on
the cell surface. The cells were often closely
associated with collagen fibers. The distribution,
morphology and reactions of the cells to vitamin
A administration in the anterior pituitary, thy-
roid gland and pancreas will now be described.

1. Anterior Pituitary

Cells with small lipid droplets and with a cen-
trally located nucleus were found in the connec-
tive tissue among endocrine cell masses (Fig. 1b).
Fluorescence microscopy revealed the occurrence
of vitamin A in the droplets of the cells (Fig. 1a).
However, these findings were conspicuous only in
the animals treated with a total dose of 400,000
and 800,000 I.U./kg vitamin A. The cells were
often located in the connective tissue space be-
tween the endocrine cells and endothelial cells of
the blood capillaries (Fig. 1c), sometimes with pe-
culiar cytoplasmic projections protruding into the
lumen (Fig. 1d).

2. Thyroid Gland

Cells with many lipid droplets emitting inten-
sive vitamin A autofluorescence occurred numer-
ously in the interfollicular connective tissue (Figs.
2a, b, c). Some cells extended their long slender
cytoplasmic processes containing the lipid drop-
lets along the basement membrane of the follicu-
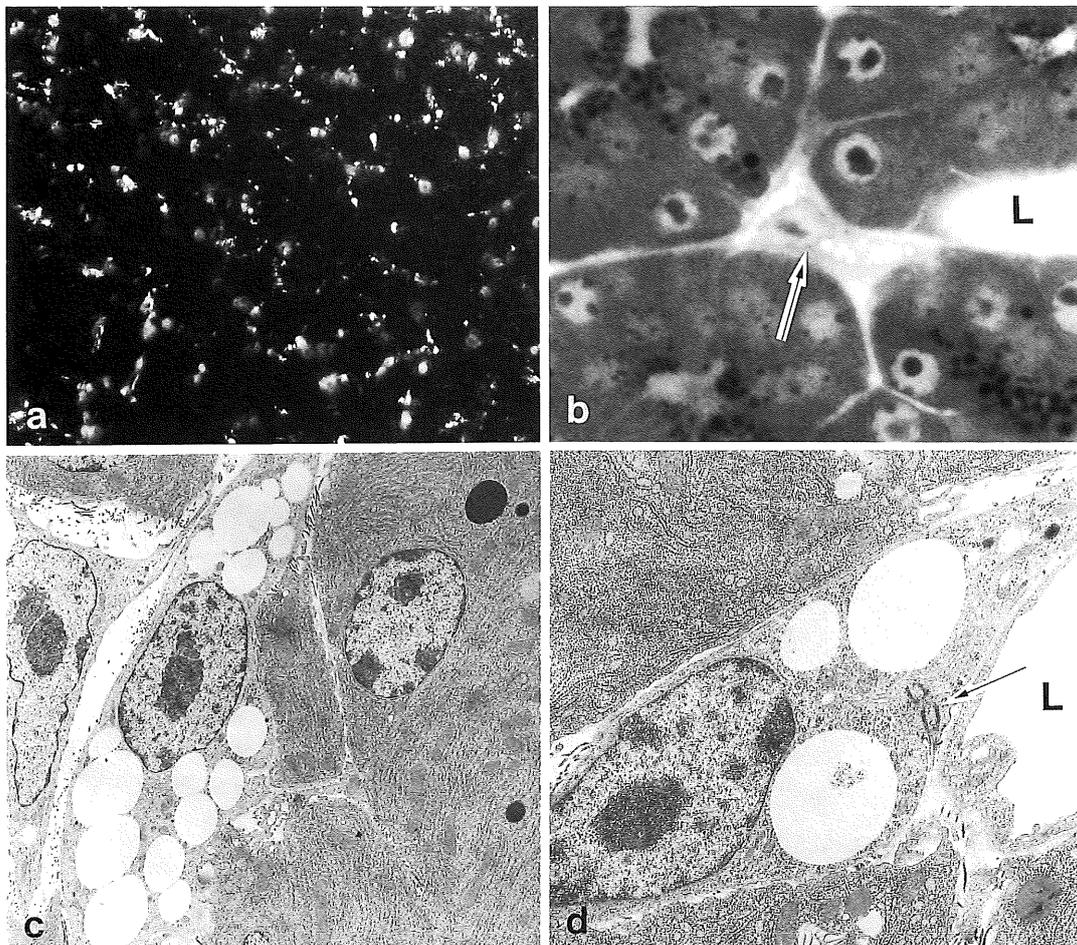
Fig. 3. Pancreas after vitamin A administration with a total dose of 800,000 I.U./kg.
Vitamin A uptake cells located in interacinar connective tissue emit vitamin A fluorescence intensively (**a**). The
cells containing numerous lipid droplets in the cytoplasm are shown in **b** (**arrow**) and **c**. **d** shows a vitamin
A uptake cell equipped with a single cilium (**arrow**), rarely encountered under an electron microscope. **L:** blood
capillary lumen, **a:** ×350, **b:** ×1,300, **c:** ×5,300, **d:** ×10,600

lar epithelial cells (Fig. 2d). These observations were possible even in the animals treated with a total dose of 200,000 I.U./kg vitamin A. The capacity of vitamin A uptake and storage in the cells of this organ was comparable to that of Ito cells in the liver[9,11].

3. Pancreas

Interstitial cells in the interacinar and interlobular connective tissue contained numerous lipid droplets in the cytoplasm emitting intensively the characteristic bright green fluorescence of vitamin A (Figs. 3a, c), which rapidly fades under ultraviolet light. The cells were detected clearly only in the animals treated with a total dose of 400,000 and 800,000 I.U./kg vitamin A. The cells were often located in the vicinity to blood capillary (Figs. 3b, d). Two of the cells examined exhibited one single cilium, though this is rarely encountered under an electron microscope (Fig. 3d). No vitamin A uptake cells were detected in the pancreatic islets.

DISCUSSION

Previously vitamin A research was focused primarily on preventing xerophthalmia and night blindness. During the past five to ten years, however, there has been a dramatic shift in emphasis[1]. The possibility was raised that vitamin A influences specific elements of the immune system against infectious disease and cancer. It was also suggested that vitamin A seems to have an antioxidant protection against oxidative free radical attack. Thus, at present, vitamin A is known as a micro-nutrient essential for immunity, cellular differentiation, maintenance of epithelial surfaces, growth, reproduction, and vision[1,12,18,19]. In relation to this information about vitamin A, it is important to elucidate the physiological functions of vitamin A uptake and its storing cells.

The studies by Yumoto (1982) and Komuro (1990) are worthy of special mention in this respect[8,26]. Yumoto described as the following: Retinyl palmitate (vitamin A) has surface-active and membranolytic effects on biological membranes, especially on lysosomal membranes. Ito cells, hepatic vitamin A uptake cells, selectively ingest exogenous, lipophilic and toxic substances such as vitamin A and Triton WR-1339, a nonionic detergent, which labilizes lysosomal membranes. A variety of carcinogenic or teratogenic substances have the action of labilizing the lysosomal membrane. Triton WR-1339 and excessive doses of vitamin A have teratogenic effects on the embryo. Ito cells may play a role in removing membranolytic, carcinogenic or teratogenic substances from the blood and are therefore a specialized type of phagocyte, distinct from leukocytes. If this hypothesis is true, the vitamin A uptake cells investigated in the present study

may reflect a special cellular component existing locally which defends each organ against toxic substances incorporated into our body, though hitherto the function of intoxication has been exclusively confined to the liver. The present results may suggest that a local defense system against toxic substances occurs in other tissues and organs than the liver and that vitamin A uptake cells play the main role in the system.

To re-evaluate fibroblasts and fibroblast-like cells, Komuro (1990) proposed categorizing the connective tissue cells into subtypes depending on their main functions: 1) fibrogenesis; 2) tissue skeleton on barrier; 3) intercellular communication system; 4) gentle contractile machinery; 5) endocrine activity; and 6) vitamin A-storing, adding that the differences in functional features among these cells are quantitative rather than qualitative, and that the degree of their changes are serial[8]. We were able here to provide data to clarify the high potential of the cells focussed on in the present work to uptake and store vitamin A. It appears that they normally conduct fibrogenesis as a chief function, but, in addition, exhibit another function of vitamin A uptake and storage when in hypervitaminosis A. In a physiological state, it was difficult to detect the vitamin A uptake cells in the above-mentioned endocrine organs. After excess vitamin A administration, the interstitial cells, corresponding to fibroblasts, demonstrated identical or closely related cytological features to Ito cells[21]. Therefore, "quiescent" fibroblasts with the capacity to uptake and store vitamin A occur normally in the endocrine organs and may be activated as typical vitamin A uptake cells when hepatic functions are impaired or overworked. On the other hand, another function of the cells may be proposed. It was postulated that Ito cells regulate blood flow by a possible contractile ability because of the localization of desmin in the cytoplasm[2,8,13,17,21,25]. Morphologically, other vitamin A uptake cells are often located in close association with the endothelium of blood capillaries. Therefore they may exert such an activity in each organ. No studies have so far been available on the occurrence of vitamin A uptake cells in the anterior pituitary and thyroid gland outside our preliminary reports[10,11]. On the other hand, there have been a few morphological studies on pancreatic vitamin A uptake cells[6,22]. Interestingly, the capacity in vitamin A uptake and storage of the cells examined in these studies seems to be very low considering the too high dosage of vitamin A, as far as we can judge from their descriptions and photographs. In contrast, avian vitamin A uptake cells in the pancreas demonstrated a high capacity to take up and store vitamin A, in spite of a lower dosage of vitamin A. Moreover, the cells were much more widely and numerously distributed in the inter-

acinar and interlobular connective tissue space, as shown in Fig. 3. These observations raise a simple question: What is the significance of species difference in these respects? We have no answer at the moment. More intensive and precise studies need to be made on avian species as model animals in the study of vitamin A uptake cells to elucidate the physiological functions, in consideration of the important roles of vitamin A as an anticancer agent and immunity enhancer.

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