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VITAMIN A: Physiological and Biochemical Processing

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INTRODUCTION

Knowledge about vitamin A (retinol) has advanced dramatically during the last few years. A major breakthrough came with the discovery of nuclear retinoid receptors that regulate gene expression by binding to short DNA sequences (retinoic acid–responsive elements) in the vicinity of target genes. Most of the extravisual functions of vitamin A seem to be mediated via these newly discovered receptors.

How does the body homeostatically regulate vitamin A, which plays such a critical role in vision and cellular growth and differentiation? The answer involves the following: (*a*) The liver, with its processing and storage of retinol, and several extracellular retinoid-binding proteins provide tissues with optimal amounts of retinol in spite of normal fluctuations in daily vitamin A intake. (*b*) A group of cellular retinoid-binding proteins and enzymes regulate intracellular metabolism and transport. And (*c*), the family of nuclear receptors mediates the ultimate actions of vitamin A in gene expression.

The aim of this article is to review the physiology and biochemistry of vitamin A with emphasis on the absorption, transport, cellular uptake, storage, and intracellular metabolism of vitamin A. Since several reviews of vitamin A metabolism have been published recently (5, 11, 12, 18, 99), here we provide an overview of the metabolism of vitamin A and highlight recent advances in the field. Another chapter in this volume discusses functional aspects of vitamin A, including the role of the nuclear retinoid receptors.

VITAMIN A INTAKE AND INTESTINAL ABSORPTION

Vitamin A is a term used for all compounds that exhibit the biological activity of retinol, whereas *retinoids* is a term that includes the natural forms of vitamin A as well as the many synthetic analogs of retinol, whether or not they have biological activity. The main dietary sources of vitamin A are provitamin A carotenoids from vegetables, preformed retinyl esters, and, to a lesser extent, retinol from animal sources.

Absorption of Carotenoids

Little quantitative data are available on the efficiency of intestinal absorption of provitamin A carotenoids (α-, β-, and γ-carotene and cryptoxanthin) and their conversion to retinol. Carotene absorption is by passive diffusion, and in humans, between 5 and 50% is absorbed (reviewed in Ref. 12). Absorption efficiency appears to be dependent on an adequate quantity of dietary fat. It is generally assumed that, in humans consuming a "normal" diet, one-sixth (on a weight basis) of dietary β-carotene and one-twelfth of other provitamin

carotenoids are absorbed and converted to retinol in the enterocytes (12). Notably in humans and some other species such as the ferret and preruminant calf, a significant fraction of the carotenoids is absorbed intact and transported via the lymph to other cells in the body.

The enzymatic mechanisms responsible for intestinal conversion of β-carotene to retinol have been a subject of recent controversy (23, 43, 59). Work by Dmitrovski (23) indicates that carotene may be cleaved both centrally, as originally reported by Goodman, Olson, and collaborators (37, 38, 73), and peripherally by two separate enzymes. The apo-carotinals formed by peripheral cleavage may subsequently be further processed to retinoic acid or to retinol. Retinal generated by central cleavage is presumably reduced to retinol by a reductase. In contrast to the earlier reports that this was accomplished by a cytosolic enzyme (73), recent work by Kakkad & Ong (58) suggests that retinal bound to the intestinal cellular retinol-binding protein type II [CRBP(II)] may be reduced by a membrane-bound microsomal enzyme. Clearly, the conversion of carotene to retinol in vivo should be further investigated.

Absorption of Retinyl Esters

Retinyl esters from the diet are hydrolyzed in the intestinal lumen by a pancreatic enzyme, and mixed micelles containing retinol deliver retinol to the enterocytes. Retinol in physiological concentrations is apparently absorbed by facilitated diffusion, whereas at pharmacological levels it can be absorbed by passive diffusion. Published data suggest that absorption of retinol is $< 75\%$, and it is apparently dependent on both quantity and quality of fat in the diet (reviewed in Ref. 12). More research is needed to determine what factors influence retinol absorption.

Esterification of Retinol in Enterocytes

Most of the retinol absorbed into the enterocytes leaves via the lymphatics as retinyl esters in chylomicrons (Figure 1). Two enzymes have been identified as being important for the esterification of retinol in enterocytes: acyl CoA:retinol acyltransferase (ARAT) (47, 48) and lecithin:retinol acyltransferase (LRAT) (66, 75). MacDonald & Ong found that retinol complexed to CRBP(II) was esterified by LRAT (66). In contrast, uncomplexed retinol in membranes may be esterified by ARAT. Blomhoff and co-workers suggested (12) that LRAT esterifies retinol during absorption of a "normal" load of retinol, and ARAT esterifies excess retinol when large doses are absorbed and CRBP(II) becomes saturated. Thus, CRBP(II) may play a critical role in the normal carrier-mediated absorption of retinol.

Recently, a retinoic acid responsive element (RARE) was detected in the promoter for CRBP(II) (69),
positively regulated by retinoic acid via the nuclear retinoid receptors. We

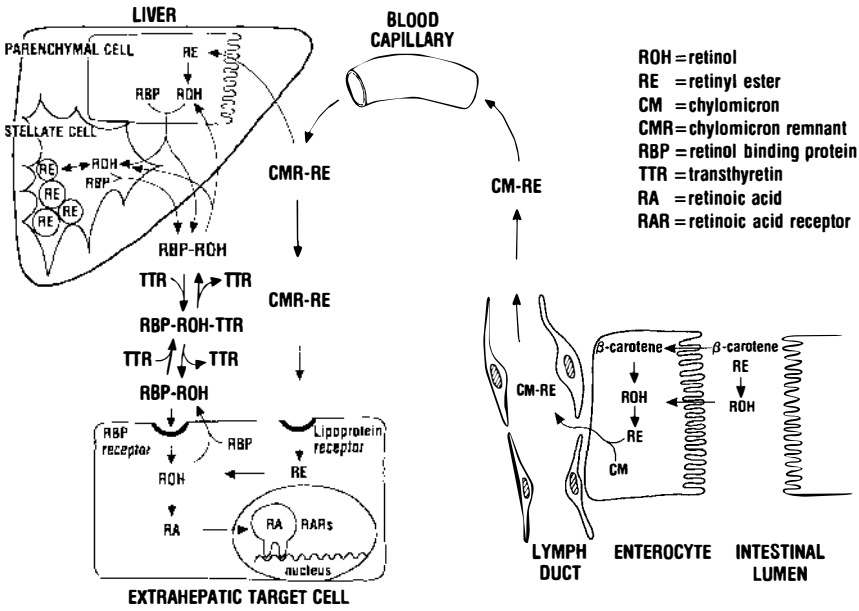

Figure 1 Schematic diagram of vitamin A absorption, transport, storage, and metabolism.

speculate that high doses of retinol in the diet may lead to an increased concentration of retinoic acid in enterocytes and an increased expression of CRBP(II).

TISSUE UPTAKE OF CHYLOMICRON REMNANT RETINYL ESTERS

In the circulation, hydrolysis of chylomicron triacylglycerols and several other processes are involved in formation of chylomicron remnants. Most retinyl esters present in the chylomicrons remain with the particle during conversion to chylomicron remnants (12).

Although chylomicron remnants are mainly cleared by the liver (see next section), extrahepatic uptake of remnants may be important in the delivery of retinol and carotenoids to extrahepatic tissues (Figure 1) such as adipose tissue, skeletal muscle, kidney, and carcass (13, 39). Recently, Hussain and coworkers reported that, although liver was the main site of chylomicron retinyl ester removal in all species examined, the bone marrow in rabbits and

marmoset monkeys and the spleen in rats, guinea pigs, and dogs were also important (56, 57). In light of the importance of retinoids for cell differentiation, chylomicrons may be an important transport complex for delivering retinol (and carotenoids) to tissues with intensive cell proliferation and differentiation such as bone marrow and spleen. We have recently shown that human myeloid leukemia cells take up retinyl esters from chylomicron remnants and that this uptake leads to both differentiation and decreased cell proliferation (17, 97).

HANDLING OF CHYLOMICRON REMNANT RETINYL ESTERS BY THE LIVER

Constituents of chylomicron remnants including retinyl esters are taken up by liver parenchymal cells (hepatocytes) via a process that appears to involve chylomicron sequestration in the space of Disse, further lipolytic processes, and then receptor-mediated uptake into the cells (67). Both the low density lipoprotein (LDL) receptor and the LDL receptor-related protein (LRP) can bind chylomicron remnants and may be involved in uptake (67). The quantitative role of these two receptors is being debated.

Once chylomicron remnant retinyl esters have been taken up by liver parenchymal cells, their observed rapid hydrolysis may be catalyzed by a plasma membrane retinyl ester hydrolase recently described by Harrison & Gad (46). Subcellular fractionation (10) and autoradiographic studies (52) suggest that chylomicron vitamin A is rapidly transferred to endosomes and then to the endoplasmic reticulum. Retinol binds to plasma retinol-binding protein (RBP), presumably in the endoplasmic reticulum, and RBP-retinol is then transferred to Golgi for secretion (5). Secretion is influenced by vitamin A status: in vitamin A–deficient animals, secretion is reduced such that plasma levels of RBP and retinol are decreased. Vitamin A repletion leads to an immediate increase in secretion of RBP-retinol from the liver (reviewed in Ref. 5).

PARACRINE TRANSFER OF RETINOL TO STELLATE CELLS IN THE LIVER

In vitamin A–sufficient rats, most of the chylomicron remnant retinyl esters taken up by hepatocytes appear to be rapidly (within 2–4 hours) transferred as retinol to perisinusoidal stellate cells in the liver (13, 14) (Figure 1). A plausible candidate for a protein carrier that mediates the transfer is RBP, since stellate cells can take up the RBP-retinol complex (15, 36) and hepatocytes secrete retinol bound to RBP (5). The transfer of retinol from hepatocytes to stellate cells is too rapid to be accounted for by a secretion of RBP-retinol from hepatocytes to the general circulation, followed by retinol

being taken up by liver stellate cells. During an in situ perfusion of rat livers, we observed that labeled retinol was transferred from hepatocytes to stellate cells. Furthermore, addition of antibodies against RBP blocked the transfer, indicating that RBP was the transport protein mediating the transfer of retinol from hepatocytes to stellate cells (9).

STORAGE OF RETINYL ESTERS IN STELLATE CELLS

In mammals, 50–80% of the body's total retinol (retinol plus retinyl esters) is normally present in the liver (18). Under most conditions, stellate cells contain about 90% of the liver total retinol (4, 6, 16, 53); the rest is in hepatocytes. Ninety-eight percent of the stellate cell vitamin A is present in the form of retinyl esters. The normal reserve of vitamin A in stellate cells is adequate to last for several months. Only when retinol concentration in the liver is very low ($<$ 1–5 nmol/g tissue) does the proportion of liver vitamin A in hepatocytes become appreciable (3, 8).

Older morphological studies by Wake (95, 96) were important in showing that stellate cells in the liver have the ability to store large amounts of retinyl esters in lipid droplets. The size and number of these droplets is dependent on the vitamin A status of the animal. Chemical analysis of lipid droplets isolated from rat liver stellate cells revealed that between 12 and 65% of the total lipid mass is retinyl esters, depending on the vitamin A status of the animal. Triacylglycerols compose between 35 and 50% of the lipid mass (72).

A small, acute load of retinol does not accumulate in liver stellate cells of vitamin A–depleted rats (13). This observation may be related to the amount of cellular retinol-binding protein type I [CRBP(I)] in these cells. Rats fed normal levels of vitamin A have large amounts of CRBP(I) in stellate cells (16). Because CRBP(I), like intestinal CRBP(II), is an effective donor of retinol for esterification by LRAT (76, 101), reduced levels of CRBP(I) in stellate cells of vitamin A–deficient rats may account for the reduced accumulation of retinyl esters.

Several reports have shown that addition of retinol or retinoic acid to different cell types results in induction of CRBP(I) (44, 98). In addition, Smith et al (88) recently showed that a retinoic acid responsive element is present in the CRBP(I) promoter, which suggests that control of CRBP(I) gene transcription by retinoic acid and retinoic acid receptors may represent a positive feedback mechanism that is important in regulating cellular uptake of retinol and retinyl ester storage.

RETINOL MOBILIZATION FROM STELLATE CELLS

Theoretically, retinol could be secreted by stellate cells directly to the general circulation or it might first be transferred to hepatocytes. Since cultured

hepatocytes have been shown to synthesize and secrete RBP, it has been assumed that hepatocytes are the exclusive site of retinol mobilization from the liver (5). As discussed below, data now available suggest that a direct mobilization of retinol from stellate cells to the general circulation occurs.

Although there is some disagreement in the literature about how much RBP stellate cells contain and whether they synthesize RBP, these cells apparently do contain the RBP necessary for exporting the vitamin (6, 51, 70). We (1) recently demonstrated that stellate cells contain mRNA for RBP, whereas Yamada et al (100) were unable to detect mRNA for RBP in stellate cells. We have also found that when stellate cells are cultured in a serum-free medium they secrete RBP-retinol. An alternative route of mobilization may also take place: Recent data suggest that apoRBP can bind to stellate cells, make a complex with stellate cell retinol, and then be released as RBP-retinol to the circulation (1). Since most of the liver RBP is found in parenchymal cells (5), one can speculate that apoRBP secreted by these cells may bind to stellate cells and mobilize retinol to the circulation (Figure 1).

An independent line of evidence supporting the possibility that stellate cells secrete RBP-retinol directly to the blood comes from a whole-body multicompartmental model of retinol dynamics in rats (M. H. Green et al, unpublished). The model suggests that stellate cells are an important site of retinol secretion into blood. The model predicted that the stellate cell retinol pool that is responsible for the secretion was small and rapidly turning over; this prediction is compatible with the relatively small amounts of RBP observed in stellate cells.

The ability of stellate cells to control storage and mobilization of retinol ensures that the blood plasma retinol concentration is close to 2 μM in spite of normal fluctuations in daily vitamin A intake. It is likely that the retinoid-regulated CRBP(I) expression, in addition to saturation of CRBP(I) and RBP by retinol, controls retinol uptake, storage, and mobilization by stellate cells.

TURNOVER AND RECYCLING OF PLASMA RETINOL

Until a decade ago, it was assumed that, once retinol left the blood plasma, it was taken up by tissues and irreversibly utilized. However, earlier work by Vahlquist (94) indicated that retinol may be cycled to the blood. This idea has recently been verified in rats (40–42, 62, 63). In fact, it is now thought that the majority of retinol that leaves the plasma is recycled, since the plasma retinol turnover rate is more than an order of magnitude greater than the utilization rate (40–42, 62). Thus, in the rat, an average retinol molecule recycles to the plasma 7–13 times before irreversible utilization, and the whole-body vitamin A utilization rate is only about 10% of the plasma retinol turnover rate. Data from tracer kinetic experiments in the rat (42) indicate that the vehicle for retinol recycling is RBP. In rats with normal versus marginal

versus nearly depleted liver vitamin A levels, kinetic studies indicate that the recycling time for retinol averages 8.4 day (41), 1.7–2.0 day (41, 42), and 0.6–0.7 day (41, 62), respectively. That is, once retinol leaves the plasma, it may take a week or more to recycle to the plasma in a normal rat. Most of this time would presumably be spent in retinyl ester pools.

In the rat, it has been estimated that ~50% of plasma retinol turnover is to kidneys (40, 62), ~20% is to liver, and the remaining 30% is to extrahepatic/extrarenal tissues. The source of RBP for retinol recycling from the kidneys and other extrahepatic tissues is not yet known. It is interesting in this regard that many extrahepatic tissues, including the kidneys, contain mRNA for RBP (68, 89). Notably, Makover et al (68) reported that adipose tissue contains relatively high concentrations of RBP mRNA. The function of adipose tissue in retinol recycling remains to be determined.

Plasma retinol turnover time, or mean transit time, is defined as the mean of the distribution of times that retinol molecules remain in plasma before leaving plasma reversibly or irreversibly. It is thus the time it takes for a mass equivalent to the plasma pool to turn over. For rats, kinetic data have been used to estimate a mean transit time of 1–3.5 hr (41, 42, 62). That is, an average retinol molecule spends 1–3.5 hr in the blood plasma before leaving the circulation. This retinol has several possible fates once it enters the interstitial spaces: Either it can be taken up by cells reversibly or irreversibly, or it can cycle to the blood from the interstitium. Based on equilibrium distributions and the mass distribution of unbound retinol, RBP-retinol, and TTR-RBP-retinol (27, 72), ~95.5% of plasma retinol is present as TTR-RBP-retinol, ~4.4% as RBP-retinol, and ~0.14% as unbound retinol. Using these distributions and considering molecular weights (286 for retinol, 21,286 for RBP-retinol, and 76,266 for TTR-RBP-retinol) and capillary permeabilities, we estimate that the vast majority of retinol leaves the circulation as RBP-retinol. TTR-RBP-retinol would leave the circulation too slowly (transit time, >18 hr), and there is too little unbound retinol (in spite of a high filtration fraction into tissues) to account for the high rates of plasma retinol turnover observed in kinetic studies (41, 42, 62). This conclusion was also drawn by Fex & Felding (27).

PLASMA RETINOL HOMEOSTASIS

In normal humans and experimental animals such as the rat, plasma retinol concentrations are maintained within a fairly narrow range despite wide fluctuations in dietary vitamin A intake. Thus it appears that plasma retinol levels are homeostatically regulated, unlike plasma retinyl esters. The control of plasma retinol is not yet well understood. It may be that the controlled variable is the pool of retinol that equilibrates between plasma RBP-retinol

(free or bound to TTR), interstitial fluid RBP-retinol, and intracellular CRBP(I)-retinol. Thus, since liver stellate cells represent the main storage site of vitamin A in the body, it is likely that regulation of stellate cell CRBP(I) expression is one of many factors involved in regulation of plasma retinol homeostasis (reviewed in Ref. 12).

Control can also be mediated in many tissues by the enzymes that esterify retinol and hydrolyze retinyl esters. See Figure 2 for a schematic representation of retinol metabolism in stellate cells (or other cells which store retinol). Note the numerous transport proteins (e.g. RBP, RBP receptor, and CRBP) and enzymes [e.g. LRAT and retinyl ester hydrolase(s)] that are potentially involved in retinol homeostasis.

Control of plasma retinol is presumably also mediated by factors that affect the balance between retinol input to plasma and retinol output from plasma. Under normal conditions, output seems to be determined mainly by physical considerations (i.e. the equilibrium distribution of free retinol, RBP-retinol, TTR-RBP-retinol, and capillary permeabilities) (reviewed in Ref. 12). On the other hand, input determinants appear to be more complex. Both apoRBP and retinol (either free or more likely transferred through membranes via RBP receptors from CRBP-retinol) must be present for RBP to bind retinol and then enter the circulation.

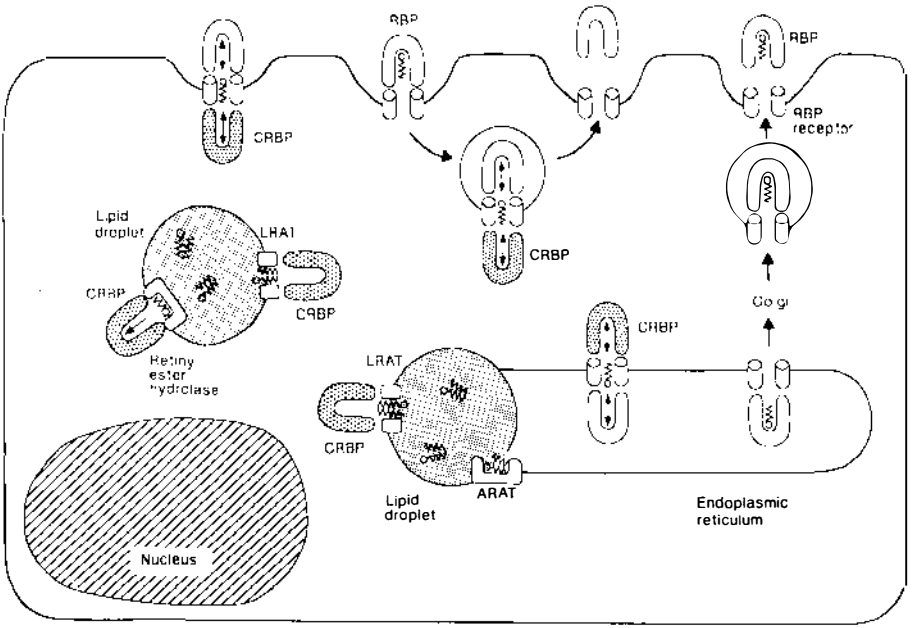

Figure 2 Hypothetical schematic diagram of retinol transport and storage in liver stellate cells.

To date, two organs have been assumed to play key roles in plasma retinol homeostasis: the liver and the kidneys. Following experiments in which retinoic acid was fed to vitamin A–depleted rats in order to spare retinol utilization, Underwood et al (93) hypothesized that vitamin A utilization resulted in the return of a "metabolite signal" to the liver and that this metabolite decreased hepatic secretion of retinol and thus plasma retinol concentrations. Recently, Gerlach & Zile (33–35) used rats with experimentally induced acute renal failure to investigate the role of the kidneys in plasma retinol homeostasis. Following nephrectomy or bilateral ligation of the renal arteries, these authors found that the plasma levels of retinol and RBP increased significantly compared with sham-operated controls. These findings are similar to observations in humans with chronic renal failure (87, 92). Gerlach & Zile concluded that the kidneys either remove or provide a specific regulatory signal to the liver that alters secretion of RBP-retinol.

Using a whole-body compartmental model of retinol kinetics in vitamin A–depleted rats (62), one can show that similar increases in plasma retinol levels would occur if the model is simulated with no output of plasma retinol to the kidneys. According to this model simulation, plasma retinol levels would increase to 41% above normal by 5 hr.

Above we noted that the controlled variable for plasma retinol homeostasis may be the pool of retinol that equilibrates between plasma TTR-RBP-retinol, RBP-retinol, interstitial fluid RBP-retinol, and intracellular CRBP-retinol (mainly stellate cell CRBP-retinol). The observation by Gerlach & Zile (34), that the injection of apoRBP into rats with bilateral ligation of the renal arteries resulted in an increase in plasma retinol, is consistent with this idea. Further studies are needed to advance our understanding of the role of various organs in plasma retinol homeostasis.

INTERCELLULAR TRANSPORT OF VITAMIN A IN VARIOUS TISSUES

Retinol-binding protein is the only retinoid-specific binding protein that has been found in plasma. However, many retinoid-binding proteins that are not found in plasma have been isolated from interstitial or other body fluids; these probably function locally to transport retinoids. An inter-photoreceptor retinoid-binding protein (IRBP) (99) has been identified in the extracellular space between the retinal pigment epithelial cells and the photoreceptor cells, where it constitutes 70% of the soluble protein. IRBP not only binds retinol and retinal but also vitamin E, fatty acids, and cholesterol. It may participate in the intercellular transport of retinoids during the visual cycle (30). Interestingly, the pineal gland, a primitive photosensitive organ, also contains IRBP (99).

Tear fluid contains retinol bound to a protein related to or identical to RBP (54). This RBP-retinol is probably a source of vitamin A for the ocular epithelia, which has an absolute requirement for vitamin A.

Several retinol-binding proteins related to but distinct from plasma RBP have been found to be secreted by the pig uterus (21). It has been suggested that RBP may carry retinol to the placenta. It has also been demonstrated that placental membranes in many species produce RBP-like proteins (64) and that the pig, sheep, and cat conceptus secrete RBP (45). The conceptus RBP is probably identical to plasma RBP. Since vitamin A is known to play a critical role in embryo development, the many RBP-like molecules produced by the conceptus and the surrounding tissues likely ensure that an optimal amount of retinol is delivered to the embryo.

Recently, Ong & Chytil observed that two proteins called epididymal-binding protein 1 and 2 (EBP1 and 2), which are secreted into the lumen of the first portion of the epididymis, were carrier proteins for retinoic acid (74). They suggested that the proteins deliver retinoic acid to sperm where it functions in sperm maturation.

CELLULAR UPTAKE OF RETINOL

The mechanism by which retinol is transferred from RBP to the plasma membrane of cells is not yet fully understood. Since a small amount of unbound retinol appears to be in equilibrium with RBP-retinol, this retinol would be available for cellular uptake by a nonspecific mechanism without involvement of membrane RBP receptors. Recent findings (28, 29, 72) suggest some nonspecific transfer of retinol from RBP-retinol to cell membranes in vivo. However, a number of other observations discussed below indicate that nonspecific partitioning is not likely to be the primary mechanism by which cells obtain retinol.

Retinal Pigment Epithelial Cells

In an autoradiographic study, Heller & Bok (50) reported that ^{125}I-RBP bound only to the choroidal surface of retinal pigment epithelial (RPE) cells. The binding was greatly inhibited by the presence of a 600-fold molar excess of unlabeled RBP. Binding to photoreceptor cells or other retinal cells was not observed. In another paper, Heller (49) presented evidence for the saturable binding of RBP to isolated RPE cells. The binding of ^{125}I-RBP was rapid (complete in about 1 min), and the level of cell-associated radioactivity was 7-fold higher at 22° than at 0°C. Since he found that unlabeled RBP-retinol could displace the cell-associated ^{125}I-RBP-retinol within 3 min, Heller concluded that RBP was not internalized by these cells.

Ottonello et al (77) more recently have shown that retinol released from

RBP after binding to RPE cells became associated with a protein of molecular weight 16,000, and they suggested that the protein might be CRBP(I). Their data indicate that a "functional link" exists between the uptake of retinol and retinol esterification, since they observed a fourfold reduction in total vitamin A incorporated when retinyl ester formation was inhibited. Since CRBP(I) seems to be involved, the enzyme responsible for the observed esterification is likely to be lecithin:retinol acyltransferase (LRAT).

Researchers in Peterson's laboratory have attempted to characterize the RBP receptor in bovine RPE cells (4a). Radiolabeled RBP was cross-linked with RPE membranes, and complexes were analyzed by electrophoresis and autoradiography. From such studies, the receptor was assumed to have a molecular weight of 63,000 (4a).

Hepatocytes and Liver Stellate Cells

That hepatocytes and stellate cells can take up both retinol and RBP from the plasma RBP-retinol transport complex in vivo has been demonstrated (15, 36). We have also applied model-based compartmental analysis to data on the kinetics of plasma [^{3}H]retinol-RBP-TTR in rats (M. H. Green et al, unpublished results). This model predicted that about half of the plasma retinol that cycled to the liver was taken up by hepatocytes and half by stellate cells.

A selective uptake of RBP by liver parenchymal and stellate cells was observed in a study by Senoo et al (82). Iodinated RBP and other ligands were injected intravenously into rats, and the uptake by liver cells was determined. They found, for example, that acetyl-LDL was recovered primarily in endothelial cells and asialoorosomucoid was recovered mainly in hepatocytes. RBP was the only protein that had appreciable recovery in both parenchymal and stellate cells.

These data agree with immunocytochemical results obtained by Senoo et al (82), in which human RBP was injected intravenously into rats and traced in cryosections of liver with gold-labeled antibodies. RBP was shown to be taken up by liver parenchymal and stellate cells but not by Kupffer cells or endothelial cells. At early times after injection, RBP was localized on the surface of liver parenchymal and stellate cells, whereas at later times it was also located in vesicles deeper in the cytoplasm.

Blood-Testis and Blood-Brain Barriers

Sertoli cells form the blood-testis barrier surrounding maturing spermatocytes. Since spermatogenesis is dependent on retinol, one would expect that Sertoli cells express RBP receptors. A saturable and specific uptake of [^{3}H]retinol-RBP was in fact recently reported by Shingleton et al (84). Since iodinated RBP was not taken up by the cells, the authors concluded that Sertoli cells take up retinol from RBP-retinol via an RBP receptor, but that

RBP is not internalized. The data also suggest that retinol taken up by the cells combines with CRBP(I) and is further processed to retinyl esters (83).

Related results were reported by MacDonald et al, who used autoradiography to study the uptake of intravenously injected ^{125}I-RBP by rat brain (65). ^{125}I-RBP was localized along the basolateral surface and inside the choroidal epithelial cells. Interestingly, high concentrations of CRBP(I) were also found in these cells. The data suggest RBP receptor-mediated uptake of retinol by the cells that constitute the blood-brain barrier and apparent further processing of retinol by CRBP(I).

Placental Brush Border Membranes

Retinol that is necessary for the developing fetus has to cross the placenta, which suggests that an RBP receptor may also be present in this tissue. Recently, Sivaprasadarao & Findlay studied binding of RBP-retinol to human placental brush border membranes (85, 86). The data are compatible with the presence of a specific receptor for RBP. Scatchard analysis of the equilibrium binding of ^{125}I-RBP revealed both high (3×10^{-9} M) and low (9×10^{-8} M) affinity binding components. The authors speculated that the higher affinity form might be converted to the lower affinity state after RBP bound and transferred its retinol or that apoRBP may have a lower affinity than RBP-retinol for the receptor.

Keratinocytes

Some investigators have not found binding of RBP to these cells. For example, in two studies (22, 55) no binding of ^{125}I-RBP to keratinocytes could be demonstrated. This is interesting in light of the fact that this cell is a classical target for retinol. Possibly some cell types express the RBP receptor while others do not.

INTRACELLULAR METABOLISM OF RETINOL

Until a few years ago, the role of the many cellular retinoid-binding proteins in intracellular retinol metabolism was neglected. Data now being presented, mainly from the laboratory of Ong and colleagues, indicate that the binding proteins direct retinoids to specific enzymes. That is, when retinoids complexed with binding proteins are added to cell homogenates, the retinoids are metabolized by other enzymes than if they are added when dissolved in an organic solvent. Three main processes are involved in the intracellular metabolism of retinol. (*a*) Retinol may be temporarily stored after conversion to retinyl esters. (*b*) Retinol may be converted to an active metabolite such as retinoic acid or retinal. (*c*) The retinol (or retinoic acid) molecule may be catabolized to a form that is excreted from the body.

Esterification of Retinol

LRAT seems to be the main intestinal enzyme esterifying retinol under normal conditions. LRAT was recently identified in liver stellate cells (7), and the high level of CRBP(I) in stellate cells points to an important role of LRAT in stellate cell retinol esterification. The product of the LRAT reaction in enterocytes is incorporated into chylomicrons while the product in liver stellate cells is instead diverted to intracellular lipid droplets. The reason for this difference is not understood, but it may be related to the different carrier proteins (CRBP(II) versus CRBP(I)).

LRAT activity (per mg protein) in the retinal pigment epithelial (RPE) cells is apparently about 1000 times higher than the activity in intestine and liver (81). Interestingly, the esterification of all-*trans* retinol by LRAT in RPE is linked to the direct conversion of all-*trans* retinyl esters to 11-*cis* retinol by an isomerase-like enzyme (20). The latter reaction couples the free energy of hydrolysis of an ester to the thermodynamically uphill trans to cis isomerization, thus providing the energy to drive the process. It will be important to determine whether LRAT in ocular cells is identical to LRAT in other cells.

Retinol is esterified in lactating mammary gland and transported as retinyl esters in milk lipid droplets to the developing organism. In this process, ARAT seems to be the most important enzyme for esterification based on the following reasoning. Randolph et al (80) showed that mammary gland contains lower levels of LRAT activity and CRBP(I) than does liver. In contrast, ARAT displayed a similar Vmax but a lower Km in mammary gland than in liver. Thus at physiological concentrations of retinol, esterification in mammary gland appears to occur predominantly by ARAT.

A gradient of retinyl esters exists in the human epidermis, with the highest concentration in the upper epidermal layer. Also in human epidermis, ARAT seems to be the predominant enzyme involved in retinol esterification (91). ARAT in keratinocytes has a more acidic pH optimum than ARAT in other tissues. The pH gradient that exists in the epidermis may thus be important for facilitating retinol esterification in the upper epidermis (91).

Activation of Retinol

It is generally assumed that 11-*cis* retinal covalently bound to opsin proteins and all-*trans* retinoic acid noncovalently bound to nuclear retinoic acid receptors (RARs) are the active retinoids in vision and in regulation of transcription, respectively. Recently, however, it was demonstrated that 9-*cis* retinoic acid binds and activates the three nuclear retinoid X receptors (RXRs), which also are ligand-dependent transcription factors (54a, 61a). Thus, retinoid isomerization may be important not only in vision but also in regulation of transcription.

Furthermore, it has been suggested that retinoic acid cannot substitute for all effects of retinol in growth regulation. Retinol is metabolized by many

cells to 14-hydroxy-4,14-*retro*-retinol, and this compound may be the mediator of these effects (19). Many other retinoids, such as 13-*cis* retinoic acid, the retinoyl glucuronides, 4-oxo and 4-hydroxy retinol and retinoic acid, may also be important for retinoid function or as intermediate products in retinoid catabolism (32). In human epidermis and in the developing chick wing bud, 3,4-didehydro-retinol, 3,4-didehydro-retinyl esters, and 3,4-didehydro-retinoic acid comprise a large proportion of the endogenous retinoids normally present (90, 91). Retinoic acid and 3,4-didehydro-retinoic acid are equipotent in evoking digit duplication in the developing wing bud (90). Much more work is needed to clarify the possible role of these metabolites in vitamin A function.

The metabolic pathway whereby retinoic acid is synthesized in situ is poorly understood. There has been a tendency to assume that retinoic acid synthesis occurs by two distinct steps, including dehydrogenation of retinol to retinal by alcohol dehydrogenases, and a further oxidation of retinal to retinoic acid. Recently, retinoic acid was shown to regulate the human alcohol dehydrogenase gene for ADH3 directly via a retinoic acid responsive element (24). This observation was thought to indicate that ADH3 may play a regulatory role in retinoic acid synthesis. Duester et al suggested that retinoic acid activation of ADH3 constitutes a positive feedback loop regulating retinoic acid synthesis (24). Physiologically, however, such a positive regulatory loop for retinoic acid synthesis may be unlikely, since large concentrations of retinoic acid are very toxic to cells.

Although several types of dehydrogenases convert retinol to retinoic acid in vitro, it is unlikely that all are physiologically important. Since it was demonstrated that retinoic acid could be synthesized using cytosol from an alcohol dehydrogenase-negative strain of deermouse, some researchers suggest that retinol and ethanol oxidation are mediated by different enzymes (60, 78).

In vivo, most intracellular retinol is bound to cellular binding proteins, and these binding proteins may be directly involved in delivering retinol to the proper enzyme in a manner similar to that discussed above for retinol esterification. In fact, recent data of Posch et al (79) suggest that retinal synthesis is supported by retinol-CRBP(I) directly rather than by unbound retinol. Furthermore, neither ethanol nor ketoconazole inhibited retinal formation from retinol-CRBP(I), which suggests that ethanol-oxidizing enzymes and cytochrome P-450 isozymes were not involved in retinoic acid biogenesis.

Other recent data indicate that retinoic acid may also be synthesized from β-carotene in organs such as intestine, liver, kidney, and lung (71). Thus, β-carotene may be a source of retinoic acid in retinoid target cells, particularly in species such as humans that are capable of accumulating high concentrations of tissue carotenoids. Also, the recent demonstration that β-

carotene dosing increases retinoic acid levels in rabbit serum is consistent with this idea (31).

Catabolism of Retinol

Several investigators have studied the catabolism of retinol by analyzing radioactive urinary, biliary, and fecal metabolites of retinol. A number of more polar metabolites are formed, and some of them have been identified (32). Rat liver microsomes may oxidize retinol to 4-hydroxy retinol and 4-oxo retinol, metabolites that are found in vivo. The cytochrome P-450 system seems to be involved in this conversion (61). Also glucuronides may be formed from retinol that is probably destined for excretion in bile and urine (2). Most of the catabolism of retinol, however, probably involves the production of retinoic acid as an intermediate. Once formed, retinoic acid cannot be converted to retinal or retinol. The catabolism of retinoic acid probably involves conjugation to retinoyl β-glucuronide and taurine, decarboxylation, oxidation at the 4 position of the cyclohexenyl ring, epoxidation, isomerization, and esterification (32).

A number of retinoids in addition to retinol and retinyl esters are present in plasma in nanomolar concentrations. These include all-*trans* retinoic acid, 13-*cis* retinoic acid, 13-*cis*-4-oxoretinoic acid, and all-*trans* retinoyl β-glucuronide (2, 25, 26, 32). The level of most of these retinoids is dependent on the intake of vitamin A and will typically increase 2–4 times after ingestion of a large amount of vitamin A (25, 26). Whether these retinoids simply reflect retinoid catabolism or whether they have a physiological role in vitamin A action is not known. However, since most of these retinoids are active in many in vitro systems in nanomolar concentrations, one should not exclude the possibility of a functional role for these plasma retinoids.

CONCLUDING REMARKS

Although advances have been made recently in our knowledge of vitamin A metabolism and function, many important topics are left for future study. For example, the absorption and metabolism of carotenoids, the mechanisms for and control of uptake of retinoids by cells, and the in situ synthesis and regulation of retinoic acid need to be studied more extensively. Although a number of proteins involved in retinol metabolism and function have been identified and their genes cloned during the last few years, many others that may be involved have not yet been cloned or characterized. Preliminary observations suggest that many other RBP-, CRBP-, and CRABP-like proteins will be discovered. Also, none of the enzymes involved in retinol metabolism, such as LRAT and ARAT, have been well characterized or cloned. In a few years, when the genes for a larger number of the proteins involved in vitamin A metabolism have been cloned and the proteins well-

characterized, an important scientific challenge will be to try to understand how these proteins work and how they are regulated. Unraveling the secrets of vitamin A transport, storage, metabolism, and action will likely continue to provide us with worthwhile and stimulating research topics for many years to come.

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