3. Metabolism, kinetics, tissue distribution, and inter- and intra-species variation

Much of our knowledge about metabolism and function of vitamin A has been derived from experimental studies with animals, particularly rats (see Section 3.2). In this handbook, recent research studies and current reviews are emphasized. The older literature can be accessed via the reviews cited.

3.1 Human studies

3.1.1 Introduction

Vitamin A is an essential nutrient for humans and other vertebrates (Blomhoff, 1994a; Olson, 1994b; Ross, 1998). Carotenoids that contain at least one unsubstituted β-ionone ring serve as precursors of vitamin A (Blomhoff, 1994; Olson, 1994b; Olson, 1998; Ross, 1998).

Vitamin A is well absorbed by the intestine, transported from the intestine to the liver and other organs in chylomicra, and stored largely in the liver as retinyl esters. Vitamin A functions in vision, cell differentiation, and embryonic development. Many other physiological processes, including growth, reproduction and the immune response, are dependent upon these cellular functions of vitamin A.

3.1.2 Absorption

Preformed vitamin A is present mainly as retinyl esters in the diet. In the small intestine, the esters are hydrolysed by esterases in pancreatic juice and in the brush border of intestinal mucosal cells. Free retinol is incorporated into lipid micelles in the gut lumen and taken up by absorptive epithelial cells of the intestine (Blomhoff, 1994a; Olson, 1994b; Ong, 1994; Ross, 1998). A specific transporter for retinol, identified in rats, may also exist in human intestinal cells (Ong, 1994). Within intestinal cells, retinol, probably as a complex with cellular retinol-binding protein type II (CRBP-II), is re-esterified by transfer of a fatty acid from the α-position of phosphatidyl choline (Ong, 1994). Vitamin A, mainly as retinyl ester, is then incorporated into chylomicra and released into the lymph (Blomhoff, 1994a; Olson, 1994b; Ross, 1998). By analogy with other species, provitamin A carotenoids are mainly cleaved oxidatively to one or two molecules of retinal, which is then enzymically reduced to retinol in intestinal cells (Devery & Milborrow, 1994; Nagao *et al.*, 1996; Olson, 1998). A minor portion of provitamin A carotenoids can be oxidized asymmetrically to β-apocarotenals, by both chemical and enzymatic routes (Krinsky *et al.*, 1993). Under conventional dietary conditions, most absorbed β-carotene is cleaved to vitamin A in the upper part of the human intestine (Parker, 1996), although the liver and other organs also possess this capacity. As β-carotene intake increases, the efficiency of absorption is reduced.

The absorption of preformed vitamin A in healthy humans is quite efficient (60–90%) in the presence of an adequate amount of dietary fat (Blomhoff, 1994a; Olson, 1994b; Ross, 1998). The efficiency of intestinal absorption of retinyl esters from supplement preparations, however, depends on the matrix or carrier present, the rate of release of vitamin A from the preparation, and the types and amounts of solubilizers and stabilizers. The daily dose of retinyl ester that induces signs of toxicity also depends on the nature of the formulation. Intestinal infections reduce the absorption efficiency to only a minor extent (Tanumihardjo *et al.*, 1996). In the absence of bile, as in cholestasis and related disorders, however, vitamin A absorption is markedly reduced (Amédée-Manesme *et al.*, 1988).

Even at doses of vitamin A as high as 200 000 IU (60 mg) in oil, the absorption efficiency is over 50% in healthy humans (Sommer & West, 1996), in contrast to many other nutrients. Indeed, such doses prevent the appearance of signs of vitamin A deficiency in vitamin A-depleted children for periods of up to six months (Underwood & Arthur, 1996).

3.1.3 Transport

Apart from chylomicra, vitamin A is transported in human plasma primarily as all-*trans*-retinol on a specific binding protein, retinol-binding protein (RBP) (Goodman, 1984; Blomhoff, 1994a; Olson, 1994b; Soprano & Blaner, 1994; Ross, 1998). The amino acid sequence and three-dimensional structure of human RBP are known (Goodman, 1984; Soprano & Blaner, 1994; Newcomer, 1995). Its molecular weight is 21 kDa, and it contains a β-barrel as an internal binding-pocket for retinol. RBP is synthesized primarily in parenchymal cells of the liver but also in other organs (Blomhoff, 1994a; Olson, 1994b; Soprano & Blaner, 1994; Ross, 1998). The plasma concentration of the 1:1 retinol–RBP complex (holo-RBP) is finely regulated. As a consequence, liver reserves of vitamin A can vary from relatively high (1 μmol/g) to relatively low (0.07 μmol/g) concentrations without an appreciable change in the plasma concentration (Blomhoff, 1994a; Olson, 1994b; Ross, 1998). The regulatory mechanism, although not fully clarified, seems to depend on three factors: (*a*) the rate of release of retinol from stored retinyl ester in liver cells, (*b*) the release of holo-RBP from liver cells, and

(*c*) feedback inhibition of the releasing mechanisms by retinoic acid and other products of metabolism (Blomhoff, 1994a; Olson, 1994b; Ross, 1998). Thus, a dose of retinoic acid lowers plasma retinol level markedly, but in a transient manner, in humans (Barua *et al.*, 1997). Furthermore, an analogue, *N*-(4-hydroxy-phenyl)retinamide (4-HPR), which is being studied as a preventive drug for breast cancer and other cancers, inhibits the release of holo-RBP from liver cells and, as a consequence, markedly lowers plasma retinol in humans (Formelli *et al.*, 1996). In the plasma, holo-RBP is present as a 1:1 complex with tetrameric transthyretin, the binding protein for thyroxine (Blomhoff, 1994a; Olson, 1994b; Ross, 1998).

The plasma concentration of retinol in young children is approximately 60% of that in adults. The level increases during adolescence, however, to reach adult values (Pilch, 1985). Mean values for a healthy population are given in Table 1. Adult males show 12–18% higher values than adult females (Pilch, 1985; Gregory *et al.*, 1990). Steady-state plasma concentrations of retinol may well be affected by genetic factors as well.

Other forms of vitamin A found in human plasma include all-*trans*-retinyl ester, all-*trans*-retinoic acid, 13-*cis*-retinoic acid, all-*trans*-4-oxoretinoic acid, 13-*cis*-4-oxoretinoic acid, all-*trans*-retinyl β-glucuronide all-*trans*-retinoyl β-glucuronide and 13-*cis*-retinoyl β-glucuronide (Blaner & Olson, 1994; Barua, 1997). The plasma concentration of all-*trans*-retinol in healthy adults is about 2 μmol/L, of retinyl ester usually <0.2 μmol/L (10% that of retinol), and that of the other forms approximately 2–20 nmol/L (0.1–1% that of retinol) (Blaner & Olson, 1994; Formelli *et al.*, 1996; Barua, 1997). Retinyl esters are found in chylomicra, in chylomicron remnants and in low-density lipoproteins, whereas retinoic acid forms a complex with albumin. The retinoid glucuronides, although much more water-soluble than vitamin A, are nonetheless bound to plasma proteins (Blaner & Olson, 1994; Formelli *et al.*, 1996; Barua, 1997). Lipoprotein lipase hydrolyses triglycerides present in chylomicra, thereby converting them to chylomicron remnants. Chylomicron remnants, which retain the retinyl esters, are mainly taken up by parenchymal cells of the liver, although other tissues can also remove them from the circulation (Blaner & Olson, 1994).

Table 1. Mean concentrations of serum vitamin A (retinol + retinyl esters) (μg/dL) in humans as a function of age[a]

Age (years)	Percentiles		
	10	50	90
3–5	24.0	35.0	49.1
6–11	27.0	36.1	49.0
12–17	32.0	44.0	59.0
18–44	37.0	54.0	75.1
45–74	42.1	60.0	85.0

[a] Values for all races and both sexes, United States population (Pilch, 1985).

3.1.3.1 Factors affecting plasma retinoid concentrations

Plasma retinol levels are homeostatically controlled. In vitamin A-sufficient persons, therefore, daily intakes of vitamin A of up to 25 000 IU have little or no effect on steady-state serum retinol concentrations (Willett *et al.*, 1983). In some studies with vitamin A-sufficient subjects, however, retinol supplements induced significant, but small, increases in serum retinol concentrations. For example, in a baseline survey of smokers and asbestos workers of both genders, subjects who routinely used commercial supplements of vitamin A (usually 5000 IU/day) showed slightly higher (8%) plasma retinol concentrations than those who did not (Goodman *et al.*, 1996). Similarly, women with low initial serum retinol concentrations who received daily supplements of 10 000 IU showed a significant 9% increase ($p<0.02$) in plasma retinol within four weeks (Willett *et al.*, 1984a). Subjects receiving larger oral supplements of vitamin A (17 000–50 000 IU) daily for 5 or 20 days also showed some increase in plasma retinol levels, but more marked increases in the plasma concentrations of retinyl esters, all-*trans*- and 13-*cis*-retinoic

acid and all-*trans*- and 13-*cis*-4-oxoretinoic acid (Eckhoff & Nau, 1990a; Buss *et al.*, 1994). Supplements caused more marked increases in plasma retinoids than the same amount of vitamin A present in calf liver (Buss *et al.*, 1994). This is clearly reflected in the data of Arnhold *et al.* (1996) (see Table 2), from human volunteers consuming a liver meal. The vitamin A was taken up and metabolized very differently to the vitamin provided in a supplement, in particular with regard to its metabolism to all-*trans*-retinoic acid (see for comparison Table 3) (Eckhoff & Nau, 1990a).

Table 2. Plasma retinoids following consumption of fried turkey liver by healthy male volunteers[a]

Retinoid		Following liver consumption		
	C_{end} (ng/mL)	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0\text{-}24\ h}$ (ng x h/mL)
Retinol	641 ± 99	800 ± 105*	9	16822 ± 1982
Retinyl palmitate[b]	32.2 ±19.1	3540 ± 1736*	4	21114 ± 7952
14-Hydroxy-4,14-retroretinol	[c]	3.7 ± 0.9	4	61.7 ± 9.0
All-*trans*-retinoic acid	0.8 ± 0.2	2.0 ± 0.5*	2	19.7 ± 1.7
All-*trans*-4-oxoretinoic acid	[c]	0.8 ± 0.2	10	14.7 ± 6.4
13-*cis*-retinoic acid	1.1 ± 0.2	21.5 ± 4.3*	4	204 ± 35.3
13-*cis*-4-oxoretinoic acid	2.4 ± 0.6	32.1 ± 4.9*	10	435 ± 68.5
9-*cis*-retinoic acid	ND[d]	2.7 ± 1.1	4	10.7 ± 3.4
9,13-di-*cis*-retinoic acid	ND[d]	17.1 ± 5.8	4	68.2 ± 21.6

[a] Values are means ± SD for C_{end}, C_{max} and $AUC_{0\text{-}24\ h}$; medians for T_{max} ($n = 10$).

[b] Retinyl palmitate data calculated with $n = 9$ due to one outlier (C_{max} = 14106 ng/mL, and $AUC_{0\text{-}24\ h}$ = 104 858 ng x h/mL).

[c] Endogenously detectable in three samples only; 1.3 ± 0.2 ng/mL (for 14-Hydroxy-4,14-retroretinol), and 0.6 ± 0.3 ng/mL (for all-*trans*-4-oxo retinoic acid).

[d] Not detectable; detection limit; 0.3 ng/mL (for 9-*cis*-retinoic acid), and 0.5 ng/mL (for 9,13-di-*cis*-retinoic acid).

* Significantly higher than C_{end} ($p < 0.001$, Student's *t*-test for paired data).

From Arnhold *et al.* (1996)

Table 3. Retinoic acid concentrations in human plasma following ingestion of a retinyl palmitate supplement

Sample	n[a]	Concentration (ng/mL plasma, mean ± SD)		
		13-*cis*-4-oxo-RA	13-*cis*-RA	All-*trans*-RA
Normal plasma	10	3.68 ± 0.99	1.63 ± 0.85	1.32 ± 0.46
Max. plasma conc. after 833 IU vitamin A/kg body weight	5	7.60 ± 1.45 [b,c]	9.75 ± 2.18 [c,d]	3.92 ± 1.40[c]

[a] Number of subjects

[b] Highest concentration measured until 6 h after dosing.

[c] Different versus normal plasma $p < 0.01$ (Student's *t*-test).

[d] Different versus maximum of all-*trans*-retinoic acid at $p < 0.01$ (Student's *t*-test).

From Eckhoff and Nau (1990a)

When the vitamin A status is poor, however, serum retinol concentrations are decreased. In such cases, daily doses of vitamin A can markedly increase serum retinol concentrations. For example, retinol concentrations increased 20% as a result of supplementation in a Chinese study (Thurnham *et al.*, 1988) and 13% in an Indian study (Jyothirmayi *et al.*, 1996). Cigarette smoking was associated inversely with plasma concentrations of carotenoids, and to a lesser degree with plasma levels of vitamins C and E, but was positively associated with plasma retinol in Afro-American women (Pamuk *et al.*, 1994). In keeping with this finding, cigarette smoke degraded the carotenoids and α-tocopherol in human plasma to a greater extent than retinol (Handelman *et al.*, 1996). Plasma retinol concentrations were positively associated with alcohol use and physical activity and negatively associated with ingestion of soy products in male Japanese smokers (Kitamura *et al.*, 1997). Adipose tissue concentrations of vitamin A were also positively associated with alcohol and with smoking, but negatively associated with body mass index in European men and women (Virtanen *et al.*, 1996). Plasma α-tocopherol concentrations generally are not depressed by long-term interventions with β-carotene and vitamin A (Goodman *et al.*, 1994).

Diseases also influence plasma retinol concentrations. Values are generally lower in cases of cancer, liver disease, protein-calorie malnutrition and infections and are generally higher in many, but not in all, kidney disorders (Goodman *et al.*, 1994; Soprano & Blaner, 1994; Olmedilla *et al.*, 1996).

3.1.4 Uptake by tissues

Chylomicron remnants contain two apolipoproteins, B48 and E, for which specific receptors exist on the surfaces of parenchymal cells of human liver. Whether such receptors are present on cells of other human tissues is less clear. The uptake of chylomicron remnants is by receptor-mediated endocytosis (Blaner & Olson, 1994; Blomhoff, 1994a; Olson, 1994b; Ross, 1998).

The uptake by tissues of retinol from holo-RBP may proceed by one of two mechanisms: (*a*) interaction with a receptor for RBP on the surface of target cells, or (*b*) dissociation of holo-RBP followed by interaction of free retinol with the membranes of target cells (Blaner & Olson, 1994). The case for a specific receptor has best been shown with bovine retinal pigment epithelial cells, but less convincingly with other tissues, whereas the rate of dissociation of holo-RBP accords with physiological rates of uptake. It is quite possible that both mechanisms occur under different physiological conditions (Blaner & Olson, 1994).

3.1.5 Tissue distribution

All tissues of the human body contain vitamin A. Its distribution in various tissues of generally healthy subjects in Iowa, United States, dying of various causes is shown in Table 4 (Raica *et al.*, 1972). The liver clearly is the major storage organ for vitamin A, although fat and muscle contain significant total amounts, albeit at much lower concentrations. The plasma contains <1% of the total vitamin A in the body of a well nourished adult. Other organs examined contain much smaller amounts. Both the concentrations of vitamin A in tissues and the total amount present in the body vary widely between individuals. The total amount of vitamin A in the body of well nourished individuals (a mean of about 260 mg; Table 4) is catabolized at a slow rate (0.5%/day). Thus, signs of vitamin A deficiency in well nourished persons who ingest a diet very low in total vitamin A appear only after a year or more (Sauberlich *et al.*, 1974).

Nonetheless, different European groups show different concentrations of total retinol (retinol + retinyl esters) in various tissues. For example, middle-aged Norwegian men showed the highest (2.3 μg total retinol/g fatty acids) and middle-aged Spanish women the lowest (0.9 μg/g) mean concentrations of total retinol in adipose tissue biopsies obtained from the buttocks in a study of 1025 subjects in nine European countries (Virtanen *et al.*, 1996). Men tended to show higher mean adipose tissue values than women, except in the Netherlands. Different adipose tissue sites show different mean levels of total retinol. Thus, the mean concentrations of total retinol in breast adipose

tissue, while higher than those in buttocks, were essentially the same between premenopausal and postmenopausal women and between those with cancer and those with benign breast disease (Zhu *et al.*, 1995). Mean values in fat were approximately 29 ± 9 mg (SD) of vitamin A/g wet weight, lower than the liver concentration but higher than that of other tissues studied (Table 4). Human colonic epithelial cells contain a mean concentration of 16 ng total retinol/10^7 cells, similar concentrations of various carotenoids and much higher concentrations of vitamin E (Nair *et al.*, 1996). The denser, less mature colonic cells contained most of these nutrients. On average, 10^9 cells are equivalent to 1 g wet weight of tissue. Thus, the mean total retinol concentration would be 1.6 μg/g tissue. Lung tissue of cancer patients contains mean total retinol concentrations of 0.15 μg/g, 60-fold higher α-tocopherol concentrations, and approximately two-fold higher carotenoid concentrations (Redlich *et al.*, 1996). Lung tissue concentrations of total retinol correlated with total retinol levels in bronchoalveolar lavage cells but not with serum values. Mean total retinol concentrations in these cancer patients tended to be lower than those found (0.91 μg/g) in presumably normal lung samples obtained at autopsy (Table 4). The retinoic acid concentration in human prostate carcinoma cells was reduced 5–8-fold relative to normal prostatic cells, whereas the retinol concentrations were the same (Pasquali *et al.*, 1996).

3.1.6 Metabolism

Within the human intestinal mucosa, retinal is readily reduced to retinol by retinal reductase and other similar enzymes. Thus, retinol is the merging point for vitamin A derived both from provitamin A carotenoids and directly from the diet. Because observations in humans are fully consistent with those from other animal species (see Section 3.2), these transformations are only briefly summarized here.

Retinol can be oxidized reversibly to retinal and then further, but irreversibly, to retinoic acid in essentially all organs (Blaner & Olson, 1994). Retinol and retinoic acid, by reaction with uridine diphosphoglucuronic acid, form

Table 4. The distribution of vitamin A (retinol and retinyl esters) in tissues of adult humans

Tissue	Mean vitamin A (μg/g)[a]	Tissue % of body weight[b]	Mean total amount (mg)[c]	% of total amount
Liver	149.00 ± 132.00	2.30	232.44	87.90
Fat	1.46 ± 1.55	18.80	18.69	7.07
Muscle	0.35 ± 0.26	42.80	10.18	3.85
Serum	0.57 ± 0.22[d]	4.90	1.90	0.72
Lung	0.91 ± 1.89	0.73	0.46	0.17
Heart	1.08 ± 1.92	0.42	0.31	0.12
Kidney	0.71 ± 0.61	0.41	0.20	0.08
Spleen	0.89 ± 0.88	0.25	0.15	0.06
Pancreas	0.52 ± 0.28	0.16	0.06	0.02
Testes	1.14 ± 1.23	0.04	0.03	0.01
Adrenal	1.26 ± 0.98	0.02	0.02	<0.01
Thyroid	0.43 ± 0.33	0.04	0.01	<0.01
Prostate	0.32 ± 0.31	0.02	<0.01	<0.01

[a] Mean values ± SD for tissues are taken from Raica *et al.* (1972).
[b] From Long, 1961. Major organs not analysed include bone (11–15%), stomach and intestine (6–10%), skin (7%) and brain (2%).
[c] Based on a reference body weight of 68 kg.
[d] Mean value ±SD, in mg/ml, for adults of both sexes, 18–74 years.

retinyl β-glucuronide and retinoyl β-glucuronide, respectively, in the liver, intestine, and other organs (Blaner & Olson, 1994; Barua, 1997). As already indicated, retinoic acid and these two glucuronides are present in low concentrations in human blood (Blaner & Olson, 1994; Formelli *et al.*, 1996; Barua, 1997). Retinol is esterified by transfer of a fatty acid from the α-position of phosphatidyl choline to yield retinyl esters, most notably in the intestinal mucosa, liver and retinal pigment epithelial (RPE) cells of the eye, but also in other tissues. The major esters formed are palmitate and stearate, with smaller amounts of oleate and of polyunsaturated fatty acids (Blaner & Olson, 1994). In the intestine, retinyl esters are incorporated into chylomicra, in the liver into vitamin A-containing globules of parenchymal cells and stellate cells, and in RPE cells into lipid aggregates. Stellate cells, the major storage cells for vitamin A, have been found in many organs of several species (Wake, 1994).

Retinoic acid can be hydroxylated at the C-4, C-16 and C-18 positions, as well as elsewhere, converted to the 5,6-epoxide, and cleaved at various points in its conjugated chain to form a variety of oxidation products. 4-Hydroxyretinoic acid can be oxidized irreversibly to its 4-oxo derivative. In humans, these oxidation products may also have some biological activity (Blaner & Olson, 1994).

The rate of catabolism of administered retinoic acid has been reported to vary with the presence of some types of carcinoma of the lung. After a large oral dose (45 mg/m^2) of retinoic acid in lipid-rich solution (Ensure®), for example, patients with squamous or large-cell carcinoma were six times more likely to have an area under the concentration–time curve (AUC) greater than 250 ng-h/mL (RR, 5.93; 95% CI, 1.3–27.2) than controls free of cancer. In contrast, these AUC values were inversely associated with adenocarcinomas of the lung (RR, 0.12; 95% CI, 0.02–0.65) (Rigas *et al.*, 1996). The most likely explanation for such differences in retinoic acid catabolism is the activity of cytochrome P450 (CYP) enzymes, which are involved in the 4-hydroxylation of retinoic acid and retinol, as well as in the activation and subsequent inactivation of carcinogens (Leo *et al.*, 1989; Rigas *et al.*, 1996). The conversion of retinol and retinoic acid to their 4-hydroxy metabolites in human liver is catalysed by the CYP2C8 isozyme (Leo *et al.*, 1989).

In RPE cells, a physiologically important enzyme, retinyl ester isomerohydrolase, converts all-*trans*-retinyl ester to 11-*cis*-retinol and a free fatty acid (Rando, 1994). Thereafter, 11-*cis*-retinol can either be esterified in the RPE or oxidized to 11-*cis*-retinal. The latter is transported on a specific protein, the interphotoreceptor retinol-binding protein (IRBP), across the inter-photoreceptor space to the outer segment of rods and cones, where it combines with opsin to form rhodopsin in the rod cells and three types of iodopsins in cone cells (Saari, 1994; Ross, 1998). All-*trans*-retinal, after being released photochemically from rhodopsin and iodopsin, is reduced to retinol, which is then ferried back to the RPE cells on IRBP. Several specific binding proteins, termed cellular retinol-binding proteins (CRBP) and cellular retinoic acid-binding proteins (CRABP), play crucial roles in the enzymatic transformations of vitamin A within cells. Several isoforms of each exist with different tissue distributions and actions. These retinoid-binding proteins may also play important regulatory roles in the overall metabolism of the vitamin (Blaner & Olson, 1994; Napoli, 1996; Ross, 1998).

3.1.7 Function

Vitamin A functions in vision, cell differentiation, immune response and embryonic development (Saari, 1994; Gudas *et al.*, 1994; Semba, 1998; Hofmann & Eichele, 1994). The function most closely associated with cancer is cell differentiation. Most, but probably not all, actions of vitamin A in cell differentiation are induced by retinoic acid via two classes of nuclear retinoid receptor, RAR and RXR (Blomhoff, 1994a; Mangelsdorf *et al.*, 1994; Olson, 1994b; Chambon, 1996; Ross, 1998). Each family has three major subtypes, termed α, β and γ. Because the distribution of each of these subtypes in different cells is different, they are presumed to have distinct cellular functions (Blomhoff, 1994a; Olson, 1994b; Ross, 1998).

Synthetic ligands selective for each of these nuclear receptors are already being tested in clinical trials (Chustecka, 1998).

The nuclear retinoid receptors generally act as heterodimers, of which the most common is the RAR–RXR dimer. To be active, RAR must bind retinoic acid (either the all-*trans* or 9-*cis* isomer), whereas RXR need not. Indeed, in the presence of either 9-*cis*-retinoic acid or a synthetic analogue as a ligand for RXR, the activity of processes dependent on the RAR–RXR dimer is reduced in some systems but is synergistically increased in other in-vitro and in-vivo models (Blomhoff, 1994a; Olson, 1994b; Elmazar *et al.*, 1997; Ross, 1998). RXR also forms hetero-dimers with nuclear receptors for triiodothyronine, 1α,25-dihydroxy-vitamin D_3 (calcitriol), peroxisome proliferator-activated receptors and others (Blomhoff, 1994b; Mangelsdorf *et al.*, 1994; Olson, 1994b; Ross, 1998). Furthermore, the ligand-bound RAR–RXR dimer can attenuate the ability of the jun-fos dimer to activate the AP-1 site of the genome, thereby reducing cell proliferation (Mangelsdorf *et al.*, 1994). Thus, the nuclear retinoid receptors and their ligands, both natural and synthetic, show profound effects on gene expression and on cell differentiation (Blomhoff, 1994b; Mangelsdorf *et al.*, 1994; Olson, 1994; Ross, 1998). The clinical implications of these interactions have been reviewed (Goss & McBurney, 1992; Jetten *et al.*, 1993; Lotan, 1996).

3.1.8 Excretion

Vitamin A (retinol and retinyl esters) in oil is well absorbed (60–90%) from the human intestine and any unabsorbed vitamin A, of course, appears in the faeces. The absorption of provitamin A carotenoids is less (<50%) and is greatly affected both by their bioavailability, which can vary more than ten-fold in different foods, and by the amount ingested (Parker, 1996; Olson, 1998). Absorbed vitamin A is ultimately converted to a variety of inactive products, as previously stated. In healthy individuals, a set of largely undefined products are excreted in the urine in relatively small amounts. During severe bacterial infections, however, holo-RBP is excreted in the urine in amounts that may exceed retinol intake by a large margin (Stephensen *et al.*, 1994). Thus, children can be rapidly depleted of vitamin A as a result of recurrent severe bacterial infections. Light or moderate infestations with intestinal parasites, such as *Ascaris*, do not seem to affect vitamin A absorption much, but heavy infestations do (Tanumihardjo *et al.*, 1996; Olson, 1995).

3.1.9 Kinetics

As noted above, ingested retinyl ester is hydrolysed in the intestinal lumen, resynthesized in the intestinal mucosal cells, and released as a component of chylomicra into the lymph. After entering the plasma, chylomicra are degraded to chylomicron remnants that are removed by the liver and other tissues. Retinyl esters taken up by the liver are hydrolysed to retinol, which is released as holo-RBP back into the plasma. The kinetics of these processes have been carefully investigated, both in rats and in humans (Green & Green, 1994).

The half-life ($t_{1/2}$) for triglyceride removal from human chylomicra is 5–8 min (Karpe *et al.*, 1997). The conversion of chylomicra (S_f > 400) to large chylomicron remnants (S_f 60–400), as measured by the presence of retinyl palmitate in these fractions, is slower (mean $t_{1/2}$ = 51 min), as expected. The kinetic analysis is complicated, however, by the presence of pools of both S_f > 400 and S_f 60–400 species with rapid and slow turn-over. Neither of these larger lipoproteins was detectably converted to smaller ones (S_f 20–60), which ostensibly are of greater concern in the formation of atherosclerotic plaques. The rate of conversion of chylomicra to large chylomicron remnants was inversely related to low-density lipoprotein (LDL)–cholesterol concentrations (Karpe *et al.*, 1997). The initial mean half-life for the clearance of retinyl palmitate-labelled chylomicra and chylomicron remnants was 19 min, within the range (10–53 min) reported in other studies (Berr, 1992). Both monoexponential ($t_{1/2}$ 19 min) and biexponential clearance patterns ($t_{1/2}$ values of 19 min and 123 min) have been noted. Uptake rates are dose-dependent, however, and are saturable at fat intakes of 70–100 g (Berr, 1992).

Investigation of the kinetics of orally ingested vitamin A requires isotopically-labelled vitamin

A to distinguish the dosed molecules from endogenous retinol in the plasma. After a single oral dose of 105 μmol [$^{13}C_3$]retinyl palmitate in coconut oil to fasting male adults, the plasma level of retinyl ester reached a peak of 4.45 μmol/L at a mean of 6.2 h after dosing (Reinersdorff *et al.*, 1996). Baseline retinyl ester concentrations in the plasma are <0.2 μmol/L. The initial half-life for retinyl ester clearance was approximately 1 h, followed by higher values (slower clearance). The composition of the plasma retinyl esters was 70% as the palmitate, 25% as the stearate, and 5% as the oleate. The retinyl ester concentrations returned to baseline values within 36 h. The [$^{13}C_3$]retinol concentrations in plasma rose slowly from 3 to 7 h and then tended to plateau. The appearance of retinol probably represents the release of holo-RBP from the liver. In this regard, the half-life of plasma RBP in healthy male adults is 11–12 h when complexed with transthyretin and 4 h when not complexed (Goodman, 1984). Most RBP (80–90%) is present as holo-RBP in healthy adults, with 96% in the complexed state (Goodman, 1984). Later, the [$^{13}C_3$]retinol concentrations in plasma slowly declined, probably as a result of three factors: (*a*) mixing with total body reserves of vitamin A, (*b*) dilution with dietary vitamin A (although a diet low in vitamin A and fat was prescribed in this study), and (*c*) irreversible loss. The study terminated at four days (Reinersdorff *et al.*, 1996).

The estimated equilibration time for vitamin A in human adults is 12–30 days (Sauberlich *et al.*, 1974; Furr *et al.*, 1989; Haskell *et al.*, 1997), whereas the estimated $t_{1/2}$ value of liver reserves is 126–140 days (Sauberlich *et al.*, 1974; Olson, 1987). Because the dose used (105 μmol) in the Reinersdorff *et al.* (1996) study was approximately 30-fold larger than the normal dietary intake, however, the kinetics observed might differ from those in individuals ingesting a conventional diet.

When [^{14}C]retinol in autologous plasma was intravenously injected into three male adults, the specific activity of retinol in the plasma decreased rapidly for 20–30 days and then tended to plateau (Green & Green, 1994). Kinetic analysis indicated an average transit time of retinol in serum of 5.4 h, an average recycling between tissues and serum of 3 times, an average turnover rate of 6.5 mg/day, and a disposal rate of 1.71 mg/day. The disposal rate (irreversible loss), however, is directly related to total body reserves. Of the retinol turnover rate, 26% was irreversibly utilized and 74% was recycled to the serum. In a devised three-compartment model, the average retinol molecule has been predicted to spend 0.86 days in the serum and 105 days in the whole body pool before being irreversibly lost (Green & Green, 1994). The clearance of retinyl esters is slower ($t_{1/2}$ = 57 min) in persons older than 50 years than in younger individuals ($t_{1/2}$ = 31 min), which may explain the higher mean fasting serum concentrations of retinyl esters (58 nmol/L versus 38 nmol/L) in older persons (Krasinski *et al.*, 1990a).

Labelled vitamin A has also been used to estimate total body reserves in humans by an isotope-dilution procedure. Based on reasonable assumptions concerning intestinal absorption efficiency, portion of the dose stored, equilibration time and catabolic rates, calculated values of total body reserves have been shown to agree well with measurements of vitamin A in liver biopsy specimens (Furr *et al.*, 1989; Haskell *et al.*, 1997).

3.2 Experimental models

3.2.1 Overview

3.2.1.1 Animal models

Most studies of vitamin A physiology have been carried out in the rat. Although far less numerous, studies have also been undertaken in the mouse, guinea-pig, rabbit, ferret, cow, pig, monkey and several non-mammalian species including the chicken and frog. The suitability of any animal model for study of vitamin A uptake, transport, storage and metabolism depends on the physiological context of the work. The processes of hepatic storage and metabolism of vitamin A in man and in other species are generally similar and, consequently, investigations using any of these animal models provides insight relevant to the human situation. In contrast, investigations of the transfer of vitamin A from the mother to the developing fetus may be relevant to humans only if carried out in higher primates, where the gross anatomy of the placenta is similar to that of the human.

The great majority of what is now known regarding vitamin A uptake from the diet, its transport in the circulation and its metabolism and storage has come from investigations carried out in the rat. Recently, arising from the development of transgenic and knock-out mouse models, the mouse has become more widely used for the study of vitamin A physiology.

Although vitamin A physiology in the mouse seems to resemble that of the rat, several striking differences between these species have been observed. One is that mouse lung contains very high concentrations of total retinol (retinol + retinyl ester) compared to the rat (see Section 3.2.6.4). In this regard, the rat closely mimics the human. This difference could raise doubts about the value of the mouse model to study processes in the lung that may be influenced by vitamin A.

It also is clear that the mouse is much more resistant than the rat towards developing vitamin A deficiency. Thus, for a weanling rat maintained continuously on a totally vitamin A-deficient diet, symptoms of vitamin A deficiency are first observed after about two to three months, whereas in the mouse, six months or even much longer are required. In some instances, mice have been maintained on a diet totally lacking vitamin A for two generations before symptoms of vitamin A deficiency were observed. Although it seems possible that the mouse accumulates larger body stores of vitamin A and/or utilizes vitamin A more efficiently, the physiological basis for this difference in sensitivity has not been established. The relevance of this difference in the context of understanding vitamin A physiology in man is not clear.

Although the general processes of vitamin A transport and metabolism are similar across species, concentrations of metabolic intermediates and fluxes through metabolic pathways can be markedly different. Thus, the concentrations of some vitamin A forms can vary substantially from species to species. Such variations are thought to account for known species differences with respect to the teratogenic effects of vitamin A (see also Section 7.2.2.2). It is possible that differences in metabolic fluxes and intermediate concentrations can also influence the chemopreventive actions of vitamin A in different species.

Because most of the published information regarding vitamin A transport and metabolism has come from investigations carried out in the rat, the following review is based largely on rat data. Differences in vitamin A physiology that have been identified between the rat and other species are highlighted as well as strain-specific differences within a species.

3.2.1.2 Vitamin A metabolism and transport

Vitamin A transport and metabolism is highly specialized and complex. These processes involve both very specific intra- and extracellular vitamin A-binding proteins which bind retinol, retinoic acid and retinal and specific enzymes. Vitamin A-binding proteins and enzymes, along with their abbreviated names and proposed physiological functions, are listed in Table 5. The vitamin A-binding proteins can be classified according to whether they bind retinol, retinoic acid or retinal and to whether they are found intra- or extracellularly.

A simplified metabolic scheme for the metabolism of vitamin A from its uptake from the diet to its activation in target cells is presented in Figure 1.

3.2.2 Intestinal uptake and metabolism of dietary vitamin A

Both preformed vitamin A and provitamin A carotenoids undergo metabolism within the intestine (Blaner & Olson, 1994). They undergo a series of metabolic conversions, extracellularly in the lumen of the intestine and intracellularly in the intestinal mucosa, which result in the preponderance of the dietary vitamin A being converted to retinol (vitamin A alcohol). The retinol, along with other dietary lipids in the intestinal mucosa, is packaged as retinyl ester in nascent chylomicra. These are secreted into the lymphatic system, and the bulk of chylomicron vitamin A is eventually taken up (as part of the chylomicron remnants) by the liver, where the majority of the body's vitamin A reserves are stored.

Absorption of vitamin A by the small intestine is markedly influenced by the other constituents of the meal or dietary supplement. An

Table 5. Some vitamin A-binding proteins and enzymes

Protein	Abbreviation	Proposed role(s)
A. Vitamin A-binding proteins		
1. Retinol — Extracellular		
Retinol-binding protein	RBP	Plasma transport of retinol
Interphotoreceptor retinol-binding protein	IRBP	Visual process
2. Retinol — Intracellular		
Cellular retinol-binding protein type I	CRBP-I	Substrate for LRAT reaction Substrate for retinol dehydrogenases Stimulate retinyl ester hydrolase Facilitate cellular uptake of retinol
Cellular retinol-binding protein type II	CRBR-II	Substrate for intestinal retinal reductase Substrate for intestinal LRAT uptake of retinol by the cell
3. Retinoic acid — Intracellular		
Cellular retinoic acid binding protein type I	CRABP-I	Facilitate oxidative metabolism of retinoic acid Delivery/inhibition of delivery of retinoic acid to nucleus
Cellular retinoic acid binding protein type II	CRAPB-II	Facilitate oxidative metabolism of retinoic acid Delivery/inhibition of delivery of retinoic acid to nucleus
Retinoic acid receptor-α	RARα	Regulate transcription of vitamin A responsive genes
Retinoic acid receptor-β	RAR-β	Regulate expression of vitamin A responsive genes
Retinoic acid receptor-γ	RAR-γ	Regulate expression of vitamin A responsive genes
Retinoid X receptor-α	RXR-α	Regulate expression of vitamin A responsive genes
Retinoid X receptor-β	RXR-β	Regulate expression of vitamin A responsive genes
Retinoid X receptor-γ	RXR-γ	Regulate expression of vitamin A responsive genes
4. Retinal — Intracellular		
Cellular retinal-binding protein	CRAIBP	Formation of the visual pigment
B. Enzymes		
Lecithin: retinol acetyltransferase	LRAT	Catalyse retinyl ester formation
Bile salt-independent retinyl ester hydrolase	BSI-REH	Catalyse retinyl ester hydrolysis
Bile salt-dependent retinyl ester hydrolase	BSD-REH	Catalyse retinyl ester hydrolysis
Brush border retinyl ester hydrolase	BB-REH	Catalyse hydrolysis of dietary retinyl ester

accompanying fat load is necessary to assure optimal vitamin A uptake, since both bile salts and free fatty acids are needed to emulsify vitamin A. For supplements, the matrix used to solubilize and/or stabilize the vitamin A can markedly influence the ability of the intestine to take up vitamin A, so that different formulations of vitamin A have markedly different bioavailabilities which must be taken into account in the design and evaluation of chemoprevention trials.

A comprehensive review of the present understanding of dietary vitamin A uptake and metabolism is provided below. All vitamin A

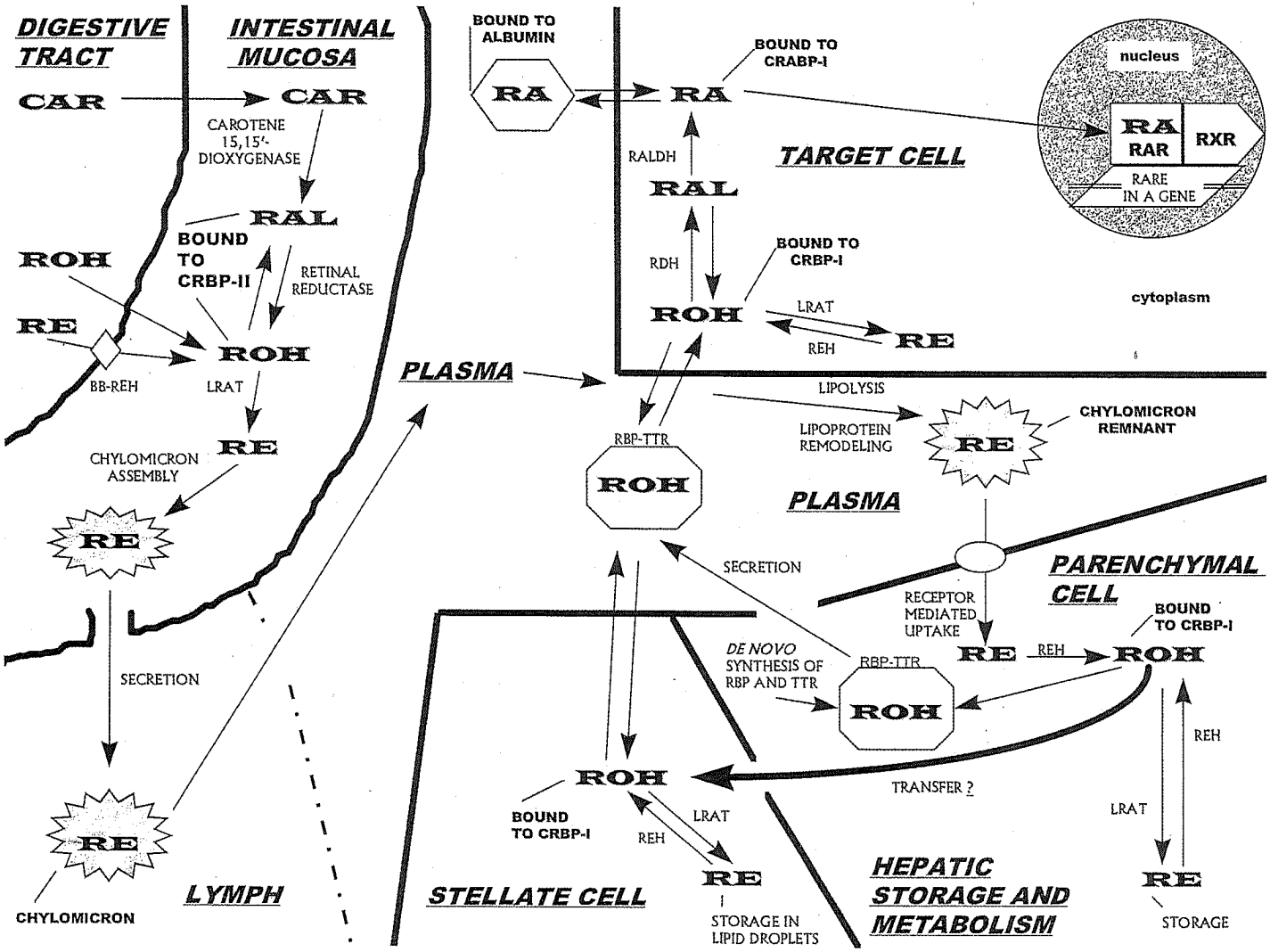

Figure 1. Simplified scheme for the transport and metabolism of retinol and retinyl esters in the digestive tract, the circulation and the liver, and within target cells, applicable to humans and most animal models.

Details of these processes are described in the text. Not all processes, especially those indicated for target cells, necessarily occur in all cells. Abbreviations: CAR, provitamin A carotenoids (including β-carotene); RAL, retinal; ROH, retinol; RE, retinyl esters; RA, retinoic acid; CRBP-I and CRBP-II, cellular retinol-binding protein, types I and II, respectively; CRABP-I, cellular retinoic acid-binding protein; LRAT, lecithin:retinol acyltransferase; BB-REH, brush border retinyl ester hydrolase; REH, retinyl ester hydrolase; RBP, retinol-binding protein; TTR, transthyretin; RAR, retinoic acid receptor; RXR, retinoid X receptor; RARE, retinoic acid response element.

which enters the body must undergo these processes. Since plasma retinol levels are tightly regulated in vitamin A-sufficient individuals, the processes of dietary vitamin A uptake and metabolism probably introduce more variability into the availability of vitamin A to target tissues than does the homeostatically regulated blood retinol pathway.

3.2.2.1 Provitamin A carotenoid uptake and metabolism

Provitamin A carotenoids like β-carotene, α-carotene and β-cryptoxanthin are absorbed intact by the mucosal cells of the proximal small intestine (Blaner & Olson, 1994). Like other neutral lipids in the diet, carotenoids are very insoluble in aqueous environments and must be emulsified with bile salts and free fatty acids within the lumen of the intestine for uptake into the mucosa (Goodman & Blaner, 1984). Within the mucosal cells, β-carotene is cleaved through the action of carotene 15,15'-dioxygenase (also known as the carotene cleavage enzyme) to retinal (vitamin A aldehyde) (Blaner & Olson, 1994). This dioxygenase is known to be cytosolic and influenced by bile salts and vitamin A levels in the diet (van Vliet *et al.*, 1996). In the rat, it is located primarily in mature jejunal enterocytes (Duszka *et al.*, 1996). Although this enzyme was first identified in the mid-1960s (Olson & Hayashi, 1965; Goodman & Huang, 1965), it has never been purified to homogeneity nor has its cDNA or genomic clone been identified. The β-carotene cleavage reaction is a specific oxidation reaction through which molecular oxygen is added across the central double bond (15,15'-double bond) yielding retinal as the sole or preponderant product (Duszka *et al.*, 1996; Nagao *et al.*, 1996). Although some studies suggest that β-carotene can undergo eccentric cleavage at any double bond within the molecule (yielding two of several different β-apocarotenals), it appears that if such cleavage occurs at all, it is not a major pathway (Duszka *et al.*, 1996; Nagao *et al.*, 1996).

Marked species differences exist with respect to carotenoid absorption and/or metabolism (Matsuno, 1991; van Vliet, 1996). No animal model perfectly reflects the human situation for β-carotene metabolism (van Vliet, 1996). Moreover, different commonly studied models show different patterns of β-carotene absorption, storage and conversion into vitamin A (van Vliet, 1996). In man, the majority of absorbed β-carotene (60–70%) is converted to retinal and the remainder is absorbed intact and deposited (or stored) in adipose tissue (Olson, 1990). In addition, humans, unlike most non-primate species, transport β-carotene primarily in the LDL fraction (van Vliet, 1996). Animal models used for study of β-carotene uptake and metabolism include the ferret, the preruminant calf, rodents, the rabbit, the pig and the chicken. The ferret is a poor converter of provitamin A carotenoids to vitamin A (Lederman *et al.*, 1998). Unlike humans, both ferrets and cows have relatively high fasting retinyl ester levels in blood (Ribaya-Mercado *et al.*, 1994; Lederman *et al.*, 1998). Rodents and chickens are very efficient carotenoid converters but they absorb intact carotenoids only when these are provided at high doses (van Vliet, 1996). The pig, in general, is a good model for digestion and absorption of carotenoids, but is not a very efficient converter of β-carotene to vitamin A (Schweigert *et al.*, 1995). Moreover, the pig seems to store most of the absorbed β-carotene in lung and not adipose tissue and liver, two major sites of β-carotene deposition in man (Schweigert *et al.*, 1995). Rabbits similarly do not store carotenoids in fat (van Vliet, 1996). Because of these interspecies differences, it is difficult to formulate from animal studies solely a unified model for β-carotene absorption and its metabolism to vitamin A that is directly applicable to man. The metabolism and conversion of carotenoids into vitamin A is considered in more detail in Volume 2 of the *IARC Handbooks of Cancer Prevention.*

Retinal formed by carotene cleavage is immediately reduced by a microsomal enzyme, retinal reductase, to retinol (Blaner & Olson, 1994). Intestinal retinal reductase requires the participation of a second cytosolic protein, cellular retinol-binding protein, type II (CRBP-II) (Ong *et al.*, 1994). CRBP-II, which binds both retinal and retinol, plays a central role in the processing of dietary vitamin A within

the mucosal cell. In the adult rat, CRBP-II is localized solely in the enterocytes of the small intestine and is thought to act solely in dietary vitamin A uptake and metabolism. It is a member of a family of closely related proteins that also includes the intracellular fatty acid-binding proteins and the intracellular retinoid-binding proteins (Ong *et al.*, 1994). CRBP-II shows the highest degree of sequence homology with the widely distributed cellular retinol-binding protein, type I (CRBP-I) but is immunologically distinct (Ong *et al.*, 1994). Retinal, when bound to CRBP-II, is the preferred substrate for reduction to retinol by intestinal retinal reductase. Retinol formed through this pathway is metabolically indistinguishable from retinol arriving in the diet as preformed vitamin A and, hence, undergoes the same subsequent metabolic events as described below for preformed vitamin A.

3.2.2.2 *Preformed vitamin A uptake and metabolism*

Preformed dietary vitamin A consists primarily of retinyl esters and retinol. Within the lumen of the small intestine, some of the esters may be hydrolysed to retinol, which, along with the retinol arriving as such in the diet, is taken up by the mucosal cells. Pancreatic lipases, such as triglyceride lipase and cholesteryl ester hydrolase (also known as carboxy ester lipase and bile-salt-dependent retinyl ester hydrolase) are able to hydrolyse retinyl esters *in vitro* and it has been assumed that these enzymes mediate the luminal hydrolysis of dietary retinyl esters (Goodman & Blaner, 1984; Blaner & Olson, 1994). However, studies in the rat have indicated that a retinyl ester hydrolase activity intrinsic to the brush border membrane of the small intestine plays a central role in this process, making a quantitatively greater contribution to the hydrolysis of dietary retinyl ester than the pancreatic enzymes (Rigtrup & Ong, 1992). The intrinsic brush border retinyl ester hydrolase activity is stimulated by both trihydroxy and dihydroxy bile salts, and preferentially hydrolyses long-chain retinyl esters like retinyl palmitate. This brush border retinyl ester hydrolase from the rat small intestine mucosa is probably identical to the brush border phospholipase B characterized earlier for this tissue (Rigtrup *et al.*, 1994).

Retinol formed through the hydrolysis of dietary retinyl esters or arriving as such in the diet is taken up by the mucosal cells. Unhydrolysed dietary retinyl ester is very poorly absorbed, if at all, from the intestinal lumen. The process of retinol uptake requires emulsification of the retinol by bile salts and free fatty acids (Goodman & Blaner, 1984). In the rat, a retinol transport protein within the plasma membrane of the enterocyte facilitates retinol uptake (Dew & Ong, 1994). Within the intestinal mucosa, all retinol is re-esterified with long-chain fatty acids (primarily palmitic, with smaller amounts of stearic, oleic and linoleic acids) through the action of the enzyme lecithin:retinol acyltransferase (LRAT). The synthesized retinyl esters are packaged along with other dietary lipids into nascent chylomicrons and secreted into the lymphatic system for uptake into the circulation. Intestinal LRAT requires retinol bound to CRBP-II as a substrate (Blaner & Olson, 1994; Ong *et al.*, 1994). Thus, CRBP-II plays a central role in directing or channelling dietary retinol to nascent chylomicra for uptake into the body. Retinal formed by carotene cleavage is also metabolically channelled, through binding to CRBP-II, towards retinyl ester formation and packaging in chylomicra. No information is available concerning the biochemical processes through which the synthesized retinyl ester is packaged by the mucosal cells into nascent chylomicra.

LRAT has been proposed to play a key role in directing the metabolism of retinol throughout the body. This enzyme catalyses the transfer of an α_1 fatty acid from the membrane-associated phosphatidyl choline to all-*trans*-retinol and has been reported to be present in the liver, eye, small intestine and testes (Blaner & Olson, 1994). It is not clear whether the LRAT species in each of these tissues are distinct gene products, but it has been established that hepatic but not intestinal LRAT is actively regulated by vitamin A nutritional status (Randolph & Ross, 1991; Matsuura & Ross, 1993). The biochemical characteristics of LRAT have been summarized by Blaner & Olson (1994). In essence, LRAT

utilizes retinol bound to CRBP-II (in intestine) or to CRBP-I (in other tissues) as its preferred substrate. The apparent K_m values of rat intestinal and hepatic LRAT for the retinol-binding protein complexes are in the low micromolar range, a concentration that is physiological (Blaner & Olson, 1994). The properties and roles of hepatic LRAT are discussed in Section 3.2.4.2. Figure 2 provides a schematic view of the uptake and metabolism of pro- and preformed vitamin A by the intestine.

3.2.3 Chylomicron delivery of postprandial vitamin A

3.2.3.1 Overview

Together with triglycerides, cholesteryl esters, phospholipids and other dietary lipids, retinyl esters are incorporated into the apolipoprotein B-48 (apoB48)-containing chylomicra and secreted into the lymphatic system. Once in the general circulation, chylomicra interact with lipoprotein lipase (LPL) bound to the luminal surface of the vascular endothelium and rapid lipolysis of the triglyceride occurs. LPL-catalysed hydrolysis gives rise to free fatty acids and a smaller lipoprotein particle termed a chylomicron remnant. The free fatty acids generated from triglyceride hydrolysis are taken up by extrahepatic tissues, including muscle and adipose tissue, where they are ultimately used as substrates for energy metabolism (Cooper, 1997). The chylomicron remnants acquire apolipoprotein E (apoE) either in the plasma or in the space of Disse, where apoE can

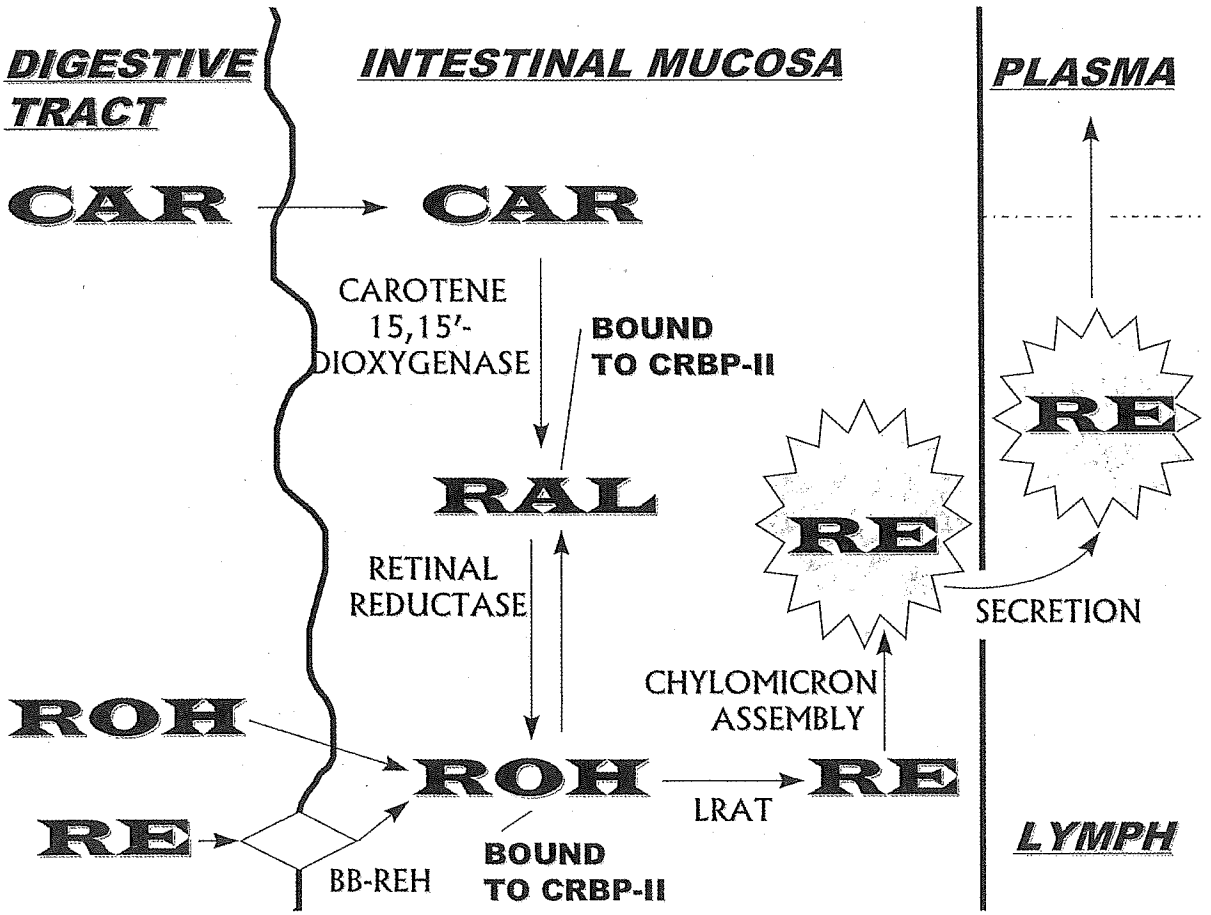

Figure 2. Simplified scheme for the uptake and metabolism of retinol and retinyl esters in the digestive tract applicable to humans and many animal models.

However, many animal species do not take up and/or metabolize carotenoids well. Details of these processes and specific species differences are described in the text.

For key to abbreviations, see Figure 1.

accumulate after its secretion from hepatocytes (Williams *et al.*, 1985; Hamilton *et al.*, 1990; Ji *et al.*, 1994, 1995). The acquisition of apoE by the remnant particles is essential for their clearance by the liver (Cooper, 1997). The importance of apoE in chylomicron remnant clearance is underscored by the fact that apoE-deficient mice clear postprandial cholesterol very slowly (Ishibashi *et al.*, 1994, 1996; Mortimer *et al.*, 1995). These mice also show very high circulating levels of total cholesterol and retinyl esters, even in the fasting state. Data accumulated from studies carried out over the past 35 years consistently show that approximately 75% of chylomicron retinyl ester is removed from the circulation by the liver and the remaining 25% by extrahepatic tissues, including skeletal muscle, adipose tissue, heart, spleen and kidney (Goodman *et al.*, 1965).

Most species seem to process and metabolize dietary lipids in a similar manner. In general, dietary vitamin A uptake and its clearance from the circulation are not thought to be very different in rats, mice, rabbits and humans. However, one major exception to this generalization can become important in disease states that lead to decreased rates of clearance of dietary lipids. Specifically, some species, including rodents, totally lack plasma cholesteryl ester transfer protein, which catalyses the transfer within the circulation of cholesteryl esters and retinyl esters from triglyceride-rich lipoproteins like chylomicra to low-density lipoprotein (LDL) and high-density lipoprotein (HDL) (Tall, 1995). Thus, in both man and rabbits, which express cholesteryl ester transfer protein, some postprandial retinyl ester is transferred to these lipoprotein fractions if lipid clearance is impaired as the result of either disease or experimental intervention (Goodman & Blaner, 1984; Blaner & Olson, 1994). The retinyl ester transferred to LDL or HDL would then be processed as a component of these lipoproteins. In most healthy individuals the amount of postprandial retinyl ester transferred by cholesteryl ester transfer protein to LDL and/or HDL is very small. Nevertheless, this process may be significant in some pathological states which involve delayed plasma lipid clearance.

3.2.3.2 Hepatic clearance

ApoE is required for uptake of chylomicron remnants by the liver, but the exact mechanisms involved are still unclear. Several distinct cell surface receptors that are able to bind apoE-containing lipoproteins may well be involved in the uptake of chylomicron remnants by hepatocytes. Among these are the LDL receptor (LDL-R), the LDL receptor-related protein (LRP) and the lipolysis-stimulated receptor (LSR) (Cooper, 1997). In addition, heparin sulfate proteoglycans (HSPG), located on the surface of hepatocytes, may also be responsible for the initial interaction of remnants with the cells of the liver (Cooper, 1997). Roles for LPL and hepatic lipase in hepatic uptake have also been suggested (Shafi *et al.*, 1994; Beisiegel *et al.*, 1991).

Gene targeting of the LDL-R has provided evidence for the involvement of the LDL-R in chylomicron remnant clearance by hepatocytes. In a mouse strain totally lacking this receptor (LDL-$R^{-/-}$), the rate of plasma clearance of chylomicron remnants was unaffected, but endocytosis of the remnant by the hepatocyte was delayed (Mortimer *et al.*, 1995; Ishibashi *et al.*, 1994, 1996; Herz *et al.*, 1995). Thus, the rate of clearance of chylomicron remnant cholesteryl ester from the circulation was comparable to that of wild-type mice, but uptake of chylomicron remnant lipids into the liver of LDL-$R^{-/-}$ mice was significantly delayed (Mortimer *et al.*, 1995; Herz *et al.*, 1995). In double mutant mice totally lacking both the LDL-R and apoE, chylomicron remnants accumulated in the circulation to levels comparable to those observed in apoE-deficient mice carrying the wild-type LDL-R alleles (Ishibashi *et al.*, 1996). Overall, these data imply that the LDL-R contributes to hepatic uptake of chylomicron remnants, but suggest that chylomicron remnants are also taken up by the liver through alternative apoE-dependent processes which are distinct from the LDL-R pathway and can compensate for absence of the LDL-R pathway.

One such alternative apoE-dependent process may involve LRP, a 600 kDa calcium-dependent multifunctional receptor protein that is a member of the LDL-R protein family. LRP is identical to the receptor reported to be

the activated receptor form of the α_2-macroglobulin receptor and therefore also is known as α_2-macroglobulin receptor/LRP. It is known to interact with various ligands that are present in the circulation (Cooper, 1997). These include apoE, activated α_2-macroglobulin, LPL, lactoferrin, receptor-associated protein (RAP) and various serum proteases. The involvement of LRP in chylomicron remnant metabolism is supported by studies using RAP, a 39 kDa protein that binds to LRP in the presence of calcium ions and upon binding suppresses binding of other ligands to LRP (Cooper, 1997). For mice totally deficient in RAP ($RAP^{-/-}$), no difference in the rate of chylomicron remnant clearance was observed; however, in double mutants lacking both LDL-R and RAP, chylomicron remnants (as assessed by apoB48 concentrations) accumulated in the circulation to high levels (Willnow *et al.*, 1995). Overexpression of RAP in mice leads to accumulation of apoB48- and apoE-containing particles in the circulation of $LDL\text{-}R^{-/-}$ mice (Willnow *et al.*, 1994). Thus, it would appear that LRP, like the LDL-R, is not essential for clearance of chylomicron remnants by the liver. However, like the LDL-R, LRP contributes to hepatic clearance of chylomicron remnants in a manner that can be compensated for through the actions of other receptors.

A third receptor proposed to be present on hepatocytes, the LSR, is distinct from the LDL-R and LRP and may also contribute to uptake of chylomicron remnants by hepatocytes (Bihain & Yen, 1992; Yen *et al.*, 1994; Mann *et al.*, 1995). The role of this protein in chylomicron remnant clearance has been studied in both human and rat cells. LSR activity seems to depend on two distinct but interacting membrane proteins (Bihain & Yen, 1992; Yen *et al.*, 1994). Free fatty acids are thought to activate the LSR by causing a conformational shift that reveals cryptic binding sides for apoE and apoB. LSR binds to lipoproteins (preferentially triglyceride-rich particles) and to RAP at high concentrations. Unlike lipoprotein particle binding to the LDL-R and LRP, binding of ligands is calcium-independent (Yen *et al.*, 1994). Whether LSR contributes to chylomicron remnant clearance *in vivo* remains to be established.

While internalization of chylomicron remnants by hepatocytes is mediated by cell surface receptors, initial binding of the remnant to the surface of the hepatocyte may involve the actions of cell-surface HSPGs, which are especially abundant in the space of Disse. Data supporting a role for HSPGs in facilitating hepatocyte removal of chylomicron remnants has been obtained from studies in mice. Following intravenous administration of heparinase to mice, the rate of removal of apoE-rich remnant particles by the liver was greatly reduced for both wild-type and $LDL\text{-}R^{-/-}$ mice (Ji *et al.*, 1995; Mortimer *et al.*, 1995). It seems reasonable to speculate that remnant binding to hepatocyte HSPGs helps increase the residence time of apoE-containing remnant particles at the cell surface, thus allowing time for the remnant to bind to receptors like the LDL-R and/or LRP.

In summary, apoE plays an essential role in the uptake of chylomicron remnants by the liver. It seems likely that initially the chylomicron remnants are sequestered in close proximity to the hepatocytes through binding to HSPGs in the space of Disse. Subsequently, the remnant is able to interact with a cell surface receptor that mediates endocytosis of the remnant particle. Both the LDL-R and LRP appear to be important cell surface receptors for the uptake of chylomicron remnants. De Faria *et al.* (1996), employing RAP or antibodies against the LDL-R, have estimated that approximately 55% of chylomicron remnants are cleared through the LDL-R-mediated pathway and approximately 25% through the LRP-mediated pathway. Ishibashi *et al.* (1996) estimated that approximately 75% of chylomicron remnants are cleared through the LDL-R pathway. Although the role of LSR in chylomicron remnant clearance by the liver has not been fully established, this receptor too may contribute substantially to the process.

3.2.3.3 Extrahepatic tissue clearance

Early work by Goodman *et al.* (1965) indicated that in rats approximately 25% of postprandial retinyl ester is taken up by extrahepatic tissues. Following intravenous injection of doses of rat mesenteric chylomicra containing [^{14}C]retinyl ester, a substantial portion of the ^{14}C-label was

detected in skeletal muscle, depot fat, heart, spleen and kidney. Neither the physiological significance nor the biochemical basis for the observation that some chylomicron or chylomicron remnant retinyl ester is taken up by extrahepatic tissues has been systematically investigated until recently. It now appears that LPL plays an important role in facilitating uptake of postprandial vitamin A by extrahepatic tissues.

Blaner *et al.* (1994) established that after most of the chylomicron triglyceride has been hydrolysed, chylomicron retinyl esters become substrates for LPL-catalysed hydrolysis. The hydrolysis of chylomicron retinyl ester enhances the uptake of chylomicron vitamin A by cultures of murine BFC-1β adipocytes (Blaner *et al.*, 1994). This led to the hypothesis that LPL plays an important role *in vivo* in facilitating clearance of dietary vitamin A by extrahepatic tissues. Study of three mouse strains having different patterns of expression of mouse and human LPL (wild-type mice, LPL-null mice overexpressing human LPL in skeletal muscle and wild-type mice overexpressing human LPL in skeletal muscle) have provided support for this hypothesis. Mice overexpressing human LPL in skeletal muscle took up approximately two-fold more chylomicron vitamin A than did wild-type mice. The data from these studies and from other studies in rats are consistent in that the level of expression of LPL activity in skeletal muscle, adipose tissue and heart directly correlates with the amount of chylomicron vitamin A taken up by the tissues. Thus, it seems that the level of LPL activity in skeletal muscle, adipose tissue and heart is a key determinant of the amount of postprandial vitamin A taken up by these tissues.

Other important findings regarding the clearance of chylomicron retinyl esters have come from studies in primates (marmosets) and rabbits (Cooper, 1997). Mahley and Hussain (1991) reported that the bone marrow of rabbits and of marmosets takes up substantial amounts of chylomicra. Chylomicron uptake by rabbit bone marrow was 50–100% of that of the liver. Unlike rabbit liver, where LPL helps facilitate uptake of the chylomicron and its remnant, rabbit bone marrow takes up chylomicron retinyl ester in the total absence of LPL activity (Hussain *et al.*, 1997). It is thus clear that bone marrow too plays a dynamic role in the overall metabolism of postprandial vitamin A.

The LXR nuclear receptor, a member of the steroid–thyroid–retinoid superfamily of receptors, is thought to be involved in regulating expression of a variety of genes believed to be important in the metabolism and transport of cholesterol and triglycerides (Janowski *et al.*, 1996). The ligand for LXR is proposed to be a sterol metabolite. LXR must form a heterodimer with RXR to be active. Thus, vitamin A may play a role, along with sterol, in regulating its own uptake and transport following a vitamin A-containing meal.

3.2.4 Hepatic storage and metabolism of vitamin A

The liver is the major organ in the body for the storage and metabolism of vitamin A. The preponderance of the body's vitamin A reserves is stored as retinyl esters in hepatic stellate cells (also called Ito cells, fat-storing cells, perisinusoidal cells and lipocytes; described more fully in Section 3.2.4.4), although other tissues including adipose tissue, lung and kidney contain significant vitamin A stores and/or are active in vitamin A metabolism (see Section 3.2.6) (Blaner & Olson, 1994). The processes of vitamin A storage and metabolism in the liver are complex and many details regarding these processes and their regulation are still not fully understood. Hepatic vitamin A metabolism is mediated by enzymes such as LRAT, which esterifies retinol with long-chain fatty acids, and retinyl ester hydrolase, which hydrolyses retinyl esters to retinol, and by CRBP-I (Blaner & Olson, 1994). To meet tissue needs for vitamin A, retinol is secreted from hepatic parenchymal cells bound to its specific plasma transport protein, RBP (Soprano & Blaner, 1994). Like hepatic vitamin A storage and metabolism, the factors and processes which regulate retinol mobilization from the liver are not fully understood. It is clear, however, that for well nourished animals the concentrations of vitamin A stored in liver are both species- and strain-dependent. Thus, hepatic vitamin A levels are different in rats, mice and rabbits, the best

studied animal models. Similarly, different strains of rats maintained on diets providing the same levels of vitamin A show different hepatic vitamin A concentrations.

3.2.4.1 Hepatic processing of dietary vitamin A

Chylomicron remnants are thought to be internalized solely by hepatocytes (Cooper, 1997). Confirming earlier work, Mortimer *et al.* (1995), used confocal microscopy to show that fluorescent-labelled cholesteryl ester derivatives arriving in chylomicron remnants accumulated solely in hepatocytes and not in other hepatic cell types. Very shortly after endocytosis by hepatocytes, the chylomicron remnant retinyl esters are hydrolysed to retinol (Blaner & Olson, 1994). Harrison *et al.* (1995) showed that the newly endocytosed retinyl esters co-localize with a retinyl ester hydrolase in early endosomes. The esters are hydrolysed to retinol at this stage of endocytosis, before the remnant particle has reached the lysosome (Harrison *et al.*, 1995). At this point in the uptake process, the metabolism of dietary vitamin A diverges from that of dietary cholesterol. The retinol formed by retinyl ester hydrolysis is bound to apo-CRBP-I, which is found in relatively high concentrations in hepatocytes (see Table 6). CRBP-I-bound retinol may be delivered to newly synthesized apo-RBP, leading to secretion of holo-RBP into the circulation. The amount of newly endocytosed retinol that is secreted from the liver depends on the vitamin A nutritional status of the animal, more of the newly arrived retinol being secreted from hepatocytes of animals with poor nutritional status (Batres & Olson, 1987). An alternative fate for newly absorbed retinol is storage as retinyl ester in hepatocytes or in stellate cells following transfer from the hepatocyte. The mechanism through which retinol is transferred from hepatocytes to hepatic stellate cells

Table 6. Distribution of retinol, retinol-binding protein (RBP), cellular retinol-binding protein type I (CRBP-I), cellular retinoic acid-binding protein type I (CRABP-I), bile-salt-dependent retinyl ester hydrolase (BSD-REH), bile-salt-independent retinyl ester hydrolase (BSI-REH), and lecithin:retinol acyltransferase (LRAT) in isolated rat liver parenchymal and stellate cells[a,b]

Parameter	Parenchymal cells	Stellate cells
Total retinol	5.2 ± 2.4 nmol/10^6 cells 2.6 ± 1.2 nmol/mg protein	293 ± 107 nmol/10^6 cells 1558 ± 569 nmol/mg protein
RBP	6.6 ± 0.3 pmol/10^6 cells 0.34 ± 0.16 pmol/mg protein	0.04 ± 0.02 pmol/10^6 cells 0.18 ± 0.09 pmol/mg protein
CRBP-I	30.1 ± 15.8 pmol/10^6 cells 16.2 ± 8.2 pmol/mg protein	15.7 ± 5.9 pmol/10^6 cells 83.3 ± 31.8 pmol/mg protein
CRABP-I	0.37 ± 0.26 pmol/10^6 cells 0.19 ± 0.22 pmol/mg protein	0.58 ± 0.37 pmol/10^6 cells 29.3 ± 20.0 pmol/mg protein
BSD-REH	826 ± 47 pmol FFA/h/10^6 cells 427 ± 24 pmol FFA/h/mg protein	1152 ± 144 pmol FFA/h/10^6 cells 6129 ± 768 pmol FFA/h/mg protein
BSI-REH	Not determined	10.98 ± 1.08 nmol FFA formed/h/mg protein
LRAT	158 ± 53 pmol retinyl ester formed/min/mg microsomal protein	383 ± 54 pmol retinyl ester formed/min/mg microsomal protein

FFA, free fatty acid

[a] Values are taken from Blaner *et al.* (1985), Blaner *et al.* (1990) and Friedman *et al.* (1993)

[b] One gram of rat liver is taken to consist of 108 × 10^6 parenchymal cells, 16 × 10^6 stellate cells, 19 × 10^6 endothelial cells, and 9 × 10^6 Kupffer cells. Parenchymal cells were determined to contain 1934 ± 161 mg protein/10^6 cells and stellate cells 188 ± 80 mg protein/10^6 cells (Blaner *et al.*, 1985).

has not been fully established. The specific events important to hepatic vitamin A storage and metabolism that occur in hepatocytes and stellate cells are summarized below for each cell type, and are represented diagrammatically in Figure 3.

3.2.4.2 Vitamin A storage and metabolism in hepatocytes

The hepatocyte is an important site of vitamin A storage and metabolism. Hepatocytes have relatively high concentrations of total retinol (retinol + retinyl ester), CRBP-I, LRAT, bile-salt-stimulated and bile-salt-independent retinyl ester hydrolases, retinol dehydrogenases and retinal dehydrogenases, as well as other enzymes that catalyse the oxidative, conjugative and/or catabolic metabolism of retinoic acid. The cellular concentrations or specific activities of some of these components in both rat hepatocytes and hepatic stellate cells are shown in Table 6.

Hepatocytes from rats fed a control diet contain total retinol concentrations of 5.2 ± 2.4 nmol retinol per 10^6 hepatocytes (2.6 ± 1.2 nmol retinol/mg hepatocyte total protein). Approximately 95–99% of this total retinol is present as retinyl esters, primarily the esters of palmitic, stearic, oleic and linoleic acids (Blaner *et al.*, 1985). It has been estimated that approximately 5–30% of the total retinol in the livers of rats maintained on a control diet is present in

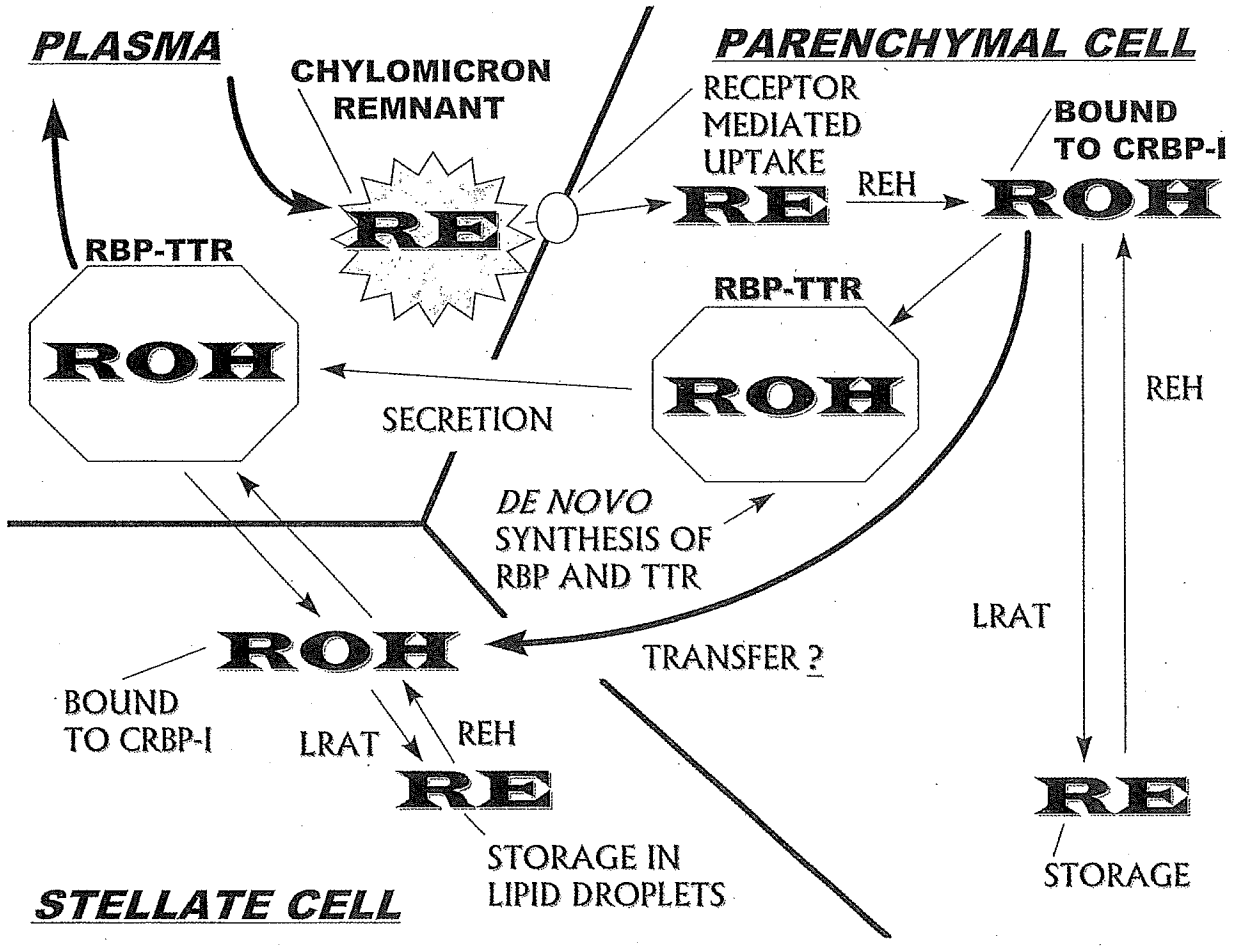

Figure 3. Simplified scheme for the uptake, transport, metabolism and storage of retinol and retinyl esters in the liver, applicable to humans and most animal models.

Details of these processes are described in the text.

For key to abbreviations, see Figure 1.

hepatocytes (Blaner *et al.*, 1985; Batres & Olson, 1987), with the remainder in stellate cells. The relative abundance of total retinol in hepatocytes is thought to be inversely related to the level of hepatic stores (Batres & Olson, 1987). Thus, as hepatic vitamin A stores decline, the proportion of vitamin A in hepatocytes increases relative to hepatic stellate cells. Since the hepatocyte is the site of RBP synthesis in the liver (Soprano & Blaner, 1994), this may imply that hepatocyte total retinol levels remain relatively constant in order to regulate circulating retinol–RBP levels.

Chylomicron remnant retinyl esters are hydrolysed through the action of a bile-salt-independent retinyl ester hydrolase that is present in the hepatocyte plasma membrane (Harrison *et al.*, 1995). This hydrolase in rats is identical to carboxylesterase ES-2, which was first described as a thiol esterase (Sun *et al.*, 1997). The activity of rat liver bile-salt-independent retinyl ester hydrolase *in vitro* is reported to be markedly influenced by the presence of *N*-(4-hydroxyphenyl)retinamide, all-*trans*-retinoic acid or 13-*cis*-retinoic acid in the assay mixture (Ritter & Smith, 1996). The enzyme was identified originally as a bile-salt-independent retinyl ester hydrolase as opposed to the bile-salt-stimulated retinyl ester hydrolase from rat liver that was characterized in the late 1970s. It had been believed that the bile-salt-stimulated retinyl ester hydrolase was the key enzyme responsible for hydrolysis of retinyl ester before its mobilization from the liver (Goodman & Blaner, 1984; Harrison, 1993). Later work demonstrated that in rats this enzyme is identical to cholesteryl ester hydrolase (Chen *et al.*, 1997a), a lipid hydrolase that is expressed in both liver and pancreas (as described in Section 3.2.2.2). Although rat hepatocytes possess both bile-salt-stimulated and bile-salt-independent retinyl ester hydrolases, the bile-salt-stimulated enzyme is not required for hepatic retinyl ester hydrolysis. The physiological actions of this enzyme in the liver are redundant with those of other enzymes. Whether the bile-salt-independent retinyl ester hydrolase plays a unique and irreplaceable role in retinyl ester hydrolysis within hepatic cells remains to be demonstrated. However, it is thought that this enzyme is important for hydrolysing chylomicron remnant retinyl ester and it may also be involved in the hydrolysis of hepatocyte retinyl ester stores to provide retinol to newly synthesized RBP.

CRBP-I concentrations in hepatocytes are relatively high, and it has been estimated that approximately 90% of the CRBP-I present in the liver is localized in hepatocytes (Blaner *et al.*, 1985). In rat liver and testis, CRBP-I is present at concentrations in excess of those of retinol, suggesting that all retinol present within these tissues is bound to CRBP-I (Harrison *et al.*, 1987). CRBP-I plays an important role in retinol metabolism (Ong *et al.*, 1994). Holo-CRBP-I has been reported to deliver retinol to LRAT for esterification (Ong *et al.*, 1994) and to provide retinol to retinol dehydrogenases which catalyse the first of two oxidation steps needed for formation of retinoic acid. Both LRAT (Blaner & Olson, 1994) and several retinol–CRBP-I-utilizing retinol dehydrogenases (Napoli, 1996) are present in hepatocytes. In addition, apo-CRBP-I has been reported to enhance hepatic bile-salt-independent retinyl ester hydrolase activity (Blaner & Olson, 1994). It also has been postulated that holo-CRBP-I delivers retinol to newly synthesized RBP for secretion from the liver into the circulation (Blaner & Olson, 1994). Finally, based on the binding characteristics of retinol to CRBP-I, it has been proposed that CRBP-I plays an important role in facilitating retinol uptake by cells from the circulating retinol–RBP complex. Thus, CRBP-I serves as an intracellular transporter of retinol, linking and facilitating the processes of retinol uptake, metabolism and mobilization within the hepatocyte.

Hepatic LRAT is present in both hepatocytes and stellate cells, but its specific activity is highest in the latter (Blaner *et al.*, 1990; Matsuura *et al.*, 1997). However, because of the large size and the number of hepatocytes, approximately 85% of the hepatic LRAT activity is present in hepatocytes (Blaner *et al.*, 1990). As outlined in Section 3.2.2.2, retinol bound to CRBP-I is the preferred substrate for hepatic LRAT, which shows an apparent K_m of 2.2 μmol/L for retinol–CRBP-I. LRAT has not been purified

from any tissue or cell and its cDNA and gene have not been cloned. Using radiation inactivation analyses, Ross and Kempner (1993) estimated that hepatic LRAT has a molecular size of approximately 52 ± 10 kDa. In rats, hepatic but not intestinal LRAT activity is regulated by vitamin A nutritional status (Randolph & Ross, 1991). Hepatic LRAT activity falls progressively during vitamin A depletion and within eight hours after repletion of vitamin A-deficient rats with retinol, hepatic LRAT activity starts to rise, reaching control levels within 24 hours after the start of repletion. Repletion with retinoic acid also increased hepatic LRAT activity in vitamin A-deficient rats, but this effect could be abolished if hepatocytes were pre-treated with inhibitors of gene transcription and protein synthesis. Taken together, these data suggest that hepatic LRAT expression is regulated by retinoic acid and consequently vitamin A availability. This provides a convenient regulatory mechanism through which retinol, in times of sufficient intake, can be converted to retinyl ester for storage or, in times of insufficient intake, can remain in the unesterified form to serve as a substrate for retinoic acid synthesis.

3.2.4.3 Transfer of vitamin A between hepatocytes and stellate cells

For vitamin A homeostasis to be maintained, interactions between hepatocytes and stellate cells must be tightly regulated. However, little is known about how hepatocytes and stellate cells communicate or about how vitamin A is transferred between the two cell types. Blaner and Olson (1994) reviewed mechanisms that have been proposed to explain the movement of vitamin A between hepatocytes and stellate cells. Although it is clear that chylomicron remnant retinyl ester must be hydrolysed before it is transferred from hepatocytes to stellate cells, the specific roles of RBP, CRBP-I and/or intercellular contacts between hepatocytes and stellate cells in the transfer process have not been definitively established. It is widely believed that vitamin A transfer involves the action of RBP and bulk movement through the extracellular space, but the possibility that movement of free retinol, retinol–CRBP-I or retinyl ester occurs via intercellular contacts between hepatocytes and stellate cells has not been ruled out (Blaner & Olson, 1994).

Early studies supporting the participation of RBP in the uptake process demonstrated that RBP is taken up by rat stellate cells both *in vivo* and *in vitro* (Blomhoff *et al.*, 1988; Senoo *et al.*, 1993). This was taken to indicate that RBP is important for the delivery of retinol from hepatocytes to stellate cells (Senoo *et al.*, 1993). Furthermore, in a perfused rat liver model, transfer of newly absorbed retinol between hepatocytes and stellate cells was effectively blocked when the animals were perfused with antibodies against RBP (Blomhoff *et al.*, 1988; Senoo *et al.*, 1990). Support for a role of RBP in the uptake process has come from studies of the transfer of RBP between co-cultured human HepG2 hepatoma cells, which are known to secrete RBP, and rat hepatic stellate cells. After co-culture for 18 hours, human RBP was identified by indirect immunolabelling on the surface, in coated pits and in vesicles in the stellate cells, suggesting movement of hepatocyte-secreted human RBP to the stellate cells. The amount of human RBP associated with the stellate cells was significantly reduced when antibodies against human RBP were added to the culture medium (Blomhoff *et al.*, 1988). The intracellular pool of vitamin A within stellate cells influences the amount of retinol that is esterified when retinol–RBP is added to the cell medium but not when free retinol is added (Trøen *et al.*, 1994). A need for RBP in intercellular transfer was not supported, however, by the finding that while stellate cells may take up RBP and retinol bound to RBP, the uptake of retinol into stellate cells does not depend on RBP (Matsuura *et al.*, 1993a,b). Thus, it remains unclear whether RBP plays an essential role in the transfer of retinol from hepatocytes to stellate cells.

3.2.4.4 Vitamin A storage and metabolism in hepatic stellate cells

In the vitamin A-sufficient adult rat, 70–95% of hepatic vitamin A is stored as retinyl ester in lipid droplets of stellate cells (Blaner & Olson, 1994). Hepatic stellate cells are non-parenchymal cells located perisinusoidally in the space of Disse, in recesses between parenchymal cells (Geerts *et al.*, 1994). They comprise about 5–8%

of total rat liver cells, and 1% of the total liver mass (Geerts *et al.*, 1994) and are the major cellular site of vitamin A storage in the body. Approximately 99% of the vitamin A present in stellate cells is present as retinyl ester. The lipid droplets are located in the cytoplasm of the stellate cells and represent the characteristic morphological feature of these cells. They are larger (up to 8 μm in diameter) than those in rat parenchymal cells (up to 2.5 μm in diameter) (Geerts *et al.*, 1994). The size and number of the lipid droplets in stellate cells is markedly influenced by dietary vitamin A intake (Wake, 1980; Kudo, 1989) and intraportal injection of retinol (Wake, 1980).

Stellate cell lipid droplets are formed through vacuolization of cisternae of the rough endoplasmic reticulum and exist in membrane-bound and non-membrane-bound forms (Wake, 1980). There is general agreement regarding the lipid composition of these droplets (Blaner, 1994). One study reported that the lipid of droplets isolated from vitamin A-sufficient rats consisted of approximately 42% retinyl ester, 28% triglyceride, 13% total cholesterol (free + ester) and 4% phospholipid (Yamada *et al.*, 1987). The retinyl esters in the droplets consisted of approximately 70% retinyl palmitate, 15% retinyl stearate, 8% retinyl oleate, 4% retinyl linoleate and smaller percentages of other long-chain retinyl esters (Yamada *et al.*, 1987). A later study showed that the lipid composition of the droplets is markedly affected in rats by dietary retinol intake, but not by dietary triglyceride (calorie) intake (Blaner & Olson, 1994).

The mechanism by which vitamin A regulates and maintains the lipid composition of the lipid droplets in stellate cells is not understood. However, it has been suggested that retinoic acid may be important (Yumoto *et al.*, 1989). Because retinoic acid has a sparing effect on hepatic retinol levels, intracellular retinoic acid may well influence hepatic retinol secretion (Shankar & DeLuca, 1988). Although the liver contains some retinoic acid (Kurlandsky *et al.*, 1995), its cellular distribution has not been determined. Because retinoic acid receptors-α, -β, and -γ (RAR-α, -β and -γ) and retinoid X receptor-α (RXR-α) are all expressed in stellate cells (Weiner *et al.*, 1992; Friedman *et al.*, 1993), retinoids may well play a role in regulating the state of differentiation and metabolism within stellate cells.

As seen in Table 6, stellate cells are highly enriched in CRBP-I and the enzymes which are able to hydrolyse (bile-salt-dependent and bile-salt-independent retinyl ester hydrolases) and to synthesize retinyl esters (LRAT). In addition, the stellate cells contain an intracellular binding protein for retinoic acid, cellular retinoic acid-binding protein, type I (CRABP-I). However, stellate cells contain very little RBP (Blaner & Olson, 1994).

In vivo, rat liver stellate cells exhibit a dual phenotype, that is, a quiescent phenotype in normal healthy liver and an activated phenotype in chronically diseased liver (Geerts *et al.*, 1994). The quiescent phenotype is characterized by lipid droplets rich in vitamin A, a low proliferative rate and low levels of collagen synthesis. In contrast, the activated or myoblast-like phenotype is distinguished by loss of vitamin A-containing lipid droplets, increased cell proliferation and increased synthesis of collagen. The activated form predominates in liver fibrosis (Friedman, 1993) and is observed in livers of rats experiencing vitamin A toxicity. Although these observations suggest a possible link between stellate cell vitamin A storage and liver disease, the underlying pathophysiological mechanisms remain unclear. It has been established that retinoic acid exacerbates rat liver fibrosis by inducing activation of latent transforming growth factor-β (TGF-β) (Okuno *et al.*, 1997), the major cytokine implicated in the pathogenesis of liver fibrosis and cirrhosis (Friedman, 1993). This action of retinoic acid on TGF-β activity could be an important process linking vitamin A toxicity and hepatic fibrosis.

In culture, stellate cells isolated from healthy control rats rapidly lose their *in vivo* quiescent phenotype and become activated. One characteristic of this activated phenotype observed in cultured rat stellate cells is the rapid loss of their capability to store retinyl ester (Trøen *et al.*, 1994). Freshly isolated rat stellate cells contain about 144 nmol retinol/mg cellular protein;

this concentration declines to 33 nmol/mg after two days in culture (Trøen *et al.*, 1994) and continues to decline until no vitamin A remains. Therefore, the many *in vitro* studies utilizing primary cultures of rat stellate cells may not be relevant for understanding normal hepatic vitamin A physiology, since these cells have the activated rather than quiescent phenotype.

3.2.4.5 Hepatic mobilization and plasma transport of retinol: retinol-binding protein

The transport of retinol from vitamin A stores in the liver to target tissues is accomplished exclusively by means of its specific plasma transport protein, RBP (Goodman, 1984; Soprano & Blaner, 1994). In the circulation, the retinol–RBP complex is bound to another plasma protein, transthyretin (TTR).

The liver is the major site of synthesis of RBP, which occurs primarily in the parenchymal cells (Goodman, 1984; Soprano & Blaner, 1994). Initial immunocytochemical studies indicated that RBP distribution within the liver was restricted to the parenchymal cells. Likewise, examination by specific and sensitive radioimmunoassay procedures of highly purified fractions of parenchymal cells, Kupffer cells, endothelial cells and stellate cells revealed RBP only in the purified parenchymal cell preparations (Blaner *et al.*, 1985). Subsequent investigations have established that RBP mRNA is localized solely in parenchymal cells and not in stellate cells (Yamada *et al.*, 1987; Weiner *et al.*, 1992; Friedman *et al.*, 1993). High resolution immunoelectron microscopy similarly did not reveal immunoreactive RBP in any hepatic cell type other than parenchymal cells (Suhara *et al.*, 1990). However, after a large dose of human RBP (corresponding to about twice the amount of RBP present in the entire circulation of a rat) was injected into the circulation of rats, a very small amount of human RBP was detected by immunohistochemical techniques in the stellate cells (Senoo *et al.*, 1990). Nevertheless, the great majority of RBP of hepatic origin, if not all, seems to be synthesized by parenchymal cells.

RBP secretion from the liver is a highly regulated process that is still not fully understood. The factors and processes that regulate RBP secretion are primarily localized to the endoplasmic reticulum (Goodman, 1984; Soprano & Blaner, 1994). It seems that retinol availability within the cell is the most critical factor regulating the secretion of RBP. In addition, other hormonal and physiological factors probably play roles in regulating the efflux of RBP from cells. It has long been known, for instance, that when retinoic acid is provided chronically in the diet to rats, plasma retinol–RBP concentrations decline by 25–50% (Shankar & DeLuca, 1988). Recent data suggest that the retinol-RBP:TTR complex forms in the hepatocyte before being secreted into the circulation (Soprano & Blaner, 1994). However, it remains uncertain what biochemical signals or processes are important for regulating the transcription of RBP, for retaining RBP within cells or conversely, for allowing the secretion of RBP. It is not yet known if the information needed to bring about secretion of RBP is internal in the RBP primary sequence or if this information resides in some other still undescribed molecule.

In vitamin A deficiency, apo-RBP accumulates in the liver to levels which are 3–10-fold higher than those observed in corresponding control livers (Goodman, 1984; Soprano & Blaner, 1994), while liver RBP mRNA levels show no difference between the two nutritional states (Soprano & Blaner, 1994). It is clear that retinol-deficiency specifically inhibits the secretion of RBP from the liver. Several biochemical studies have shown that retinol deficiency largely prevents the movement of newly synthesized RBP from the endoplasmic reticulum to the Golgi apparatus (Suhara *et al.*, 1990). RBP concentrations in endoplasmic reticulum fractions isolated from retinol-deficient rat livers were substantially elevated over levels in similar fractions from normal rat livers (Soprano & Blaner, 1994). In cultured hepatocytes prepared from retinol-deficient rats, pulse-labelled RBP accumulated in the endoplasmic reticulum and was not secreted into the cell medium. Interestingly, the transit of RBP through the endoplasmic reticulum of cultured hepatocytes prepared from normal rats is relatively slow compared to the transit times for albumin and transferrin. Studies investigating the sub-

cellular localization of plasma RBP in normal, retinol-deficient, and retinol-repleted retinol-deficient rats using electron microscopic techniques have provided additional information regarding the RBP secretory pathway (Suhara *et al.*, 1990). In the normal liver parenchymal cell, RBP was localized in synthetic and secretory structures, including endoplasmic reticulum, Golgi complex and secretory vesicles. This distribution changed markedly with retinol depletion. A heavy accumulation of RBP in the endoplasmic reticulum accompanied by a marked decrease in RBP-positive Golgi complex and secretory vesicles was observed in the parenchymal cells of retinol-deficient rats. After repletion of deficient rats with retinol, the RBP from the endoplasmic reticulum appeared to move rapidly through the Golgi complex and the secretory vesicles to the surface of the cell.

3.2.4.6 Hepatic retinoic acid formation and metabolism

The liver is a site for both synthesis of retinoic acid from retinol and oxidative metabolism of retinoic acid. This oxidative metabolism may be activating in nature, since some retinoic acid metabolites such as the 4-hydroxy and 4-oxo derivatives are active in transactivation assays (Mangelsdorf *et al.*, 1994; Hofmann & Eichele, 1994; Gudas *et al.*, 1994) or may be catabolic in nature, generating products which will be eliminated from the body (Blaner & Olson, 1994).

(a) Retinoic acid formation

Many studies of the formation of retinoic acid from retinol have been carried out using enzyme preparations from rat liver, as described in detail in Section 3.2.7.1. As outlined below, hepatocytes are especially rich in both cytosolic and membrane-bound enzymes that catalyse the oxidation of retinol to retinal (Blaner & Olson, 1994). In addition, hepatocytes are rich in retinal dehydrogenases that catalyse oxidation of retinal to retinoic acid (Blaner & Olson, 1994). It is clear that many different enzymes in the liver are able to catalyse the oxidation of retinol or retinal *in vitro*, but there is as yet no consensus regarding the physiological relevance of each of these in intact animals.

An important question for understanding the metabolism of vitamin A within the intact organism concerns whether retinoic acid is specifically synthesized in some tissues for export into the circulation to other tissues. Although there is very little information addressing this possibility, it is assumed by some investigators that some of the retinoic acid synthesized in the liver is secreted into the circulation for delivery to other tissues. Considering the great capacity of hepatic enzymes to catalyse retinol and retinal oxidation *in vitro*, this may be reasonable, but it remains to be established experimentally.

(b) Retinoic acid metabolism

The liver also possesses enzymatic machinery that is able to metabolize retinoic acid. Metabolites of all-*trans*-retinoic acid generated *in vivo* include 13-*cis*-retinoic acid, 9-*cis*-retinoic acid, retinoyl β-glucuronide, 5,6-epoxyretinoic acid, 4-hydroxyretinoic acid, 4-oxoretinoic acid and 3,4-didehydroretinoic acid (Blaner & Olson, 1994). Some of these metabolites may be active in mediating retinoic acid function, whereas others are probably catabolic products destined for export from the body. The formation of many of these metabolites seems to be catalysed by enzymes of the cytochrome P450 system. Aspects of retinoic acid metabolism and the role of the cytochrome P450 system are described in Section 3.2.7.3.

3.2.5 Plasma transport of vitamin A

In the fasted state, the predominant form of vitamin A in the circulation is retinol, bound to RBP. It is thought that retinol accounts for more than 99% of the vitamin A present in the circulation of fasted rats (Goodman, 1984; Soprano & Blaner, 1994). However, low levels are also present of all-*trans*-retinoic acid, 13-*cis*-retinoic acid, glucuronides of both retinoic acid and retinol, some retinyl ester bound to lipoproteins and possibly some other metabolites of retinol and retinoic acid. The same seems to hold for most other animal species which have been investigated, although the relative abundance of each of the vitamin A metabolites may vary among species. Nevertheless, these other

forms of vitamin A in the circulation may be important sources of vitamin A for some tissues under some physiological and/or pathological conditions. The different vitamin A species present in the fasting circulation are considered separately below and in Section 7.2.

3.2.5.1 Retinol delivery to target tissues

The sole plasma transport protein for retinol is RBP. This is a single polypeptide chain with a molecular weight of about 21 000 and has one binding site for one molecule of all-*trans*-retinol. In the blood, RBP circulates as a 1:1 molar complex with another serum protein, transthyretin (formerly called prealbumin). Studies of transthyretin-deficient mice have demonstrated that the formation of the complex reduces the glomerular filtration and renal catabolism of RBP. Normal levels of serum retinol and RBP in healthy well nourished Caucasian populations are about 2–3 μM (Goodman, 1984; Soprano & Blaner, 1994). The levels are slightly lower (1–2 μM) in the circulations of well nourished rats, mice and rabbits (Goodman, 1984; Folman *et al.*, 1989; Soprano & Blaner, 1994; Wei *et al.*, 1995). Since essentially all retinol in the circulation is bound to RBP, serum retinol and RBP levels are highly correlated in well nourished humans as well as vitamin A-sufficient rats and mice (Goodman, 1984). Erythrocytes contain only very small amounts of vitamin A (Bieri *et al.*, 1979). Treatment of humans and rats with *N*-(4-hydroxyphenyl)retinamide markedly lowers serum retinol concentrations (Formelli *et al.*, 1996).

Circulating levels of retinol–RBP remain very constant except in response to extremes in vitamin A, protein, calorie and zinc nutrition, or hormonal factors or stress or as a consequence of some disease states (Goodman, 1984; Soprano & Blaner, 1994). The physiological process responsible for maintaining and regulating retinol–RBP levels in the circulation are not well characterized. Plasma retinol–RBP levels could be maintained through regulation of RBP synthesis and/or secretion from the hepatocyte, through regulation of retinol–RBP plasma clearance and catabolism, or through a combination of these mechanisms. RBP synthesis and secretion from hepatocytes is a regulated process that is still not fully understood (Soprano & Blaner, 1994). Hormonal and physiological factors probably play roles in regulating the efflux of RBP from hepatocytes, but it remains uncertain which biochemical signals or processes are important for retaining RBP within hepatocytes or conversely, for allowing the secretion of RBP. Similarly, there is very little information regarding the catabolism of RBP or its regulation. It is believed that the kidney plays an important role, since human patients and rats with chronic renal failure show elevated plasma RBP levels (Goodman, 1984). However, it is not understood how the kidney or other organs influence or regulate RBP turnover.

Plasma retinol homeostasis in the rat has been extensively studied (Sundaresan, 1977; Keilson *et al.*, 1979; Underwood *et al.*, 1979; Lewis *et al.*, 1981, 1990; Green *et al.*, 1985, 1987; Gerlach & Zile, 1990, 1991), leading to the conclusion that a feedback control mechanism regulates mobilization and/or release of retinol–RBP from hepatic stores. It has been proposed that in response to peripheral tissue needs for vitamin A, a signal is sent from the periphery to the liver to regulate retinol–RBP release (Vahlquist *et al.*, 1973; Sundaresan, 1977; Green *et al.*, 1985, 1987; Lewis *et al.*, 1990). Although this feedback signal has not been identified, circulating retinoic acid levels may provide such a signal (Sundaresan, 1977; Keilson *et al.*, 1979; Underwood *et al.*, 1979; Lewis *et al.*, 1981). A regulatory linkage between plasma retinol and retinoic acid levels is suggested by the finding that plasma retinol levels are lower in animals receiving relatively high amounts of retinoic acid in the diet (Keilson *et al.*, 1979; Lewis *et al.*, 1981; Shankar & DeLuca, 1988). Others have suggested that apo-RBP levels (Vahlquist *et al.*, 1973; Sundaresan, 1977; Green *et al.*, 1985, 1987; Lewis *et al.*, 1990) or a modified form of circulating RBP (Keilson *et al.*, 1979; Underwood *et al.*, 1979) may serve as such a signal. At present, though, the identity and nature of such a signal from the periphery to the liver remain elusive.

Even though data have been lacking, there also have been suggestions that the magnitude of vitamin A uptake from the diet may play some role in influencing or possibly regulating

plasma retinol–RBP levels (Keilson *et al.*, 1979; Lewis *et al.*, 1990). Among other possibilities, Underwood *et al.* (1979) suggested that the rate of release of retinol from retinyl esters in the liver from newly absorbed vitamin A may be important for regulating plasma retinol levels. Lewis *et al.* (1990) suggested that when RBP synthesis is not compromised, the rate-limiting factor for retinol secretion from the liver is the appropriate positional availability of unesterified retinol near the intracellular site of RBP synthesis. They also suggested that an acute influx of a reasonable load of diet-derived vitamin A will increase hepatocyte retinol–RBP secretion up to some saturation point at which apo-RBP availability becomes rate-limiting.

The biochemical mechanism through which cells take up retinol from plasma RBP is central for understanding vitamin A metabolism and actions within cells, but has not yet been identified. The possible existence of a cell surface receptor for RBP has been extensively explored (Soprano & Blaner, 1994), but only very limited data characterizing a plasma membrane protein implicated as an RBP receptor have appeared. Thus, even though many cellular systems have been characterized as having cell surface RBP-binding activity, the biochemical nature of such a putative plasma membrane receptor remains unclear. Other studies failed to demonstrate a cell surface receptor for RBP in isolated cells or found that such a receptor is not necessary to ensure the cellular uptake of retinol from RBP. Overall, the recent reports concerning this topic can be divided into two groups based on the nature of the studies. One group consists of reports which characterize the binding (or lack of binding) of RBP to tissue minces, cultured cells or plasma membrane vesicles and the uptake of retinol from RBP by them. The second group examines retinol transfer from RBP (or other proteins able to bind retinol) to liposomes or isolated membrane systems. Two opposing views have been derived from these studies. One proposes the involvement of a cell surface receptor for RBP in the process of internalization of retinol by cells. The other view suggests that retinol is internalized by cells from RBP through a non-receptor-mediated uptake process. It is also possible that some cell types require retinol through a cell surface receptor whereas others do not; these two uptake possibilities can possibly coexist within the body.

In hypervitaminosis A (vitamin A toxicity), it is postulated that toxicity arises due to the inability of RBP to pick up and transport vitamin A 'leaking' from overfilled hepatic storage sites. Essentially, the available RBP is overwhelmed with excessive vitamin A and is unable to bind all the retinol in need of uptake and transport. The retinol not bound to RBP would then be free to associate with lipoproteins which non-specifically deliver it to cells and subcellular locations that do not usually process retinol. The retinol-containing lipoproteins would accumulate within cellular lysosomes. Here the retinol is thought to have a disrupting effect on the lysosomal membranes, causing release of lysosomal enzymes into the cell that could be the underlying cause of the pathology associated with hypervitaminosis A (Goodman, 1984).

In hypovitaminosis A (vitamin A deficiency), plasma retinol–RBP levels drop only after hepatic total retinol stores become depleted. Studies in the rat indicate that RBP is synthesized by the hepatocyte normally, but, in the absence of retinol, the apo-RBP is not secreted from the hepatocyte (Goodman, 1984). In hypovitaminosis A, apo-RBP levels in the liver rise. If retinol becomes available again, this RBP rapidly binds the retinol and is immediately secreted into the circulation for delivery to target tissues.

3.2.5.2 Plasma retinoic acid

In plasma, retinoic acid circulates bound to albumin (Blaner & Olson, 1994). The fasting plasma level of retinoic acid is very low, in the range of 1–14 nmol/L in humans (about 0.2–0.7% of plasma retinol levels) (De Leenheer *et al.*, 1982; Eckhoff & Nau, 1990a; Arnhold *et al.*, 1996) and 1–7 nmol/L in rats (Cullum & Zile, 1985; Napoli *et al.*, 1985; Tzimas *et al.*, 1995). It is not known if the retinoic acid present in the circulation arises solely from the diet (i.e., is of intestinal origin) or if some arises through export of retinoic acid from tissues which synthesize it from retinol. The possibility that the kidney is a site of synthesis and

export of retinoic acid has been raised (Bhat *et al.*, 1988a,b); however, at present, no data are available to support the possibility that some tissues (except for the intestine after dietary intake of carotenoids or retinoids) are able to provide retinoic acid to the circulation for delivery to other tissues.

Retinoic acid is taken up efficiently by cells. No cell surface receptor specific for the all-*trans* acid is known. Retinoic acid, although fully ionized in aqueous solutions at pH 7.4, is uncharged when within a lipid environment (Noy, 1992a,b). In the uncharged state, it moves rapidly between the outer and inner leaflets of the plasma membrane and can thus traverse cellular membranes and rapidly enter the cell.

The concentration of retinoic acid in the blood can be markedly influenced by recent uptake of vitamin A. For example, in monkeys different vitamin A supplement formulations had marked effects on plasma levels of retinol, retinyl esters, all-*trans*-, 13-*cis*-, all-*trans*-4-oxo- and 13-*cis*-4-oxoretinoic acid and retinyl-β-glucuronide and retinoyl-β-glucuronide. Plasma levels of these compounds were much higher when the vitamin A was in a Tween 20-containing aqueous preparation as compared to a soybean oil-based vehicle (Eckhoff *et al.*, 1991a). The vitamin A present in a liver meal (see Section 3.1.3.1 and Tables 2 and 3) is handled differently by humans as compared to vitamin A present in a supplement, in particular with regard to the metabolism of all-*trans*-retinoic acid (see also Section 7.2.2).

Kurlandsky *et al.* (1995) explored the contribution of plasma retinoic acid to tissue pools of this compound in chow-fed male rats, and reported the tissue levels (Table 7). Using a steady-state tracer kinetic approach, these authors determined how much of the retinoic acid in each tissue was derived from the circulation. For the liver and brain, more than 75% was derived from the circulation (88.4% in brain and 78.2% in liver). The seminal vesicles, epididymis, kidney, epididymal fat, perinephric fat, spleen and lungs derived, respectively, 23.1%, 9.6%, 33.4%, 30.2.%, 24.5%, 19.0% and 26.7% of their all-*trans*-retinoic acid from the circulation. In the pancreas and eyes, only 2.3% and 4.8%, respectively, was contributed by the circulation. The testes did not take up any (<1%) retinoic acid from the circulation. The authors also reported a fractional catabolic rate for retinoic acid in plasma of 30.4 plasma pools/h and an absolute catabolic rate for retinoic acid of 640 pmol/h. These rates are very rapid compared with those of the only other naturally occurring form of vitamin A studied under normal physiological conditions, all-*trans*-retinol (Lewis *et al.*, 1990). Very little 9-*cis*- or 13-*cis*-retinoic acid was detected in any of these tissues. These data demonstrate that plasma all-*trans*-retinoic acid is a significant source of all-*trans*-retinoic acid for some, but not all, tissues; this suggests, by inference, that tissues have different capacities for the conversion of retinol to retinoic acid.

In rabbits, the β-carotene content of the diet influences serum levels of retinoic acid (Folman *et al.*, 1989). In female rabbits given graded doses of β-carotene in their diet for nine weeks, β-carotene intake was associated with higher serum concentrations of retinoic acid.

*3.2.5.3 Plasma 13-*cis*-retinoic acid*

Cullum and Zile (1985) reported that 13-*cis*-retinoic acid is an endogenous retinoid present in the intestinal mucosa, intestinal muscle and

Table 7. All-*trans*-retinoic acid concentrations in various rat tissues [a,b]

Tissue	All-*trans*-retinoic acid (pmol/g tissue)
Liver	11.3 ± 4.7
Brain	6.8 ± 3.3
Testis	10.7 ± 2.7
Seminal vesicles	12.0 ± 7.0
Epididymis	4.2 ± 1.6
Kidney	8.3 ± 4.0
Pancreas	29.3 ± 16.3
Epididymal fat	15.7 ± 12.3
Perirenal fat	12.7 ± 8.7
Spleen	12.7 ± 12.0
Eyes	125 ± 37.3
Plasma	1.8 ± 0.7[c]

[a] From Kurlandsky *et al.* (1995)
[b] Each value is given as the mean ± 1 standard deviation for separate measurements employing eight individual 400–450 g male Sprague-Dawley rats.
[c] pmol/mL plasma.

plasma of vitamin A-sufficient rats. When a dose of all-*trans*-retinoic acid was administered by intrajugular injection into vitamin A-depleted rats, 13-*cis*-retinoic acid appeared in the plasma and small intestine within 2 min after dosing. The endogenous plasma concentrations of all-*trans*- and 13-*cis*-retinoic acid in vitamin A-sufficient rats were reported to be 9.7 and 3.0 nmol/L, respectively. The authors concluded that 13-*cis*-retinoic acid is a naturally occurring metabolite of all-*trans*-retinoic acid. Napoli *et al.* (1985) similarly demonstrated that 13-*cis*-retinoic acid is a naturally occurring form of retinoic acid in rat plasma. Bhat and Jetten (1987) demonstrated that cultures of rabbit tracheal epithelial cells can convert all-*trans*-retinoic acid to 13-*cis*-retinoic acid, and Tang and Russell (1990) confirmed that 13-*cis*-retinoic acid is an endogenous component in human serum. Fasting serum levels of all-*trans*- and 13-*cis*-retinoic acid determined in 26 human volunteers ranged from 3.7 to 6.3 nmol/L and from 3.7 to 7.2 nmol/L, respectively (Tang & Russell, 1990), levels similar to those observed for the rat. Plasma levels of all-*trans*- and 13-*cis*-retinoic acid levels rose 1.3- and 1.9-fold, respectively, above fasting levels, in human subjects who had received a physiological dose of retinyl palmitate (Tang & Russell, 1991). Similarly, Eckhoff *et al.* (1991b) demonstrated that 13-*cis*-retinoic acid is an endogenous component of human plasma and that administration of an oral dose of retinyl palmitate elevated plasma levels of 13-*cis*-retinoic acid.

3.2.5.4 Plasma 9-cis-retinoic acid

The existence of 9-*cis*-retinoic acid in cells was reported simultaneously by Levin *et al.* (1992) and Heyman *et al.* (1992). Using a nuclear receptor-dependent ligand-trapping technique to identify 9-*cis*-retinoic acid, Levin *et al.* (1992) demonstrated that this stereoisomer is an activating ligand for RXR-α in COS-1 cells. Heyman *et al.* (1992) similarly reported that 9-*cis*-retinoic acid is a ligand for the human nuclear retinoid receptor, RXR-α and estimated that the concentrations of 9-*cis*-retinoic acid in mouse liver and kidney were 13 and 100 pmol/g tissue, respectively. Mangelsdorf *et al.* (1992) showed that 9-*cis*-retinoic acid is able to transactivate gene expression through the actions of mouse RXR-α, RXR-β and RXR-γ.

3.2.5.5 Retinoid glucuronides

When all-*trans*-retinoic acid is orally administered to rats, all-*trans*-retinoyl β-glucuronide is secreted into the bile in significant amounts (Blaner & Olson, 1994). This metabolite is synthesized from retinoic acid and uridine diphosphoglucuronic acid in the liver, intestine, kidney and other tissues by several of the 40 or more identified microsomal β-glucuronyl transferases (Genchi *et al.*, 1996, 1998). Of various tissues, the intestinal mucosa seems to be the most active in synthesizing and retaining retinoyl β-glucuronide. When 13-*cis*-retinoic acid is administered, all-*trans*-retinoyl β-glucuronide is a major metabolite in rats *in vivo*. Isomerization to all-*trans*-retinoic acid probably, but not necessarily, occurs before conjugation. The extent of retinoyl β-glucuronide formation from retinoic acid, as assessed from pharmacokinetic measurements, is dependent on both the isomer administered and the species, although retinoyl β-glucuronide is formed in all species studied. Orally administered retinoyl β-glucuronide is hydrolysed only slowly *in vivo* in vitamin A-sufficient rats, but more rapidly in vitamin A-deficient rats (Kaul & Olson, 1998).

Retinol is also conjugated with glucuronic acid *in vivo* and *in vitro* to form retinyl β-glucuronide (Blaner & Olson, 1994). Like retinoyl β-glucuronide, retinyl β-glucuronide is present in human plasma at a mean concentration of 6.8 nmol/L (Blaner & Olson, 1994). Retinyl β-glucuronide is hydrolysed *in vivo* to retinol, however, which is esterified and stored in the liver.

3.2.5.6 Other metabolites of retinol

In 1990, Buck *et al.* showed that human lymphoblastoid cells in culture are dependent for growth on a constant supply of retinol. In the absence of retinol, these cells perished within days. Retinoic acid was unable to prevent cell death. Employing sensitive trace-labelling techniques, Buck *et al.* (1991a) did not detect

retinoic acid and 3,4-didehydroretinoic acid as metabolites of retinol in activated B lymphocytes. Nevertheless, B lymphocytes formed several other metabolites of retinol, which were able to sustain B cell growth in the absence of an external source of retinol (Buck *et al.*, 1991a). Buck *et al.* (1991b) found one of these active metabolites to be optically active 14-hydroxy-4,14-retro-retinol, identified as a direct biosynthetic product of retinol in 5/2 lymphoblastoid cells. This metabolite was isolated, by reverse-phase HPLC, from cells of the lymphoblastoid line 5/2 which were grown in the presence of [^{3}H]retinol–RBP complex; it was active in sustaining the growth of cells of the lymphoblastoid line 5/2 (and of T cell lines) in the absence of retinol. In confirmation of their initial studies, retinoic acid was not active in these growth assays. Thus, in addition to the retinoic acid pathway, a second pathway of retinol metabolism mediates the actions of retinoids in cellular growth and differentiation, at least in specific cells. In mouse skin, retinol was also converted to 14-hydroxy-4,14-retro-retinol (Sass *et al.*, 1996).

Another metabolite of all-*trans*-retinol proposed to have biological activity is all-*trans*-4-oxoretinol. Achkar *et al.* (1996) identified this metabolite as being essentially involved in maintaining F9 cell differentiation after its induction with all-*trans*-retinoic acid. The possible importance of 4-oxoretinol *in vivo* remains to be established.

3.2.5.7 Lipoprotein-bound retinyl ester

Low levels of retinyl ester can be found in the very low-density lipoprotein (VLDL), LDL and HDL plasma fractions from fasting humans (Krasinski *et al.*, 1990b). It is possible that this arises through the actions of cholesteryl ester transfer protein, which can transfer retinyl ester between triglyceride-rich chylomicrons and other lipoprotein fractions (Goodman & Blaner, 1984; Krasinski *et al.*, 1990b; Blaner & Olson, 1994; Tall, 1995). Alternatively, human hepatocytes may package and secrete some retinyl ester in nascent VLDL.

In the rabbit, the liver does not secrete retinyl ester in VLDL; all of the retinyl ester present in the circulation appears to ultimately arise from transfer of chylomicron retinyl ester to other lipoprotein fractions (Thompson *et al.*, 1983). However, this is not the case for all animal species. Fasting blood of some species, especially ferrets and dogs, contains high levels of retinyl ester in VLDL, LDL and HDL (Wilson *et al.*, 1987; Schweigert, 1988; Ribaya-Mercado *et al.*, 1994; Lederman *et al.*, 1998). In both dogs and ferrets, retinyl ester is the major form of vitamin A in the fasting circulation. In dogs, lipoprotein-bound retinyl ester in the fasting circulation may arise from hepatic secretion of retinyl ester in VLDL (Wilson *et al.*, 1987).

Overall, different species show very distinct patterns of distribution of retinyl ester in fasting blood. The level of retinyl ester present in fasting rodent and rabbit blood closely resembles the level in fasting human blood. However, since rodents do not express cholesteryl ester transfer protein and since rabbits do not secrete retinyl ester in VLDL, one must apply caution when trying to extrapolate from these species to man.

3.2.6 Extrahepatic vitamin A storage and metabolism

Peripheral tissues other than the liver play important roles in the storage and mobilization of retinol. Four lines of evidence support this conclusion. (*a*) Kinetic modelling studies (Lewis *et al.*, 1981, 1990; Green *et al.*, 1985, 1987) initially suggested that, in both vitamin A-sufficient and -deficient rats, retinol is extensively recycled among the liver, plasma, interstitial fluid and peripheral tissues. A multicompartment model of whole-body retinoid metabolism (Green *et al.*, 1985) provides an excellent means for understanding the dynamics of retinoid transport and storage in liver and peripheral tissues. According to this model, extrahepatic tissues of rats with normal plasma retinol levels but with very low total liver stores (< 0.35 μmol of vitamin A), should contain 44% of whole-body vitamin A. (*b*) The sole mechanism for plasma transport of retinol from the liver to tissues is by complexing with RBP (see Section 3.2.5.1). Although most RBP is thought to be synthesized in the liver, Soprano *et al.* (1986) have demonstrated that a variety of tissues, including the kidney, lung, heart, spleen,

skeletal muscle and adipose tissue express RBP; thus, these tissues may well play a role in the recycling of retinol back to the liver. (*c*) Retinol and retinyl esters as well as enzymes that are able to esterify retinol and to hydrolyse retinyl esters are found in most peripheral tissues. (*d*) Many rat tissues, including lung, kidney and small intestine, contain cells which morphologically and structurally resemble hepatic stellate cells (Nagy *et al.*, 1997). These extrahepatic stellate-like cells contain lipid droplets which resemble those of hepatic stellate cells and both their size and number increase in response to administration of excess dietary vitamin A. Limited biochemical data suggest that these cells contain relatively high concentrations of vitamin A, which increase in response to dietary vitamin A intake (Nagy *et al.*, 1997), supporting a role for these 'extrahepatic stellate cells' in vitamin A storage and metabolism.

Figure 4 represents the transport and metabolism of retinol and its metabolites within target cells.

Table 8 provides a summary of total retinol levels reported in the literature for adipose tissue, kidney, testis, lung, bone marrow and eye cups for some species. For some tissues, the range of total retinol levels reported by different investigators is large. Presumably, this reflects species and strain differences and the physiological status (age, sex and dietary

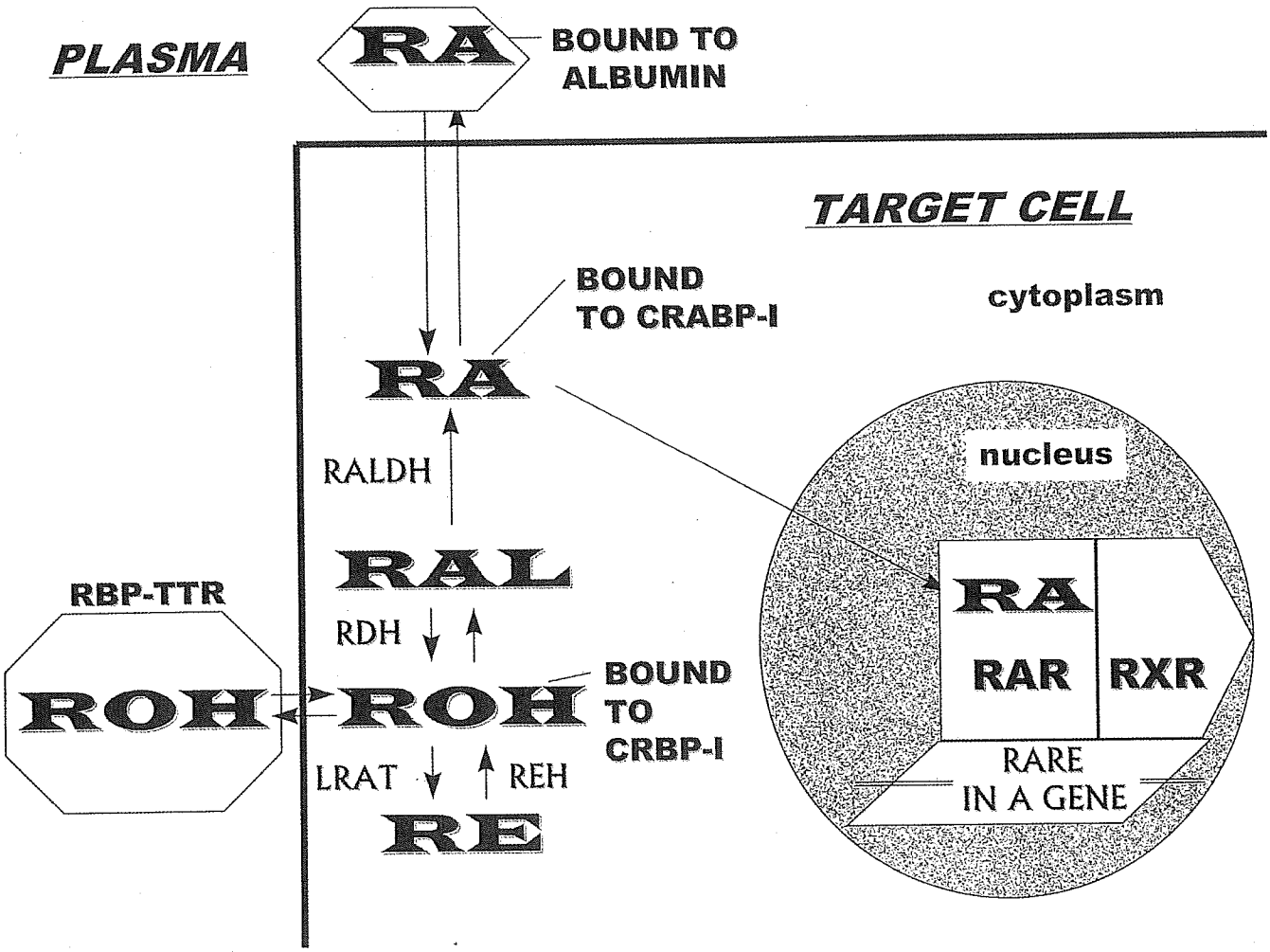

Figure 4. Simplified scheme for the transport and metabolism of retinol and retinyl esters within target cells, applicable to humans and most animal models.

Details of these processes are described in the text. Not all processes necessarily occur in all cells. For key to abbreviations, see Figure 1.

Table 8. Reported total retinol levels in extrahepatic tissues for various animal species

Tissue	Species	Vitamin A concentration (nmol/g tissue)	Reference
Adipose tissue	Rat	20 ± 8	Tsutsumi *et al.* (1992)
	Rabbit	8.0	Blaner *et al.* (1993)
	Ferret	34 ± 4	Ribaya-Mercado *et al.* (1992)
Kidney	Rat	40.9	Bhat & Lacroix (1983)
	Rat	6.6	Napoli *et al.* (1984)
	Rat	10.0	Gerlach *et al.* (1989)
	129sv mouse	1.4 ± 0.7	Wei *et al.* (1995)
	Ferret	87.1 ± 14.4	Schweigert *et al.* (1995)
Testis	Rat	20.3	Bhat & Lacroix (1983)
	Rat	4.2	Gerlach *et al.* (1989)
	129sv mouse	2.8 ± 1.4	Wei *et al.* (1995)
Lung	Rat	21.5	Bhat & Lacroix (1983)
	Newborn rat pups	53.6	Shenai & Chytil (1990)
	C57BL mouse	700 – 1400	Ribaya-Mercado *et al.* (1992)
Bone marrow	Rabbit	13	Blaner *et al.* (1993)
	Rabbit	2.3	Skrede *et al.* (1993)
Eye	Eye cup of 129sv mouse	1.9 ± 0.6	Wei *et al.* (1995)

status) of the animals employed. A difficulty in compiling Table 8 arose from differences in how investigators report tissue vitamin A levels. Some report concentration of retinol (vitamin A) per g tissue wet weight, as given in Table 8. However, other investigators have reported tissue retinol levels per g tissue dry weight or per mg tissue total protein and consequently, it is not always possible to reconcile all of the reported tissue vitamin A concentrations.

3.2.6.1 Adipose tissue

Vitamin A has long been known to be present in adipose tissue (Moore, 1957). Tsutsumi *et al.* (1992) reported similar retinoid levels in six anatomically different adipose depots(inguinal, dorsal, mesenteric, epididymal, perinephric, and brown adipose tissue) of chow-fed rats, averaging approximately 20 nmol retinol/g adipose tissue. About two thirds of the retinoid present in adipose tissue was reported to be present as retinol and the remainder as retinyl ester. In these studies, hepatic vitamin A levels were approximately 520 nmol/g liver. Because adipose tissue and liver in mature rats represent approximately 15% and 4%, respectively, of the total body mass, the total amount of vitamin A in adipose tissue was approximately 14% of that in the liver. Thus, adipose tissue contributes substantially to total body retinoid stores. Tsutsumi *et al.* (1992) also identified the adipocyte (and not the stromal-vascular cells) as the cellular site of retinoid storage in adipose tissue. Adipocytes from the epididymal, perinephric and brown adipose depots contained between 2 and 3 nmol retinol per 10^6 cells. Primary rat hepatocytes contain between 0.35 and 1.2 nmol vitamin A per 10^6 cells (Blaner *et al.*, 1985; Yamada *et al.*, 1987), whereas rat liver stellate cell isolates contain 38 nmol vitamin A per 10^6 cells (Yamada *et al.*, 1987). Thus, rat adipocytes and liver parenchymal cells contain

similar levels of vitamin A. From this perspective, the adipocyte should be considered as important for retinoid storage.

Zovich *et al.* (1992) demonstrated that murine BFC-1β preadipocytes during their differentiation to adipocytes take up and esterify retinol, primarily to retinyl palmitate and oleate. Whether retinol esterification in adipocytes occurs via an acyl coenzyme-A-dependent or -independent process (involving the actions of LRAT) has not been defined. However, the esterification process seems to depend on the state of adipocyte differentiation in BFC-1β cells. This is also true for expression of RBP, which is not expressed in undifferentiated preadipocytes, but highly expressed in BFC-1β adipocytes. LPL, which is synthesized and secreted by BFC-1β adipocytes and binds to the surface of these cells, is active in hydrolysing chylomicron retinyl ester after much of the chylomicron triglyceride has been first hydrolysed (Blaner *et al.*, 1994). Therefore LPL may play a role in facilitating uptake of postprandial vitamin A by extrahepatic tissues. Hormone-sensitive lipase, a cAMP-stimulated lipase which also hydrolyses both triglycerides and cholesteryl esters, catalyses the hydrolysis of the retinyl esters present in BFC-1β adipocytes (Wei *et al.*, 1997). Hydrolysis of adipocyte retinyl ester catalysed by hormone-sensitive lipase seems to be regulated through cAMP-signalling pathways. Taken together, these results demonstrate that the adipocyte is dynamically involved in vitamin A uptake, storage and metabolism.

The role of adipose tissue in retinol storage has also been investigated in other species. Perinephric and epididymal fat from rabbits fed a control diet have been reported to contain 8.0 and 8.7 nmol total retinol/g tissue, respectively (Blaner *et al.*, 1993). For ferrets receiving control diets, subcutaneous adipose tissue is reported to contain 21.4 ± 6.4 nmol retinyl ester and 6.8 ± 3.1 nmol retinol/g tissue (Ribaya-Mercado *et al.*, 1992). In ferrets receiving the same diet supplemented with β-carotene (80 μg/g wet weight) for three weeks, subcutaneous adipose tissues contained less retinyl ester (13.4 ± 2.3 nmol/g) and more retinol (20.6 ± 1.8 nmol/g) than the control-fed ferrets (Ribaya-Mercado *et al.*, 1992). The mechanistic basis for this effect of β-carotene on total retinol levels in ferret adipose tissue was not established.

3.2.6.2 Kidney

Bhat and Lacroix (1983) reported that retinyl ester levels in normal rat kidney tissue are 40.9 nmol/g tissue. The retinyl esters consisted of 94.2% palmitate, 3.8% stearate, 1.0% linoleate and 1.0% palmitoleate. The concentration of 9-*cis*-retinol in rat kidney was reported to be approximately 10% of that of all-*trans*-retinol. The kidney of vitamin A-deficient rats given a single intraperitoneal injection of vitamin A (consisting of 53 μg retinol) accumulated progressively more vitamin A as the liver was progressively depleted (Bhat, 1997). This finding, along with the identification and localization of a retinal dehydrogenase, which is able to oxidize either 9-*cis*- or all-*trans*-retinal to the corresponding retinoic acid isomer in the kidney, led these investigators to propose that the kidney is able to synthesize all-*trans*-retinoic acid and 9-*cis*-retinoic acid from the all-*trans*- and 9-*cis*-retinol present in the tissue. They further proposed that the newly synthesized retinoic acid isomers are carried from the kidney in the blood to fill the needs of other tissues for retinoic acid (Bhat *et al.*, 1988a,b; Bhat, 1997).

Napoli *et al.* (1984) reported rat kidney retinol levels of 6.6 nmol/g of tissue. Of the total retinol localized in kidney, 74% was present as retinyl ester, with the remainder as retinol. In rats maintained on an α-tocopherol-free diet, the levels of retinol remained approximately the same (5.2 nmol retinol/g tissue), but retinyl esters markedly decreased (2.2 nmol/g tissue or only 30% of the total vitamin A present). A bile-salt-dependent retinyl ester hydrolase in normal rat kidney homogenates has a specific activity in the range 154–180 pmol/h/mg protein (Napoli *et al.*, 1984; Napoli & Beck, 1984). This hydrolase was inhibited by addition of 250 μmol/L α-tocopherol to the assay mixture (Napoli *et al.*, 1984). A microsomal bile-salt-independent retinyl ester hydrolase, which is unable to hydrolyse cholesteryl oleate, is also present in rat kidney (Napoli *et al.*, 1989).

Gerlach *et al.* (1989) reported that kidney from vitamin A-sufficient rats contains an

average of approximately 10 nmol vitamin A/g dry weight of tissue. Of the total vitamin A present, half each consisted of non-esterified retinol and of various retinyl esters.

Ribaya-Mercado *et al.* (1992) reported that kidney from ferrets fed a control diet contains 87.1 ± 14.4 nmol retinyl ester/g tissue and 15.1 ± 9.8 nmol retinol/g tissue. Maintaining ferrets on the same diet supplemented with β-carotene (80 μg/g diet) for three weeks led to an approximately four-fold reduction in renal retinyl ester content and 2.3-fold increase in renal retinol content. Kidney from five-week-old female pigs is reported to contain 61.5 ± 11.5 nmol total retinol/g tissue (Schweigert *et al.*, 1995). The relative distribution of this total retinol as retinyl ester and retinol was not reported.

3.2.6.3 Testis

Rajguru *et al.* (1982) demonstrated by autoradiographic techniques that vitamin A is primarily localized in three cellular sites within the adult rat testis, namely, the macrophages of the interstitial tissue, the lipid droplets of the Sertoli cells, and the spermatids in association with Golgi saccules. In rats receiving a control diet, the testes have been reported to contain retinyl ester at a concentration of 20.3 nmol/g tissue (Bhat & Lacroix, 1983). Approximately 98% was retinyl palmitate, the remainder consisting of the stearate, linoleate and palmitoleate (Bhat & Lacroix, 1983). Gerlach *et al.* (1989) reported that rat testis contained approximately 4.2 nmol vitamin A/g dry weight of tissue, retinol accounting for approximately 50% of the total vitamin A (retinol + retinyl ester) present. Studies by Chaudhary and Nelson (1985, 1986), exploring the metabolism of all-*trans*-retinyl acetate in rat testes, demonstrated that non-esterified retinol accounts for approximately 50% of the total vitamin A present in the testes, with the remainder present primarily as retinyl palmitate.

When retinol was provided to rat Sertoli cell cultures as [^{3}H]retinol–RBP:TTR, retinol was rapidly taken up and largely converted to retinyl ester (Bishop & Griswold, 1987). Within 28 h after the labelled retinol was applied, 83% of the labelled retinoids found in the Sertoli cells was accounted for as retinyl ester (64% of the ester was the palmitate). The endogenous retinol concentrations in isolated rat Sertoli cells averaged 75 ± 13 pmol/mg cellular protein.

Although both the acyl coenzyme-A-dependent and -independent retinyl ester synthase activities are present in rat Sertoli cell microsomes, LRAT is the physiologically important activity in the Sertoli cell (Shingleton *et al.*, 1989). A bile-salt-dependent retinyl ester hydrolase has also been reported to be present in rat testis homogenates, with a specific activity of 38–97 pmol/h/mg protein (Napoli *et al.*, 1984).

3.2.6.4 Lung

Bhat and Lacroix (1983) reported retinyl ester levels of 21.5 nmol vitamin A/g tissue in lungs of vitamin A-sufficient rats. Retinyl palmitate accounted for 62% of the total retinyl ester and the remainder was mainly retinyl stearate (Bhat & Lacroix, 1983). Napoli *et al.* (1984) reported substantially higher values; namely total vitamin A levels of 53.6 nmol/g tissue, with approximately 81% of the retinol present as retinyl ester. The palmitate and oleate together accounted for 53% of lung retinyl ester (Napoli *et al.*, 1984).

Within 24 h of the oral administration of retinol to pregnant rats, the concentrations of retinyl esters in the lungs of fetuses and of newborn pups were significantly (1.7–7.1-fold) higher than in the lungs of the control group (Shenai & Chytil, 1990). In untreated rats, prenatal levels of retinyl ester ranged between 7 and 14 nmol/g tissue, and postnatal levels, from birth to 14 days, consistently averaged around 3.5 nmol/g tissue. In contrast, after pregnant mothers were given a single intragastric dose of 52 μmol retinyl palmitate, retinyl ester levels in prenatal lungs ranged between 28 and 38 nmol/g tissue. During a 14-day postnatal period, however, vitamin A concentrations continuously declined. Thus, vitamin A concentrations in fetal lung clearly are responsive to prenatal administration of a single large dose of retinol to the mother.

Zachman *et al.* (1992) reported retinyl palmitate levels in alveolar Type II cells of 21 ± 3.5 pmol/mg protein and 8 ± 4.2 pmol/mg protein in cells isolated from control and vitamin A-deficient rats, respectively. When added to a monolayer of Type II cells, [^{3}H]retinol was

converted, in a time-dependent manner, to retinyl palmitate. Interestingly, cultured Type II cells are also able to synthesize retinoic acid from exogenous retinol (Zachman *et al.*, 1992).

Nagy *et al.* (1997) have reported that rat lung contains stellate-like cells which seem to take up and store vitamin A when rats are fed excess vitamin A. This finding is consistent with an earlier report of the isolation in high yield and high purity of retinol-storing cells from rat lung and their subsequent culture *in vitro* (Okabe *et al.*, 1984). These isolated cells apparently possess the overall morphology, including lipid droplets, that is characteristic of the retinol-storing cells found in lung tissues.

Levels of total retinol in lung tissue from three-month-old male C57BL mice fed a chow diet range between 700 and 1400 nmol of retinol per g tissue wet weight, compared with liver levels of 2100–2800 nmol. In these mice, the total retinol concentration in lung, when normalized per g tissue weight, is between 25 and 50% of that in the liver. Since the weight of a mouse lung is approximately 50% of the weight of the liver from the same animal, this implies that lung tissue of mice contains approximately 12.5 to 25% of the total retinol that is present in the mouse liver. Male Sprague-Dawley rats three months of age maintained on a chow diet have lung concentrations of total retinol ranging between 7 and 17.5 nmol retinol per g wet weight and liver levels ranging between 350 and 1050 nmol retinol per g wet weight. In the rat, when normalized per tissue weight, the lung contains approximately 1 to 5% of the total retinol present in the liver (Nagy *et al.*, 1997).

In ferrets receiving a control diet, retinyl ester concentrations in lung have been reported to be 4.3 ± 0.9 nmol/g tissue and lung retinol levels 0.31 ± 0.15 nmol/g tissue (Ribaya-Mercado *et al.*, 1992). Supplementation of the diet with β-carotene for three weeks resulted in an approximately two-fold increase in both lung retinyl ester and retinol concentrations.

3.2.6.5 Bone marrow

As outlined in Section 3.2.3.3, bone marrow of rabbits and of primates takes up substantial amounts of chylomicra. Chylomicron uptake by rabbit bone marrow is 50–100% of that of the liver. Rabbit bone marrow contains some stored retinol (Blaner *et al.*, 1993; Skrede *et al.*, 1993), one report indicating a level of 13 nmol total retinol/g tissue and another 2.3 nmol total retinol/g tissue. These reports agree that most if not all of the retinol present in bone marrow is unesterified. By comparison, rabbit perinephric and epididymal fat were reported to contain 8.0 and 8.7 nmol total retinol/g tissue, respectively (Blaner *et al.*, 1993) and the mean total retinol level for control rabbit liver (n = 3) was 429 nmol/g tissue (Skrede *et al.*, 1993). Interestingly, rabbit bone marrow also expresses RBP (Blaner *et al.*, 1993). Thus, bone marrow may well play a dynamic role in the overall metabolism of vitamin A.

It has been proposed that the vitamin A stored in bone marrow is important for maintaining normal blood cell differentiation (Twining *et al.*, 1996). Rats receiving a totally vitamin A-deficient diet reached a state where both hepatic and serum retinol concentrations were less than 1% of those of rats fed a control diet, but bone marrow from the vitamin A-deficient rats contained four times more retinol than that of control-fed animals. This suggests that bone marrow vitamin A stores are among the last tissue stores to be mobilized in the face of inadequate vitamin A intake. The vitamin A sequestered in the bone marrow of vitamin A-deficient rats may be important for the survival of the animal, since it will be needed to maintain the differentiation of myeloid cells to neutrophils.

3.2.6.6 Eye

The eye is an important site for vitamin A metabolism and action. Retinyl ester levels in cells of fresh retinal pigment epithelium (RPE) from human eyes range between 3.5 and 14 nmol per 10^6 cells (Flood *et al.*, 1983). This retinyl ester consists of 11-*cis*-retinyl palmitate, 11-*cis*-retinyl stearate, all-*trans*-retinyl palmitate (the major component), all-*trans*-retinyl stearate and all-*trans*-retinyl oleate. The post-mortem human pigment epithelium–choroid contains 36 ± 25 nmol vitamin A/g tissue (7.9 ± 4.5 nmol vitamin A/eye) (Blaner & Olson, 1994). Retinas contained 15.3% of the retinoid

present in the whole pigment epithelium–choroid complex. Most of the retinoid in the eye was esterified (98.3% in the pigment epithelium–choroid complex; 79.3% in the retina).

The lacrimal gland contains retinyl esters and both acyl-coenzyme A:retinol acyltransferase (ARAT) and retinyl ester hydrolase activities (Blaner & Olson, 1994). By cannulation of the lacrimal gland ducts of rabbits and rats, retinol was shown to be present in the tear fluid. When microsomes prepared from rabbit lacrimal gland were incubated with [^{3}H]retinol in the presence of an acyl-coenzyme A generating system, a mixture of retinyl esters, including the laurate, linoleate, palmitate and stearate, was formed. In the presence of 180 μmol/L [^{3}H]retinol and 100 μmol/L palmitoyl-coenzyme A, retinyl palmitate was synthesized at 175–220 pmol/mg/min and the reaction displayed Michaelis–Menten kinetics. Thus, the lacrimal gland seems to synthesize retinyl esters via an acyl-coenzyme A-dependent process. A bile-salt-independent retinyl ester hydrolase which is present in microsomes of rat lacrimal gland has a pH optimum of 7 and a maximum specific activity of 1073 pmol/mg/h.

A crucial step in the visual cycle is the isomerization of all-*trans*- to 11-*cis*-retinol, which occurs primarily, if not solely, in the RPE (Shi & Olson, 1990; Winston & Rando, 1998). All-*trans*-retinol is first acylated by LRAT, followed by a concerted hydrolysis and isomerization reaction to yield 11-*cis*-retinol (Winston & Rando, 1998).

In addition to having a complex pattern of vitamin A metabolism, the eye is possibly the most sensitive organ with regard to vitamin A availability. An early symptom of vitamin A deficiency is night-blindness. Well nourished patients receiving *N*-(4-hydroxyphenyl)retinamide (4HPR) also display night-blindness as a side-effect of the drug. 4HPR-induced night-blindness appears to arise from a competition between 4HPR and retinol for apo-RBP in liver cells, thereby markedly reducing the plasma concentration of holo-RBP (Ritter & Smith, 1996).

3.2.6.7 Other tissues and species

Many other tissues take up and store retinol. In addition to those discussed above, Gerlach *et al.* (1989) reported that trachea, intestine and spleen of vitamin A-sufficient rats contain retinyl esters. In the guinea-pig, Biesalski (1990) found significant concentrations of retinyl esters in the kidney, lung, testes, epididymis, vas deferens, trachea, nasal mucosa, tongue and inner ear. It seems likely that other tissues will also be found to store vitamin A. These local stores may be used to meet both local tissue demands as well as total body needs.

3.2.7 Retinoic acid metabolism

Although it is generally accepted that all-*trans*- and 9-*cis*-retinoic acid facilitate most of the actions of vitamin A in mammalian tissues, other forms of vitamin A, including all-*trans*-3,4-didehydroretinoic acid and 4-oxo-all-*trans*-retinoic acid are reported to bring about RAR interaction with RXRs and may be important for facilitating retinoid actions in birds and amphibians *in vivo* (Hofmann & Eichele, 1994; Mangelsdorf *et al.*, 1994). Each of these vitamin A forms, however, must be derived from all-*trans*-retinol. A metabolic scheme for the formation of these active retinoid forms from all-*trans*-retinol is given in Figure 5. The pathways shown are for the most part hypothetical, since data supporting some of the conversions are extremely limited. Only the enzymatic processes responsible for the formation of all-*trans*-retinal from all-*trans*-retinol and for the oxidation of all-*trans*-retinal to all-*trans*-retinoic acid have been much studied. Even the processes by which all-*trans*-retinoic acid is enzymatically formed within tissues by oxidation of all-*trans*-retinol have not been unequivocally established (Duester, 1996; Napoli, 1996). The currently prevailing hypothesis is that retinol is first oxidized to retinal, which in turn is oxidized to retinoic acid, a process analogous to the oxidation of ethanol to acetaldehyde and on to acetic acid. This process is represented diagramatically in Figure 5.

3.2.7.1 Retinoic acid formation in tissues

It has long been known that the relatively non-specific alcohol dehydrogenase of liver can catalyse the oxidation of retinol to retinal (Blaner & Olson, 1994; Duester, 1996: Napoli,

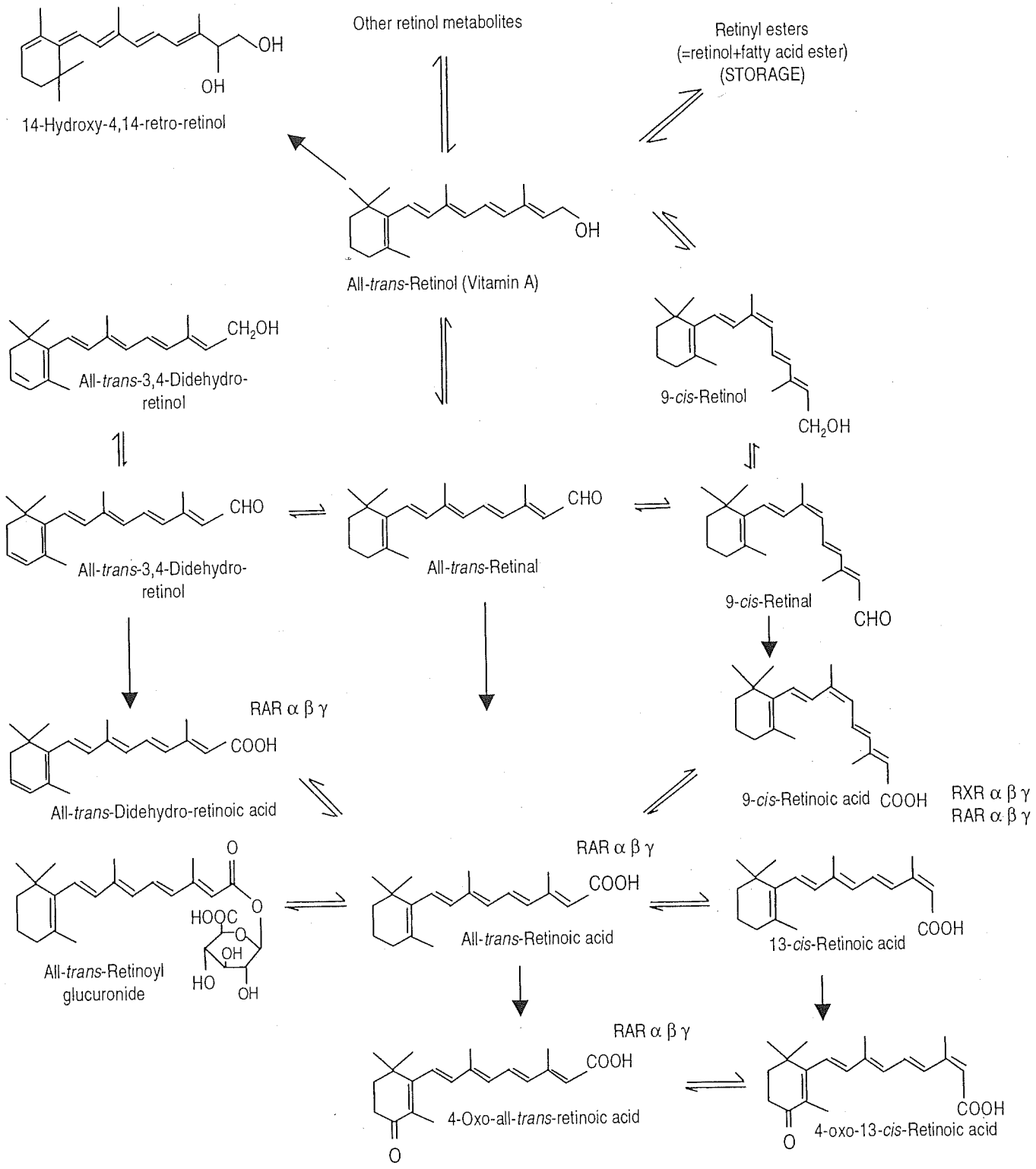

Figure 5. Hypothetical scheme for the metabolism of all-*trans*-retinol.
Although all of the metabolite interconversions indicated in this scheme have not been unequivocally demonstrated experimentally, all of these transformations of vitamin A species have been postulated in the literature to take place in some living organism. The reader should especially focus on the metabolic transformations described in the body of the text. These are the most extensively studied transformations and involve the oxidative metabolism of retinol to retinoic acid and the oxidative and conjugative transformations of retinoic acid to more polar metabolites. Less well understood are the *cis-trans* isomerizations proposed in the figure.

1996) and that aldehyde oxidase can convert retinal to retinoic acid (Duester, 1996; Napoli, 1996). Because retinoic acid in tiny amounts shows such potent physiological actions, however, one must question the role of abundant enzyme systems (such as alcohol dehydrogenase, aldehyde dehydrogenase and aldehyde oxidase), which possess relatively broad substrate specificities, in forming retinoic acid. This issue is now a focus of much research and debate.

(a) Oxidation of retinol

The enzymes which catalyse the formation of retinal from retinol have not been unequivocally established. Members of two distinct families of enzymes have been proposed as being important. Several members of the family of cytosolic alcohol dehydrogenases catalyse retinol oxidation *in vitro* (Boleda *et al.*, 1993; Duester, 1996; Napoli, 1996), and much circumstantial evidence supports the idea that some of these are importantly involved in retinoic acid formation *in vivo*. Members of a second family of enzymes, the short-chain alcohol dehydrogenase family, have also been proposed as being physiologically relevant for catalysing retinol oxidation (Duester, 1996; Napoli, 1996). The members of this enzyme family, which are known to catalyse retinol oxidation, are present in cells and tissues at relatively low concentrations and are associated with membrane fractions. Several of the short-chain alcohol dehydrogenases which oxidize retinol prefer as a substrate, over unbound retinol, retinol bound to vitamin A-binding proteins (CRBP-I or cellular retinal-binding protein (CRalBP)) (Saari, 1994; Duester, 1996; Napoli, 1996). This is unlike the cytosolic alcohol dehydrogenases, which require unbound retinol as substrate (Duester, 1996; Napoli, 1996). It remains unclear whether all or only some of the enzymes reported to be important for retinoic acid formation are indeed physiologically essential.

Both class I and class IV alcohol dehydrogenases catalyse the oxidation of all-*trans*-retinol to all-*trans*-retinal (Duester, 1996; Napoli, 1996). In developing mouse embryos, the pattern of expression of a class IV alcohol dehydrogenase overlaps both temporally and spatially with the pattern of retinoic acid distribution (Ang *et al.*, 1996). A class IV alcohol dehydrogenase purified from rat stomach is able to catalyse both the oxidation of omega-hydroxy fatty alcohols and of free retinol (Boleda *et al.*, 1993). Nevertheless, since most retinol within a cell is bound to CRBP-I and since class I and IV alcohol dehydrogenases only catalyse the oxidation of free retinol, these authors were not convinced as to whether this class IV alcohol dehydrogenase is physiologically important for retinoic acid formation (Duester, 1996; Napoli, 1996).

Other reports indicate that oxidation of all-*trans*-retinol to all-*trans*-retinal is catalysed by microsomal enzymes that use all-*trans*-retinol bound to CRBP-I as substrate. Napoli and colleagues have cloned and characterized three microsomal retinol dehydrogenases from rat liver (termed retinol dehydrogenase, type I, type II and type III). Each of these recognizes all-*trans*-retinol bound to CRBP-I as substrate (Posch *et al.*, 1991; Boerman & Napoli, 1995; Chai *et al.*, 1995, 1996). Since most retinol within cells is bound to CRBP-I, this substrate specificity suggests that these enzymes are physiologically relevant for retinol oxidation. Sequence analysis indicates that these enzymes are 82% identical to each other and are members of the class of short-chain alcohol dehydrogenases. Each requires $NADP^+$ as an electron acceptor and is expressed most prominently in liver. Retinol dehydrogenase type I is the best studied isoform and is reported to be present also in kidney, brain, lung and testis, but at levels less than 1% of that in liver (Boerman & Napoli, 1995). Retinol dehydrogenase type II is expressed in kidney, brain, lung and testis at levels which are 25, 8, 4 and 3%, respectively, of that observed in liver, whereas retinol dehydrogenase type III is expressed only in liver (Chai *et al.*, 1996). Interestingly, retinol dehydrogenase type I does not oxidize 9-*cis*-retinol (Posch *et al.*, 1991; Boerman & Napoli, 1995). The substrate specificity of the type II and III enzymes for different retinol isomers has not been reported (Chai *et al.*, 1995, 1996). The properties of these three enzymes suggest that they are physiologically

involved in the oxidation of all-*trans*-retinol to all-*trans*-retinal.

17β- and 3α-hydroxysteroid dehydrogenases cloned from rat and human prostate share a high degree of primary sequence homology with rat retinol dehydrogenase type I, suggesting that the microsomal retinol dehydrogenases might also use hydroxysteroids as substrates (Biswas & Russell, 1997). Indeed, recombinant rat retinol dehydrogenase type I and recombinant protein generated from the newly cloned human homologue of this enzyme both catalyse the oxidation of 5α-androstan-3,17-diol to dihydrotestosterone, with the same apparent K_m value 0.1 μM (Biswas & Russell, 1997) (as compared to approximately 2 μM for retinol–CRBP-I for rat retinol dehydrogenase type I (Boerman & Napoli, 1995)). Thus, the microsomal retinol dehydrogenases may play important roles both in the generation of active forms of vitamin A and in the generation of active steroids.

In ocular tissue, the interconversion of retinol and retinal is an essential part of the visual cycle. Several membrane-bound dehydrogenases catalyse this oxidation–reduction process (Saari, 1994). One such enzyme, present in the rod outer segments, catalyses the interconversion of all-*trans*-retinol and all-*trans*-retinal. In the eye, soluble alcohol dehydrogenases, although present, may not play a major role in retinoid metabolism (Saari, 1994).

In the RPE, a different membrane-bound dehydrogenase, which is a member of the short-chain alcohol dehydrogenase family, has been reported to catalyse the stereospecific interconversion of 11-*cis*-retinol and 11-*cis*-retinal (Saari, 1994). Interestingly, 11-*cis*-retinal bound to CRalBP is reduced reversibly by this RPE enzyme to 11-*cis*-retinol (Saari, 1994). Simon *et al.* (1995) and Driessen *et al.* (1995) independently described the isolation, cloning and characterization of this stereospecific 11-*cis*-retinol dehydrogenase from bovine RPE. Like the enzymes characterized by Napoli and colleagues (Boerman & Napoli, 1995; Chai *et al.*, 1995, 1996), this dehydrogenase is a member of the family of short-chain alcohol dehydrogenases. However, unlike Napoli's enzymes, it does not employ all-*trans*-retinol as a substrate and requires NAD^+ and not $NADP^+$ as an electron acceptor. The bovine 11-*cis*-retinol dehydrogenase shows a 54% amino acid sequence homology with rat liver retinol dehydrogenase type II (Chai *et al.*, 1995, 1996). Northern blot analysis of total RNA from bovine RPE, liver, kidney, adrenal, lung, testis, brain and muscle indicates that the 11-*cis*-retinol dehydrogenase is present only in the RPE, supporting the idea that this enzyme is important for providing 11-*cis*-retinal for visual pigment formation (Simon *et al.*, 1995; Driessen *et al.*, 1995).

Edwards *et al.* (1992) reported that cultured rabbit Müller cells are able to synthesize retinoic acid from [^{3}H]retinol. [^{3}H]Retinoic acid initially accumulated slowly, but by 30 min, retinoic acid was rapidly released into the medium. Extracellular retinoic acid exceeded the intracellular amount after 30 min of incubation. Thus, some cells of the vertebrate retina have the capacity to synthesize retinoic acid from retinol and to release retinoic acid into the medium.

Shih and Hill (1991) have reported that an NADPH-dependent oxidase is present in rat liver microsomes, with an optimal pH between 8.2 and 8.7, which converts retinol to retinal. This oxidase was induced by 3-methylcholanthrene and inhibited by citral, ketoconazole and α-naphthoflavone, but was unaffected by the dehydrogenase inhibitor pyrazole. This enzyme seems to be distinct from previously characterized cytosolic and microsomal enzymes.

(b) Oxidation of retinal

Lee *et al.* (1991a) explored the ability of the 13 aldehyde dehydrogenases known at the time to be present in mouse tissues to catalyse the oxidation of all-*trans*-retinal to all-*trans*-retinoic acid. Three of the six aldehyde dehydrogenases present in mouse liver cytosol were able to catalyse this oxidation. One of these, ALDH-2, was estimated to catalyse about 95% of the oxidation of retinal to retinoic acid in the liver. The apparent K_m of ALDH-2 for all-*trans*-retinal was 0.7 μM. None of the aldehyde dehydrogenases present in the particulate fractions of mouse liver were able to catalyse retinal oxidation significantly. Based on these data, the

authors concluded that the enzymes responsible for retinoic acid formation from retinal are cytosolic, NAD-linked, substrate-non-specific dehydrogenases.

Other investigators have demonstrated that cytosol preparations from rat kidney, testis and lung can catalyse the oxidation of retinal to retinoic acid (Bhat *et al.*, 1988a,b). The enzyme responsible seems to be an oxidase, inasmuch as the oxidative formation of retinoic acid was stimulated by the addition of NADPH and blocked by inhibitors of aldehyde oxidase (Bhat *et al.*, 1988a). An enzymatic activity present in rat liver cytosol catalyses the formation of retinoic acid from retinal, showing linear kinetics with respect to protein concentration (0–2.4 mg/mL) and time (0–30 min), a broad pH maximum of 7.7 to 9.7, and an apparent K_m of 0.25 mmol/L for all-*trans*-retinal (Hupert *et al.*, 1991).

An aldehyde dehydrogenase present at high levels in the basal forebrain of mice has been reported to catalyse the formation of retinoic acid (McCaffery & Dräger, 1995; Zhao *et al.*, 1996). This enzyme, now termed RALDH-2, is expressed very early in embryonic development and levels of expression decline later in development (McCaffery & Dräger, 1995; Niederreither *et al.*, 1997). In mouse embryos, a teratogenic dose of all-*trans*-retinoic acid at embryonic day 8.5 results in downregulation of expression of this enzyme (Niederreither *et al.*, 1997). Bhat and Lacroix have purified and characterized a cytosolic retinal dehydrogenase from rat kidney which is NAD^+-dependent and catalyses the oxidation of both all-*trans*- and 9-*cis*-retinal to the corresponding retinoic acid isomer (Labrecque *et al.*, 1993, 1995). A similar retinal dehydrogenase has been partially purified from rat liver cytosol and characterized by El Akawi and Napoli (1994). This enzyme also catalyses the oxidation of both all-*trans*- and 9-*cis*-retinal in an NAD^+-dependent manner. Presence of CRBP-I decreases the rate of all-*trans*-retinoic acid synthesis by the rat liver retinal dehydrogenase (El Akawi & Napoli, 1994). Thus, these and other studies (Blaner & Olson, 1994) strongly support a role for cytosolic retinal dehydrogenases in the formation of retinoic acid *in vivo*.

Roberts *et al.* (1992) reported that CYP1A2 and CYP3A6 from rabbit liver microsomes oxidize retinal to retinoic acid. No work exploring the roles of cytosolic retinal dehydrogenases in formation of retinoic acid in the rabbit has been reported. Thus, it is possible that retinoic acid formation and metabolism in other mammalian species may differ from that in rodents or humans.

*(c) 9-*cis*-Retinoic acid formation*

9-*cis*-Retinoic acid acting through RARs and RXRs is an essential vitamin A form for regulating retinoid-responsive gene activity. The genes regulated by retinoids are very diverse and are involved in regulating a wide array of cellular functions. Thus, any factor which influences 9-*cis*-retinoic acid availability to or within a cell will have a broad impact on retinoic acid signalling pathways and cellular responses.

There is still only very limited information on how 9-*cis*-retinoid isomers are formed. For the visual process, the isomerization of all-*trans*-retinoids to 11-*cis*-retinoids is catalysed by a specific enzyme and the isomerization takes place at the level of the retinols and not the aldehydes (Saari, 1994). Since the first reports in 1992 that 9-*cis*-retinoic acid is a ligand for the RXRs, several studies have explored possible pathways for 9-*cis*-retinoic acid formation. Urbach and Rando (1994) reported that membranes prepared from bovine liver catalyse non-enzymatically the isomerization of all-*trans*-retinoic acid to 9-*cis*-retinoic acid. This isomerization depends on free sulfhydryl groups present in the microsomes and does not involve the participation of an enzyme. Hébuterne *et al.* (1995) reported that 9-*cis*-β-carotene serves as a precursor for 9-*cis*-retinoic acid *in vivo* in the rat. The rate of cleavage of 9-*cis*-β-carotene, however, is only 6–7% of that of all-*trans*-β-carotene (Nagao & Olson, 1994). Furthermore, since rats maintained on carotenoid-free diets display normal health, the conversion of 9-*cis*-β-carotene to 9-*cis*-retinoic acid cannot be an essential pathway for formation of this retinoic acid isomer. The ability of retinal dehydrogenases in rat kidney (Labrecque *et al.*, 1993, 1995) and rat liver (El Akawi & Napoli, 1994) to catalyse the oxidation of 9-*cis*-retinal to 9-*cis*-retinoic acid was taken to suggest that a pathway starting with 9-*cis*-retinol may be important for 9-*cis*-retinoic acid formation. The presence of 9-*cis*-

retinol in rat kidney at approximately 10% of the level of all-*trans*-retinol (Labrecque *et al.*, 1993, 1995) supports this possibility. Although each of these reports is individually convincing, it still is unclear whether any or all of these possibilities are important for 9-*cis*-retinoic acid formation *in vivo*.

An NAD-dependent retinol dehydrogenase which specifically oxidizes 9-*cis*-retinol but not all-*trans*-retinol has recently been cloned from a human mammary tissue cDNA library and characterized upon its expression in CHO cells (Mertz *et al.*, 1997). This enzyme, a member of the short-chain alcohol dehydrogenase enzyme family, is expressed in adult human mammary tissue, kidney, liver and testis. Mertz *et al.* (1997) proposed that this enzyme may play an important role in the synthesis of 9-*cis*-retinoic acid. Interestingly, this enzyme, termed 9-*cis*-retinol dehydrogenase, is expressed in kidney, a tissue which has been reported to contain significant quantities of 9-*cis*-retinol (Labrecque *et al.*, 1993, 1995). However, it remains to be established whether this enzyme is physiologically important for generating active forms of vitamin A like 9-*cis*-retinoic acid.

A second *cis*-retinol dehydrogenase, also a member of the short-chain alcohol dehydrogenase family, has been described by Napoli and colleagues (Chai *et al.*, 1997). The cDNA for this mouse liver enzyme, termed the *cis*-retinol/3α-hydroxysterol short-chain dehydrogenase, encodes a 317-amino acid-containing enzyme which recognizes 9-*cis*- and 11-*cis*-retinol, 5α-androstan-3α,17β-diol and 5α-androstan-3α-ol-17-one as substrates. The apparent K_m values for these substrates indicate that the *cis*-retinol dehydrogenase has a greater affinity for the sterol substrates. This mouse enzyme, which is most similar to mouse retinol dehydrogenase isozymes types 1 and 2 (86% and 91% homology, respectively), uses NAD^+ as its preferred cofactor. It is expressed in liver, kidney, small intestine, heart, RPE, brain, spleen, testis and lung. Chai *et al.* (1997) proposed that this multifunctional enzyme is important for generating both 9-*cis*-retinoic acid and bioactive androgens. It is possible that this enzyme provides a link between the actions of vitamin A and androgens.

(d) Summary

The extent to which soluble, relatively nonspecific, enzymes such as alcohol dehydrogenase, aldehyde dehydrogenase or aldehyde oxidase are involved in the enzymatic formation of retinoic acid from retinol is unclear. The oxidation of retinol to retinal is most likely catalysed by a microsomal enzyme or enzymes, which use retinol bound to CRBP-I as substrate. Whether the oxidation of retinal to retinoic acid is also CRBP-I-dependent or is catalysed by a soluble aldehyde dehydrogenase and/or aldehyde oxidase is still uncertain. Nonetheless, multiple enzymatic activities clearly are involved in the conversion of retinol to retinoic acid.

3.2.7.2 Synthesis of retinoic acid from carotenoids within tissues

In 1988, Napoli and Race reported that cytosol preparations from rat tissues could catalyse the formation of retinoic acid from β-carotene. The rate of retinoic acid synthesis from 10 μmol/L β-carotene ranged from 120 to 224 pmol/h/mg protein for intestinal cytosol, and from 334 to 488 pmol/h/mg protein for cytosols prepared from kidney, lung, testes and liver. Retinol that was generated during β-carotene metabolism was determined not to be the major substrate for retinoic acid synthesis. Retinal was not detected as a free intermediate in this process, but retinal might be tightly bound by the enzyme. Alternatively, β-carotene might be oxidized to a 15,15'-enediol before dioxygenase cleavage, by analogy with the conversion of catechol to *cis,cis*-muconic acid. Other mechanisms for producing retinoic acid from β-carotene without yielding retinal are possible, but as yet no intermediates have been characterized.

Wang *et al.* (1991) reported that homogenates of liver, lung, kidney and fat from monkey, ferret and rat formed retinoic acid upon incubation with 2 μmol/L β-carotene, with a pH optimum of 7.0. Because citral, which inhibits the conversion of retinal to retinoic acid, did not affect retinoic acid formation from β-carotene or β-apocarotenals, Wang *et al.* (1992) concluded that retinoic acid is formed through a biochemical process that does not involve retinal as an intermediate.

3.2.7.3 Metabolism of retinoic acid

Metabolites of all-*trans*-retinoic acid generated *in vivo* include 13-*cis*-retinoic acid, 9-*cis*-retinoic acid, retinoyl β-glucuronide, 5,6-epoxyretinoic acid, 4-hydroxyretinoic acid, 4-oxoretinoic acid, and 3,4-didehydroretinoic acid (Blaner & Olson, 1994). Some of these metabolites retain activity in mediating retinoic acid function, whereas others seem to be inactive catabolic products.

The cytochrome P450 system is active in metabolizing retinoic acid. Roberts *et al.* (1979) reported that the formation of polar metabolites of retinoic acid was catalysed by an activity present in the microsomal fraction of rat intestine and liver homogenates. This activity required NADPH and oxygen, and was strongly inhibited by carbon monoxide. In addition, the activity was markedly induced by retinoids, but only to a minor extent by phenobarbital or 3-methylcholanthrene. Roberts *et al.* (1979) concluded that the enzyme responsible for the formation of polar metabolites of retinoic acid is a member of a class of mixed function oxidases containing the cytochrome P450s. Leo *et al.* (1984) reported that rats fed a diet containing a 100-fold excess of retinyl acetate, for two to three weeks, showed an increase in hepatic microsomal cytochrome P450 content. When microsomes were isolated from the livers of the treated rats, the conversion of all-*trans*-retinoic acid to more polar metabolites, including 4-hydroxy- and 4-oxoretinoic acid, was enhanced. Purified rat liver CYP2C7 and CYP2B1 catalysed the conversion of retinoic acid to polar metabolites, including 4-hydroxy-retinoic acid (Leo *et al.*, 1984). The isozyme CYP2C8 of human liver microsomes oxidizes retinoic acid to 4-hydroxyretinoic acid and 4-oxoretinoic acid (Leo *et al.*, 1989). Roberts *et al.* (1992) reported that many rabbit liver CYP isoforms, including 2A4, 1A2, 2E1, 2E2, 2C3, 2G1 and 3A6, catalyse the 4-hydroxylation of retinoic acid, as well as of both retinol and retinal, but not the conversion of 4-hydroxy-retinoids to the corresponding 4-oxoretinoids. Van Wauwe *et al.* (1992) showed that oral administration of a dose (40 mg/kg body weight) of liarozole, a 1-substituted imidazole derivative which inhibits cytochrome P450 activity, enhances the endogenous plasma concentrations of retinoic acid from less than 1.7 nmol/L to 10–15 nmol/L. Thus, the cytochrome P450 system seems to play an important role in retinoic acid metabolism and homeostasis.

Novel cytochrome P450s of the CYP26 family (termed P450RA from P19 cells and P450RAI from the zebrafish), which are able to metabolize retinoic acid, were recently cloned (Fujii *et al.*, 1997; White *et al.*, 1997a). One cDNA clone (P450RA) was obtained from a subtraction library prepared from retinoic acid-treated and untreated murine P19 embryonic carcinoma cells (Fujii *et al.*, 1997). When expressed, the murine P450RA cDNA catalysed the oxidation of all-*trans*-retinoic acid to 5,8-epoxy-all-*trans*-retinoic acid. Both 13-*cis*- and 9-*cis*-retinoic acid are also substrates for P450RA. This cytochrome P450 is expressed in a stage- and region-specific fashion in mouse development, but expression does not appear to be inducible by excess retinoic acid. In the adult mouse, P450RA is expressed only in liver (Fujii *et al.*, 1997). White *et al.* (1997a) reported the isolation and characterization of a cDNA for cytochrome P450RAI expressed during gastrulation of the zebrafish. P450RAI was expressed normally during gastrulation and in a defined pattern in epithelial cells of the regenerating caudal fin in response to administration of exogenous all-*trans*-retinoic acid. When the cDNA for P450RAI was expressed in COS-1 cells, all-*trans*-retinoic acid was rapidly metabolized to more polar metabolites, two of which were identified as 4-oxo-all-*trans*-retinoic acid and 4-hydroxy-all-*trans*-retinoic acid. P450RAI, which is induced upon retinoic acid exposure, catalyses the oxidative metabolism of retinoic acid. It is tempting to speculate that the oxidative metabolism catalysed by P450RAI and P450RA is an important and common mechanism through which cells and tissues regulate levels of this active form of vitamin A; however, this possibility requires further studies.

Livers from aryl hydrocarbon receptor-null ($AHR^{-/-}$) mice possess total retinol levels which are approximately three-fold higher than those of wild-type mice (Andreola *et al.*, 1997). In addition, $AHR^{-/-}$ mice show a reduced capability to oxidize retinoic acid and significantly decreased hepatic mRNA levels for both retinal dehydrogenase types 1 and 2. Interestingly,

expression of P450RAI was not different in AHR-deficient and wild-type mice. These results strongly suggest that the aryl hydrocarbon receptor plays an important role in vitamin A homeostasis within the body. This is in keeping with observations that mice and rats given 2,3,7,8-tetrachlorodibenzo-*p*-dioxin show a rapid decline in hepatic total retinol levels (Brouwer *et al.*, 1985; Chen *et al.*, 1992a). Thus, it would appear that there is a direct link between xenobiotic exposure and metabolism and vitamin A metabolism and homeostasis in mice and rats. Although the potential pathological consequences of such a link are clear, the underlying biochemical processes and mechanisms still need to be resolved.

A direct role for CRABP-I in the oxidative metabolism of retinoic acid has been proposed by Fiorella and Napoli (1991). Microsomal enzymes of rat testes catalyse the conversion of CRABP-I-bound all-*trans*-retinoic acid to 3,4-didehydro-, 4-hydroxy-, 4-oxo-, 16-hydroxy-4-oxo- and 18-hydroxy-retinoic acids. Thus, CRABP-I may well play a direct role in the oxidative metabolism of all-*trans*-retinoic acid. Furthermore, the binding of all-*trans*-retinoic acid to CRABP may provide a mechanism for discriminating metabolically between all-*trans*- and 13-*cis*-retinoids.