Factors affecting vitamin A transport in animals and man

By J. GLOVER, *Department of Biochemistry, University of Liverpool, PO Box* 147, *Liverpool L*69 3*BX*

The fat-soluble vitamin A has, of necessity, to be transported in the body by carrier proteins since it functions in an aqueous environment. At the same time, control of the distribution of the alcohol form, via specific retinol-binding proteins (RBP), provides additional physiological advantages. The concentration of the amphiphilic molecule is more easily limited in tissue fluids where, if free, it could damage or lyse cell membranes indiscriminately (Dingle & Lucy, 1962) and the presence of a binding site on the carrier protein for specific acceptors in functional cell membranes enables the vitamin to be directed to its targets selectively (Heller, 1975) and in controlled amounts.

Bulk transport of the vitamin during its absorption from the intestine to the liver takes place mainly as retinyl esters within the lipid phase of low-density lipoproteins (Ganguly, 1960), whereas the physiologically-active retinol circulates attached to a hydrophobic cleft on plasma retinol-binding protein (holoRBP) (21 000 MW) synthesized and secreted by the liver (Kanai *et al.* 1968). In plasma, holoRBP forms a 1:1 complex with thyroxine-binding prealbumin (TBPA) (54 000 MW); the formation of this larger complex is regarded as important in preventing the rapid loss of the holoRBP by ultrafiltration through the kidney and it also increases the stability of the retinol bound to RBP (Raz *et al.* 1970). A third binding site on the latter enables it to bind to the membrane acceptors of target cells so that the retinol can be released to cross the membrane and be taken up by cellular retinol-binding protein (CRBP) (14 600 MW) (Bashor *et al.* 1973; Chytil & Ong, 1978) for delivery to its site of action within the cell.

This complex transport system for retinol is affected (i) by malnutrition, such as protein and zinc deficiencies, (ii) by hormonal changes affecting protein synthesis and metabolism and (iii) by diseases of the intestine, liver and kidney which directly affect either the absorption and metabolism of retinol or the synthesis and metabolism of RBP. The analysis of blood plasma for retinol or RBP or both provides a guideline to the immediate supply of vitamin available but gives no indication as to the extent of reserves within the liver store. Some understanding of the factors influencing or controlling the supply of retinol in plasma is necessary for a sound interpretation of the concentration values for both retinol and RBP which are found in animals undergoing normal growth and development, in order that values outside the expected ranges may be recognized as being abnormal.

Factors affecting storage and release of vitamin A

A number of factors known to affect retinol concentration in plasma are listed in Table 1 and some of these will be discussed in relation to knowledge regarding each stage of retinol transport and metabolism. Biochemical (Linder *et al.* 1971)

https://doi.org/10.1079/PNS19830004 Published online by Cambridge University Press

and histological (Wake, 1980) evidence show that retinol is taken up mainly by two types of cells within the rat liver—the main parenchymal cells (78% of cell volume) and the perisinusoidal stellate cells (1·4%)—with only small amounts appearing in the Kupffer cells (2·1%). Retinol is held in high concentration mainly as the ester in lipid globules within the stellate or fat-storage cells, whereas the concentration in the parenchymal cells is much lower. In studies on Japanese quail livers (R. McEwan, J. Fennis & J. Glover, unpublished observations) the concentration of total retinol/μg DNA in the parenchymal cells was only 1·3–10% of that in the lower density lipocytes; both cell types, however, contained a small percentage (<15%) of unesterified retinol. The manner in which the vitamin can be transferred between the two cell types is not yet clear. The above evidence is in keeping with information from isotope equilibration studies that there are several pools of the vitamin in liver and that it requires at least 7–10 d before a dose of labelled retinol becomes uniformly distributed between the liver reserves and the plasma fraction (Rietz *et al.* 1974; Hughes *et al.* 1976).

Cell fractionation studies have been carried out in many laboratories to determine how the vitamin is distributed within hepatocytes. The largest proportion of the vitamin is in the ester form (Ganguly, 1960; Glover *et al.* 1974) enclosed in a lipoprotein aggregate with a particle size from sedimentation analysis $>1{\cdot}6\times10^6$ MW (Heller, 1979) and isolated from the cytosol fraction. The complex contains 66% lipid, 30% protein and 4% carbohydrate. Extraction of the lipid enables the proteins to be resolved into a number of components, and the three major ones have polypeptide chains varying in size from 2×10^4 MW to $1{\cdot}1\times10^5$ MW. Retinyl palmitate hydrolase was found to be associated with this complex (Chen & Heller, 1979). Furthermore, triolein hydrolase and cholesterol oleate hydrolase were also present (Harrison *et al.* 1979). The particle also sediments with the crude nuclear and other membrane fractions but is not enriched in purified nuclear or plasma membrane components. The cytosol fraction from 105 000 *g* supernatant contains 30–35% of the hydrolase activity.

HoloRBP, however, is synthesized in the rough endoplasmic reticulum or microsomal fraction, but up to 23% has been found in the Golgi apparatus (Harrison *et al.* 1979; Smith *et al.* 1982) and this RBP is known to carry retinol (Glover *et al.* 1974). It is presumed that holoRBP, as with other proteins, is secreted via the Golgi apparatus and the microtubule system of the cell since colchicine, which interacts with tubulin, inhibits the secretion of RBP into plasma (Kershaw, 1978; Smith *et al.* 1980). The actual position at which the native

Table 1. *Factors affecting transport of vitamin A*

Nutritional	Hormonal	Pathological
Dietary supply of	Control by	Diseases affecting
Vitamin A (or provitamin A)	Glucocorticoids	Liver
Good quality protein	Hypothalamo–pituitary axis	Kidney
Zinc	Sex hormones	Intestine
Other lipids in diet		Endocrine glands

https://doi.org/10.1079/PNS19830004 Published online by Cambridge University Press

apoRBP picks up its retinol is not yet clear. However, since holoRBP is found in the Golgi apparatus, it seems likely that retinol becomes attached either at this stage or immediately beforehand.

Since the release of retinol from the ester fraction involves a hydrolase and carrier proteins, it is apparent that the nutritional status of the animal with respect to both retinol and protein can have a marked effect on the amount of the holoprotein which can be released into plasma.

Effects of vitamin A deficiency on plasma RBP

It has long been established that vitamin A deficiency in experimental rats as well as in children lowers the plasma concentration of retinol and RBP in plasma. Table 2 summarizes some of the plasma values for holo- and total RBP obtained in survey studies, carried out to assess the vitamin A status of preschool children in several countries compared with normal controls or similar groups of children treated with a massive dose of vitamin A. These values illustrate two points.

First, a deficiency of retinol itself reduces the plasma level of retinol as would be expected, but this is mediated by a lowering of the concentration of holoRBP as

Table 2. *Retinol-binding protein (RBP) in plasma of protein and vitamin A deficient preschool children and of control groups*

(Mean values with standard errors)

			RBP (μg/ml)				
			Total		Holo		Apo•
Country	Group	*n*	Mean	SEM	Mean	SEM	Mean
Indonesia	Deficient	30	16·0	1·0	2·2	0·8	13·8
(Bogar)	Controls (K)	10	20·3	2·2	10·4	1·4	9·9
	Controls (L)	37	29·7	1·2	19·0	1·2	10·7
South India	Deficient (X1†)	17	20·8	1·8	4·8	1·4	16·0
(Madurai)	Deficient (X2†)	31	13·0	1·0	2·0	0·5	12·0
	Deficient (X3†)	7	12·9	1·4	3·7	1·5	9·2
	Treated‡	5	28·0	3·0	26·0	2·5	2·0
West Africa	Deficient	4	24·7	1·1	10·6	3·6	14·1
(Niamey)	Treated‖	5	25·0	2·8	14·5	2·8	10·5
Thailand	Kwashiorkor	8	10·0	2·0	3·0	1·0	7·0
(Chiang-Mai)	Kwashiorkor–marasmus	15	15·0	3·0	3·0	2·0	12·0
	Marasmus	4	27·0	15·0	11·0	8·0	16·0
	Treated‖	21	81·3	8·0	50·0	5·0	31·3
England							
(Liverpool)	Normal	7	33·0	3·0	29·0	1·0	4·0

K, Lower socioeconomic group.
L, Higher socioeconomic group.
•By difference of total RBP-holoRBP.
†Xerophthalmia index.
‡Examined 3 months post-treatment with 60 000 μg retinyl palmitate.
‖Examined 2–4 weeks post-treatment with 30 000 μg retinyl palmitate.

https://doi.org/10.1079/PNS19830004 Published online by Cambridge University Press

was first demonstrated in the rat (Muto *et al.* 1972). There is also a corresponding elevation of the pool of native apoRBP which accumulates in the liver.

Data from experiments on groups of rats in our own laboratory are given in Table 3. They show that the amount of native apoRBP which accumulates in the livers of a group of weanling rats placed on a vitamin A deficient diet for 5 weeks is about five times the steady-state amount found in normal controls. A third group, rendered vitamin A deficient but maintained for several weeks on retinoic acid, accumulated just less than two times that of the control group. The reason as to why less should accumulate in the latter is not yet clear but the synthesis of RBP is certainly maintained in the prolonged absence of retinol.

When the vitamin A deficient rats are given a massive dose of retinol the accumulated material in their livers is secreted promptly into the plasma and causes the plasma holoRBP level to rise to a peak, well above normal, within 2–3 h of dosing, before dropping back to a much lower level representative of that which can be maintained by the protein synthetic capacity of the liver for RBP at that time (Muhilal & Glover, 1974). The site of accumulation of the protein within the cells is still not completely clear. Studies on the distribution of RBP between cell fractions from liver homogenates of vitamin A deficient and normal rats (Smith *et al.* 1982) indicate that the pool of accumulated native RBP increases in the microsomes prior to the Golgi apparatus. The accumulated protein is immunologically indistinguishable from normal RBP and does not appear to carry an additional signal peptide for attachment, say, to the endoplasmic reticulum (Smith *et al.* 1982).

Second, the amount and quality of the dietary proteins are important to maintain optimal synthesis of RBP, which contains a high proportion of aromatic and essential amino acids (Kanai *et al.* 1968; Peterson, 1971).

Protein deficiency and RBP synthesis

The effects of severe protein–energy malnutrition (PEM) in lowering serum vitamin A and other lipids have been examined in many laboratories. It was especially noted, however, that the reduced serum vitamin A concentrations of some kwashiorkor patients upon admission to hospital were rapidly corrected by treatment with an improved protein diet practically devoid of vitamin A (Arroyave

Table 3. *Effect of vitamin A deficiency on retinol-binding protein (RBP) in plasma and liver of young rats*

Group*		*n*	Plasma holoRBP (μg/ml) Mean	SEM	Liver total RBP (μg/g wet tissue) Range	Mean
A	Normal controls	3	28	1	17–23	19
B	Retinol deficient	3	3	1	46–169	101
C	Deficient + retinoic acid	4	0		26–49	34

*Basal retinol deficient diet of Lewin *et al.* (1970) was used for all groups. Group A was supplemented with retinol from the outset, and group C with retinoic acid after 6 weeks.

https://doi.org/10.1079/PNS19830004 Published online by Cambridge University Press

et al. 1961). The implication was drawn that the livers of these patients must have contained sufficient reserves of retinol which could not be distributed properly owing to an impairment of the vitamin transport system. This has been confirmed by examining changes in the secretion of retinol, RBP and TBPA in similar groups of PEM children (Smith, Goodman, Arroyave *et al.* 1973). It was demonstrated that in eleven patients out of thirty-three, the provision of improved dietary protein and food energy intake, but without supplemental vitamin A, restored their serum retinol and the capacity of the livers to secrete RBP and other plasma proteins. Indeed, the holoRBP concentration in PEM children tends to rise above normal for a few weeks after treatment before settling back to normal levels again (Smith, Goodman, Arroyave *et al.* 1973; Ingenbleek *et al.* 1975; Large *et al.* 1980).

Again, it has been observed that rice protein, given to rats at the same concentration as a more nutritionally balanced soya-protein diet, was less effective in promoting the synthesis of RBP, but supplementation of the rice diet with lysine and methionine restored its capacity to support normal RBP synthesis (Glover & Muhilal, 1976). Thus, the normal distribution of vitamin A depends not only on the provision of retinol but also on the availability of a well-balanced pool of dietary amino acids to maintain RBP synthesis. Other effects of protein deficiency might involve reductions in the concentration of the enzymes or proteins contained in the lipoprotein aggregates which retain retinyl esters, so affecting the mechanism of release of the retinol from them or even the capacity of the liver to take up a massive therapeutic dose of vitamin A quickly. The levels of retinyl ester hydrolase or synthetase as well as CRBP could all be affected. These have not yet been examined.

Zinc deficiency

The report that Zn deficient rats given adequate vitamin A had low plasma vitamin A levels, which could be restored to normal values with Zn therapy, indicated that Zn was involved in retinol metabolism (Smith, McDaniel *et al.* 1973) and recalled the previous observations of Stevenson & Earle (1956) that massive doses of vitamin A acetate were unable to raise the low plasma concentrations of vitamin A in swine with parakeratosis. Vallee and co-workers (1957, 1959) suggested that the deficiency of Zn, which is a cofactor of alcohol dehydrogenase, may be partly involved in altered retinol metabolism. A number of recent studies have now confirmed that plasma retinol and RBP levels are 30–50% lower than normal in Zn deficient rats (Smith *et al.* 1974). The effects seem to be operative at two levels. There appears to be (i) a general reduction of growth with food restriction and of plasma protein synthesis, in particular with RBP being affected more severely than others (Smith *et al.* 1974), and (ii) the mobilization of free retinol itself also appears to be affected, possibly through an effect on the retinyl ester hydrolase and other factors associated with the lipoprotein aggregate.

Recently, low concentrations of Zn were observed in PEM children from 2 to 8 years of age (Shingwekar *et al.* 1979) and supplementation of their diet with 40 mg

Zn/d raised their retinol and RBP levels significantly within 5 d. Since vitamin A was not administered, there must have been sufficient reserves to load the increased amount of RBP secreted in these children.

The heterogeneity of holoRBP

It was clear from studies in many laboratories on the isolation of pure RBP that not all of the material obtained was fully saturated with retinol. At least two major forms were recognized; most of the holoRBP in normal plasma is complexed (1:1) with TBPA to form a 75 000 MW species which can be separated from the residual small amount of protein which remains free (approximately 21 000 MW) and does not carry retinol (Raz *et al.* 1970; Peterson, 1971). This apoprotein can also be separated from the native holoprotein by electrophoresis since it carries an additional negative charge (Glover, 1973). In vitamin A deficient children or rats the concentration of holoRBP is often reduced to zero, but invariably some apoprotein is present which must be released from the liver in spite of complete retinol deficiency. When samples of fresh plasma from such deficient cases are shaken in vitro with retinol, none appears to be taken up by this apoRBP (as shown in Table 4) whereas freshly extracted native holoRBP readily becomes resaturated under the same conditions. Purified RBP from which retinol has been extracted, becomes denatured in vitro, but the site of the denaturation of the apoprotein in retinol deficient subjects has not been clearly identified. Since the administration of retinol to deficient animals permits the rapid release of the accumulated apoRBP within their livers as the holoprotein, most of this material must have accumulated there in the native form. Consequently, the denatured apoprotein in the plasma of deficient subjects must have been formed immediately prior to or just after its release from the liver. If the latter is the case, the change may be catalysed following interaction of the native protein with membrane receptors on functional tissue cells.

A high concentration of apoRBP has also been observed in Japanese quail at the time of egg-laying when retinol is being actively transferred into the yolk (Heaf *et al.* 1980). This material is also incapable of taking up fresh retinol as can be seen

Table 4. *Inability of apoRBP in normal serum to take up retinol*

(Mean values)

	Human RBP (μg/ml)			Quail RBP (μg/ml)		
Treatment	Total	Holo	Apo*	Total	Holo	Apo*
None	85	73	12	139	90	49
Plus retinol	—	73	12	—	90	49
Extracted†	—	37	48	—	63	76
Extracted† then recharged	—	75	10	—	82	57

*By difference of total RBP − holoRBP.

†Partially extracted by shaking with heptane under nitrogen for 2 h to minimize the possibility of denaturing freshly formed apoprotein.

https://doi.org/10.1079/PNS19830004 Published online by Cambridge University Press

from information included in Table 4. Thus the holoprotein becomes denatured quickly after releasing its retinol and the high concentration of apoRBP reflects the very high rate of utilization of retinol. This would also appear to be the case in seasonal breeding animals during gonadal development (see below) and during lactation in ewes when both holo- and apoprotein concentrations in plasma are raised above the minimal mid-summer level. Again, a relatively high concentration of the apoprotein (~40% of total immunoreactive material) persists in some chronic PEM children for several weeks after treatment with a massive dose of retinol (30 000 μg) and a good diet (Large *et al.* 1980), compared with the amount (<15%) found in normal children replete with vitamin A (see Table 2). The difference may perhaps be partially explained on the basis that a faster utilization of retinol occurs during the 'catch-up' growth recovery period.

It should, therefore, not be assumed that assays of total RBP give some measure of the retinol concentration on the basis that an equimolar amount of retinol is always present, because some of the protein is liable to be denatured.

Furthermore, it is equally true that analysis for total retinol present in plasma by direct colour test does not give a true assessment of the concentration of RBP present on the basis of the binding of 1 mol retinol/mol protein.

Changes in plasma RBP at different stages of growth and development

It is well-known that vitamin A is required for growth and in normal growing animals there is a correlation between vitamin A utilization and growth rate (Rechcigl *et al.* 1962) so that in physiological situations where accelerated growth of tissues occurs, one should expect an increase in the supply and utilization of retinol. This does, in fact, happen in most situations examined so far. A cyclic 1·5- to 2·5-fold seasonal increase in holoRBP of ewes and wethers occurs in September to November during their period of gonadal development (Glover *et al.* 1976). This is consistent with the previous observations of Thompson *et al.* (1964) that retinol is specifically required for reproduction. A cyclic 2-fold increase in holoRBP during gonadal development has also been observed in birds (Japanese quail) in the spring (Glover *et al.* 1980). In each species the concentration falls again with the regression of the tissues. The changes in RBP were paralleled by the changing diameter of the cloacal gland in the male Japanese quail and by egg laying in females as a measure of gonadal development. The effects are induced by changing length of daylight or photoperiod, since the process can be accelerated by putting birds under artificial lighting where the normal annual cycle is condensed to 4 months (Heaf *et al.* 1982). They are mediated by the hypothalamo–pituitary endocrine system but the final hormone acting on the liver which alters the rate of RBP synthesis in the liver has still to be clarified. It may be cortisol or other glucocorticoids which have been shown capable of stimulating the synthesis of RBP in liver cell cultures in vitro (Smith *et al.* 1978, 1982). Experiments on groups of young rats show that a similar increase in holoRBP occurs during puberty as their growth rate accelerates and the gonads develop (Kershaw, 1978).

The level in the immature male and female rats immediately after weaning at day 21 is about 20–25 μg/ml holoRBP until day 36, when it begins to rise, reaching a peak of 60–70 μg/ml in the male by day 55, before returning to the normal adult level of 40–45 μg/ml a week later. In the female the increase is less marked, with peak values of 50 μg/ml being reached on day 54. These increases correlate with the increased growth rate of the rats and, in particular, with the development of their gonads expressed as a percentage of body-weight (see Fig. 1).

Similarly, the mean plasma levels of RBP in groups of young boys and girls remains around 28–30 μg/ml up to 10 years of age, after which it begins to rise during puberty to around 40 μg/ml at 16 years (Peterson *et al.* 1974) with the boys tending to have slightly higher values (Michaelsson *et al.* 1976), corresponding with the period of rapid growth as shown by Tanner *et al.* (1976). Thus increased growth of the animal is associated with a higher concentration of RBP in plasma.

Influence of photoperiod on holoRBP

It is interesting that the growth rate of children is significantly faster in the summer months than in the winter (Tanner, 1962) and this raises the question about the importance of length of exposure of animals to daylight in relation to RBP as well as to vitamin D formation. Since increasing photoperiod raises the

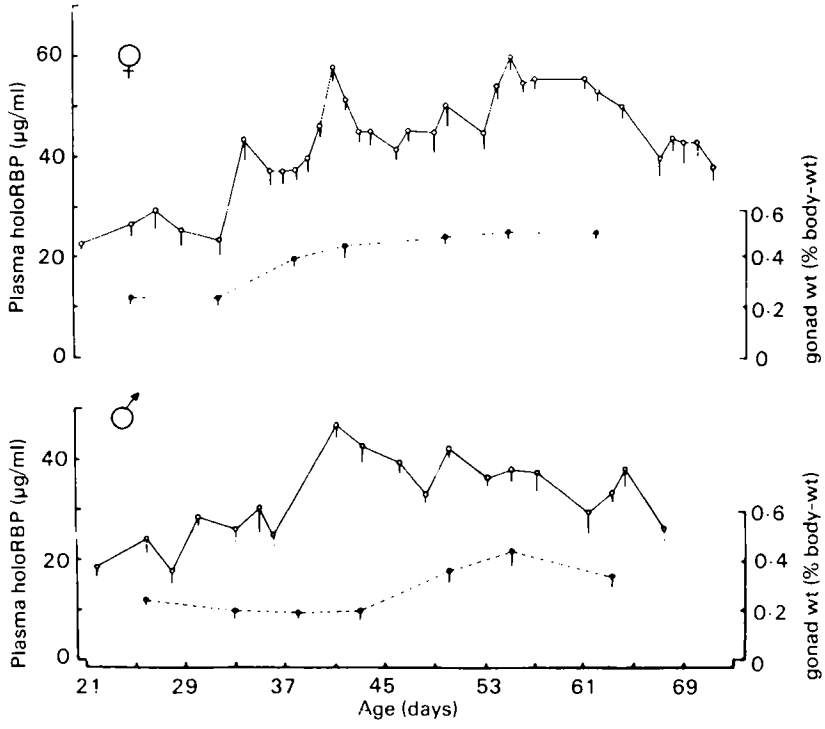

Fig. 1. The increase in plasma holoRBP concentration in weanling male and female rats with gonadal development as they mature. The holoRBP values (- -○—) are means of determinations on groups of eight rats. The gonad weights (- —●— -) are means of groups of four rats and each value is expressed as a percentage of body-weight.

https://doi.org/10.1079/PNS19830004 Published online by Cambridge University Press

mean plasma level of holoRBP in birds, the possibility should be considered that holoRBP may follow a circadian type rhythym in polyoestrous species. The plasma holoRBP concentration was examined every 2 h in groups of rats under 14 h light/10 h dark photoperiod conditions and changes were observed which showed that the plasma concentration was at its lowest level immediately after the dark period and rose to a peak in the late afternoon. The results are summarized in Table 5 and show that there is a significant difference between the morning and evening values. These changes may reflect changes in the hypothalamo–pituitary axis mediated by light. It is important in comparative studies, therefore, to take samples at the same time of day. However, in longer term studies the plasma holoRBP concentration varied in individual animals between minimal and maximal values even at the same time of day over a period of 7–9d. This was not correlated with diet and has not yet been explained (Kershaw, 1978).

Uptake of retinol into functional cells

Free retinol in micellar dispersion within the intestine can readily penetrate the mucosal cell outer membrane when it is trapped in the microvilli; however, the mechanism of the transfer of retinol attached to RBP from intercellular fluid into vitamin A dependent cells is not so clear. Retinol bound to RBP exchanges directly with excess retinol in aqueous dispersion in vitro with a half-life of 2·7 h (Muhilal & Glover, 1974) so the ligand would not be released quickly enough just through random collisions with plasma membranes.

Peterson and colleagues (1974) studied the uptake of [^{3}H]retinol from labelled holoRBP using isolated intestinal mucosal cells of the monkey. They found that retinol is readily taken up from the holoRBP, but the protein itself does not enter the cells because when it is coupled to Sepharose beads before incubation the retinol is still taken up although at a reduced rate. Again, native apoRBP, but not denatured protein, added to the incubation mixture, competed with holoRBP to reduce the uptake of retinol, indicating that a specific receptor for the protein was involved.

This view was independently confirmed by Heller (1975) in studies on bovine retina. Using [^{125}I]RBP he demonstrated that the protein bound to receptors on the pigment epithelium membrane. These receptors were later shown autoradiographically to be specifically located only on the membrane surface facing the choroid

Table 5. *Diurnal changes in plasma holoRBP concentration in the rat*

Group/*n*	Photoperiod (light (h)/ dark (h))	Sampling		Plasma holoRBP (μg/ml)		Statistical significance
		Frequency	Time (hours)	Mean	SEM	*P*
I/6	8/16	Daily for 11 days	09.00	31·8	1·0	<0·05
			17.00	34·4	0·8	
II/3	14/10	Every 2 h for	04.00–08.00	33·8	1·4	<0·001
		1st day	18.00–22.00	45·9	1·0	
		Every 2 h for	04.00–08.00	36·5	1·0	<0·001
		2nd day	18.00–22.00	43·9	1·0	

https://doi.org/10.1079/PNS19830004 Published online by Cambridge University Press

layer. The protein remained outside the cell, but the retinol was found in the cytosol partly as free retinol and partly bound to a high-molecular-weight protein ($>1{\cdot}5\times10^6$). A smaller amount of the retinol was attached to a low-molecular-weight protein (15 000 daltons), now considered to be CRBP. Similarly, using chick pigment epithelial cells, Wiggert & Chader (1975) observed that retinol bound to a similar intracellular low-molecular-weight acceptor protein.

The presence of membrane receptors for RBP has been confirmed for bovine retina (Saari & Futterman, 1976) and the testis (Bhat & Cama, 1979; McGuire *et al.* 1981). In their studies with [^{3}H]retinol and [^{125}I]RBP in the rat, McGuire *et al.* (1981) observed that the receptors were attached to interstitial cells. [^{125}I]RBP did not bind to sertoli cells when injected into the testis or when incubated with cultures of these cells in vitro.

Thus the carrier protein specifically binds to a membrane receptor to ensure that the retinol is delivered to the particular functional cell and transferred across the membrane to an intracellular acceptor protein. CRBP may fill this role.

Mechanism of transfer of retinol

The method by which retinol is released from the holoprotein has not yet been established. The fact that the apoprotein can no longer bind retinol or associate with TBPA shows that some change in structure has taken place following the release of retinol. The release of retinol must occur fairly quickly because in studies on Japanese quail it has been observed that an amount of free retinol, equivalent to that in the bloodstream at any one time, is deposited in the yolk of the egg every 24 h (Heaf *et al.* 1980). During the egg-laying period, the concentration of apoprotein increases in Japanese quail plasma and makes up to 50% of the total RBP. This material is denatured and carries an additional negative charge and does not bind to TBPA. This seems to confirm that the holoRBP, in transferring retinol into the tissue, changes its conformation or structure so that it is also enabled to leave the binding site on the receptor.

Various mechanisms have been proposed for this. An early suggestion that possibly the COOH terminal amino acid (arginine) was cleaved off holoRBP (Rask *et al.* 1971) had to be revised in the light of the knowledge that the apoprotein has the same basic polypeptide chain as holoRBP following hydrolysis (Fex & Hansson, 1979) and that the COOH terminal amino acid is leucine and not arginine (White *et al.* 1972; Fex & Hansson, 1979; Rask *et al.* 1979). After determining the full amino acid sequence of the protein, Rask and colleagues (1981) suggested that it may be possible for a serine residue at position 8 to be phosphorylated. The sequence of amino acids around that position is very similar to that seen in other polypeptides which can be phosphorylated such as phosphorylase kinase. Furthermore, they have demonstrated that human RBP can become phosphorylated in vitro using [γ-^{32}P]ATP and the catalytic subunit of cyclic-AMP-stimulated protein kinase. However, protein kinases normally operate intracellularly, whereas RBP attaches only to the outer face of the membrane to be

released back into the intercellular fluid and plasma again. If such a phosphorylation occurred it would yield a product with an increased negative charge and explain the higher electrophoretic mobility of apoRBP as opposed to that of the holoprotein. Since the apoprotein secreted in the urine of patients suffering from renal disorders appears to be the same as that in plasma as far as amino acid composition, mobility and inability to bind TBPA are concerned (Fex & Hansson, 1979), it would be interesting to know if phosphate was present on any of this material.

An increase in negative charge, however, can readily arise through the loss of the amide group from any of the many glutamine or asparagine residues which may be on or near the surface of the molecule. Some of these are readily lost artifactually at physiological pH, even during the chromatographic isolation of the protein from plasma. RBP preparations known to be homogeneous with regard to size and molecular weight are, nevertheless, heterogeneous when subjected to isoelectric focussing. Some preparations have been resolved into seven to nine components depending on the number of amide groups lost in processing compared with the native protein (Glover, 1973).

Thus the conformation of the protein could be readily changed when it acquires an additional negative charge following the loss of an amide group. An enzyme capable of carrying out this transformation in other plasma proteins (Mycek & Waelsch, 1960), peptidyltransglutaminase, has been isolated from liver and purified (Connellan *et al.* 1971). It is now known to be more widespread and binds to fibronectin (Yamada *et al.* 1981) which links the tissue cell membrane with collagen fibres and may be close enough to deamidate RBP, change its conformation and release the retinol for transfer across the membrane.

Once retinol penetrates the cell it can be collected by CRBP and transported to the active site. The latter protein has a slightly higher affinity for retinol than holoRBP (Ong & Chytil, 1978) and permits specific interaction of retinol with the nucleus in vitro (Takase *et al.* 1979).

REFERENCES

Arroyave, G., Wilson, D., Mendez, J., Behar, M. & Scrimshaw, N. S. (1961). *Am. J. clin. Nutr.* **9**, 180.
Bashor, M. M., Toft, D. O. & Chytil, F. (1973). *Proc. Natl. Acad. Sci. USA* **70**, 3483.
Bhat, M. K. & Cama, H. R. (1979). *Biochem. biophys. Acta* **587**, 273.
Chen, C. C. & Heller, J. (1979). *Arch. Biochem. Biophys.* **198**, 572.
Chytil, F. & Ong, D. E. (1978). *Vitam. Horm.* **36**, 1.
Connellan, J. M., Chung, S. I., Whetzel, N. K., Bradley, L. M. & Folk, J. E. (1971). *J. biol. Chem.* **246**, 1093.
Dingle, J. T. & Lucy, J. A. (1962). *Biochem. J.* **84**, 611.
Fex, G. & Hansson, B. (1979). *Eur. J. Biochem.* **94**, 307.
Ganguly, J. (1960). *Vitam. Horm.* **18**, 387.
Glover, J. (1973). *Vitam. Horm.* **31**, 1.
Glover, J., Heaf, D. J. & Large, S. (1980). *Br. J. Nutr.* **43**, 357.
Glover, J., Jay, C., Kershaw, R. C. & Reilly, P. E. B. (1976). *Br. J. Nutr.* **36**, 137.
Glover, J., Jay, C. & White, G. H. (1974). *Vitam. Horm.* **32**, 215.

Glover, J. & Muhilal, H. (1976). *Int. J. Vitam. Nutr. Res.* **46**, 239.
Harrison, E. H., Smith, J. E. & Goodman, DeW. S. (1979). *J. Lipid Res.* **20**, 760.
Heaf, D. J., El-Sayed, M., Phythian, B., Carroll, J. & Glover, J. (1982). *J. Endocr.* **92**, 163.
Heaf, D. J., Phythian, B., El-Sayed, M. & Glover, J. (1980). *Int. J. Biochem.* **12**, 439.
Heller, J. (1975). *J. biol. Chem.* **250**, 6549.
Heller, J. (1979). *Arch. Biochem. Biophys.* **198**, 562.
Hughes, D. R., Rietz, P., Vetter, W. & Pitt, G. A. J. (1976). *Int. Z. Vitam. Forsch.* **46**, 231.
Ingenbleek, Y., van den Schrieck, H. G., de Nayer, P. & de Visscher, M. (1975). *Metabolism* **24**, 633.
Kanai, M., Raz, A. & Goodman, DeW. S. (1968). *J. clin. Invest.* **47**, 2025.
Kershaw, R. C. (1978). Factors controlling retinol-binding protein concentration. PhD Thesis, University of Liverpool.
Large, S., Neal, G., Glover, J., Thanangkul, O. & Olson, R. E. (1980). *Br. J. Nutr.* **43**, 393.
Lewin, D. R., Thompson, J. N., Pitt, G. A. J. & Howell, J. McC. (1970). *Int. J. Vitam. Res.* **270**, 40.
Linder, M. C., Anderson, G. H. & Ascarelli, I. (1971). *J. biol. Chem.* **246**, 5538.
McGuire, B. W., Orgebin-Crist, M.-C. & Chytil, F. (1981). *Endocrinology*, **108**, 658.
Michaelsson, G., Vahlquist, A., Juhlin, L., Mellbin, T. & Bratt, L. (1976). *Scand. J. clin. Lab. Invest.* **36**, 827.
Muhilal, H. & Glover, J. (1974). *Br. J. Nutr.* **32**, 549.
Muto, Y., Smith, J. E., Milch, P. O. & Goodman, DeW. S. (1972). *J. biol. Chem.* **247**, 2542.
Mycek, M. J. & Waelsch, H. (1960). *J. biol. Chem.* **235**, 3513.
Ong, D. E. & Chytil, F. (1978). *J. biol. Chem.* **253**, 828.
Peterson, P. A. (1971). *J. biol. Chem.* **246**, 34.
Peterson, P. A., Nilsson, S. F., Ostberg, L. & Vahlquist, A. (1974). *Vitam. Horm.* **32**, 181.
Rask, L., Anundi, H., Böhme, J., Eriksson, U., Ronne, H., Sege, K. & Peterson, P. A. (1981). *Ann. N.Y. Acad. Sci.* **359**, 79.
Rask, L., Anundi, H. & Peterson, P. A. (1979). *FEBS Lett.* **104**, 55.
Rask, L., Vahlquist, A. & Peterson, P. A. (1971). *J. biol. Chem.* **246**, 6638.
Raz, A., Shiratori, T. & Goodman, DeW. S. (1970). *J. biol. Chem.* **245**, 1903.
Rechcigl, M., Berger, S., Loosli, J. K. & Williams, H. H. (1962). *J. Nutr.* **76**, 435.
Rietz, P., Wiss, O. & Weber, F. (1974). *Vitam. Horm.* **32**, 237.
Saari, J. C. & Futterman, S. (1976). *Exp. Eye Res.* **22**, 425.
Shingwekar, A. G., Mohanram, M. M. & Reddy, V. (1979). *Clin. Chim. Acta* **93**, 97.
Smith, F. R., Goodman, DeW. S., Arroyave, G. & Viteri, F. (1973). *Am. J. clin. Nutr.* **26**, 982.
Smith, F., Goodman, DeW. S., Zaklama, M. S., Gabr, M. K., El Maraghy, S. & Patwardhan, V. N. (1973). *Am. J. clin. Nutr.* **26**, 973.
Smith, J. C., McDaniel, F. G., Fan, F. F. & Halstead, J. A. (1973). *Science* **181**, 954.
Smith, J. E., Borek, C. & Goodman, DeW. S. (1978). *Cell* **15**, 865.
Smith, J. E., Borek, C. & Goodman, DeW. S. (1982). *Ann. N.Y. Acad. Sci.* **359**, 171.
Smith, J. E., Brown, E. D. & Smith, J. C. (1974). *J. Lab. clin. Med.* **84**, 692.
Smith, J. E., Deen, D. D., Sklan, D. & Goodman, DeW. S. (1980). *J. Lipid Res.* **21**, 229.
Stevenson, J. W. & Earle, I. P. (1956). *J. Anim. Sci.* **15**, 1039.
Takase, S., Ong, D. E. & Chytil, F. (1979). *Proc. Natl. Acad. Sci. USA* **76**, 2204.
Tanner, J. M. (1962). In *Protein Metabolism* [F. Goss, editor]. Berlin: Springer-Verlag.
Tanner, J. M., Whitehouse, R. H., Marubini, E. & Resele, L. (1976). *Annls Hum. Biol.* **3**, 109.
Thompson, J. N., Howell, J. McC. & Pitt, G. A. J. (1964). *Proc. R. Soc., B.* **115**, 510.
Vallee, B. L., Wacker, W. E. C., Bartholomay, A. F. & Hoch, F. L. (1957). *N. Engl. J. Med.* **257**, 1055.
Vallee, B. L., Wacker, W. E. C., Bartholomay, A. F. & Hoch, F. L. (1959). *Ann. Int. Med.* **50**, 1077.
Wake, K. (1980). *Int. Rev. Cytol.* **66**, 303.
White, G. H., Weston, S. M. & Glover, J. (1972). *FEBS Lett.* **27**, 107.
Wiggert, B. O. & Chader, G. J. (1975). *Exp. Eye Res.* **21**, 143.
Yamada, K. M., Akiyama, S. K. & Hayashi, M. (1981). *Biochem. Soc. Trans.* **9**, 506.

Printed in Great Britain