Retinol homeostasis in lambs given low and high intakes of vitamin A

BY SUSAN DONOGHUE, DAVID S. KRONFELD

Section of Nutrition, Department of Clinical Studies, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA 19348, USA

AND DAVID SKLAN

Hebrew University of Jerusalem, PO Box 12, Rehovot, 76-100, Israel

(*Received 17 January 1983 – Accepted 25 February 1983*)

1. Four groups of lambs were fed on a low-carotene basal diet. One group received no supplemental vitamin A (mildly deficient). Remaining groups were supplemented daily with vitamin A acetate equivalent to 100 (control) 9000 (mildly intoxicated) and 18000 (severely intoxicated) μg retinol/kg body-weight. After 16 weeks lambs received a bolus of [15-^{3}H]retinol intravenously; blood, urine and faeces were sampled for 48 h.

2. Plasma retinol was complexed to a protein of 20000 molecular weight (MW), which in turn was complexed to a protein of 65000 MW; these proteins correspond respectively to retinol-binding protein and prealbumin. Plasma retinol concentration reached plateau values in intoxicated lambs, but plasma retinyl ester concentrations increased rapidly when liver contents of both retinol and retinyl esters exceeded approximately 10 and 100 mg respectively and kidney contents of both retinol and retinyl esters exceeded 30 μg. Labelled compounds, more polar than retinol, were found in plasma; their concentration increased tenfold in intoxicated lambs within 48 h.

3. Plasma retinol transport rates were 0·1, 10·5 and 11·8 times control values, and clearance rates were 0·3, 14·1 and 14·3 times control values in mildly-deficient, and mildly- or severely-intoxicated lambs respectively. Turnover of retinol increased rapidly when liver contents of retinol and retinyl esters exceeded approximately 10 and 100 mg respectively and kidney contents of both retinol and retinyl esters exceeded approximately 30 μg. Plasma clearance of retinyl esters was unchanged with intake. Faecal excretion of tracer increased linearly with plasma retinol clearance.

4. Our findings identify several variables that appear to be involved in retinol homeostasis, including plasma retinol clearance and excretion.

Vitamin A is stored primarily in liver as retinyl esters (Futterman & Andrews, 1964). These esters are hydrolysed to retinol and complexed to a specific binding protein, retinol-binding protein (RBP), before release into plasma, where holoRBP is further complexed to prealbumin. This system has been extensively studied in rats (Goodman, 1980) and humans (Kanai *et al.* 1968). However, in ruminants conflicting information has been reported. HoloRBP is reportedly complexed to prealbumin in goats (Sreekrishna & Cama, 1979) but not in cattle (Heller, 1975). While holoRBP concentrations have been determined in sheep (Glover *et al.* 1976), binding of the complex to prealbumin has not been reported.

Plasma retinol concentration appears to be highly regulated over a wide range of vitamin A intakes although the mechanisms of this control are not well defined. Recent studies in hypovitaminotic A rats have suggested extrahepatic control factors, possibly retinol utilization rates, which determine the plasma retinol set point and a schematic model has been proposed (Underwood *et al.* 1979).

The present study was designed to investigate the plasma retinol transport system in lambs receiving four different intakes of vitamin A and to determine some of the factors which regulate plasma retinol concentration.

https://doi.org/10.1079/BJN19830093 Published online by Cambridge University Press

Table 1. *Composition (g/kg) of low-carotene diets**

Diet... Ingredients	Starter	Basal (weeks 1–16)
White maize meal	705	200
Oats	0	200
Soya-bean meal	200	150
Beet pulp	75	394
Limestone	8	0
Dicalcium phosphate	7	0
Molasses	0	50
Monosodium phosphate	0	1
Trace mineral salt†	5	5

* Each concentrate contained 21·1 μg cholecalciferol/kg.

† IOFIXT T-M; Morton Salt Co., Chicago, IL, containing (g/kg): 4·75 sodium chloride, 0·025 zinc, 0·02 manganese, 0·0125 iron, 0·0005 copper, 0·0001 iodine, 0·0001 cobalt.

MATERIALS AND METHODS

Sixteen 2-week old female crossbred lambs were purchased from commercial dealers and housed individually in metal crates. All were administered antibiotics for 10 d, wormed with thiobendazole and vaccinated against tetanus and enterotoxemia. Lambs were given a commercial sheep milk-substitute and a low-carotene starter ration (Table 1). At 5 weeks of age the lambs were weaned and gradually changed to a low-carotene diet of lower energy and protein densities (Table 1). Lambs were offered the diet at a daily rate of 30 g/kg body-weight. The diets met National Research Council recommended intakes for energy, protein, calcium phosphorus and cholecalciferol ((US) National Research Council, 1975). All lambs received two injections of α-tocopherol and selenium and had free access to plain salt and fresh water.

At 5 weeks of age the lambs were randomly selected to be in one of four groups: mildly-vitamin A-deficient (three lambs), control (three lambs), mildly-vitamin A-intoxicated (five lambs) and severely-vitamin A-intoxicated (five lambs). The groups received retinyl acetate daily in their food equivalent to 0, 100, 9000, and 18000 μg retinol/kg body-weight. Lambs were maintained for 16 weeks. Feed intake was monitored daily, and body-weights were recorded weekly.

After 16 weeks and following catheterization of urinary bladders and blood sampling, all lambs were injected via the jugular vein with a bolus (0·5 mCi) of [15-^{3}H]retinol (14·3 Ci/mmol) (New England Nuclear, Boston, MA) in 1 ml ethanol followed by a saline (9 g sodium chloride/l)–ethanol wash. Sequential heparinized blood samples were obtained from the other catheterized jugular vein for 48 h. Urine and faeces were collected every 6 h and frozen for subsequent determination of radioactivity. Lambs had free access to water and the vitamin A-free diet until sacrifice.

After 48 h all lambs were killed by an intravenous injection of pentobarbitol sodium. Livers and kidneys were removed, weighed, ground and frozen for subsequent analysis.

Concentrations of retinol and retinyl esters were determined by the method of Neeld & Pearson (1963) for plasma and Bunnell *et al.* (1954) for tissues, following separation by alumina column chromatography (DeLuca *et al.* 1969). Colorimetric determinations were made on a spectrophotometer. Recoveries were determined using retinol and retinyl acetate standards (Sigma Chemical Company, St Louis, MO) and mean (with SE) values were 90·4 (3·8) and 88·4 (6·9) % respectively. Lipid-soluble compounds more polar than retinol were

https://doi.org/10.1079/BJN19830093 Published online by Cambridge University Press

determined by elution of retinol and retinyl esters from an alumina column with 20% diethyl ether in heptane followed by diethyl ether.

Faeces were ground and extracted with an acid chloroform–methanol (2:1 v/v) solution (Folch *et al.* 1957) utilizing an internal standard to correct for quenching.

Radioactivity was determined with a liquid scintillation counter. Standards for counting were prepared from portions of tracer dose diluted in non-radioactive plasma. Extracts from blood, tissues and faeces, and whole urine, were counted following addition of counting solution (Aquasol II, New England Nuclear) with internal standards used for determination of quenching when appropriate.

Plasma proteins associated with retinol transport were characterized by ultracentrifugation and gel chromatography. Plasma samples (1 ml) were adjusted to densities of 1·006, 1·063 and 1·21 g/ml by the addition of potassium bromide and separated by centrifugation for 40 h at 105000 ***g*** (Model L, Rotor Model 65; Beckman Instruments Inc.). The upper and lower zones were aspirated into a syringe, following tube puncture by a needle. A portion (1 ml) was removed, added to the counting solution and radioactivity determined. Chromatography was performed on a Sephacryl S-300 column (Pharmacia Fine Chemicals, Piscataway, NJ) with 0·05 M-Tris-hydrochloric acid buffer, pH 8·0. Apo-ferritin, horseradish peroxidase (*EC* 1 . 11 . 1 . 7), bovine serum albumin, ovalbumin, myoglobin and cytochrome *c* were used as standard proteins. Radioactivity and fluorescence were monitored.

The tracer data were analysed by fitting multi-exponential equations to curves of labelled plasma retinol or retinyl esters (% dose/100 ml) and their specific activity (% dose/μg) against time (Gurpide & Mann, 1970; Kronfeld & Ramberg, 1981). These equations were not used as a basis for compartmental modelling, which would require various biological assumptions, but simply as a means of estimating areas subtended by curves, i.e. time integrals of specific activity. Plasma retinol transport rate (μg/h) was calculated as the reciprocal of this area; it represents the sum of all flows of retinol into or out of the plasma. Retinol clearance rate from plasma (100 ml/h) was calculated by dividing plasma retinol transport rate by plasma retinol concentration; it represents the volume of plasma from which retinol was removed per unit time (Donoghue *et al.* 1979). Student's *t* test was used to determine significance of difference between groups (Snedecor & Cochran, 1967).

Correlations between certain pairs of variables were tested for best fits to linear, exponential, logarithmic and power functions (Model HP-97; Hewlett Packard, Corvallis, OR).

RESULTS

Clinical status, feed intake and growth. At the end of the 16-week experiment the mildly-deficient and mildly-intoxicated lambs appeared healthy but slightly smaller than controls. The severely-intoxicated lambs appeared unthrifty and depressed.

Feed intake was not affected by dietary vitamin A, mean (with SE) values (g/kg body-weight per day) being 26·7 (1·2), 26·6 (1·7), 27·4 (0·4) and 27·4 (0·4) for the deficient, control, mildly-intoxicated and severely-intoxicated lambs respectively. However, growth was depressed in mildly-deficient and intoxicated lambs ($P < 0·05$) and means (with SE) were 56 (16), 133 (9), 113 (15) and 40 (16) g/d respectively for the four groups.

Plasma transport system. On gel filtration of plasma from control lambs, approximately 95% of retinol fluorescence was found at a molecular weight of approximately 85000 (Fig. 1). Part of the retinol fluorescence could be transferred to a molecular weight of approximately 20000 by treatment of plasma with 3 M-guanidine hydrochloride and carrying out the chromatography with 1·5 M-guanidine in the eluting buffer. Following injection of labelled retinol, the chromatographic profile of radioactivity was identical to that of retinol fluorescence in less than 60 min.

https://doi.org/10.1079/BJN19830093 Published online by Cambridge University Press

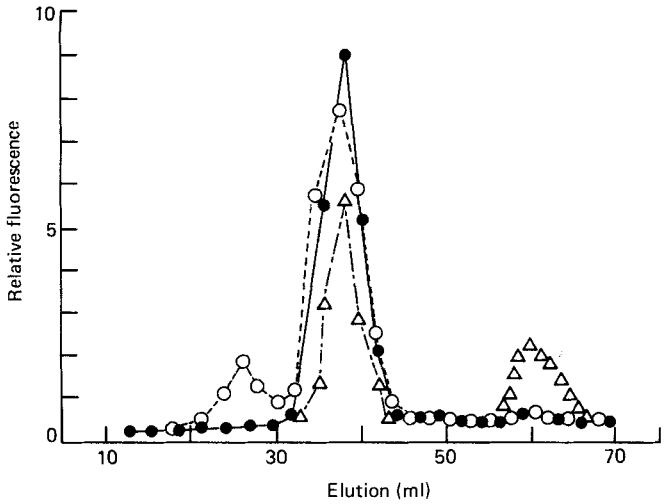

Fig. 1. Gel filtration of plasma from lambs given high- and low-vitamin A intakes, on a column of Sephacryl S-300 (480 × 18 mm) with 50 mM-Tris-hydrochloric acid, pH 8·0. Plasma (1 ml) was loaded on to the columm and fractions of 2·2–2·4 ml were collected. Albumin eluted at 43 ml and myoglobin at 66 ml. Relative fluorescence was determined with excitation at 330 nm and emission at 470 nm. Values represent plasma from a control lamb (●), mildly-intoxicated lamb (○) and the control plasma following treatment with guanidine (△).

Table 2. *Distribution of tracer in ultracentrifuged plasma adjusted to various densities from a control and a severely-vitamin A-intoxicated lamb (percentage radioactivity)*

		Density (g/ml)					
		1·006		1·063		1·21	
Lamb	Time (min)	Top	Bottom	Top	Bottom	Top	Bottom
Control	10	7·1	92·9	9·0	91·0	29·5	70·5
	20	8·2	91·8	9·1	90·9	24·1	75·9
	60	7·2	92·8	3·1	96·9	3·4	96·6
	48 h	7·2	92·8	7·4	92·6	17·4	82·6
Intoxicated	48 h	39·3	60·7	40·1	59·9	52·5	47·5

Ultracentrifugation of plasma from a control lamb demonstrated that over 70% of label was transferred to the 1·21 g/ml bottom fraction within 10 min and over 95% was in this portion by 60 min after injection (Table 2).

Mildly-deficient lambs had a chromatographic profile similar to that of controls. Enhancement of fluorescence was found at high molecular weights, above 500000 in lambs on high-vitamin A-intakes. This fraction contained predominantly retinyl esters. Ultracentrifugation of plasma from these animals determined that over 50% of the radioactivity resided in the 1·21 g/ml top fraction, with smaller portions in top fractions of 1·006 and 1·063 g/ml (Table 2).

Plasma and tissue vitamin A. Plasma total vitamin A increased with increasing vitamin A intake (Table 3). This response was due mainly to increases in plasma retinyl ester levels, which were four and six times higher in the mildly- and severely-intoxicated lambs respectively.

https://doi.org/10.1079/BJN19830093 Published online by Cambridge University Press

Table 3. *Effect of vitamin A intake on plasma and tissue levels of vitamin A*
(Mean values with their standard errors)

	Vitamin A intake (μg/kg body-wt per d)							
	0		100		9000		18000	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Plasma total vitamin A (μg/100 ml)	12·7*	0·6	40·4	13·9	55·7*	5·2	71·1*	8·9
Plasma retinyl esters (% total)	16·6	4·8	13·3	6·0	37·1	13·6	44·5*	11·9
Liver retinol (log μg/organ)	1·89*	0·16	4·12	0·34	4·78	0·11	4·90	0·12
Liver retinyl esters (log μg/organ)	2·04*	0·12	4·42	0·37	5·45*	0·11	5·55*	0·18
Kidney retinol (log μg/organ)	0·66*	0·11	1·26	0·14	2·09*	0·12	2·25*	0·34
Kidney retinyl esters (log μg/organ)	0·65*	0·03	1·35	0·08	2·40*	0·08	2·46*	0·42

* Significantly different from control (100 μg/kg) value: $P < 0·05$.

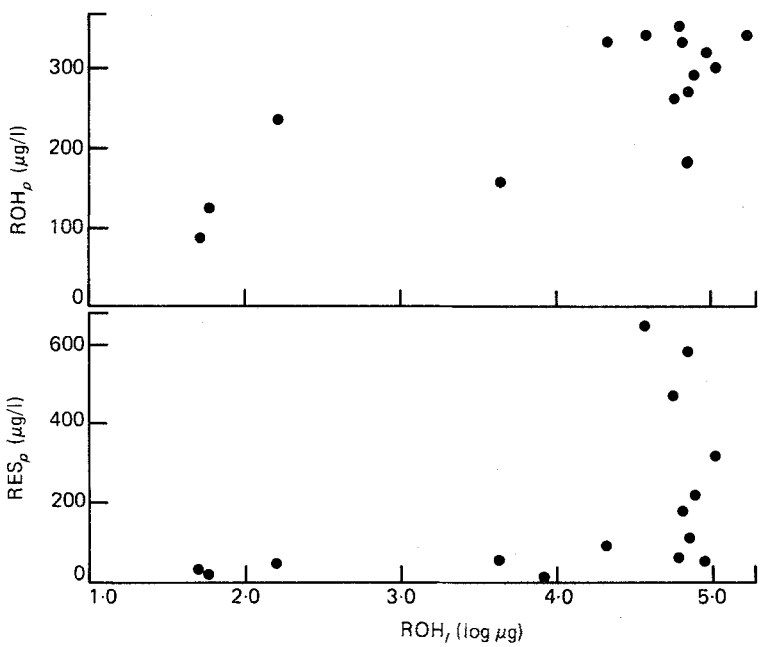

Fig. 2. Plasma retinol (ROH$_p$; μg/l) and retinyl ester (RES$_p$; μg/l) concentrations in relation to hepatic retinol (ROH$_l$; log μg) content in lambs. Best fits of these results are provided in Table 7 (p. 246).

https://doi.org/10.1079/BJN19830093 Published online by Cambridge University Press

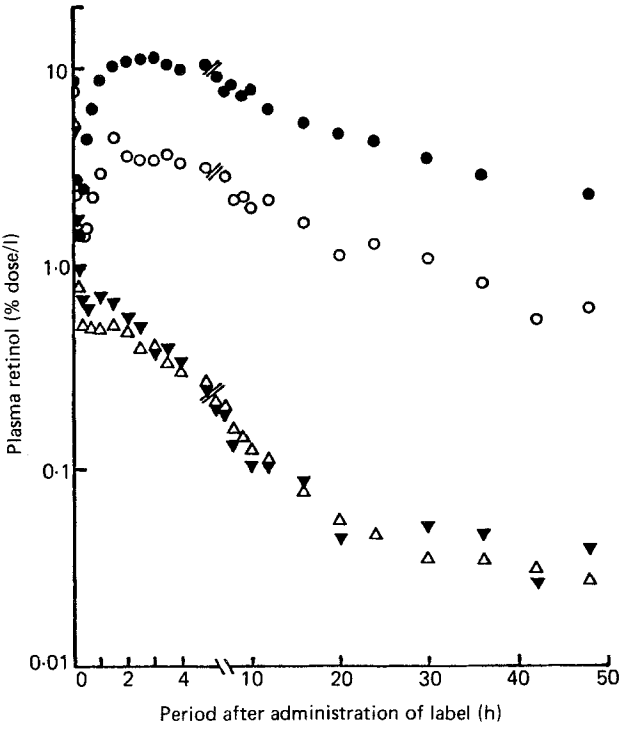

Fig. 3. Amounts of labelled retinol in plasma over 48 h for all mildly-deficient (●), control (○), mildly intoxicated (△) and severely-intoxicated (▼) lambs.

Table 4. *Best-fitting exponential equations for curves of extracted plasma* [15-^3H]*retinol* (*radioactivity of plasma retinol, % dose/100 ml*)

$$A_t = A_1 \exp -\alpha_1 t + A_2 \exp -\alpha_2 t + A_3 \exp -\alpha_3 t + A_4 \exp -\alpha_4 t$$

Vitamin A intake	Lamb	Intercepts				Exponents			
		A_1	A_2	A_3	A_4	α_1	α_2	α_3	α_4
Mildly-deficient	114	0·890	−1·83	0·512	0·834	7·33	1·44	0·021	0·167
	115	1·102	−1·84	0·927	0·655	13·06	1·05	0·083	0·010
	116	1·315	−3·52	2·21	0·918	11·04	0·96	0·546	0·050
Control	117	0·420	−0·650	0·304	0·157	4·88	1·14	0·073	0·020
	128	0·581	−0·380	0·275	0·126	20·17	0·90	0·274	0·034
	110	1·858	−0·514	0·348	0·266	28·90	1·27	0·118	0·022
Mildly-intoxicated	119	0·525	−0·029	0·064	0·0052	18·89	1·07	0·189	0·018
	120	0·662	−0·064	0·084	0·0164	35·76	0·87	0·264	0·029
	121	0·770	−0·009	0·072	0·0085	21·18	1·05	0·206	0·023
	122	0·287	—	0·052	0·0031	15·32	—	0·430	0·019
	123	0·624	−0·016	0·062	0·0116	21·92	1·91	0·228	0·058
Severely-intoxicated	112	1·07	—	0·176	0·0244	25·55	—	0·368	0·021
	124	0·370	−0·057	0·106	0·0092	24·14	0·50	0·267	0·028
	125	0·318	—	0·056	0·0082	24·83	—	0·240	0·040
	126	0·343	—	0·047	0·0038	19·56	—	0·408	0·022
	127	0·289	−0·023	0·062	0·0059	21·74	1·34	0·222	0·013

t = time (min).

https://doi.org/10.1079/BJN19830093 Published online by Cambridge University Press

Table 5. *Effect of vitamin A intake on plasma retinol and retinyl ester transport, clearance and excretion of radioactivity in* 48 *h*

(Mean values with their standard errors)

	Vitamin A intake (μg/kg body-wt per d)							
	0		100 (control)		9000		18000	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Plasma transport (mg/h)								
Retinol	0·062	0·035	0·490	0·200	5·15*	0·62	5·78*	1·70
Retinyl esters	6·1	4·3	6·7	2·7	33·3†	11·6	60·7†	25·3
Plasma clearance (l/h)								
Retinol	0·34	0·11	1·29	0·52	18·2*	3·4	18·4*	4·8
Retinyl esters	152	86	146	19	158	17	162	19

Significance of difference from control: † $0{\cdot}10 > P > 0{\cdot}05$, * $P < 0{\cdot}05$.

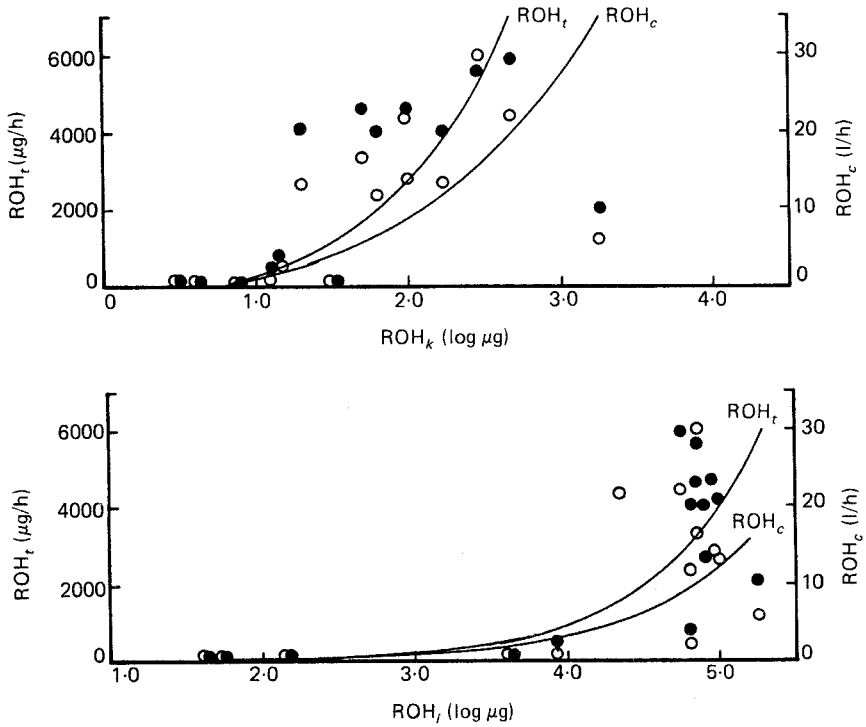

Fig. 4. Rates of plasma retinol transport (ROH$_t$; μg/h; (●)) and clearance (ROH$_c$; l/h; (○)) of lambs given high- and low-vitamin A intakes. ROH$_t$ and ROH$_c$ increased with increasing hepatic (ROH$_l$) and renal (ROH$_k$) retinol levels. Equations and levels of significance of best fits are provided in Table 7 (p. 246).

https://doi.org/10.1079/BJN19830093 Published online by Cambridge University Press

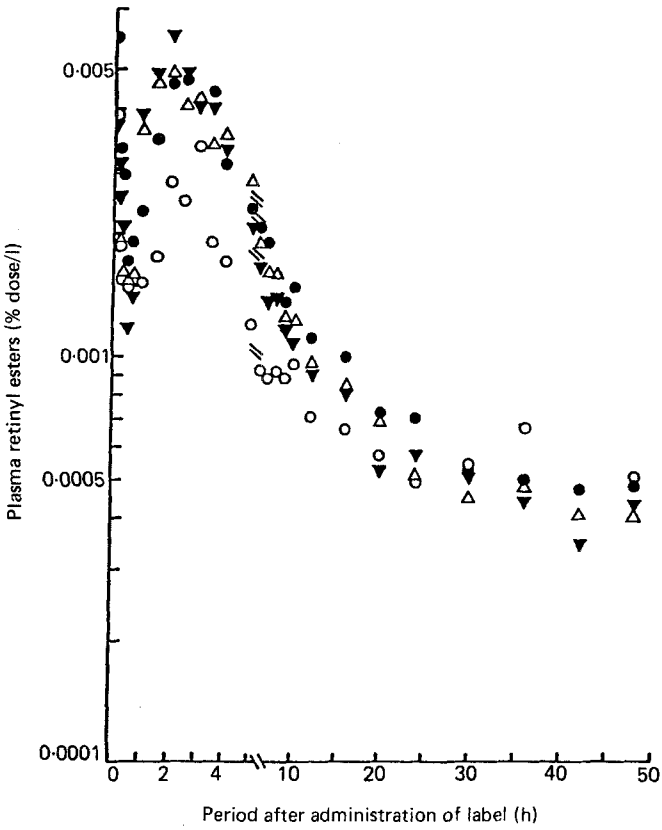

Fig. 5. Amounts of labelled retinyl esters in plasma over 48 h for all mildly-deficient (●), control (○), mildly-intoxicated (△) and severely-intoxicated (▼) lambs.

Hepatic total vitamin A levels increased with intake, especially the retinyl ester fraction which increased ten and thirteen times the control values in the mildly- and severely-intoxicated lambs respectively (Table 3). Retinol and retinyl ester levels also increased in kidneys with increasing vitamin A intake (Table 3).

Retinol and retinyl ester concentrations in plasma were related to their contents in liver and kidney. At high tissue levels, plasma retinol concentrations remained relatively constant while plasma retinyl ester concentrations increased markedly (Fig. 2). When hepatic retinol rose above approximately 10 mg, for example, plasma retinol concentration approached a plateau but retinyl ester concentrations began to rise rapidly (Fig. 2). Similar responses of plasma concentrations of retinol and retinyl esters were observed when hepatic contents of retinyl esters were high and exceeded approximately 100 mg (not shown). A similar response was observed when renal contents of both retinol and retinyl esters exceeded approximately 30 μg.

Plasma vitamin A transport and clearance. Amounts of labelled plasma retinol were plotted *v.* time (Fig. 3). Radioactivity rapidly disappeared from blood for 20 min after injection of tracer, followed by a gradual increase for several hours. This response decreased with increasing vitamin A intake (Fig. 3). For the mildly deficient, control, four of the mildly-intoxicated and two of the severely-intoxicated lambs, four exponentials were

https://doi.org/10.1079/BJN19830093 Published online by Cambridge University Press

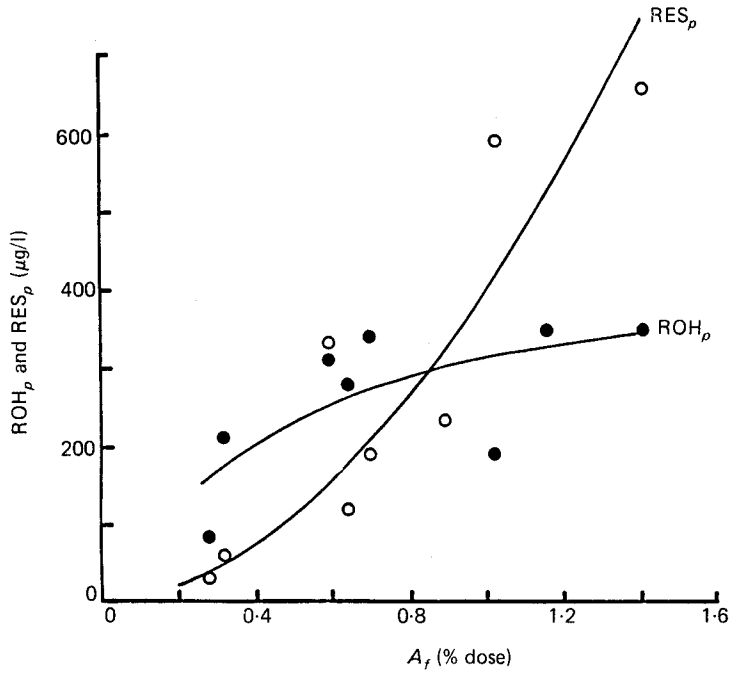

Fig. 6. Plasma concentrations of retinol (ROH$_p$; μg/l; ●) and retinyl esters (RES$_p$; μg/l; ○) in relation to cumulative faecal excretion of radioactivity (A_f; %dose) in lambs given low- and high-vitamin A intakes. Equations and levels of significance of best fits are provided in Table 7 (p. 246).

necessary and sufficient to fit the data; for the remaining animals, the negative exponential was unnecessary (Table 4).

Plasma retinol transport and clearance were calculated from these curves; they were significantly affected by vitamin A intake (Table 5). Retinol transport was 0·1, 10·5 and 11·8 times the control values in the mildly-deficient and mildly- or severely-intoxicated lambs respectively, while clearance of retinol from plasma was 0·3, 14·1 and 14·3 times control rates. Retinol transport and clearance increased with increasing hepatic and renal levels of vitamin A; this increase became very rapid when hepatic and renal vitamin A levels exceeded approximately 10 mg and 30 μg, respectively (Fig. 4).

Retinyl esters in plasma (% dose/l) were also plotted *v.* time and rates of transport and clearance were calculated from exponential equations fitted to these curves (Fig. 5). A much smaller percentage of the dose was located in this component than in the retinol fraction. There were no differences in tracer disappearance curves between groups. Plasma transport of retinyl esters averaged 0·92, 5·0 and 9·1 times the control value for the mildly-deficient and the mildly- and severely-intoxicated lambs respectively (Table 5). Increased transport rates of the latter two groups were due to increased plasma retinyl ester concentrations, however, as clearance rates were unchanged from controls (Table 5).

Tracer excretion. Cumulative radioactivity excreted in faeces and urine for 48 h was determined in nine and eleven lambs respectively. Mean (with SE) values for cumulative excretion of label in urine after 48 h were 7·3 (5·2) (*n*2), 6·1 (*n*1), 2·5 (0·5) (*n*4) and 2·4 (1·1) (*n*4) % dose for the mildly-deficient, control, mildly- and severely-intoxicated lambs, respectively.

Mean (with SE) values for cumulative excretion of radioactivity in faeces were 0·3 (*n*1)

https://doi.org/10.1079/BJN19830093 Published online by Cambridge University Press

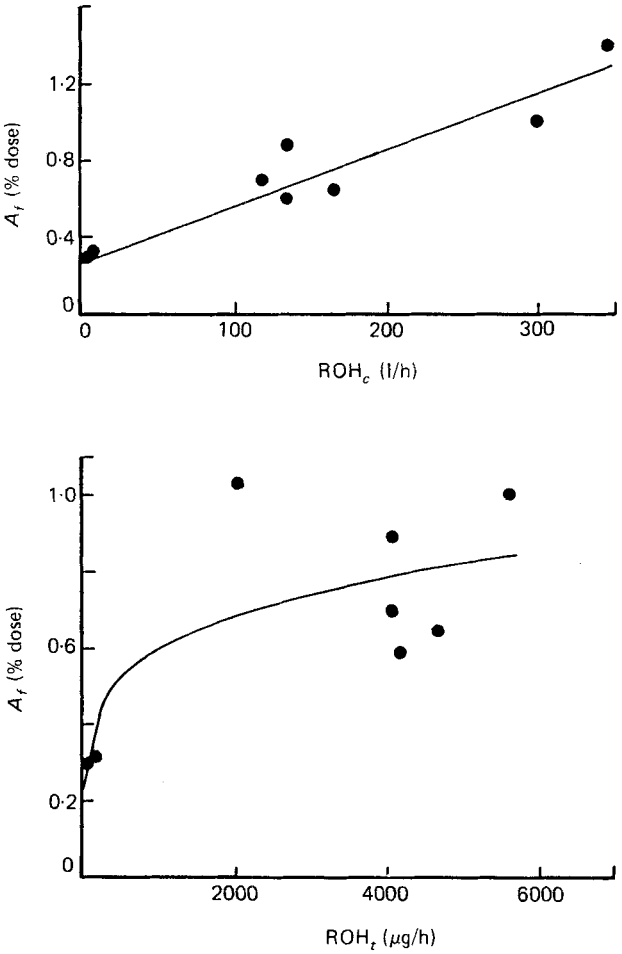

Fig. 7. Cumulative faecal radioactivity (A_f; % dose) in relation to rates of plasma retinol transport and clearance (ROH_t (μg/h) and ROH_c (l/h) respectively). Equations and levels of significance of best fits are provided in Table 7 (p. 246).

0·3 (*n*1), 0·9 (0·1) (*n*3) and 1·0 (0·2) (*n*4) % dose for the mildly-deficient, control, mildly- and severely-intoxicated lambs respectively. At higher cumulative faecal radioactivity, the plasma concentration of retinol approached a plateau but that of retinyl esters continued to rise (Fig. 6). Cumulative faecal radioactivity was related linearly to plasma retinol clearance (Fig. 7), but it approached a plateau at plasma retinol transport rates from 2500 to 6000 μg/h.

Tissue retention of tracer. Percentage of dose as retinol and retinyl esters remaining in liver and kidney after 48 h differed between control and deficient groups (Table 6). In liver from deficient lambs, labelled retinol was 11% of the control value while mildly- and severely-intoxicated lambs retained 66 and 77% of control values respectively. The level of labelled retinyl esters increased with increasing vitamin A intake (Table 6).

Tissue dose retention in kidney was different from that in liver in that the percentage of renal H^3-labelled retinol in mildly-deficient lambs was over five times higher than in controls, while percentage amounts remaining in intoxicated groups were similar.

https://doi.org/10.1079/BJN19830093 Published online by Cambridge University Press

Table 6. *Effect of vitamin A intake on tissue retention and esterification of tracer*

	Vitamin A intake (μg/kg body-wt per d)							
	0		100 (control)		9000		18000	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Liver radioactivity (% dose)								
Retinol	0·69*	0·04	6·22	0·95	4·13	0·64	4·78	0·74
Retinyl esters	0·25*	0·01	8·62	0·55	8·79	1·36	13·45	3·61
Retinol:retinyl esters	2·8*	0·0	0·7	0·1	0·5‡	0·1	0·4†‡	0·1
Kidney radioactivity (% dose)								
Retinol	0·227*	0·050	0·039	0·016	0·020	0·005	0·026	0·014
Retinyl esters	0·076*	0·010	0·018	0·009	0·021	0·005	0·027	0·017
Retinol:retinyl esters	2·9	0·2	2·6	1·0	1·2‡	0·2	1·3‡	0·2

Significance of difference from control: † $0{\cdot}10 > P > 0{\cdot}05$, * $P < 0{\cdot}05$.
‡ The ratios of labelled retinol:retinyl esters in mildly- and severely-intoxicated lambs differed significantly between liver and kidney ($P < 0{\cdot}05$).

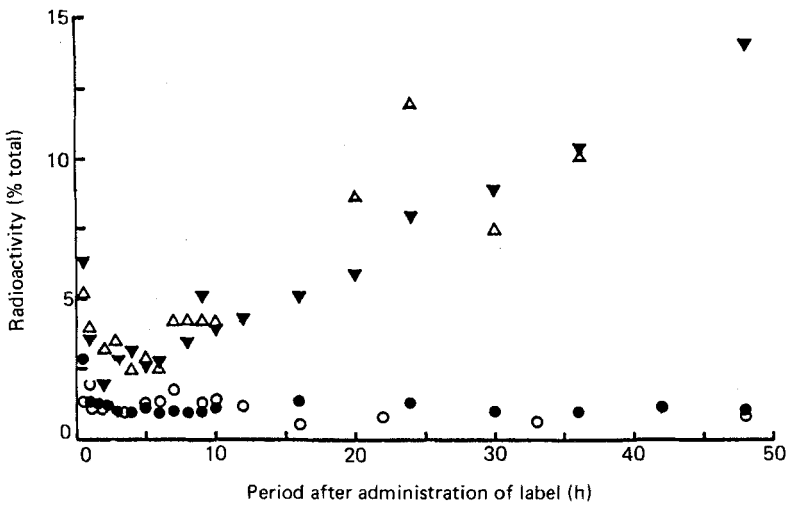

Fig. 8. Distribution of radioactivity with period after administration of label for all sheep given high- and low-vitamin A intakes. Labelled compounds more polar than retinol increased in mildly- (△) and severely-(▼) vitamin A-intoxicated lambs tenfold compared with mildly-vitamin A-deficient (●) and control (○) lambs.

Polar vitamin A metabolites. The percentage dose of lipid-soluble plasma metabolites more polar than retinol increased markedly with time in lambs on excessive vitamin A intakes (Fig. 8). Levels were over ten times higher than those found in control lambs.

Mathematical relationships. Twelve significant mathematical correlations were found to be best fits (Table 7). These relationships are empirical and imply no physiological assumptions.

https://doi.org/10.1079/BJN19830093 Published online by Cambridge University Press

Table 7. *Best-fitting linear, exponential, logarithmic or power functions for certain paired variables*

Equation	r^2	Significance ($P <$)
$ROH_p = 7{\cdot}55\ (ROH_l)^{0{\cdot}89}$	0·70	0·001
$RES_p = 0{\cdot}49\ e^{0{\cdot}74(ROH_l)}$	0·46	0·01
$ROH_p = 20{\cdot}1\ (ROH_k)^{0{\cdot}54}$	0·48	0·005
$RES_p = 0{\cdot}79\ e^{1{\cdot}58(ROH_k)}$	0·66	0·001
$ROH_t = 2{\cdot}02\ e^{1{\cdot}52(ROH_l)}$	0·84	0·001
$ROH_t = 298\ (ROH_k)^{3{\cdot}22}$	0·71	0·001
$ROH_c = 0{\cdot}23\ e^{1{\cdot}26(ROH_l)}$	0·76	0·001
$ROH_c = 13{\cdot}6\ (ROH_k)^{2{\cdot}76}$	0·69	0·001
$ROH_p = 31{\cdot}2 + 11{\cdot}3\ \ln\ (A_f)$	0·48	0·05
$RES_p = 28{\cdot}2\ (A_f)^{1{\cdot}42}$	0·55	0·05
$A_f = 0{\cdot}30 + 0{\cdot}003\ (ROH_c)$	0·90	0·001
$A_f = 0{\cdot}16\ (ROH_t)^{0{\cdot}19}$	0·69	0·025

ROH, retinol; RES, retinyl ester; A, cumulative radioactivity.
Subscripts refer to plasma concentration (μg/100 ml) (p); total amount (log μg) in liver (l) or kidney (k); total amount (% dose) in faeces (f); rates of transport (t) or clearance (c).

DISCUSSION

Our findings indicate that the retinol transport system in sheep plasma is similar to that described for man (Kanai *et al.* 1968), rat (Goodman, 1980) and goat (Sreekrishna & Cama, 1979). The present results demonstrate retinol binding to a protein of approximately 20000 molecular weight, which in turn is complexed to another protein of approximately 65000 molecular weight. These proteins correspond to RBP and prealbumin as described for other species. The present ultracentrifugation results demonstrated that plasma radioactivity was distributed in the 1·21 g/ml bottom fraction within a relatively short period of time after administration to control lambs. This observation is consistent with binding of labelled retinol to RBP complexed with prealbumin, as reported for rats (Mallia *et al.* 1975). When considered with the labelled plasma retinol disappearance curves (Fig. 3), these findings suggest that the tracer rapidly entered a physiological plasma compartment, probably via hepatic uptake, followed by binding to RBP and re-entry into blood as a RBP–prealbumin complex.

In the vitamin A intoxicated lambs, ultracentrifugation demonstrated radioactivity in the 1·21 g/ml top fraction, like the profile reported for hypervitaminotic A rats (Mallia *et al.* 1975). Moreover, we examined other densities and found radioactivity in fractions of densities of 1·006 and 1·063 g/ml (Table 2).

Plasma vitamin A levels increased with intake due mainly to elevation in the retinyl esters fraction (Table 3). This has been observed previously in man (Smith & Goodman, 1976), rat (Mallia *et al.* 1975) and horse (Donoghue *et al.* 1981*b*). Defective metabolism of hepatic RBP due to liver saturation at high intakes of vitamin A was suggested to be a factor involved in elevation of plasma retinyl ester concentrations (Mallia *et al.* 1975). Plasma retinol concentration plateaued at high levels of liver retinol and retinyl esters, while plasma retinyl esters continued to increase markedly. The plasma esters may arise from tissue stores or depressed hepatic uptake of intestine-derived esters.

Transport and clearance of plasma retinol were enhanced by increasing vitamin A intake. This finding agrees with our previous study of mature hypervitaminotic A ewes in which total plasma vitamin A (retinol and retinyl esters) transport and clearance rates increased

https://doi.org/10.1079/BJN19830093 Published online by Cambridge University Press

eight- and twofold respectively (Donoghue *et al.* 1979). The greater increase of clearance rate (fourteenfold for retinol in the present study *v.* twofold for total vitamin A in the previous study) is probably due to fractionation of plasma vitamin A into retinol and retinyl esters, as the latter portion exhibited unchanged clearance rates in vitamin A intoxication. In the present study, relationships of retinol transport and clearance to hepatic and renal vitamin A levels suggest that tissue thresholds, probably approximately 10 mg retinol and 100 mg retinyl esters in liver and 30 μg each of retinol and retinyl esters in kidney, may affect plasma retinol homeostasis. At these levels, turnover of plasma retinol increased markedly (Fig. 4) while plasma retinyl esters increased in concentration only (Fig. 2 and Table 5).

The present results also suggest that an excretory pathway of vitamin A is responsive to changes in vitamin A intake. The linear relationship of faecal excretion of tracer with clearance of retinol from plasma indicates that this route may play a role in vitamin A homeostasis.

Increases in plasma vitamin A metabolites more polar than retinol with hypervitaminosis A (Fig. 7) have not been reported previously. The response may reflect increased retinol metabolism with high vitamin A intake. Elevated levels of polar metabolites may contribute to the syndrome of vitamin A intoxication.

Vitamin A intake affected several criteria of kidney vitamin A metabolism, such as total amounts of renal vitamin A; certain of these criteria differed from our findings for liver, for example percentage dose retained in the tissue and the ratio of labelled retinol:retinyl esters. The differences from liver could be explained by variation in the extent of tissue vitamin A saturation; however, other evidence supports a role for kidney in vitamin A homeostasis distinct from that of the liver. In the present study we found a higher extent of correlation of plasma retinyl esters with renal vitamin A than with hepatic vitamin A, but an opposite effect for plasma retinol and the tissues (Table 7). In addition, we found a threshold of approximately 30 μg for both renal retinol and retinyl esters, above which plasma retinol turnover increased more rapidly and plasma retinyl ester concentrations rose. Although results are limited, urinary excretion of the tracer was apparently independent of vitamin A intake or kidney levels of labelled retinol and retinyl esters. Urinary excretion of the label was also unchanged in rats with varied liver vitamin A stores given a loading dose of labelled retinyl acetate (Reitz *et al.* 1974). This may indicate that urinary excretion of retinol or its derivatives is highly regulated. While retinol, bound to RBP, is filtered by glomeruli (Petersen *et al.* 1973), lipoprotein-bound retinyl esters would be too large for filtration and would escape this aspect of renal control. Recycling of retinol from the kidney following tubular reabsorption has been suggested (Petersen *et al.* 1973) and implied schematically (Underwood *et al.* 1979) but never demonstrated. This process has become more feasible following the recent finding of a retinol-esterifying enzyme in lamb kidney (Donoghue *et al.* 1981*a*).

The present findings confirmed and estimated the magnitude of several mechanisms that are involved in the regulation of plasma retinol concentration. Plasma retinol clearance increased with vitamin A intake, thereby tending to moderate any tendency of plasma retinol concentration to rise. In contrast, plasma retinyl ester clearance remained unchanged with increasing vitamin A intake, allowing plasma retinyl ester concentration to rise. Kidney storage, excretion and, perhaps, recycling of retinol and its derivatives also appeared to be related to plasma concentrations of retinol and retinyl esters. The present findings support the schematic model of vitamin A homeostasis (Underwood *et al.* 1979) and strengthen the proposed role of the kidney. An addition to that model is the indication of an adaptive faecal excretory pathway for retinol derivatives.

Schemes for retinol metabolism and homeostasis proposed previously (Underwood *et al.*

https://doi.org/10.1079/BJN19830093 Published online by Cambridge University Press

1979) have not been quantitatively simulated mathematical models. The significant empirical relationships listed in Table 7 should be of value in the development of such models in the future, although such a physiological model has not been proposed in this paper.

This study was supported by USPHS, NIH Grant HD11273 and BASF, Ludwigshafen.

REFERENCES

Bunnell, R. H., Rousseau, J. E. Jr, Eaton, H. D. & Beall, G. (1954). *Journal of Dairy Science* **37**, 1473–1476.
Deluca, H. F., Zile, M. H. & Neville, P. R. (1969). In *Lipid Chromatographic Analysis*, pp. 345–448 [G. V. Marinetti, editor]. New York: Marcel Dekker, Inc.
Donoghue, S., Johnson, K., Donawick, W. J. & Sklan, D. (1981*a*). *Journal of Dairy Science* **64**, 2419–2421.
Donoghue, S., Kronfeld, D. S., Berkowitz, S. J. & Copp, R. L. (1981*b*). *Journal of Nutrition* **111**, 365–374.
Donoghue, S., Kronfeld, D. S. & Ramberg, C. F. (1979). *Journal of Dairy Science* **62**, 326–332.
Folch, J., Lees, M. & Stanley, G. H. S. (1957). *Journal of Biological Chemistry* **226**, 497–509.
Futterman, S. & Andrews, J. S. (1964). *Journal of Biological Chemistry* **239**, 4077–4080.
Glover, J., Jay, C., Kershaw, R. C. & Reilly, P. E. B. (1976). *British Journal of Nutrition* **36**, 137–141.
Goodman, D. S. (1980). *Federation Proceedings* **39**, 2716–2722.
Gurpide, E. & Mann, J. (1970). *Journal of Clinical Endocrinology* **30**, 707–718.
Heller, J. (1975). *Journal of Biological Chemistry* **250**, 6549–6554.
Kanai, M., Raz, A. & Goodman, D. S. (1968). *Journal of Clinical Investigation* **47**, 2025–2044.
Kronfeld, D. S. & Ramberg, C. F. (1981). *Proceedings of the Nutrition Society* **40**, 129–138.
Mallia, A. K., Smith, J. E. & Goodman, D. S. (1975). *Journal of Lipid Research* **16**, 180–188.
National Research Council (1975). *Nutrient Requirements of Sheep*. Washington DC: National Academy of Sciences.
Neeld, J. B. Jr, & Pearson, W. N. (1963). *Journal of Nutrition* **79**, 454–462.
Petersen, P. A., Rask, L., Ostberg, L., Andersson, L., Kamwendo, F. & Pertoft, H. (1973). *Journal of Biological Chemistry* **248**, 4009–4022.
Reitz, P., Wiss, O. & Weber, F. (1974). *Vitamins and Hormones* **32**, 237–249.
Smith, F. R. & Goodman, D. S. (1971). *Journal of Clinical Investigation* **50**, 2426–2436.
Smith, F. R. & Goodman, D. S. (1976). *New England Journal of Medicine* **294**, 805–808.
Smith, J. E., Borek, C. & Goodman, D. S. (1978). *Cell*, **15**, 865–873.
Snedecor, G. W. & Cochran, W. G. (1967). *Statistical Methods*, p. 59. Ames, Iowa: Iowa State University Press.
Sreekrishna, K. & Cama, H. R. (1979). *Biochimica et Biophysica Acta* **581**, 136–141.
Underwood, B. A., Loerch, J. D. & Lewis, K. C. (1979). *Journal of Nutrition* **109**, 796–806.

Printed in Great Britain

https://doi.org/10.1079/BJN19830093 Published online by Cambridge University Press